and buffalo are roughly similar to those in bovine milk but those in human milk are very different, as will be discussed in section 4.13. β-Lg and α-la are synthesized in the mammary gland and are milk-specific; most of the other proteins in whey originate from blood or mammary tissue.

Since the 1930s, several methods have been developed for the isolation of homogeneous whey proteins, which have been crystallized (McKenzie, 1970, 1971). Today, homogeneous whey proteins are usually prepared by ion-exchange chromatography on DEAE cellulose.

4.7 β-Lactoglobulin

4.7.1 Occurrence and microheterogeneity

β-Lactoglobulin is a major protein in bovine milk, representing about 50% of total whey protein and 12% of the total protein of milk. It was among the first proteins to be crystallized, and since crystallizability was long considered to be a good criterion of homogeneity, β-lg, which is a typical globular protein, has been studied extensively and is very well characterized (reviewed by McKenzie, 1971; Hambling, McAlpine and Sawyer, 1992).

β-Lg is the principal whey protein (WP) in bovine, ovine, caprine and buffalo milks, although there are slight interspecies differences. Some years ago, it was believed that β-lg occurred only in the milks of ruminants but it is now known that a closely related protein occurs in the milks of the sow, mare, kangaroo, dolphin, manatee and other species. However, β-lg does not occur in human, rat, mouse or guinea-pig milks, in which α-la is the principal WP.

Four genetic variants of bovine β-lg, designated A, B, C and D, have been identified in bovine milk. A fifth variant, which contains carbohydrate, has been identified in the Australian breed, Droughtmaster. Further variants occur in the milks of yak and Bali cattle. Genetic polymorphism also occurs in ovine β-lg.

4.7.2 Amino acid composition

The amino acid composition of some β-lg variants is shown in Table 4.4. It is rich in sulphur amino acids which give it a high biological value of 110. It contains 2 moles of cystine and 1 mole of cysteine per monomer of 18 kDa. The cysteine is especially important since it reacts, following heat denaturation, with the disulphide of κ-casein and significantly affects rennet coagulation and the heat stability properties of milk; it is also responsible for the cooked flavour of heated milk. Some β-lgs, e.g. porcine, do not contain a free sulphydryl group. The isoionic point of bovine β-lgs is *c.* pH 5.2.

4.7.3 *Primary structure*

The amino acid sequence of bovine β-lg, consisting of 162 residues per monomer, is shown in Figure 4.22.

4.7.4 *Secondary structure*

β-Lg is a highly structured protein: optical rotary dispersion and circular dichroism measurements show that in the pH range 2–6, β-lg consists of 10–15% α-helix, 43% β-sheet and 47% unordered structure, including β-turns.

4.7.5 *Tertiary structure*

The tertiary structure of β-lg has been studied in considerable detail using X-ray crystallography. It has a very compact globular structure in which the β-sheets occur in a β-barrel-type structure or calyx (Figure 4.23). Each monomer exists almost as a sphere with a diameter of about 3.6 nm.

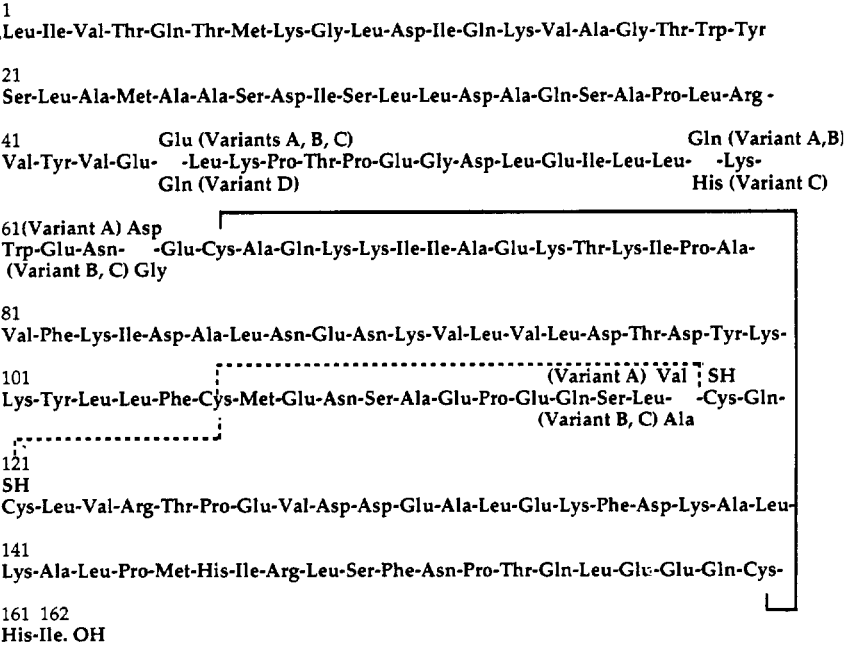


Figure 4.22 Amino acid sequence of bovine β-lactoglobulin, showing amino acid substitutions in genetic polymorphs and the intramolecular disulphide bonds (–, - - -) (from Swaisgood, 1982).

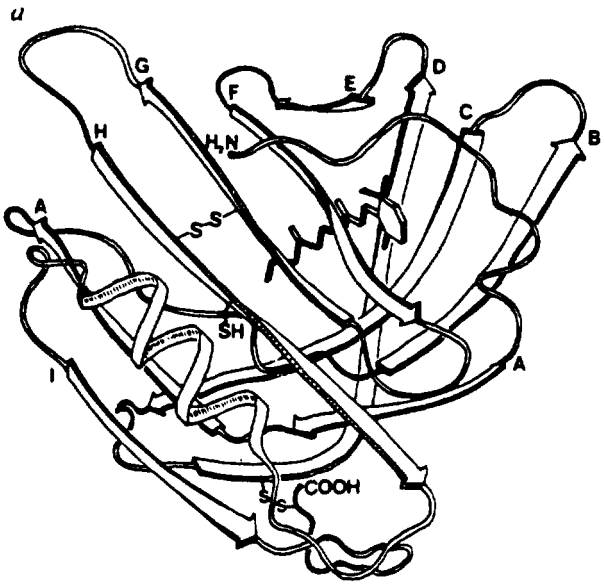


Figure 4.23 Schematic representation of the tertiary structure of bovine β-lactoglobulin, showing the binding of retinol; arrows indicate antiparallel β-sheet structures (from Papiz *et al.*, 1986).

4.7.6 Quaternary structure

β-Lg shows interesting association characteristics. Early work indicated that the monomeric molecular mass of bovine β-lg was 36 kDa but it was shown by Timasheff and co-workers that below pH 3.5, β-lg dissociates to monomers of 18 kDa. Between pH 5.5 and 7.5, all bovine β-lg variants form dimers of molecular mass 36 kDa but they do not form mixed dimers, i.e. a dimer consisting of A and B monomers, possibly because β-lg A and B contain valine and alanine, respectively, at position 178. Since valine is larger than alanine, it is suggested that the size difference is sufficient to prevent the proper fit for hydrophobic interaction. Porcine and other β-lgs that contain no free thiol do not form dimers; lack of a thiol group is probably not directly responsible for the failure to dimerize.

Between pH 3.5 and 5.2, especially at pH 4.6, bovine β-lg forms octamers of molecular mass 144 kDa. β-Lg A associates more strongly than β-lg B, possibly because it contains an additional aspartic acid instead of glycine (in B) per monomer; the additional Asp is capable of forming additional hydrogen bonds in the pH region where it is undissociated. β-Lg from Droughtmaster cattle, which has the same amino acid composition as bovine β-lg A but is a glycoprotein, fails to octamerize, presumably due to stearic hinderance by the carbohydrate moiety.

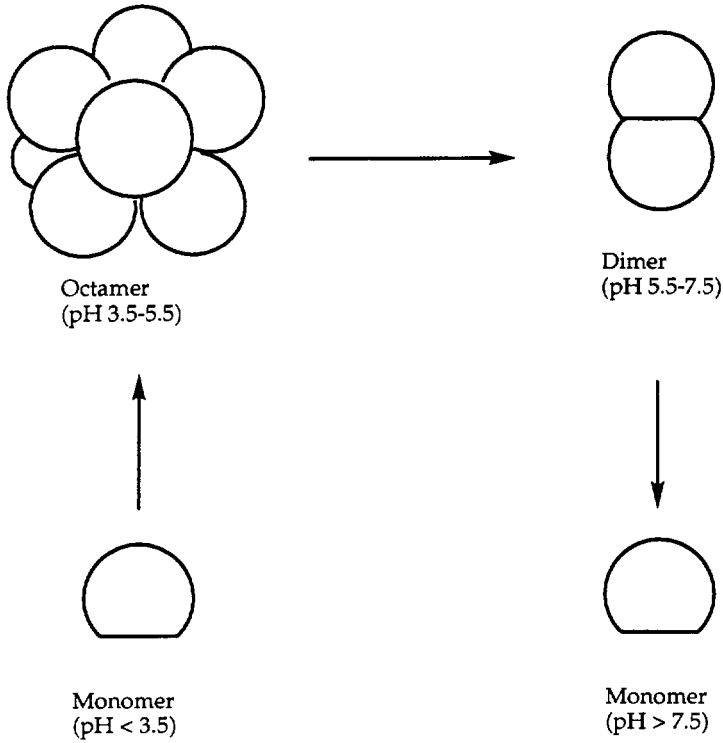


Figure 4.24 Effect of pH on the quaternary structure of β-lactoglobulin.

Above pH 7.5, bovine β-lg undergoes a conformational change (referred to as the N ⇄ R transition), dissociates to monomers and the thiol group becomes exposed and active and capable of sulphydryl–disulphide interchange. The association of β-lg is summarized in Figure 4.24.

4.7.7 Physiological function

Since the other principal whey proteins have a biological function, it has long been felt that β-lg might have a biological role; it appears that this role may be to act as a carrier for retinol (vitamin A). β-Lg can bind retinol in a hydrophobic pocket (see Figure 4.23), protect it from oxidation and transport it through the stomach to the small intestine where the retinol is transferred to a retinol-binding protein, which has a similar structure to β-lg. β-Lg is capable of binding many hydrophobic molecules and hence its ability to bind retinol may be incidental. Unanswered questions are how retinol is transferred from the core of the fat globules, where it occurs in milk, to β-lg and how humans and rodents have evolved without β-lg.

β-Lg also binds free fatty acids and thus it stimulates lipolysis (lipases are inhibited by free fatty acids); perhaps this is its physiological function. BSA also binds hydrophobic molecules, including fatty acids; perhaps BSA serves a similar function to β-lg in those species lacking β-lg.

4.7.8 Denaturation

Denaturation of whey proteins is of major technological significance and will be discussed in Chapter 9.

4.8 α-Lactalbumin

α-Lactalbumin (α-la) represents about 20% of the proteins of bovine whey (3.5% of total milk protein); it is the principal protein in human milk. It is a small protein with a molecular mass of *c.* 14 kDa. Recent reviews of the literature on this protein include Kronman (1989) and Brew and Grobler (1992).

4.8.1 Amino acid composition

The amino acid composition is shown in Table 4.4. α-La is relatively rich in tryptophan (four residues per mole). It is also rich in sulphur (1.9%) which is present in cystine (four intramolecular disulphides per mole) and methionine; it contains no cysteine (sulphydryl groups). The principal α-la's contain no phosphorus or carbohydrate, although some minor forms may contain either or both. The isoionic point is *c.* pH 4.8 and minimum solubility in 0.5 M NaCl is also at pH 4.8.

4.8.2 Genetic variants

The milk of Western cattle contains only α-la B but Zebu and Droughtmaster cattle secrete two variants, A and B. α-La A contains no arginine, the one Arg residue of α-la B being replaced by glutamic acid.

4.8.3 Primary structure

The primary structure of α-la is shown in Figure 4.25. There is considerable homology between the sequence of α-la and lysozymes from many sources. The primary structures of α-la and chicken egg white lysozyme are very similar. Out of a total of 123 residues in α-la, 54 are identical to corresponding residues in lysozyme and a further 23 residues are structurally similar (e.g. Ser/Thr, Asp/Glu).

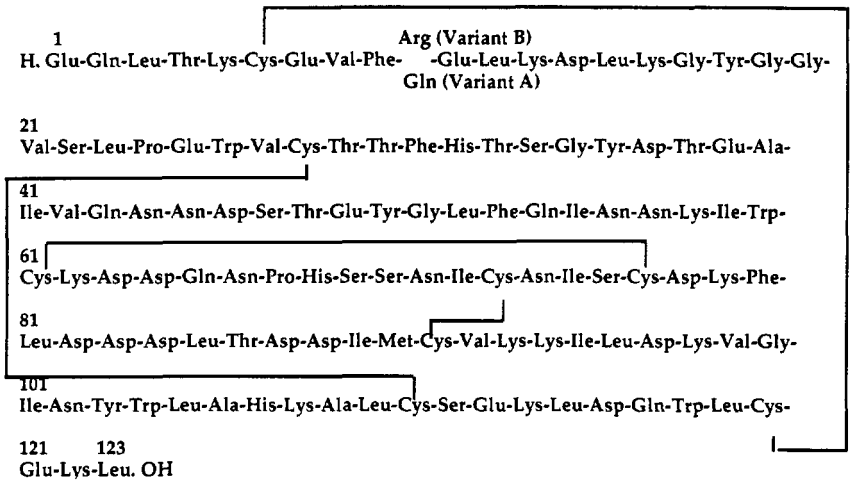


Figure 4.25 Amino acid sequence of α-lactalbumin showing intramolecular disulphide bonds (_) and amino acid substitutions in genetic polymorphs (from Brew and Grobler, 1992).

4.8.4 Secondary and tertiary structure

α-La is a compact globular protein, which exists in solution as a prolate ellipsoid with dimensions of 2.5 × 3.7 × 3.2 nm. It consists of 26% α-helix, 14% β-structure and 60% unordered structure. The metal binding and molecular conformational properties of α-la were discussed in detail by Kronman (1989). The tertiary structure of α-la is very similar to that of lysozyme. It has been difficult to crystallize bovine α-la in a form suitable for X-ray crystallography but work on the detailed structure is at an advanced stage (Brew and Grobler, 1992).

4.8.5 Quaternary structure

α-La associates under a variety of environmental conditions but the association process has not been well studied.

4.8.6 Other species

α-La has been isolated from several species, including the cow, sheep, goat, sow, human, buffalo, rat and guinea-pig. Some minor interspecies differences in the amino acid sequence and properties have been reported. The milks of sea mammals contain very little or no α-la.

4.8.7 *Biological function*

One of the most interesting characteristics of α-lactalbumin is its role in lactose synthesis:

$$\text{UDP-D-Galactose} + \text{D-glucose} \xrightarrow[\text{synthetase}]{\text{lactose}} \text{lactose} + \text{UDP}$$

Lactose synthetase, the enzyme which catalyses the final step in the biosynthesis of lactose, consists of two dissimilar protein subunits, A and B; the A protein is UDP-galactosyl transferase while the B protein is α-la. In the absence of B protein, the A protein acts as a non-specific galactosyl transferase, i.e. it transfers galactose from UDP-galactose to a range of acceptors, but in the presence of B protein it becomes highly specific and transfers galactose only to glucose to form lactose (K_M for glucose is reduced approximately 1000-fold). α-Lactalbumin is, therefore, a 'specifier protein' and its action represents a unique form of molecular control in biological reactions. α-La from the milks of many species are effective modifier proteins for the UDP-galactosyl transferase of bovine lactose synthetase. How it exercises its control is not understood, but it is suggested that the synthesis of lactose is controlled directly by α-lactalbumin which, in turn, is under hormonal control (Brew and Grobler, 1992). The concentration of lactose in milk is directly related to the concentration of α-la; milks of marine mammals, which contain no α-la, contain no lactose. Since lactose is the principal constituent in milk affecting osmotic pressure, its synthesis must be controlled rigorously and this is the presumed physiological role of α-la. Perhaps each molecule of α-la regulates lactose synthesis for a short period and is then discarded and replaced; while this is an expensive and wasteful use of an enzyme component, the rapid turnover affords a faster response should lactose synthesis need to be altered, as in mastitic infection, when the osmotic pressure of milk increases due to an influx of NaCl from the blood (Chapter 2).

4.8.8 *Metal binding and heat stability*

α-La is a metallo-protein; it binds one Ca^{2+} per mole in a pocket containing four Asp residues (Figure 4.26); these residues are highly conserved in all α-la's and in lysozyme. The Ca-containing protein is quite heat stable (it is the most heat stable whey protein) or more correctly, the protein renatures following heat denaturation (denaturation does occur at relatively low temperatures, as indicated by differential scanning calorimetry). When the pH is reduced to below about 5, the Asp residues become protonated and lose their ability to bind Ca^{2+}. The metal-free protein is denatured at quite low temperatures and does not renature on cooling; this characteristic has been exploited to isolate α-la from whey.

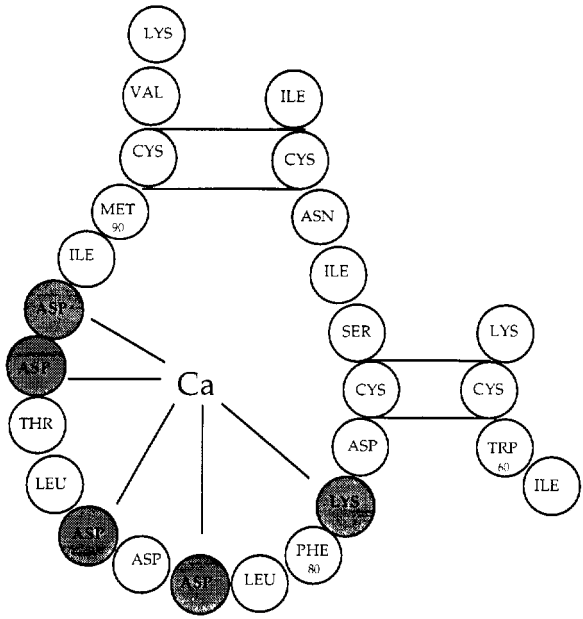


Figure 4.26 Calcium-binding loop in bovine α-lactalbumin (modified from Berliner *et al.*, 1991).

4.9 Blood serum albumin

Normal bovine milk contains a low level of blood serum albumin (BSA) ($0.1–0.4\,g\,l^{-1}$; 0.3–1.0% of total N), presumably as a result of leakage from blood. BSA is quite a large molecule (molecular mass *c.* 66 kDa; 582 amino acids); its amino acid sequence is known. The molecules contain 17 disulphides and one sulphydryl. All the disulphides involve cysteines that are relatively close together in the polypeptide chain, which is therefore organized in a series of relatively short loops, some of which are shorter than others (Figure 4.27). The molecule is elliptical in shape and is divided into three domains.

In blood, BSA serves various functions but it is probably of little significance in bovine milk, although it does bind metals and fatty acids; the latter characteristic may enable it to stimulate lipase activity.

4.10 Immunoglobulins (Ig)

Mature milk contains $0.6–1\,g\ Ig\,l^{-1}$ (*c.* 3% of total N) but colostrum contains up to $100\,g\,l^{-1}$, the level of which decreases rapidly postpartum (Figure 4.2).

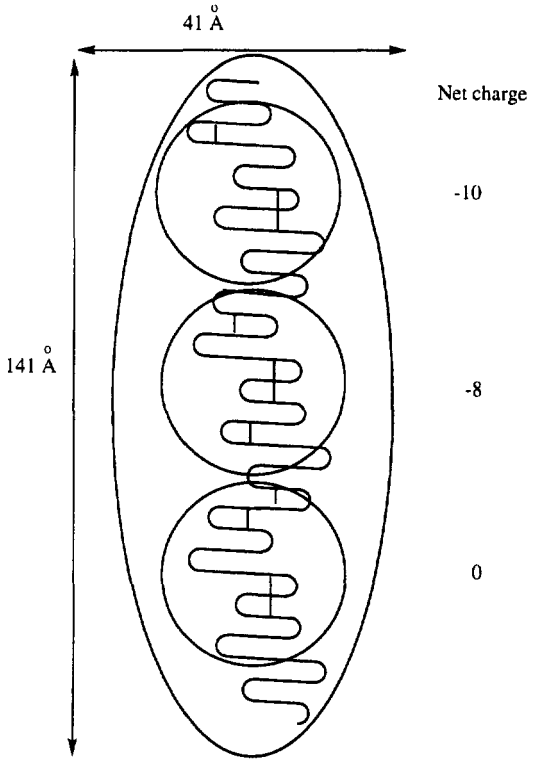


Figure 4.27 Model of the bovine serum albumin molecule.

Igs are very complex proteins which will not be reviewed here. Essentially, there are five classes of Ig: IgA, IgG, IgD, IgE and IgM. IgA, IgG and IgM are present in milk. These occur as subclasses, e.g. IgG occurs as IgG_1 and IgG_2. IgG consists of two long (heavy) and two shorter (light) polypeptide chains linked by disulphides (Figure 4.28). IgA consists of two such units (i.e. eight chains) linked together by secretory component (SC) and a junction (J) component, while IgM consists of five linked four-chain units (Figure 4.29). The heavy and light chains are specific to each type of Ig. For a review of immunoglobuins in milk, see Larson (1992).

The physiological function of Ig is to provide various types of immunity in the body. The principal Ig in bovine milk is IgG_1 while in human milk it is IgA. The calf (and the young of other ruminants) is born without Ig in its blood serum and hence is very susceptible to infection. However, the intestine of the calf is permeable to large molecules for about 3 days postpartum and therefore Ig is absorbed intact and active from its mother's milk; Igs from colostrum appear in the calves blood within about 3 h of

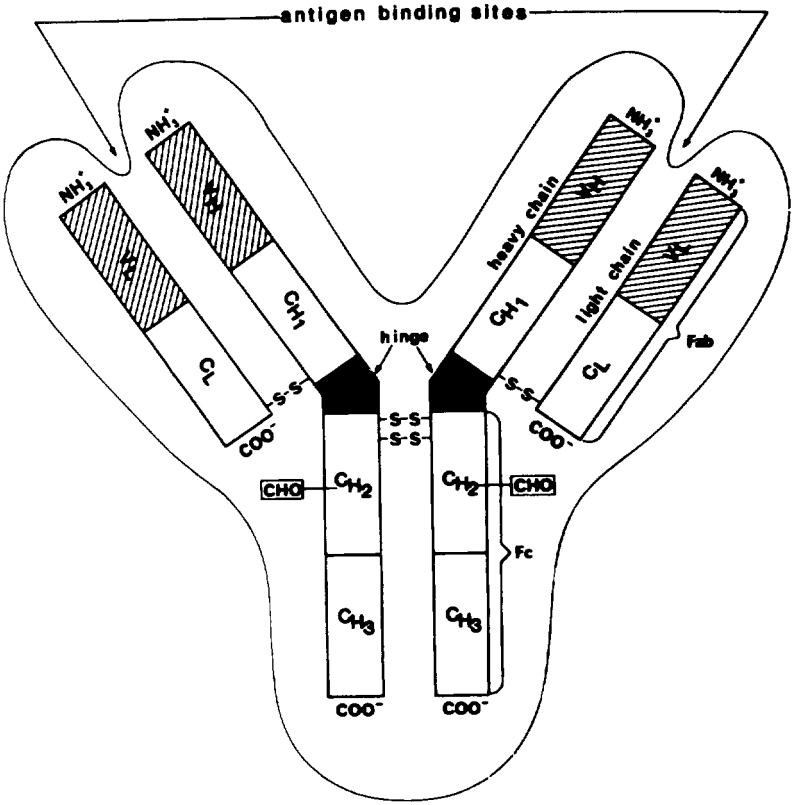


Figure 4.28 Model of the basic 7S immunoglobulin (Ig) molecule showing two heavy and two light chains joined by disulphide bonds: V, variable region; C, constant region; L, light chain; H, heavy chain; 1, 2 and 3 subscripts refer to the three constant regions of the heavy chains; CHO, carbohydrate groups; Fab refers to the (top) antigen-specific portion of the Ig molecule; Fc refers to the cell-binding effector portion of the Ig molecule (from Larson, 1992).

suckling and persist for about 3 months, although the calf is able to synthesize its own Ig within about 2 weeks. It is, therefore, essential that a calf should receive colostrum within a few hours of birth, otherwise it will probably die. The human baby obtains Ig *in utero* and hence, unlike the calf, is not as dependent on Ig from milk (in fact its intestine is impermeable to Ig). However, the Ig in human colostrum is beneficial to the baby, e.g. it reduces the risk of intestinal infections.

As regards the type and function of Ig in colostrum, mammals fall into three groups (Figure 4.30) – those like the cow (i.e. other ruminants), those like the human, and some, e.g. the horse, with features of the other two groups (Larson, 1992).

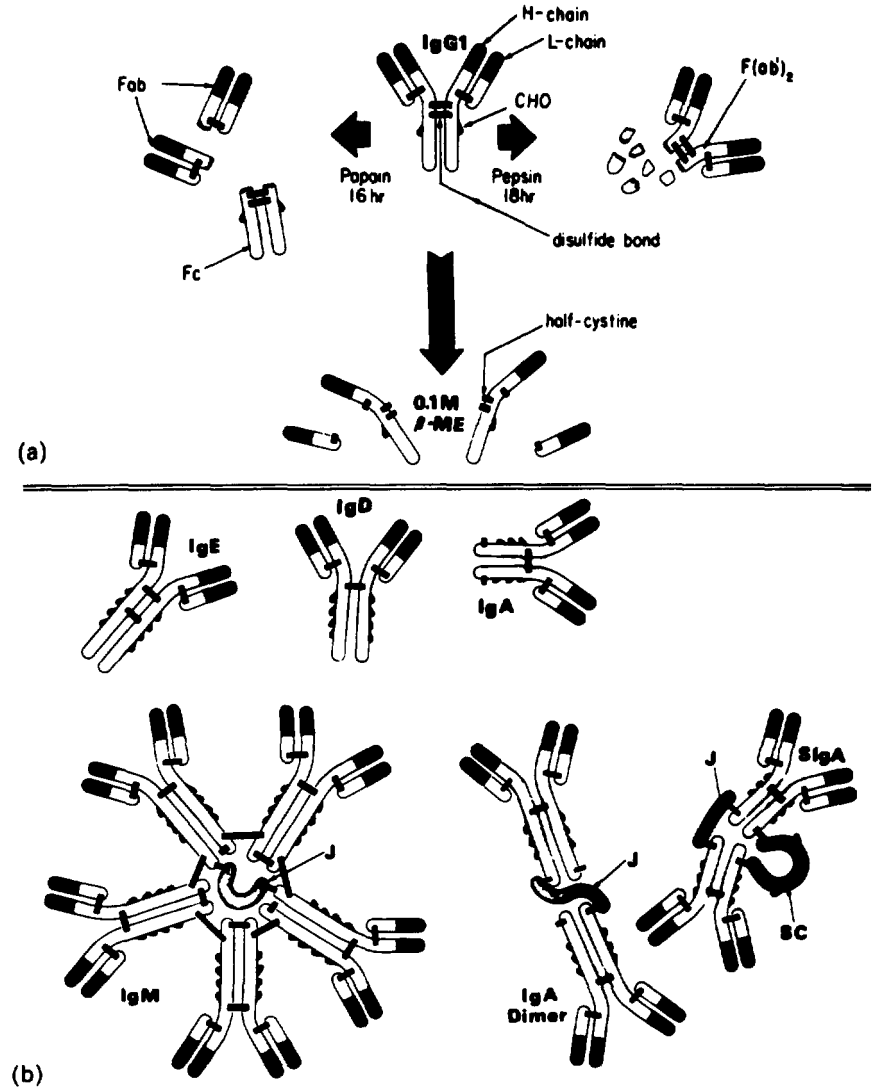


Figure 4.29 Models of IgG, IgA, IgD, IgE and IgM. (a) Structural model of IgG_1 before and after fragmentation by pepsin and papain and reduction with a sulphydryl reagent. Solid black chain portion = variable regions; light chain portion = constant regions. Small black lines represent disulphide and half-cystine (—SH) groups. Small black dots in Fc regions represent attached carbohydrate groups. The various parts of the model are labelled. (b) The structure of four classes of immunoglobulins are shown with monomeric IgA, dimeric IgA and secretory IgA. Location of the J-chain, secretory component (SC) and carbohydrate is approximate. (From Larson, 1992.)

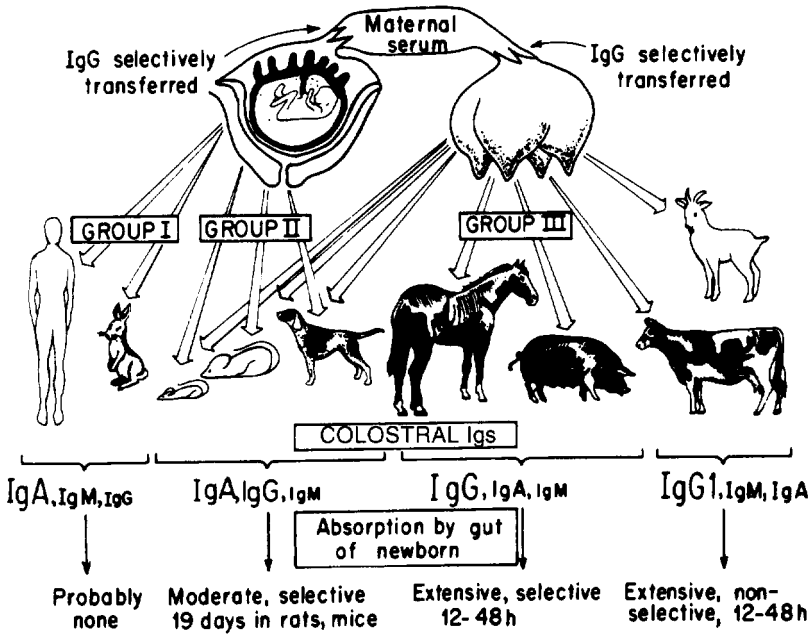


Figure 4.30 Transfer of maternal immunoglobulins to the foetus and neonate of representative mammalian species. Group I species transfer Ig *in utero* before birth. Group II species transfer Ig both *in utero* before birth and via colostrum after birth. Group III species transfer Ig only via colostrum after birth. The size of the immunoglobulin notation (IgA, IgM, IgG, IgG_1) indicates the relative percentage composition of the immunoglobulins in colostrum. Species in group II may have IgG as the predominant Ig in colostrum. Significant IgG_2 also may be present in the colostrum of some Group III species. The relative absorption of immunoglobulins in the gut of the neonate is also shown. (From Larson, 1992.)

4.11 Minor milk proteins

Milk contains numerous minor proteins, including perhaps 60 indigenous enzymes, some of which, e.g. lipase, proteinase, phosphatases and lactoperoxidase, are technologically important (Chapter 8). Most of the minor proteins have biological functions and probably play very significant roles (section 4.16).

4.12 Non-protein nitrogen

Nitrogen soluble in 12% TCA is referred to as non-protein nitrogen (NPN), of which milk contains 250–300 mg l^{-1}, i.e. 5–6% of total milk nitrogen. The NPN is a very heterogeneous fraction (Table 4.7).

Table 4.7 Non-protein nitrogen of cow's milk

Component	N (mg l^{-1})
Ammonia	6.7
Urea	83.8
Creatinine	4.9
Creatine	39.3
Uric acid	22.8
α-Amino nitrogen	37.4
Unaccounted	88.1

The 'unaccounted' N includes some phospholipids, amino sugars, nucleotides, hippuric acid and orotic acid. The α-amino N includes free amino acids and small peptides; almost a complete range of amino acids, including ornithine, has been identified in milk, but glutamic acid predominates.

All the components of NPN are present in blood, from which they are probably transferred into milk. The technological and nutritional significance of NPN is not known but the amino acids are likely to be important for the nutrition of starter micro-organisms, especially of weakly proteolytic strains. Urea, which is the principal component of the NPN (6 mmol l^{-1}), is strongly correlated with the heat stability of milk; the urea content of milk from cows on pasture is twice as high as that from cows on dry feed and hence the heat stability of the former is considerably higher. The level of NPN in freshly drawn milk is fairly constant but it does increase on ageing, especially if significant growth of psychrophilic bacteria, which may be strongly proteolytic, occurs.

4.13 Comparison of human and bovine milks

As mentioned in section 4.1, milk is species-specific, designed to meet the nutritional and physiological requirements of the young of that species. There are about 4300 species of mammal but the milks of only about 170 have been analysed, and data for only about 40 of these are considered reliable. Not surprisingly, human and bovine milks have been studied most intensely. In many respects, the milks of these two species are at the opposite ends of a spectrum. It will be apparent from the foregoing discussion that the proteins in human and bovine milks differ markedly, both qualitatively and quantitatively. Some of the more important differences are summarized in Table 4.8. At least some of these differences are probably nutritionally and physiologically important. It is perhaps ironic that human babies are the least likely of all species to receive the milk intended for them.

Table 4.8 Some important differences between bovine and human milk proteins

Constituent	Bovine	Human
Protein concentration (%)	3.5	1
Casein : NCN	80 : 20	40 : 60
Casein types	$\alpha_{s1} = \beta > \alpha_{s2} = \kappa$	$\beta > \kappa > \alpha_{s1}$[a]
β-Lactoglobulin	50% of NCN	None
Lactotransferrin	Trace	20% of total N
Lysozyme	Trace	Very high (6% TN; 3000 × bovine)
Glycopeptides	Trace	High
NPN (as % TN)	3	20
Taurine	Trace	High
Lactoperoxidase	High	Low
Immunoglobulins (Ig) (colostrum)	Very high	Lower
Ig type	$IgG_1 > IgG_2 > IgA$	$IgA > IgG_1 > IgG_2$

NCN, Non-casein nitrogen; NPN, non-protein nitrogen; TN, total nitrogen.
[a]A low level of α_{s1}-casein has recently been demonstrated in human milk (Martin *et al.*, 1996).

4.14 Synthesis and secretion of milk proteins

The synthesis and secretion of milk proteins have been studied in considerable detail; reviews include Mercier and Gaye (1983), Mepham (1987) and Mepham *et al.* (1992).

4.14.1 Sources of amino acids

Arteriovenous (AV) difference studies and mammary blood flow measurements (Chapter 1) have shown that in both ruminants and non-ruminants, amino acids for milk protein synthesis are obtained ultimately from blood plasma but that some interconversions occur. The amino acids can be divided into two major groups:

1. those for which uptake from blood is adequate to supply the requirements for milk protein synthesis and which correspond roughly to the essential amino acids (EAA); and
2. those for which uptake is inadequate, i.e. the non-essential amino acids (NEAA).

Studies involving AV difference measurements, isotopes and perfused gland preparations indicate that the EAA may be subdivided into those for which uptake from blood and output in milk proteins are almost exactly balanced (Group I) and those for which uptake significantly exceeds output (Group II). Group II amino acids are metabolized in the mammary gland and provide amino groups, via transamination, for the biosynthesis of those

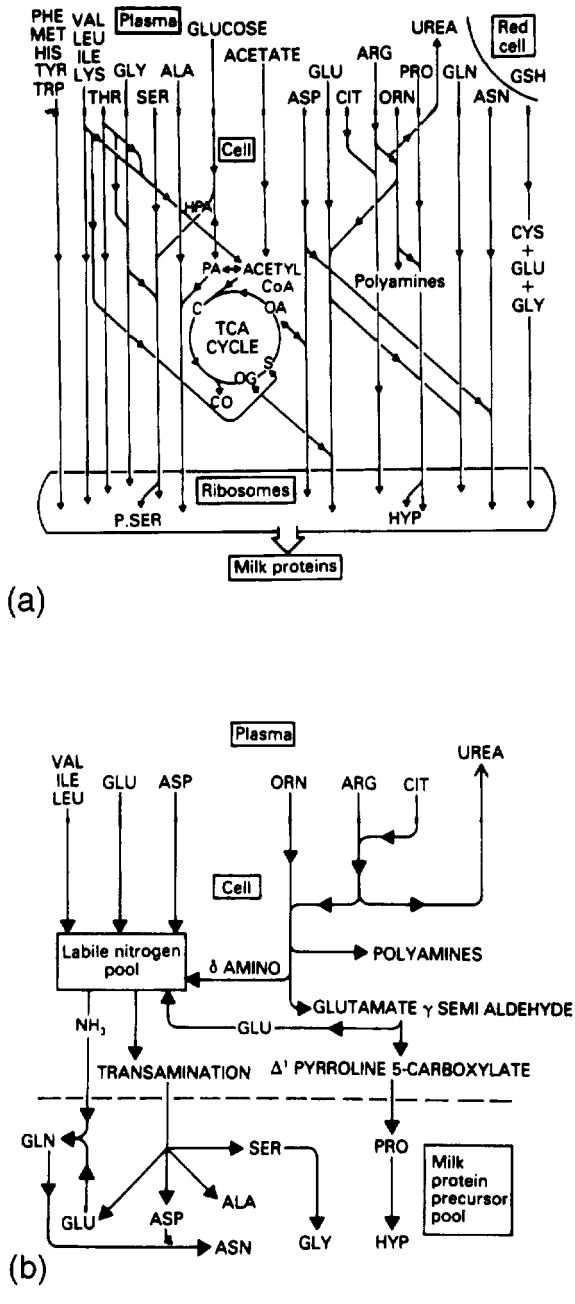


Figure 4.31 Summary diagrams of amino acid metabolism in mammary tissue. (a) Amino acid carbon interrelationships, (b) amino acid nitrogen interrelationships (from Mepham, Gaye and Mercier, 1982).

amino acids for which uptake from blood is inadequate (Group III), their carbon skeletons are oxidized to CO_2. Considered as a whole, total uptake and output of amino acids from blood are the major, or sole, precursors of the milk-specific proteins (i.e. the caseins, β-lactoglobulin and α-lactalbumin).

- Group I amino acids: methionine, phenylalanine, tyrosine, histidine and tryptophan.
- Group II amino acids: valine, leucine, isoleucine, lysine, arginine and threonine.
- Group III amino acids: aspartic acid, glutamic acid, glycine, alanine, serine, cysteine/cystine, proline.

The interrelationships between the carbon and nitrogen of amino acids are summarized in Figure 4.31.

4.14.2 Amino acid transport into the mammary cell

Since the cell membranes are composed predominantly of lipids, amino acids (which are hydrophilic) cannot enter by diffusion and are transported by special carrier systems. In the case of mammary cells, the carrier system(s) has not yet been elucidated.

4.14.3 Synthesis of milk proteins

Synthesis of the major milk proteins occurs in the mammary gland; the principal exceptions are serum albumin and some of the immunoglobulins, which are transferred from the blood. Polymerization of the amino acids occurs on ribosomes fixed on the rough endoplasmic reticulum of the secretory cells, apparently by a method common to all cells.

The primary blueprint for the amino acid sequence of proteins is contained in deoxyribonucleic acid (DNA) within the cell nucleus. The requisite information is transcribed in the nucleus to ribonucleic acid (RNA) of which there are three types: messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). These are transferred to the cytoplasm where each plays a specific role in protein synthesis.

Protein synthesis actually takes place in the ribosomes of the rough endoplasmic reticulum (RER) which contain rRNA. There is a specific tRNA for each amino acid, with which it forms an acyl complex:

$$\text{Amino acid} + \text{tRNA} + \text{ATP} \xrightarrow[\text{amino acyl-tRNA synthetase}]{Mg^{2+}} \text{amino acyl-tRNA} + \text{AMP} + \text{PPi}$$

There is a specific amino acyl-tRNA synthetase for each amino acid; these enzymes have two specific binding sites, one for the amino acid and the

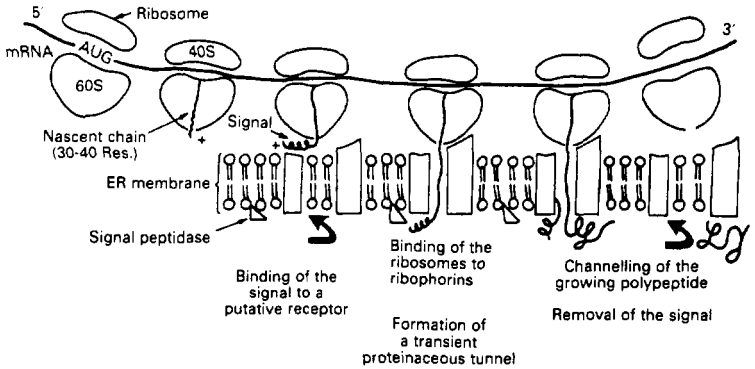
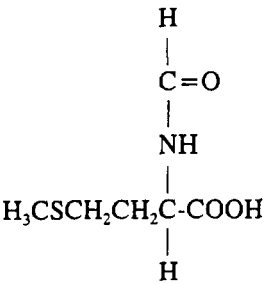


Figure 4.32 Schematic representation of ribosomes attached to mRNA showing the growing polypeptides and a proposed mechanism for cotranslational crossing of the RER membrane (from Mercier and Gaye, 1983).

second for the appropriate tRNA. The specificity of the tRNAs is determined by the sequence of the anticodon which recognizes and hydrogen bonds with the complementary codon of the mRNA. Interaction between the tRNA and the appropriate amino acid occurs in the cytoplasm but the remaining reactions in protein synthesis occur in the ribosomes, which are complex structures of rRNA and a number of proteins (including enzymes, initiators and controlling factors). The ribosomes of animal cells have diameters of about 22 nm and a sedimentation coefficient of 80S; they consist of two principal subunits: 60S and 40S. mRNA passes through a groove or tunnel between the 60S and 40S subunits; while in the groove, mRNA is protected from the action of ribonuclease (Figure 4.32).

The information for the amino acid sequence is contained in the mRNA. Synthesis commences at the correct codon of the mRNA because a special amino acid derivative, *N*-formyl methionine:



is bound to a specific special codon and it forms the temporary *N*-terminal residue of the protein; *N*-formyl methionine is later hydrolysed off, together

with a short hydrophobic signal peptide, exposing the permanent *N*-terminal residue. The acyl amino acid-tRNA is bound to the mRNA just outside the ribosome by becoming attached to its corresponding codon; presumably, a full range of amino acid-tRNAs are available in the environment but only the tRNA with the appropriate anticodon is bound. GTP and a number of specific cytoplasmic protein factors are required for binding.

In the ribosome, the amino group of the newly bound amino acid reacts through nucleophilic substitution with the C-terminal carbonyl carbon of the existing peptide, and in the process the peptide is transferred to the newly bound tRNA, releasing the tRNA just vacated. Condensation is catalysed by peptidyl transferase, which is part of the ribosomal subunit.

For the next cycle, a new acyl amino acid–tRNA is bound to the mRNA, the ribosome tracks along the mRNA and the emptied tRNA is ejected. As the polypeptide is elongated it assumes its secondary and tertiary structure (Figure 4.32).

The factors controlling termination of synthesis are poorly understood; it is known that there is a specific ribosomally bound **protein release factor** which promotes the hydrolysis of the linkage between the tRNA and the newly formed protein.

A strand of mRNA is long enough to accommodate several ribosomes along its length, e.g. the mRNA for haemoglobin (150 amino acid residues/molecule) contains 450 nucleotides and is *c.* 150 nm long; since each ribosome is about 20 nm in diameter, 5–6 ribosomes can be accommodated. The ribosomes are connected to each other by the mRNA strand, forming a polysome (polyribosome) which can be isolated intact if adequate care is taken. Each ribosome in a polysome is at a different stage in the synthesis of a protein molecule, thereby utilizing the mRNA more efficiently (Figure 4.32).

Milk proteins are destined to be exported from the cell. Like other exported proteins, translocation through cell membranes is facilitated by a **signal sequence**, a sequence of 15–29 amino acids at the amino terminal of the growing polypeptide chain. This sequence causes the ribosome to bind to the ER membrane, in which a 'channel' forms, allowing the growing chain to enter the ER lumen (Figure 4.32). Subsequently, the signal sequence is cleaved from the polypeptide by **signal peptidase**, an enzyme located on the luminal side of the ER membrane.

4.14.4 Modifications of the polypeptide chain

In addition to proteolytic processing (i.e. removal of the signal peptide sequence), the polypeptide is subject to other covalent modifications: *N*- and *O*-glycosylation and *O*-phosphorylation. After synthesis and transportation across the ER lumen, the proteins pass to the Golgi apparatus and thence,

via secretory vesicles, to the apical membrane. Covalent modification must therefore occur at some point(s) along this route. Such modifications may be either **co-translational** (occurring when chain elongation is in progress) or **post-translational**. Proteolytic cleavage of the signal peptide is co-translational and this seems to be the case also for *N*-glycosylation, in which dolichol-linked oligosaccharides are enzymatically transferred to asparaginyl residues of the chain when these are present in the sequence code, Asn–X–Thr/Ser (where X is any amino acid except proline). The large oligosaccharide component may be 'trimmed' as it traverses the secretory pathway. Formation of disulphide bonds between adjacent sections of the chain, or between adjacent chains (as in κ-casein), may also be partly co-translational.

By contrast, *O*-glycosylation and *O*-phosphorylation appear to be post-translational events. Glycosylation of the principal milk-specific glycoprotein, casein, is believed to be effected by membrane-bound glycosyltransferases (three such enzymes have been described) located in the Golgi apparatus. *O*-Phosphorylation involves transfer of the γ-phosphate of ATP to serine (or, less frequently, threonine) residues, occurring in the sequence, Ser/Thr–X–A (where X is any amino acid residue and A is an acidic residue, such as aspartic or glutamic acid or a phosphorylated amino acid). Phosphorylation is effected by **casein kinases** which are located chiefly in the Golgi membranes. In addition to the correct triplets, the local conformation of the protein is also important for phosphorylation of Ser since not all serines in caseins in the correct sequence are phosphorylated. Some serine residues in β-lg occur in a Ser–X–A sequence but are not phosphorylated, probably due to extensive folding of this protein.

The Golgi complex is also the locus of casein micelle formation. In association with calcium, which is actively accumulated by Golgi vesicles, the polypeptide chains associate to form submicelles, and then micelles, prior to secretion.

4.14.5 Structure and expression of milk protein genes

The structure, organization and expression of milk protein genes are now understood in considerable detail. This subject is considered to be outside the scope of this book and the interested reader is referred to Mepham *et al.* (1992). Such knowledge permits the genetic engineering of milk proteins with respect to the transfer of genes from one species to another, the overexpression of a particular desirable protein(s), the elimination of certain undesirable proteins, changing the amino acid sequence by point mutations to modify the functional properties of the protein or transfer of a milk protein gene to a plant or microbial host. This topic is also considered to be outside the scope of this text and the interested reader is referred to Richardson *et al.* (1992).

4.14.6 Secretion of milk-specific proteins

Following synthesis in the ribosomes and vectorization into the ER lumen, the polypeptides are transferred to Golgi lumina. The route of transfer from ER to Golgi has not been established with certainty. It is possible that lumina of the ER and Golgi apparatus are connected, or that small vesicles bud from the ER and subsequently fuse with the Golgi membranes. In either case, casein molecules aggregate in the Golgi cisternal lumina in the form of micelles.

Lumina at the nuclear face of the Golgi apparatus (Figure 4.33) are termed *cis* cisternae; those at the apical face *trans* cisternae. Proteins appear to enter the complex at the *cis* face and progress, undergoing post-translational modification, towards the *trans* face. Transfer between adjacent Golgi cisternae is thought to be achieved by budding and subsequent fusion of vesicles.

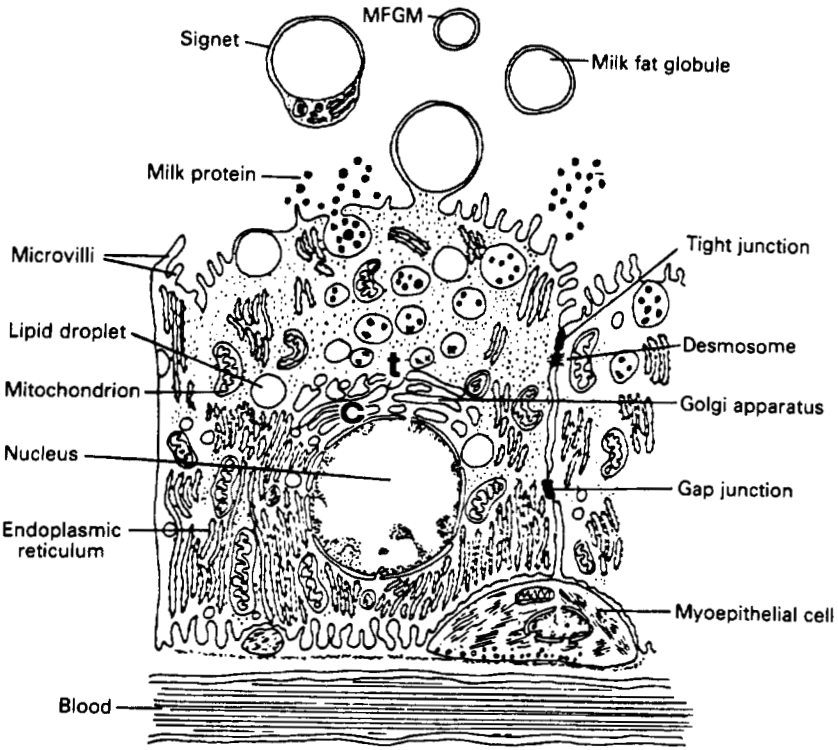


Figure 4.33 Schematic representation of a mammary secretory cell as interpreted from electron micrographs; *c*, *cis* face of Golgi apparatus; *t*, *trans* face (from Mepham, 1987).

In the apical cytosol there are numerous protein-containing secretory vesicles (Figure 4.33). EM studies suggest that they move to the apical plasmalemma and fuse with it, releasing their contents by **exocytosis.** Current ideas on intracellular transport of vesicles suggest participation of cytoskeletal elements–microtubules and microfilaments. In mammary cells, these structures are orientated from the basal to the apical membrane, suggesting that they may act as 'guides' for vesicular movement. Alternatively, vesicle transport may involve simple physical displacement as new vesicles bud from the Golgi complex, or an 'electrophoretic' process, dependent on a transcellular potential gradient.

Secretory vesicles seem to become attached to the cytoplasmic face of the apical plasmalemma. The vesicles have a distinctive coat on their outer surface which appears to react with appropriate receptors on the apical membrane, forming a series of regularly spaced bridges. Presumably, these bridges, and the contiguous vesicle and apical membrane material, are subsequently eliminated and the vesicular contents released, but the process seems to be very rapid and it has proved difficult to visualize the details of the sequence by EM. However, secretory vesicle membrane becomes incorporated, however briefly, into the apical membrane as a consequence of exocytosis.

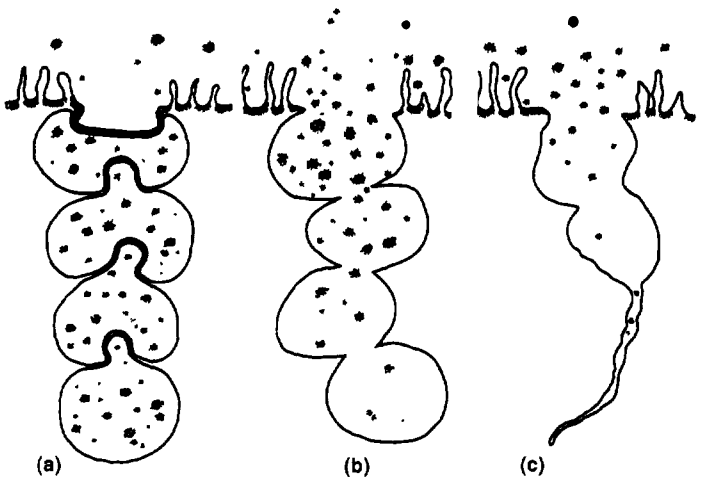


Figure 4.34 Schematic representation of one apparent mechanism for exocytotic release of secretory vesicle contents. (a) Vesicles assemble into a chain through ball-and-socket interaction. The exit vesicle interacts with apical plasma membrane via a vesicle depression. (b) Linked vesicles fuse together, apparently by disintegration of membrane in areas of fusion, resulting in the formation of a continuum with the alveolar lumen. (c) Emptying of the vesicular chain appears to result in collapse and subsequent fragmentation of the membrane. (From Keenan and Dylewski, 1985.)

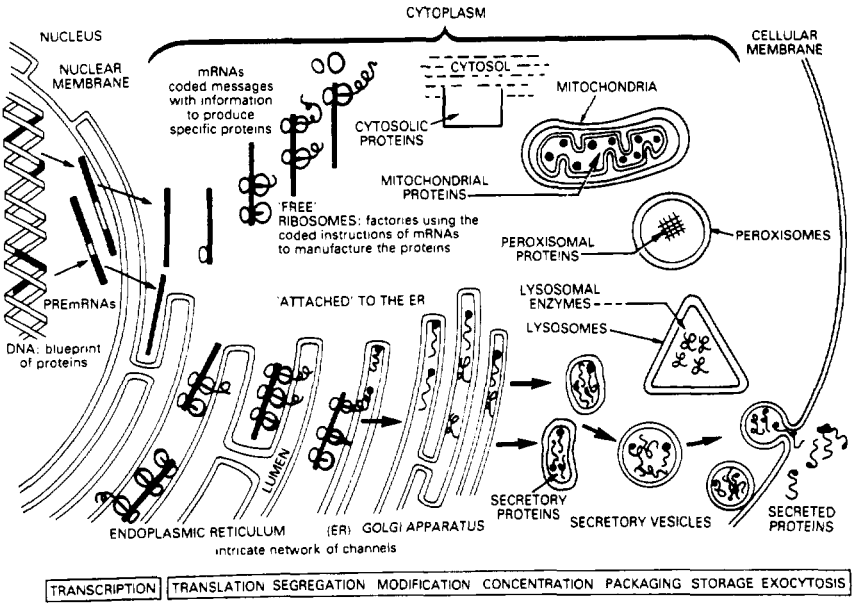


Figure 4.35 Schematic representation of the intracellular transport of proteins in mammary cells (from Mepham, Gaye and Mercier, 1982).

Alternatively, protein granules are transported through the lumina of a contiguous sequence of vesicles, so that only the most apical vesicle fuses with the apical membrane (Figure 4.34). The process has been called **compound exocytosis**.

Thus, the synthesis and secretion of milk proteins involves eight steps: transcription, translation, segregation, modification, concentration, packaging, storage and exocytosis, as summarized schematically in Figure 4.35.

4.14.7 Secretion of immunoglobulins

Interspecies differences in the relative importance of colostral Igs are discussed in section 4.10. The IgG of bovine colostrum is derived exclusively from blood plasma. It is presumed that cellular uptake involves binding of IgG molecules, via the Fc fragment (Figure 4.28), to receptors situated in the basal membranes; just prior to parturition, there is a sharp increase in the number of such receptors showing a high affinity for IgG_1, which is selectively transported into bovine colostrum. The intracellular transport route has not been described with any degree of certainty, but the most

likely scheme appears to involve vesicular transport, followed by exocytosis at the apical membrane.

IgA in colostrum is derived partly from intramammary synthesis and partly by accumulation in the gland after being transported in the blood from other sites of synthesis. In either case, IgA molecules are transported into the secretory cells across the basal membrane by means of a large, membrane-bound form of secretory component, which acts as a recognition site. It is presumed that, following **endocytosis**, the sIgA complex (Figure 4.29) is transported to the apical membrane of the secretory cell where, following cleavage of a portion of the complex, the mature sIgA complex is secreted by exocytosis.

4.15 Functional milk proteins

The term 'Functional Properties of Proteins' in relation to foods refers to those physicochemical properties of a protein which affect the functionality of the food, i.e. its texture (rheology), colour, flavour, water sorption/binding and stability. Probably the most important physicochemical properties are solubility, hydration, rheology, surface activity and gelation, the relative importance of which depends on the food in question; these properties are, at least to some extent, interdependent.

The physical properties of many foods, especially those of animal origin, are determined primarily by their constituent proteins, but those properties are not the subject of this section. Rather, we are concerned with isolated, more or less pure, proteins which are added to foods for specific purposes. The importance of such proteins has increased greatly in recent years, partly because suitable technology for the production of such proteins on a commercial scale has been developed and partly because a market for functional proteins has been created through the growth of fabricated foods, i.e. foods manufactured from more or less pure ingredients (proteins, fats/oils, sugars/polysaccharides, flavours, colours). Perhaps one should view the subject the other way round, i.e. fabricated foods developed because suitable functional proteins were available. Some functional proteins have been used in food applications for a very long time, e.g. egg white in various types of foamed products or gelatine in gelled products. The principal functional food proteins are derived from milk (caseins and whey proteins) or soybeans; other important sources are egg white, blood, connective tissue (gelatine) and wheat (gluten).

Probably because of the ease with which casein can be produced from skim milk, essentially free of lipids, lactose and salts, by rennet or isoelectric coagulation and washing of the curd, acid and rennet caseins have been produced commercially since the beginning of this century. However, until relatively recently, they were used for industrial applications, e.g. in glues,

plastics, fibres or dye-binders for paper glazing. Although some casein is still used for industrial applications, at least 80% of world production is now used in foods. This change has occurred partly because cheaper and possibly better materials have replaced casein for industrial applications while growth in the production of fabricated foods has created a demand for functional proteins at higher prices than those available for industrial-grade products. Obviously, the production of a food-grade protein requires better hygienic standards than industrial proteins; the pioneering work in this area was done mainly in Australia and to a lesser extent in New Zealand in the 1960s.

Although heat-denatured whey protein, referred to as lactalbumin, has been available for many years for food applications, it was of little significance, mainly because the product is insoluble and therefore has limited functionality. The commercial production of functional whey protein became possible with the development of ultrafiltration in the 1960s. Whey protein concentrates (WPCs) produced by ultrafiltration are now of major commercial importance, with many specific food applications. Superior whey protein products (whey protein isolates, WPI) are being produced on a limited scale by chromatography, although their substantially higher cost has limited their production.

As discussed in section 4.16, many of the whey proteins have interesting biological and physical properties. It is now possible to isolate individual whey proteins on a commercial scale in a relatively pure form; it is likely that in the immediate future such purified whey proteins will be readily available for specific applications.

4.15.1 Industrial production of caseins

There are two principal established methods for the production of casein on an industrial scale: isoelectric precipitation and enzymatic (rennet) coagulation. There are a number of comprehensive reviews on the subject (e.g. Muller, 1982; Fox, 1989; Mulvihill, 1992; Fox and Mulvihill, 1992) which should be consulted for references.

Acid casein is produced from skim milk by direct acidification, usually with HCl, or by fermentation with a *Lactococcus* culture, to *c.* pH 4.6. The curds/whey are cooked to about 50°C, separated using inclined perforated screens or decanting centrifuges, washed thoroughly with water (usually in counter-flow mode), dewatered by pressing, dried (fluidized bed, attrition or ring dryers) and milled. A flow diagram of the process and a line diagram of the plant are shown in Figures 4.36 and 4.37.

Acid casein is insoluble in water but soluble caseinate can be formed by dispersing the casein in water and adjusting the pH to 6.5–7.0 with NaOH (usually), KOH, $Ca(OH)_2$ or NH_3 to produce sodium, potassium, calcium or ammonium caseinate, respectively (Figure 4.38). The caseinates are

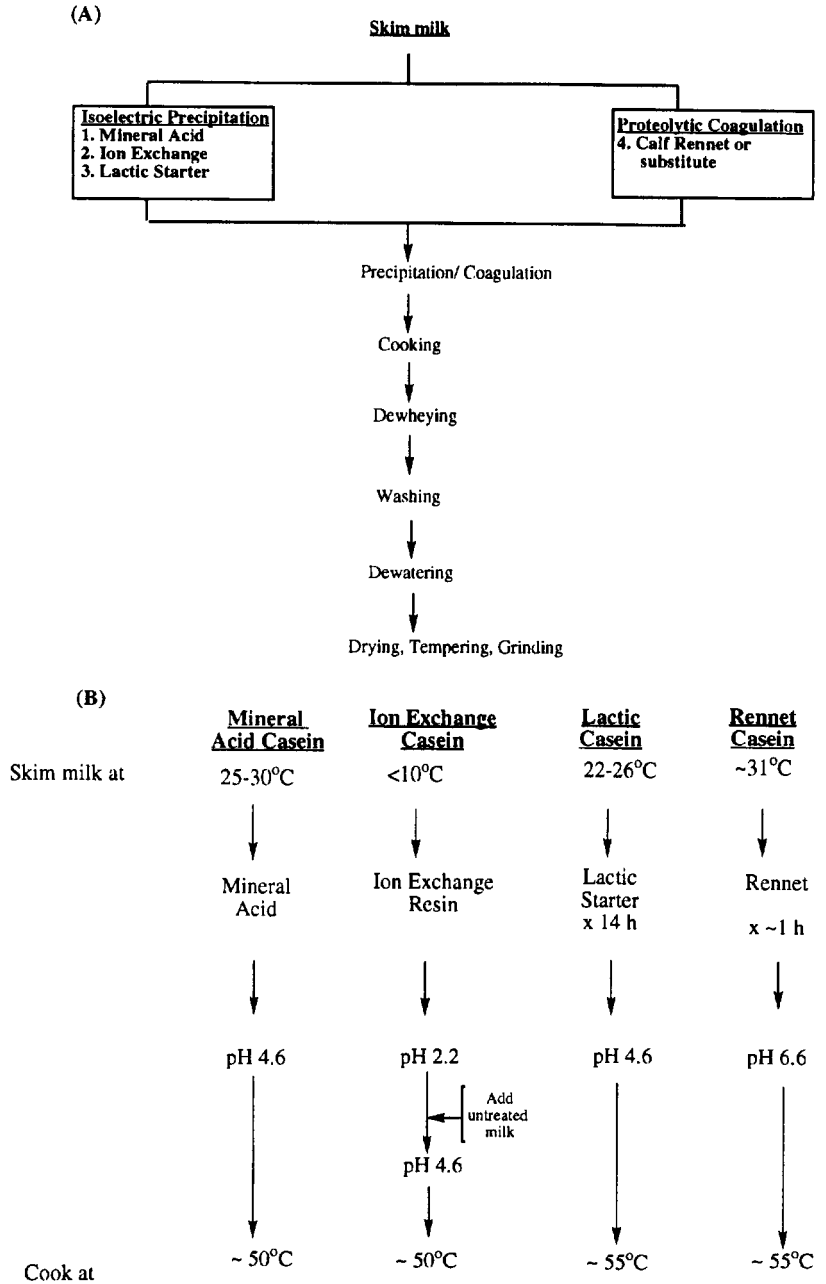


Figure 4.36 (a) Line diagram of industrial processes for the manufacture of acid and rennet casein. The conditions (time, temperature and pH) of precipitation are shown in (b). (Modified from Mulvihill, 1992.)

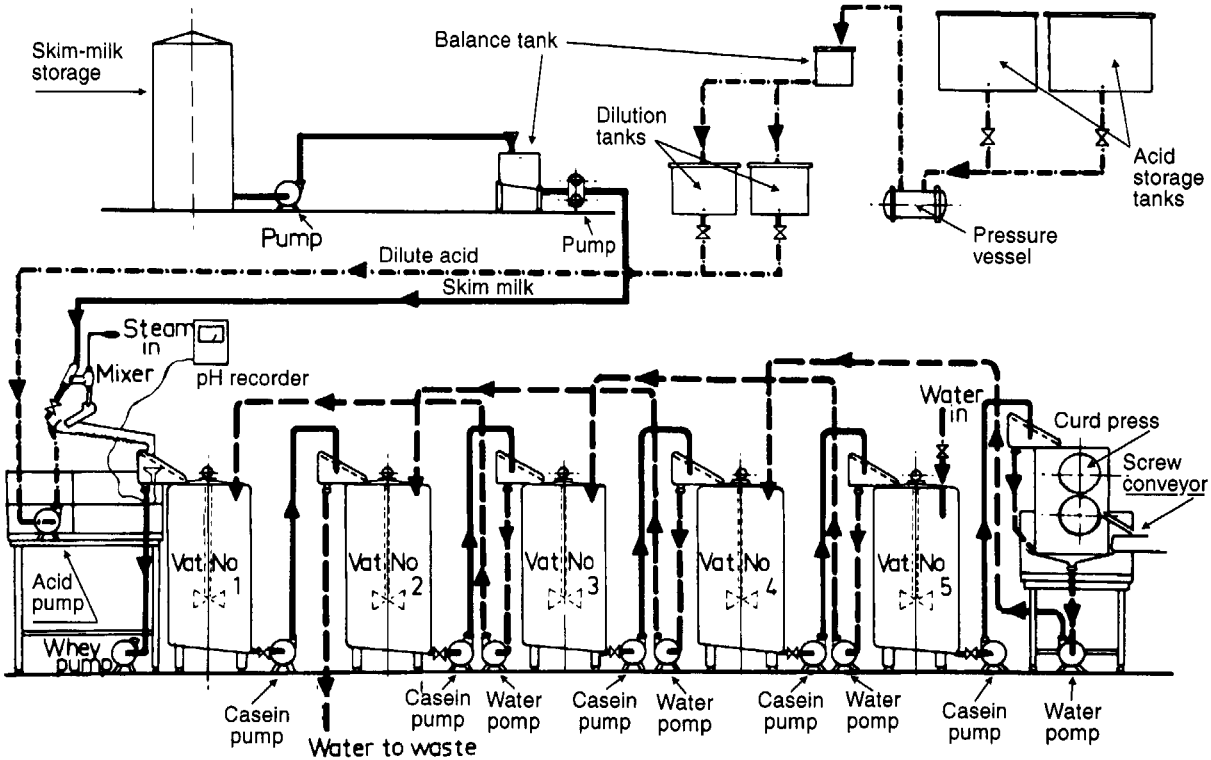


Figure 4.37 Line diagram of an acid casein manufacturing plant: – casein flow lines; --- water flow lines; and -·-·- acid flow lines (from Muller, 1982).

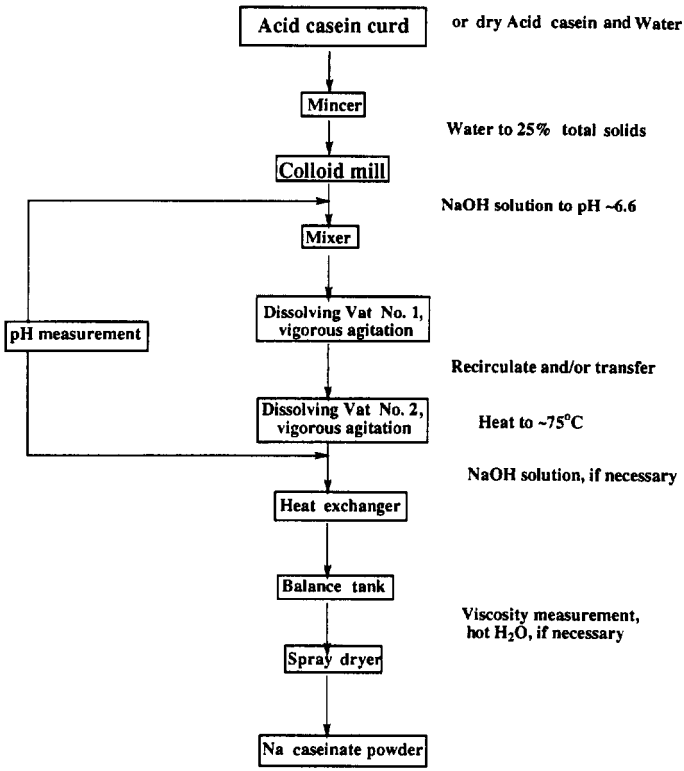


Figure 4.38 Protocol for the manufacture of sodium caseinate (from Mulvihill, 1992).

usually spray dried. Caseinates form very viscous solutions and solutions containing only about 20% casein can be prepared; this low concentration of protein increases drying costs and leads to a low-density powder. Calcium caseinate forms highly aggregated colloidal dispersions.

Direct acidification with HCl or *in situ* production of lactic acid by a mesophilic lactic starter still dominate in the production of acid casein. A relatively recent development in the production of acid casein is the use of ion exchangers for acidification. In one such method, a portion of the milk is acidified to approximately pH 2 at 10°C by treatment with a strong acid ion exchanger and then mixed with unacidified milk in proportions so that the mixture has a pH of 4.6. The acidified milk is then processed by conventional techniques. A yield increase of about 3.5% is claimed, apparently due to the precipitation of some proteose–peptones. The resulting whey has a lower salt content than normal and is thus more suitable for further processing. The elimination of strong acid reduces the risk of corrosion by the chloride ion (Cl^-) and hence cheaper equipment may be

used. However, in spite of these advantages, this process has not been widely accepted and, as far as we are aware, is used only in France, where it was developed. In other proposed methods, deproteinated whey or milk ultrafiltration permeate, acidified by ion exchangers, is used to acid-precipitate casein from skim milk or skim-milk concentrate. Apparently, these methods have not been commercialized.

Rennet casein is produced from skim milk by treatment with certain proteolytic enzymes, known as rennets. The rennet coagulation of milk and related aspects are discussed in Chapter 10. Apart from the coagulation mechanism, the protocol for the production of rennet casein is essentially similar to that for acid casein.

Rennet casein is insoluble in water or alkali but can be solubilized by treatment with polyphosphates. Most rennet casein is used in the manufacture of cheese analogues, the recipe for which includes calcium chelators, e.g. polyphosphates (Chapter 10).

4.15.2 Novel methods for casein production

Cryoprecipitation. When milk is frozen and stored at about $-10°C$, the ionic strength of the liquid phase increases with a concomitant increase in $[Ca^{2+}]$ and a decrease in pH (to approximately 5.8) due to precipitation of calcium phosphates with the release of hydrogen ions (H^+) (Chapter 5). These changes destabilize the casein micelles which precipitate when the milk is thawed.

Cryodestabilization of casein limits the commercial feasibility of frozen milk, which may be attractive in certain circumstances. However, cryodestabilized casein might be commercially viable, especially if applied to milks concentrated by ultrafiltration, which are less stable than normal milk. Cryodestabilized casein may be processed in the usual way. The product is dispersible in water and can be reconstituted as micelles in water at 40°C. The heat stability and rennet coagulability of these micelles are generally similar to those of normal micelles and casein produced by cryodestabilization may be suitable for the production of fast-ripening cheeses, e.g. Mozzarella or Camembert, when the supply of fresh milk is inadequate. As far as we are aware, casein is not produced commercially by cryodestabilization.

Precipitation with ethanol. The casein in milk coagulates at pH 6.6 on addition of ethanol to about 40%; stability decreases sharply as the pH is reduced, and only 10–15% ethanol is required at pH 6. Ethanol–precipitated casein may be dispersed in a micellar form and has very good emulsifying properties. The commercial production of ethanol-precipitated casein is probably economically viable but the process is not being used commercially.

Membrane processing. The use of ultrafiltration (UF) for the production of whey protein concentrates (WPCs) is now well established (p. 223). Obviously, UF or diafiltration (DF) can be used to prepare products enriched in total milk protein. Products with protein concentrations up to 85% have been produced and assessed for a range of functionalities and applications (Fox and Mulvihill, 1992).

The development of large-pore membranes facilitates the separation of whey proteins from casein micelles by microfiltration (MF). Membranes used in MF have cut-offs in the range 0.01–10 μm, and therefore casein micelles may be in the permeate or retentate streams, depending on the pore size of the MF membranes chosen. MF with large-pore membranes very effectively removes bacteria and somatic cells from milk and may also be used to remove lipoprotein complexes from whey prior to the production of WPCs with improved functionality. The preparation of micellar casein by MF is still at the exploratory stage.

High-speed centrifugation. The casein micelles may be sedimented by centrifugation at greater than 100 000 g for 1 h, which is widely used on a laboratory scale. A combination of ultrafiltration and ultracentrifugation has been proposed for the industrial production of 'native' phosphocaseinate. Almost all the casein in skim milk and UF retentates, containing 3.0–17% protein, can be sedimented by centrifugation at greater than 75 000 g for 1 h at 50°C.

'Native' casein. An exciting new development is the production of 'native' casein. Few details on the process are available at present but it involves electrodialysis of skim milk at 10°C against acidified whey to reduce the pH to about 5; the acidified milk is centrifuged and the sedimented casein dispersed in water, concentrated by UF and dried. The product disperses readily in water and is claimed to have properties approaching those of native casein micelles.

4.15.3 Fractionation of casein

As discussed in section 4.4, individual caseins may also be isolated on a laboratory scale by methods based on differences in solubility in urea solutions at around pH 4.6, by selective precipitation with $CaCl_2$ or by various forms of chromatography, especially ion-exchange or reverse-phase high performance liquid chromatography (RP-HPLC). Obviously, these methods are not amenable to scale-up for industrial application.

There is considerable interest in developing techniques for the fractionation of caseins on an industrial scale for special applications. For example:

- β-Casein has very high surface activity and may find special applications as an emulsifier or foaming agent.

- Human milk contains β- and κ-caseins but only a low level of α_{s1}-casein; hence β-casein should be an attractive ingredient for bovine milk-based infant formulae.
- κ-Casein, which is responsible for the stability of casein micelles, might be a useful additive for certain milk products.
- As discussed in section 4.16.7, all the principal milk proteins contain sequences which have biological properties when released by proteolysis; the best studied of these are the β-caseinomorphins. The preparation of biologically active peptides requires purified proteins.

Methods with the potential for the isolation of β-casein on a large scale, leaving a residue enriched in α_{s1}-, α_{s2}- and κ-caseins, have been published. The methods exploit the temperature-dependent association characteristics of β-casein, the most hydrophobic of the caseins. Up to 80% of the β-casein may be recovered from sodium caseinate by UF at 2°C; the β-casein may be recovered from the permeate by UF at 40°C (Figure 4.39). MF at 2°C has been used to isolate casein from milk or sodium caseinate. It is not known whether these methods are being used commercially.

Casein dissociates from the micelles when milk or a dispersion of casein micelles at pH $\geqslant$ 6.7 is heated to at least 90°C; in the former, the dissociated κ-casein is complexed with whey proteins. The functional properties of κ-casein–β-lg complexes isolated by centrifugation of heated milk have been reported by Singh, Fox and Cuddigan (1993).

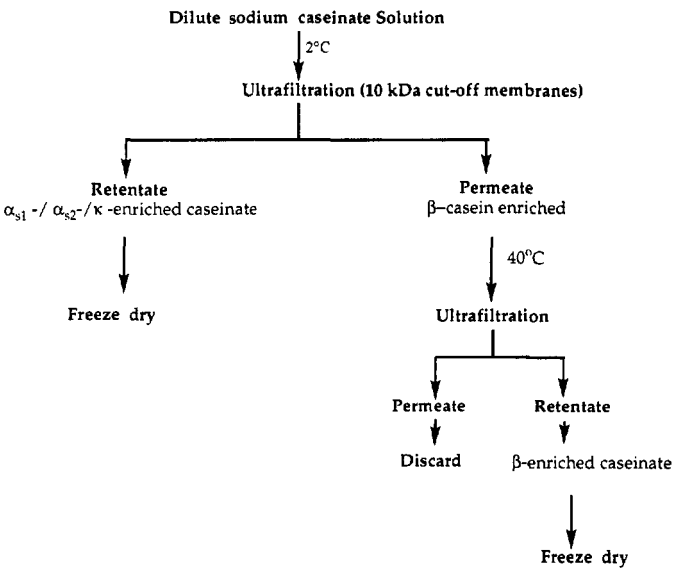


Figure 4.39 Method for preparing α_{s1}-/α_{s2}-/κ- and β-casein-enriched fractions by ultrafiltration (from Murphy and Fox, 1991).

4.15.4 Functional (physicochemical) properties of caseins

Solubility. Solubility is an important functional property *per se*, i.e. in fluid products, and is essential for other functionalities since insoluble proteins can not perform useful functions in foods. The caseins are, by definition, insoluble at their isoelectric points, i.e. in the pH range *c.* 3.5–5.5; the insolubility range becomes wider with increasing temperature. Insolubility in the region of the isoelectric point is clearly advantageous in the production of acid casein and is exploited in the production of two major families of dairy products, i.e. fermented milks and fresh cheeses. However, such insolubility precludes the use of casein in acid liquid foods, e.g. protein-enriched fruit juices or carbonated beverages. Acid-soluble casein can be prepared by limited proteolysis or by interaction with certain forms of pectin.

Rheological properties. Viscosity, an important physicochemical property of many foods, can be modified by proteins or polysaccharides. The caseins form rather viscous solutions, a reflection of their rather open structure and relatively high water-binding capacity. While the high viscosity of caseinate may be of some importance in casein-stabilized emulsions, it causes production problems; for example, due to very high viscosity, not more than about 20% protein can be dissolved even at a high temperature. The low protein content of caseinate solution increases the cost of drying and results in low-density powders which are difficult to handle.

Hydration. The ability of proteins to bind and hold water without syneresis is critical in many foods, e.g. comminuted meat products. Although the caseins are relatively hydrophobic, they bind *c.* 2 g $H_2O\,g^{-1}$ protein, which is typical of proteins. Hydration increases with increasing pH and is relatively independent of NaCl concentration, which is especially important in the efficacy of casein in meat-based products. The water-holding capacity of sodium caseinate is higher than that of calcium caseinate or micellar casein.

Gelation. One of the principal functional applications of proteins is the formation of gels. In milk, caseins undergo gelation when the environment is changed in one of several ways, but the most important are rennet-induced coagulation for cheese or rennet casein manufacture (which is discussed in Chapter 10) or on acidification to the isoelectric point (pH 4.6), which is exploited in the preparation of fermented milk products and isoelectric casein. In addition, casein may be gelled or coagulated by organic solvents, prolonged heat treatments or during storage of heat-sterilized products; these changes are usually negative. Heat-induced gelation is used in the preparation of many food products but, as discussed in Chapter 9,

the caseins are remarkably heat stable and do not undergo thermally-induced gelation except under extremely severe conditions; their stability is of course a major advantage in milk processing.

Surface activity. Probably the outstanding property of caseins, as far as their functionality in foods is concerned, is their surface activity, which makes them good foaming agents and especially good emulsifiers. Surface-active agents are molecules with hydrophilic and hydrophobic regions which can interact with the aqueous and nonaqueous (air or lipid) phases of emulsions and foams, thus reducing the interfacial or surface tension.

Caseins are among the most surface-active proteins available to food technologists, β-casein being particularly effective. To exhibit good surface activity, a protein must possess three structural features:

1. It should be relatively small, since the rate of migration to the interface is inversely proportional to the molecular mass. In actual food processing operations, the rate of diffusion is not particularly important since the production of emulsions and foams involves a large imput of work with vigorous agitation which moves the protein rapidly to the interface.
2. The molecule must be capable of adsorbing at the oil–water or air–water interface and hence must have relatively high surface hydrophobicity; the caseins, especially β-casein, meet this requirement very well.
3. Once adsorbed, the molecule must open and spread over the interface; thus, an open, flexible structure is important. The caseins, which have relatively low levels of secondary and tertiary structures and have no intramolecular disulphide bonds, can open and spread readily.

In practice, while the caseins are very good emulsifiers and foam readily, the resultant foams are not very stable, possibly because the lamella of the foam bubbles are thin and drain rapidly in contrast to the thicker foams formed by egg albumin.

4.15.5 Applications of caseins

World production of casein and caseinates is about 250 000 tonnes per annum. While some casein is still used for industrial applications, the vast majority is used in foods, in which it has numerous applications, as summarized in Table 4.9.

4.15.6 Whey proteins

Many whey proteins possess interesting functional, nutritional, physiological or pharmaceutical properties. Unfortunately, all the proteins in whey are present at low concentrations and hence are relatively expensive to produce, although at least some of them are capable of carrying high production

Table 4.9 Applications of milk proteins in food products (modified from Mulvihill, 1992)

Bakery products	
Caseins/caseinates/co-precipitates	
Used in:	Bread, biscuits/cookies, breakfast cereals, cake mixes, pastries, frozen cakes and pastries, pastry glaze
Effect:	Nutritional, sensory, emulsifier, dough consistency, texture, volume/yield
Whey proteins	
Used in:	Bread, cakes, muffins, croissants
Effect:	Nutritional, emulsifier, egg replacer
Dairy products	
Caseins/caseinates/co-precipitates	
Used in:	Imitation cheeses (vegetable oil, caseins/caseinates, salts and water)
Effect:	Fat and water binding, texture enhancing, melting properties, stringiness and shredding properties
Used in:	Coffee creamers (vegetable fat, carbohydrate, sodium caseinate, stabilizers and emulsifiers)
Effect:	Emulsifier, whitener, gives body and texture, promotes resistance to feathering, sensory properties
Used in:	Cultured milk products, e.g. yoghurt
Effect:	Increase gel firmness, reduces syneresis
Used in:	Milk beverages, imitation milk, liquid milk fortification, milk shakes
Effect:	Nutritional, emulsifier, foaming properties
Used in:	High-fat powders, shortening, whipped toppings and butter-like spreads
Effect:	Emulsifier, texture enhancing, sensory properties
Whey proteins	
Used in:	Yoghurt, Quarg, Ricotta cheese
Effect:	Yield, nutritional, consistency, curd cohesiveness
Used in:	Cream cheeses, cream cheese spreads, sliceable/squeezable cheeses, cheese fillings and dips
Effect:	Emulsifier, gelling, sensory properties
Beverages	
Caseins/caseinates/co-precipitates	
Used in:	Drinking chocolate, fizzy drinks and fruit beverages
Effect:	Stabilizer, whipping and foaming properties
Used in:	Cream liqueurs, wine aperitifs
Effect:	Emulsifier
Used in:	Wine and beer industry
Effect:	Fines removal, clarification, reduce colour and astringency
Whey proteins	
Used in:	Soft drinks, fruit juices, powdered or frozen orange beverages
Effect:	Nutritional
Used in:	Milk-based flavoured beverages
Effect:	Viscosity, colloidal stability

Table 4.9 (*Continued*)

Dessert products	
Caseins/caseinates/co-precipitates	
Used in:	Ice-cream, frozen desserts
Effect:	Whipping properties, body and texture
Used in:	Mousses, instant puddings, whipped toppings
Effect:	Whipping properties, film former, emulsifier, imparts body and flavour
Whey proteins	
Used in:	Ice-cream, frozen juice bars, frozen dessert coatings
Effect:	Skim-milk solids replacement, whipping properties, emulsifying, body/texture
Confectionary	
Caseins/caseinates/co-precipitates	
Used in:	Toffee, caramel, fudges
Effect:	Confers firm, resilient, chewy texture; water binding, emulsifier
Used in:	Marshmallow and nougat
Effect:	Foaming, high temperature stability, improve flavour and brown colour
Whey proteins	
Used in:	Aerated candy mixes, meringues, sponge cakes
Effect:	Whipping properties, emulsifier
Pasta products	
Used in:	Macaroni, pasta and imitation pasta
Effect:	Nutritional, texture, freeze–thaw stability, microwaveable
Meat products	
Caseins/caseinates/co-precipitates	
Used in:	Comminuted meat products
Effect:	Emulsifier, water binding, improves consistency, releases meat proteins for gel formation and water binding
Whey proteins	
Used in:	Frankfurters, luncheon meats
Effect:	Pre-emulsion, gelation
Used in:	Injection brine for fortification of whole meat products
Effect:	Gelation, yield
Convenience foods	
Used in:	Gravy mixes, soup mixes, sauces, canned cream soups and sauces, dehydrated cream soups and sauces, salad dressings, microwaveable foods, low lipid convenience foods
Effect:	Whitening agents, dairy flavour, flavour enhancer, emulsifier, stabilizer, viscosity controller, freeze–thaw stability, egg yolk replacement, lipid replacement
Textured products	
Used in:	Puffed snack foods, protein-enriched snack-type products, meat extenders
Effect:	Structuring, texturing, nutritional

Table 4.9 (*Continued*)

Pharmaceutical and medical products
Special dietary preparations for
Ill or convalescent patients
Dieting patients/people
Athletes
Astronauts
Infant foods
Nutritional fortification
'Humanized' infant formulae
Low-lactose infant formulae
Specific mineral balance infant foods
Casein hydrolsates: used for infants suffering from diarrhoea, gastroenteritis, galactosaemia, malabsorption, phenylketonuria
Whey protein hydrolysates used in hypoallergic formulae preparations
Nutritional fortification
Intraveneous feeds
Patients suffering from metabolic disorders, intestinal disorders for postoperative patients
Special food preparations
Pateints suffering from cancer, pancreatic disorders of anaemia
Specific drug preparations
β-caseinomorphins used in sleep or hunger regulation or insulin secretion
Sulphonated glycopeptides used in treatment of gastric ulcers
Miscellaneous products
Toothpastes
Cosmetics
Wound treatment preparations

costs. Whey protein processing is relatively new and has become possible through the development of new technologies, especially ultrafiltration.

Whole whey protein products. Probably the first whey protein product was lactalbumin, prepared by heat denaturation of the proteins in acid or rennet whey (Figure 4.40) usually at about 90°C and about pH 6. Approximately 80% of the nitrogenous compounds in whey coagulate under these conditions and are recovered by centrifugation or filtration and spray or roller dried. Since the proteins in lactalbumin are extensively denatured and insoluble, they are essentially non-functional and are used mainly for nutritional fortification of foods; lactalbumin is produced on a limited scale. Lactalbumin with improved solubility may be produced by heating acidified (approximately pH 2.5) whey at about 90°C. The yield of aggregated protein may be increased by adding $FeCl_3$, although this reduces solubility. A number of variations of this principle have been published and the functional properties of the products are well characterized (see Fox and Mulvihill, 1992; Mulvihill, 1992). The extent to which these methods are used commercially is not known.

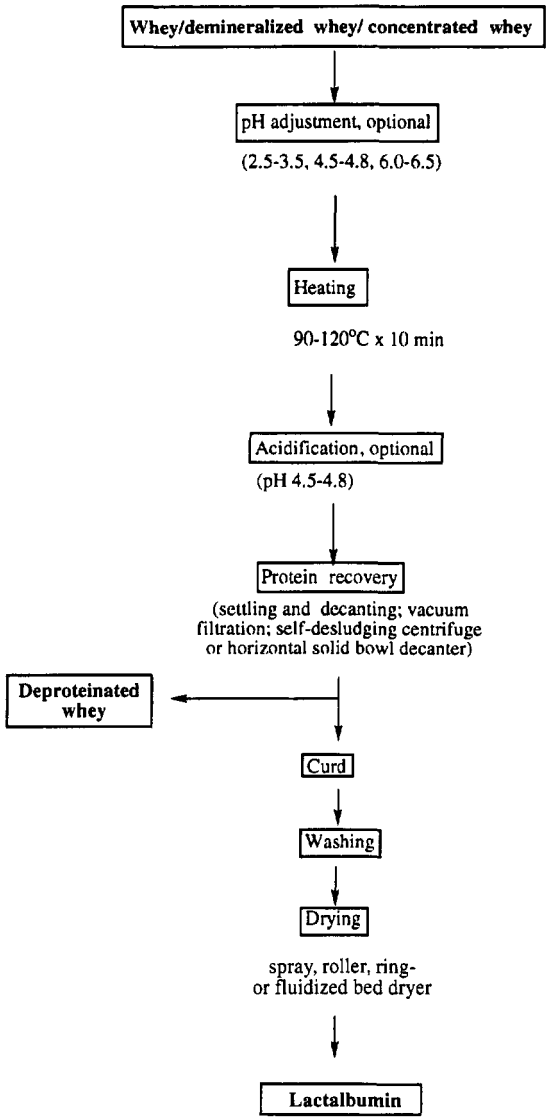


Figure 4.40 Protocol for the manufacture of lactalbumin; the pH, temperature and duration of heating vary (from Mulvihill, 1992).

The major breakthrough in the commercial production of whey proteins occurred with the development of ultrafiltration which facilitated the production of whey protein concentrates (WPCs; 30–80% protein) in which the proteins are mainly in the native state and therefore highly functional. The commercial production of WPCs by UF is now widespread; functional

properties and applications have been thoroughly described (e.g. Marshall, 1982; Morr, 1989; de Wit, 1989a, b).

The presence of even a low level of lipids impairs the functionality of WPCs and reduces flux rates during UF processing. Both problems are minimized by clarifying the whey prior to UF, e.g. by adding $CaCl_2$ to whey (to $1.2\,g\,l^{-1}$), adjusting the pH to 7.3 and heating to 50°C; the flocculent calcium phospholipoprotein complexes formed are allowed to settle and the clear supernatant siphoned off, or removed by centrifugation or microfiltration.

Whey proteins complex with, and are precipitated by, several polyionic compounds which may be used to prepare WPCs (see Marshall, 1982). The most effective of these are polyphosphates which can be removed from the resolubilized protein by precipitation with Ca^{2+}, electrodialysis, ion-exchange or gel filtration. Polyphosphate-precipitated WPCs are commercially available.

The use of ion-exchange resins (Figure 4.41) offers an effective method for the preparation of high-quality whey protein products, referred to as whey protein isolates (WPI), containing 90–95% protein (see Marshall, 1982; Mulvihill, 1992). Although the functional properties of WPI are superior to those of WPCs on an equiprotein basis (due to lower levels of lipids, lactose and salts), their production is rather limited, due to higher production costs.

Fractionation of whey proteins. Techniques for the isolation of individual whey proteins on a laboratory scale by salting-out, ion-exchange chromatography and/or crystallization have been available for about 40 years. Owing to the unique functional, physiological or other biological properties of many of the whey proteins, there is an economic incentive for their isolation on an industrial scale. For example, β-lg, the principal whey protein in bovine milk, produces better thermoset gels than α-la. However, human milk does not contain β-lg, which is the most allergenic of the bovine milk proteins for the human infant; therefore, α-la would appear to be a more appropriate protein for the preparation of infant formulae than total whey protein.

A number of methods have been developed for the separation of α-la and β-lg (Figure 4.42). Probably the most commercially feasible of these exploits the low heat stability of calcium-free α-la to precipitate it from whey, leaving β-lg, BSA and Ig in solution. α-La loses its calcium on acidification to about pH 5.0, aggregates on heating to about 55°C and can be recovered by centrifugation, filtration or microfiltration.

α-La and β-lg are insoluble in pure water at their isoelectric points (around pH 5); β-lg requires a higher ionic strength for solubility than α-la, a characteristic which may be exploited to fractionate α-la and β-lg. When UF-concentrated whey is acidified to pH 4.65 and demineralized by elec-

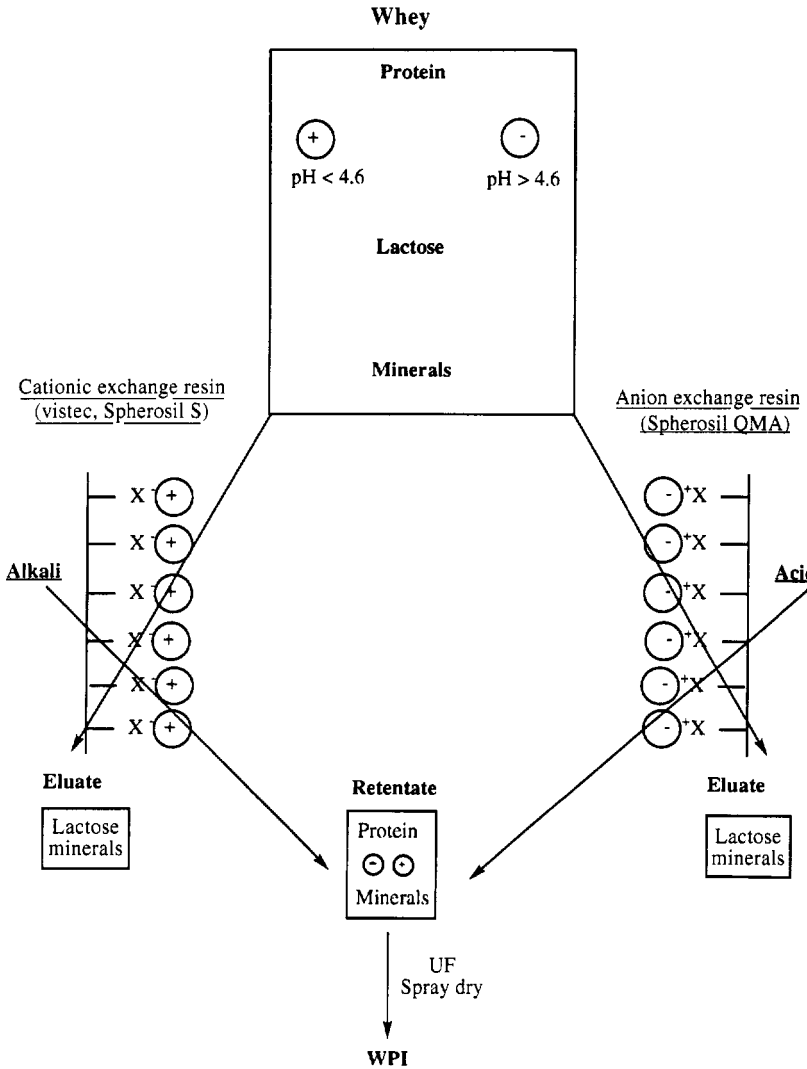


Figure 4.41 Production of whey protein isolate (WPI) by ion exchange adsorption (from Mulvihill, 1992).

trodialysis to less than about 0.02% ash, β-lg precipitates and can be removed by centrifugation with a yield of more than 90%.

The ion exchangers used to recover total whey protein (WPI) may also be used to fractionate whey proteins. All the whey proteins are adsorbed initially on Spherosil QMA resin but on continued passage of whey through

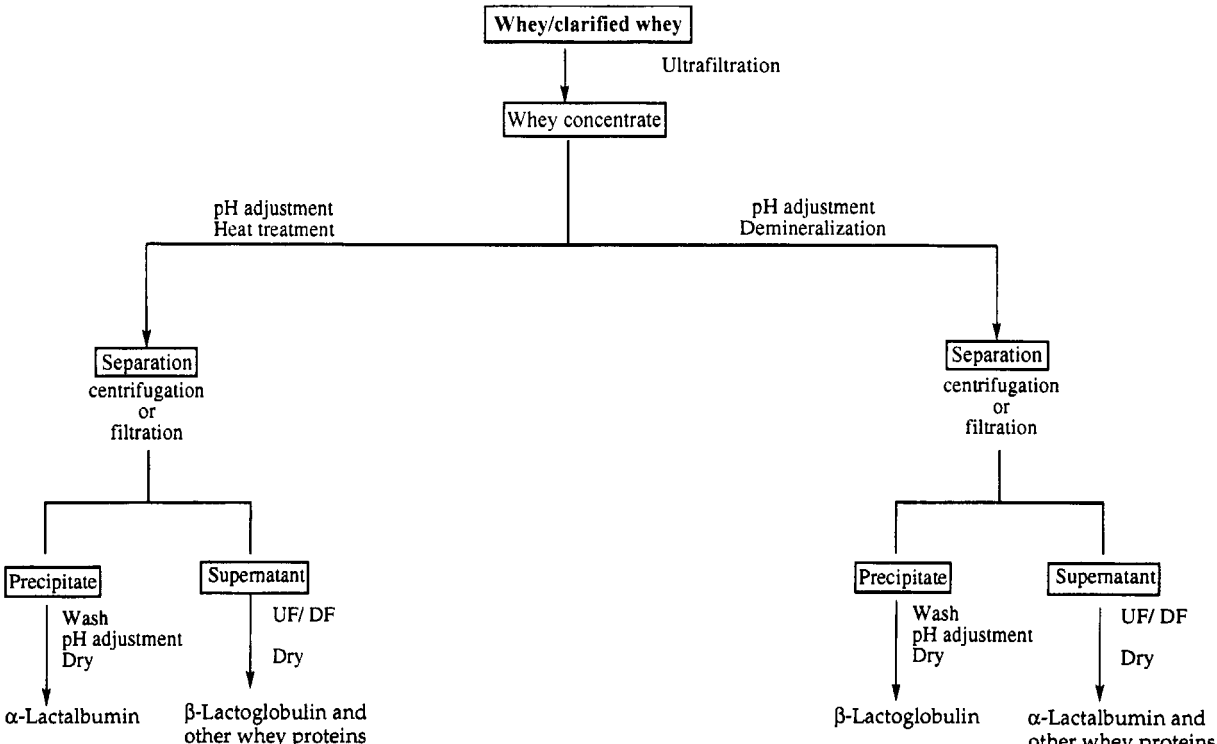


Figure 4.42 Methods for the fractionation of α-lactalbumin and β-lactoglobulin.

the column, β-lg, which has a higher affinity for this resin than the other proteins, displaces α-la and BSA, giving a mixture of these proteins in the eluate; a highly purified β-lg can be obtained by eluting the protein-saturated column with dilute HCl.

4.15.7 Casein–whey protein co-precipitates

Following denaturation, the whey proteins coprecipitate with the caseins on acidification to pH 4.6 or addition of $CaCl_2$ at 90°C, to yield a range of products known as casein–whey protein co-precipitates (Figure 4.43). The main attraction of such products is an increase in yield of about 15%, but the products also have interesting functional properties. However, they have not been commercially successful.

New forms of co-precipitate, referred to as soluble lactoprotein or total milk protein, with improved solubility, have been developed recently (Figure 4.44). By adjusting the milk to an alkaline pH before denaturing the whey proteins and co-precipitating them with the caseins at pH 4.6, the function-

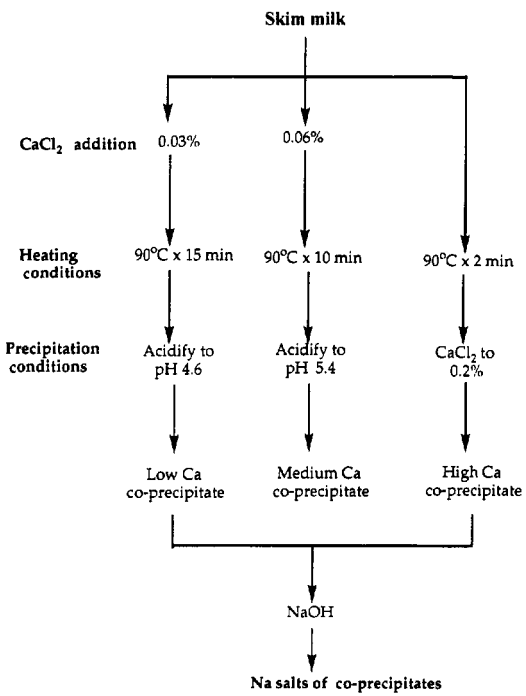


Figure 4.43 Protocols for the manufacture of conventional casein–whey protein co-precipitates (from Mulvihill, 1992).

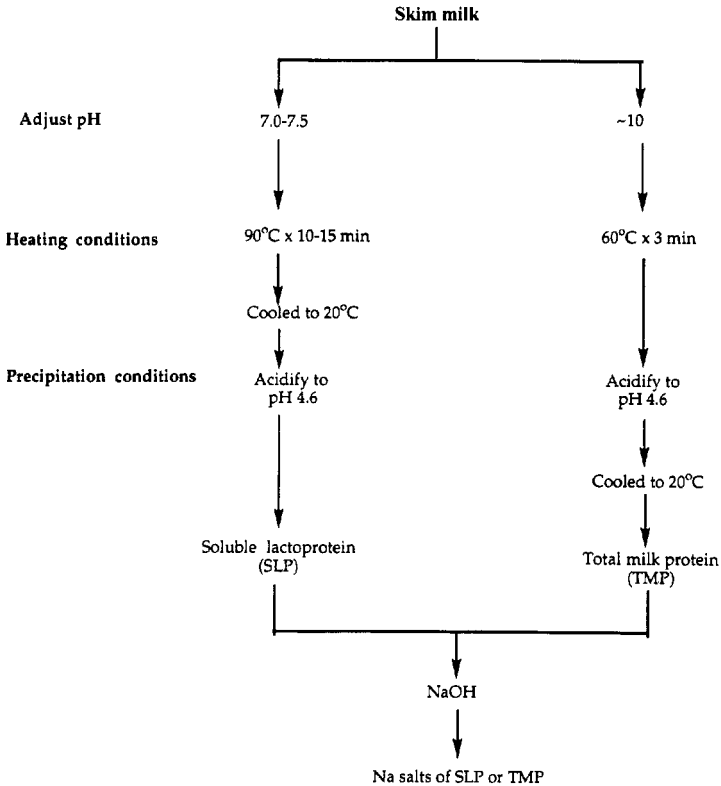


Figure 4.44 Protocols for the manufacture of soluble lactoprotein and total milk proteins (from Mulvihill, 1992).

ality of the caseins is not adversely affected; probably, the denatured whey proteins do not complex with the casein micelles at the elevated pH.

4.16 Biologically active proteins and peptides in milk

Milk contains a wide range of biologically active proteins/peptides, e.g. indigenous enzymes (perhaps 60), vitamin-binding proteins, metal-binding proteins, immunoglobulins, various growth factors and peptide hormones. Many of these proteins may eventually find commercial application as isolation procedures are improved but, at present, three are of commercial interest, viz., lactoperoxidase, lactotransferrin and immunoglobulins. In addition, all the principal milk proteins contain sequences which when released on proteolysis exhibit biological activity. The subject has been reviewed by Fox and Flynn (1992).

4.16.1 Lactoperoxidase

Lactoperoxidase (LPO) is a broad-specificity peroxidase present at high concentrations in bovine milk but at low levels in human milk. LPO, which has been isolated and well characterized (Chapter 8), has attracted considerable interest owing to its antibacterial activity in the presence of H_2O_2 and thiocyanate (SCN^-); the active species is hypothiocyanate ($OSCN^-$) or other higher oxidation species. Milk normally contains no indigenous H_2O_2, which must be added or produced *in situ*, e.g. by the action of glucose oxidase or xanthine oxidase; it is usually necessary to supplement the indigenous SCN^-. Commercial interest in LPO is focused on:

1. activation of the indigenous enzyme for cold pasteurization of milk or protection of the mammary gland against mastitis; and
2. addition of isolated LPO to calf or piglet milk replacers to protect against enteritis, especially when the use of antibiotics in animal feed is not permitted.

LPO, which is positively charged at neutral pH, can be isolated from milk or whey by ion-exchange chromatography which has been scaled up for industrial application. These methods isolate LPO together with lactotransferrin (Lf) which is also cationic at neutral pH. LPO and Lf can be resolved by chromatography on CM-Toyopearl or by hydrophobic interaction chromatography on Butyl Toyopearl 650 M (see Fox and Mulvihill, 1992).

4.16.2 Lactotransferrin

The transferrins are a group of specific metal-binding proteins, the best characterized of which are serotransferrin (present in blood plasma, milk, spinal fluid and semen), ovotransferrin (conalbumin; present in avian and reptile egg white) and lactotransferrin (present in milk, pancreatic juice, tears and leucocytes).

Human colostrum and milk contain 6–8 mg ml^{-1} and 2–4 mg ml^{-1} lactotransferrin, respectively, representing about 20% of the total protein in the latter; bovine colostrum and milk contain about 1 and 0.02–0.35 mg ml^{-1}, respectively. The concentration of lactotransferrin in human milk decreases slightly during lactation but appears to increase slightly in bovine milk and very markedly during the dry period.

Lactotransferrin binds iron very strongly, which suggests two roles for this protein: iron absorption and protection against enteric infection in the neonate. Because the concentration of Lf in human milk is considerably higher than that in bovine milk, there is considerable interest in supplementing bovine milk-based infant formulae with Lf. The concentration of Lf in milk increases markedly during mastitic infections, suggesting that it may have a protective role in the mammary gland. The structure and function of

Lf have been reviewed by Lönnerdal and Iyer (1995) and Hutchens and Lönnerdal (1996).

Lactotransferrins have been isolated from the milks of several species, including human and bovine, and some have been well characterized, including determination of their amino acid sequence. Some of the isolation procedures have industrial-scale potential; the preparations obtained from such procedures usually contain both Lf and LPO.

4.16.3 Immunoglobulins

The occurrence, significance and interspecies aspects of immunglobulin in milk were described in section 4.10. Classically, Ig is prepared by salting-out, usually with ammonium sulphate [$(NH_4)_2SO_4$]. This method is effective but expensive and current commercial products are usually prepared by ultra-filtration of colostrum or milk from hyperimmunized cows. Some recently developed methods for the isolation of Ig, sometimes with Lf, use mono-clonal antibodies, metal chelate or gel filtration chromatographies (see Fox and Mulvihill, 1992).

Ig-rich preparations are commercially available for the nutrition of calves and other neonatal animals. Although human infants do not absorb Ig from the intestine, Igs still play an important defensive role by reducing the incidence of intestinal infection. While breast feeding is best for healthy full-term infants, it is frequently impossible to breast-feed pre-term or very-low-birth-weight infants, who may be fed on banked human milk. Such infants have high protein and energy requirements which may not be met by human milk and consequently special formulae have been developed. A 'milk immunological concentrate', prepared by diafiltration of acid whey from colostrum and early lactation milk from immunized cows, for use in such formulae has been described; the product contains approximately 75% protein, 50% of which is Ig, mainly IgG_1 and not IgA, which is predominant in human milk.

The development of Ig in cows agains human pathogens, e.g. rotavirus, an important cause of illness in children, is considered to be an attractive approach in human medicine. The Ig could be administered in milk or as a concentrate prepared from milk.

4.16.4 Vitamin-binding proteins

Milk contains specific binding proteins for retinol (vitamin A), vitamin D, riboflavin (vitamin B_2), folate and cyanocobalamin (vitamin B_{12}). Such proteins may improve the absorption of these vitamins by protecting and transferring them to receptor proteins in the intestine, or they may have antibacterial activity by rendering vitamins required by intestinal bacteria unavailable. The activity of these proteins is reduced or destroyed by heat treatments.

4.16.5 Growth factors

The term 'growth factor' is applied to a group of potent hormone-like polypeptides which play a critical role in the regulation and differentiation of a variety of cells acting through cell membrane receptors. The milk and, especially, colostrum from several species contain several growth factors, including insulin-like growth factors (IGF1, IFG2), transforming growth factors ($TGF_{\alpha 1}$, $TGF_{\alpha 2}$, TGF_{β}), mammary-derived growth factors (MDGF I, MDGF II), fibroblast growth factors, platelet-derived growth factor (PDGF) and bombasin.

The source of these polypeptides may be blood plasma, mammary gland or both. The biological significance of these growth-promoting activities in colostrum and mature milks is not yet clear. In terms of possible physiological significance, two potential targets may be considered, i.e. the mammary gland or the neonate. In general, most attention has focused on the latter. It is not known whether the factors in milk that possess the capacity to promote cell proliferation (1) influence growth of mammary tissue, (2) promote the growth of cells within the intestines of the recipient neonate, or (3) are absorbed in a biologically active form and exert an effect on enteric or other target organs.

Methods using ultrafiltration and chromatography have been developed for the concentration of growth factors from whey. In addition to possible food (nutritceutical) applications for such growth factors, a major potential application is in tissue cultures, for which foetal bovine serum is used as a source of growth factors. However, the supply of foetal bovine serum is limited, unreliable, expensive and of variable quality. Whey-derived growth factors have the potential to have a major impact on the biotechnological and pharmaceutical industries for the production of vaccines, hormones, drugs, monoclonal antibodies, and the production of tissue, especially skin for treatment of burns, ulcers and lacerations.

4.16.6 Bifidus factors

Special types of growth factors are those that promote the growth of bifidobacteria. It has been recognized for many years that breast-fed babies are more resistant to gastroenteritis than bottle-fed babies. This is undoubtedly a multifactorial phemonenon, including better hygiene, more appropriate milk composition, several antibacterial systems (especially immunoglobulins, lysozyme, lactotransferrin, vitamin-binding proteins and lactoperoxidase, which are discussed above), and a lower intestinal pH. The mean pH of the faeces of breast-fed babies is 5.1 while that of bottle-fed babies is 6.4; the low pH of the former may be due partly to the lower buffering capacity of human milk compared to bovine milk, due to its lower content of protein and phosphate, and partly to differences in the intestinal microflora of breast-fed and bottle-fed infants. Bifidobacteria represent

about 99% of the faecal microflora of breast-fed infants. These bacteria also represent a high proportion of the microflora of bottle-fed infants but several other genera, e.g. *Bacteroides*, *Clostridium* and coliforms, also occur at high numbers. Furthermore, the predominant species of *Bifidobacterium* in breast-fed infants is *B. bifidum*, with lesser numbers of *B. longum;* the faecal microflora of bottle-fed infants is dominated by *B. longum*, with lower numbers of *B. bifidum*, *B. infantis*, *B. adolescentis* and *B. breve*.

The preponderance of *B. bifidum* in the faeces of breast-fed infants is due to the presence of stimulatory factors in human milk. The most important of these are N-acetylglucosamine-containing saccharides, referred to as bifidus factor I, which is present at high levels in human milk and colostrum and bovine colostrum but at very low concentrations in the milk of cows, goats and sheep. Human milk also contains several non-dialysable bifidus-promoting factors which are glycoproteins, referred to as bifidus factor II. Many of the glycoproteins have been isolated and characterized (see Fox and Flynn, 1992).

Bifidobacterium spp. are also stimulated by lactulose, a derivative of lactose (Chapter 2) which is not related to bifidus factors I and II.

4.16.7 Milk protein hydrolysates

Several methods have been described for the production, characterization and evaluation of milk protein hydrolysates tailored for specific applications in the health-care, pharmaceutical, baby food and consumer product areas (see Fox and Mulvihill, 1992, for references).

Several peptides with specific properties may be prepared from milk proteins, either *in vivo* or *in vitro*; some may have commercial potential and can be produced on a relatively large scale by preparative ion-exchange chromatography.

Macropeptides from κ-casein. These peptides represent the C-terminal region of κ-casein (residues 106–169) which is released by rennet during the manufacture of cheese or rennet casein (Chapter 10). The (glyco)macropeptides are released into the whey which contains 1.2–1.5 g l^{-1}, and from which they can be readily recovered, e.g. by anion exchange using Spherosil QMA resin. The peptides contain no Phe, Tyr, Trp or Cys; the absence of aromatic amino acids makes the macropeptides suitable for the nutrition of patients suffering from phenylketonuria.

Phosphopeptides. It is claimed that phosphopeptides prepared from casein hydrolysates stimulate the absorption of Ca in the intestine, but views on this are not unanimous. Such peptides are resistant to proteolysis due to the high density of negative charges; they have been detected in the small intestine of the rat and may pass intact through the intestinal wall. Since

phosphopeptides also bind iron it has been proposed that casein phosphopeptide–Fe complexes are useful supplements for dietary iron but their influence on the bioavailability of iron is ambiguous.

Caseinomorphins. Several peptides with opioid activity have been isolated from enzymatic digests of milk proteins (see Fox and Flynn, 1992). Such peptides were first isolated from enzymatic digests of casein and characterized as a family of peptides containing 4–7 amino acids with a common N-terminal sequence, H.Tyr.Pro.Phe.Pro-, and 0–3 additional residues (Gly, Pro, Ile), i.e. residues 60–63/6 of β-casein, and hence were called caseinomorphins (β-CM) 4 to 7, respectively. β-CM-5 is the most effective of these peptides, which are 300–4000 times less effective than morphine. β-CMs are very resistant to enzymes of the gastrointestinal tract (GIT) and appear in the contents of the small intestine following ingestion of milk. β-CN f60–70 also has weak opiate activity but may be hydrolysed to smaller, more active β-CMs by peptidases in the brush border of the GIT.

The sequence 51–57 of human β-casein, Tyr.Pro.Phe.Val.Glu.Pro.Ile, corresponds to bovine β-CN f60–66 (i.e. Tyr.Pro.Phe.Pro.Gly.Pro.Ile) and has weak opioid activity. Peptides corresponding to human β-casein residues 41–44 and 59–63 also have weak opioid activity. Exorphines have also been isolated from hydrolysates of α_{s1}-casein (f90–95 and f90–96; Arg.Tyr.Leu.Gly.Tyr.Leu (Glu)), κ-casein (f35–41, 57–60 and 25–34), α-lactalbumin (f50–53, Tyr.Gly.Leu.Phe), β-lactoglobulin (f102–105, Tyr.Leu.Leu.Phe) and lactotransferrin (Tyr.Leu.Gly.Ser.Gly.Tyr, Arg.Tyr.Tyr.Gly.Tyr and Lys.Tyr.Leu.Gly.Pro.Gln.Tyr).

Thus, all the major milk proteins contain sequences which, when liberated by gastrointestinal proteinases, possess opioid activity. These peptides are very resistant to proteolysis by gastrointestinal proteinases and, because of their high hydrophobicity, can be absorbed intact from the intestine. They possess physiological activity *in vitro* but their activity *in vivo* is as yet uncertain.

Immunomodulating peptides. Enzymatic digests of human caseins contain immunomodulating peptides which stimulate the phagocytic activity of human macrophages *in vitro* and exert a protective effect *in vivo* in mice against *Klebsiella pneumoniae* infection. Two of the peptides were characterized as H.Val.Glu.Pro.Ile.Pro.Tyr (β-CN f54–59) and H.Gly.Leu.Phe (origin not identified).

Platelet-modifying peptide. The undecapeptide, H.Met.Ala.Ile.Pro.Pro.Lys.Lys.Asn.Gln.Asp.Lys (residues 106–116 of bovine κ-casein) inhibits the aggregation of ADP-treated blood platelets; its behaviour is similar to that of the structurally related C-terminal dodecapeptide (residues 400–411) of human fibrinogen γ-chain. κ-CN f106–116 is produced from the (glyco)-

macropeptide, κ-CN f106–169, formed by the action of chymosin. Shorter peptides, κ-CN f106–112 and 113–116, have similar but weaker effects on platelet aggregation. A peptide with similar properties has been isolated from a hydrolysate of lactotransferrin.

Angiotensin converting enzyme (ACE) inhibitor. ACE is a dipeptidylaminopeptidase (EC 3.4.15.1) which cleaves dipeptides from the C-terminus of peptides. It converts angiotensin I to the potent vasoconstrictor, angiotensin II, and inactivates the vasodilator, bradykinin. The dodecapeptide, H.Phe.Phe.Val.Ala.Pro.Phe.Pro.Glu.Val.Phe.Gly.Lys, i.e. α_{s1}-CN f23–34, from tryptic hydrolysates of casein inhibits ACE. The C-terminal sequence of α_{s1}-casein, H.Thr.Thr.Met.Pro.Leu.Tyr, α_{s1}-CN f194–199, also has ACE inhibitory activity. Peptides from the sequence 39–52 of human β-casein, especially H.Ser.Phe.Gln.Pro.Gln.Pro.Leu.Ile.Tyr.Pro (β-CN f43–52), also have ACE inhibitory activity.

Calmodulin-binding peptides. Peptides that inhibit calmodulin-dependent cyclic nucleotide phosphodiesterase have been isolated from peptic digests of α_{s1}-casein (α_{s1} plus α_{s2}) and identified as α_{s2}-CN f164–179, α_{s2}-CN f183–206 and α_{s2}-CN f183–207. The physiological significance of these peptides is unknown.

Bacteriocidal peptides from lactotransferrin (Lf). The bactericidal properties of Lf, presumed to be due to iron-binding, were discussed in section 4.16.2. It has been reported that a number of bactericidal peptides are formed when Lf is heated at 120°C for 15 min, especially at pH 2, at which the degree of hydrolysis is about 10%. The effectiveness of these peptides is not related to iron-binding properties, i.e. their bactericidal properties are retained in Fe-rich media in which Lf is ineffective.

Potent antibacterial peptides can also be produced by hydrolysis of Lf by pepsins and some other acid proteinases. The low molecular weight peptides in the peptic hydrolysates were at least eight times more potent than Lf, were effective against a wider range of bacteria than Lf and retained their potency in the presence of added iron, unlike native Lf.

References

Berliner, L.J., Meinholtz, D.C,. Hirai, Y. *et al.* (1991) Functional implications resulting from disruption of the calcium binding loop in bovine α-lactalbumin. *J. Dairy Sci.*, **74**, 2394–402.

Bernhart, F.W. (1961) Correlation between growth-rate of the suckling of various species and the percentage of total calories from protein in the milk. *Nature*, **191**, 358–60.

Brew, K. and Grobler, J.A. (1992) α-Lactalbumin, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 191–229.

Brunner, J.R., Ernstrom, C.A., Hollis, R.A. *et al.* (1960) Nomenclature of the proteins of bovine milk – first revision. *J. Dairy Sci.*, **43**, 901–11.

de Wit, J.N. (1989a) Functional properties of whey proteins, in *Developments in Dairy Chemistry*, Vol. 4: *Functional Milk Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 285–321.

de Wit, J.N. (1989b) The use of whey protein products, in *Developments in Dairy Chemistry* Vol. 4: *Functional Milk Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 323–45.

Eigel, W.N., Butler, J.E., Ernstrom, C.A. *et al.* (1984) Nomenclature of proteins of cow's milk: fifth revision. *J. Dairy Sci.*, **67**, 1599–631.

Farrell, H.M. Jr (1988) Physical equilibria: proteins, in *Fundamentals of Dairy Chemistry*, 3rd edn (ed. N.P. Wong), van Nostrand Reinhold, New York, pp. 461–510.

Fox, P.F. (ed.) (1989) *Developments in Dairy Chemistry*, Vol. 1: *Proteins*, Applied Science Publishers, London.

Fox, P.F. and Flynn, A. (1992) Biological properties of milk proteins, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 255–84.

Fox, P.F. and Mulvihill, D.M. (1992) Developments in milk protein processing. *Food Sci. Technol. Today*, **7**, 152–61.

Hambling, S.G., McAlpine, A.S. and Sawyer, L. (1992) β-Lactoglobulin, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 141–90.

Holt, C. (1992) Structure and stability of bovine casein micelles. *Adv. Prot. Chem.*, **43**, 63–151.

Holt, C. (1994) The biological function of casein, in *Yearbook 1994, The Hannah Institute*, Ayr, Scotland, pp. 60–8.

Holt, C. and Sawyer, L. (1993) Caseins as rheomorphic proteins: Interpretation of primary and secondary structures of α_{s1}-, β- and κ-caseins. *J. Chem. Soc. Faraday Trans.*, **89**, 2683–92.

Hutchens, T.W. and Lönnerdal, B. (1996) *Lactoferrin: Structure and Function*, Chapman & Hall, London.

Jakob, E. and Puhan, Z. (1992) Technological properties of milk as influenced by genetic polymorphism of milk proteins – a review. *Int. Dairy J.*, **2**, 157–78.

Jenness, R., Larson, B.L., McMeekin, T.L. *et al.* (1956) Nomenclature of the proteins of bovine milk. *J. Dairy Sci.*, **39**, 536–41.

Keenan, T.W. and Dylewski, D.P. (1985) Aspects of intracellular transit of serum and lipid phases of milk. *J. Dairy Sci.*, **68**, 1025–40.

Kronman, M.J. (1989) Metal-ion binding and the molecular conformational properties of α-lactalbumin. *Crit. Rev. Biochem. Mol. Biol.*, **24**, 565–667.

Kumosinski, T.F. and Farrell, H.M. Jr. (1994) Solubility of proteins: salt-water interactions, in *Protein Functionality in Food Systems*, (eds N.S. Hettiarachchy and G.R. Ziegler), Marcel Dekker, New York, pp. 39–77.

Kumosinski, T.F., Brown, E.M. and Farrell, H.M. Jr (1993a) Three-dimensional molecular modeling of bovine caseins: An energy-minimized β-casein structure. *J. Dairy Sci.*, **76**, 931–45.

Kumosinski, T.F., Brown, E.M. and Farrell, H.M. Jr (1993b). Three-dimensional molecular modeling of bovine caseins: a refined, energy-minimized κ-casein structure. *J. Dairy Sci.*, **76**, 2507–20.

Larson, B.L. (1992). Immunoglobulins of the mammary secretions, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 231–54.

Lönnerdal, B. and Iyer, S. (1995) Lactoferrin: Molecular structure and biological function. *Ann. Rev. Nutr.*, **15**, 93–110.

McKenzie, H.A. (ed.) (1970) *Milk Proteins: Chemistry and Molecular Biology*, Vol. 1, Academic Press, New York.

McKenzie, H.A. (1971) β-Lactoglobulin, in *Milk Proteins, Chemistry and Molecular Biology*, Vol. II, (ed. H.A. McKenzie), Academic Press, New York, pp. 257–330.

McMahon, D.J. and Brown, R.J. (1984) Composition, structure and integrity of casein micelles: A review. *J. Dairy Sci.*, **67**, 499–512.

Marshall, K.R. (1982) Industrial isolation of milk proteins: whey proteins, in *Developments in Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Applied Science Publishers, London,. pp. 339–73.

Martin, P., Brignon, G., Furet, J.P. and Leroux, C. (1996) The gene encoding α_{s1}-casein is expressed in human mammary epithelial cells during lactation. *Lait*, **76**, 523–35.

Mepham, T.B. (1987) *Physiology of Lactation*, Open University Press, Milton, Keynes, UK.
Mepham, T.B., Gaye, P. and Mercier, J.C. (1982) Biosynthesis of milk proteins, in *Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 115–56.
Mepham, T.B., Gaye, P., Martin, P. and Mercier, J.-C. (1992) Biosynthesis of milk proteins, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 491–543.
Mercier, J.C. and Gaye, P.C. (1983) Milk protein syntheses, in *Biochemistry of Lactation*, (ed. T.B. Mepham), Elsevier, Amsterdam, pp. 177–227.
Morr, C.V. (1989) Whey proteins: manufacture, in *Developments in Dairy Chemistry*, Vol. 4: *Functional Milk Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 245–84.
Muller, L.L. (1982) Manufacture of casein, caseinates and casein co-precipitates, in *Developments in Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Applied Science Publishers, London, pp. 315–37.
Mulvihill, D.M. (1992) Production, functional properties and utilization of milk proteins, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 369–404.
Murphy, J.F. and Fox, P.F. (1991) Fractionation of sodium caseinate by ultrafiltration. *Food Chem.*, **39**, 27–38.
Ng-Kwai-Hang, K.F. and Grosclaude, F. (1992) Genetic polymorphism of milk proteins, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 405–55.
Ono, T. and Obata, T. (1989) A model for the assembly of bovine casein micelles from F2 and F3 subunits. *J. Dairy Res.*, **56**, 453–61.
Papiz, M.Z., Sawyer, L., Eliopoulos, E.E. *et al.* (1986) The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature*, **324**, 383–5.
Richardson, T., Oh, S., Jimenez-Flores, R. *et al.* (1992) Molecular modeling and genetic engineering of milk proteins, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 545–77.
Rollema, H.S. (1992) Casein association and micelle formation, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 111–40.
Rose, D., Brunner, J.R., Kalan, E.B. et al. (1970) Nomenclature of the proteins of cow's milk: third revision. *J. Dairy Sci.*, **53**, 1–17.
Schmidt, D.G. (1982) Association of caseins and casein micelle structure, in *Developments in Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Applied Science Publishers, London, pp. 61–86.
Singh, H., Fox, P.F. and Cuddigan, M. (1993) Emulsifying properties of protein fractions prepared from heated milk. *Food Chem.*, **47**, 1–6.
Swaisgood, H.E. (ed.) (1975) *Methods of Gel Electrophoresis of Milk Proteins*, American Dairy Science Association, Champaign, IL, 33 pp.
Swaisgood, H.E. (1982) Chemistry of milk proteins, in *Developments in Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Applied Science Publishers, London, pp. 1–59.
Swaisgood, H.E. (1992) Chemistry of the caseins, in *Advanced Dairy Chemistry*, Vol. 1: *Proteins*, (ed. P.F. Fox), Elsevier Applied Science, London, pp. 63–110.
Thompson, M.P., Tarassuk, N.P., Jenness, R. *et al.* (1965) Nomenclature of the proteins of cow's milk—second revision. *J. Dairy Sci.*, **48**, 159–69.
Visser, H. (1992) A new casein micelle model and its consequences for pH and temperature effects on the properties of milk, in *Protein Interactions*, (ed. H. Visser), VCH, Weinheim, pp. 135–65.
Walstra, P. and Jenness, R. (1984) *Dairy Chemistry and Physics*, John Wiley & Sons, New York.
Whitney, R. McL., Brunner, J.R., Ebner, K.E. *et al.* (1976) Nomenclature of cow's milk: Fourth revision. *J. Dairy Sci.*, 59, 795–815.

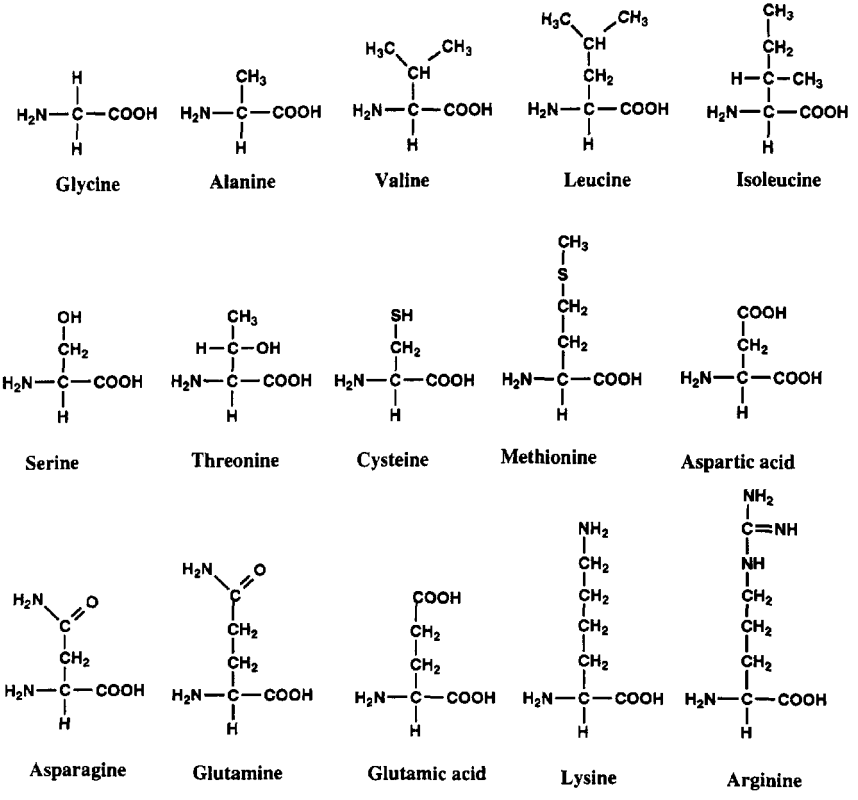
Suggested reading

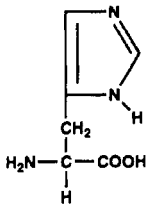
Fox, P.F. (1982) Developments in *Dairy Chemistry*, Vol. 1: *Proteins*, Applied Science Publishers, London.
Fox, P.F. (ed.) (1989) Developments in *Dairy Chemistry*, Vol. 4: *Functional Proteins*, Elsevier Applied Science Publishers, London.

Fox, P.F. (ed.) (1992) *Advanced Dairy Chemistry*, Vol. 1: *Milk Proteins*, Elsevier Applied Science Publishers, London.
Kinsella, J.E. (1984) Milk proteins: Physicochemical and functional properties. *CRC Crit. Rev. Food Sci. Nutr.*, **21**, 197–262.
Lönnerdal, B. and Iyer, S. (1995) Lactoferrin: Molecular structure and biological function. *Ann. Rev. Nutr.*, **15**, 93–110.
McKenzie, H.A. (ed.) (1970) *Milk Proteins: Chemistry and Molecular Biology*, Vol. 1, Academic Press, New York.
McKenzie, H.A. (ed.) (1971) *Milk Proteins: Chemistry and Molecular Biology*, Vol. 2, Academic Press, New York.
Mepham, T.B. (ed.) (1983) *Biochemistry of Lactation*, Elsevier, Amsterdam.
Walstra, P. and Jenness, R. (1984) *Dairy Chemistry and Physics*, John Wiley & Sons, New York.
Webb, B.H., Johnson, A.H. and Alford, J.A. (1974) *Fundamentals of Dairy Chemistry*, 2nd edn, AVI Publishing, Westport, CT.
Wong, N.P., Jenness, R., Keeney, M. and Marth, E.H. (1988) *Fundamentals of Dairy Chemistry*, 3rd edn, AVI Publishing, Westport, CT.

Appendices

Appendix 4A Structures of amino acids found in proteins





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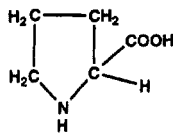

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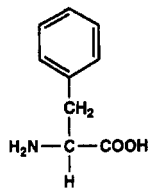

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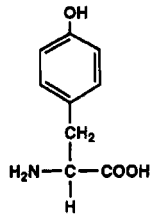
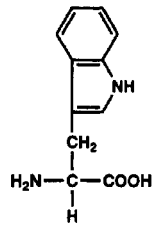


Tyrosine



Tryptophan