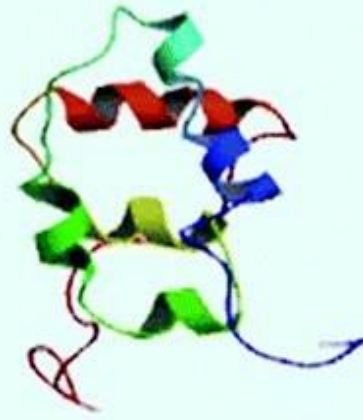


α_5 -Casein

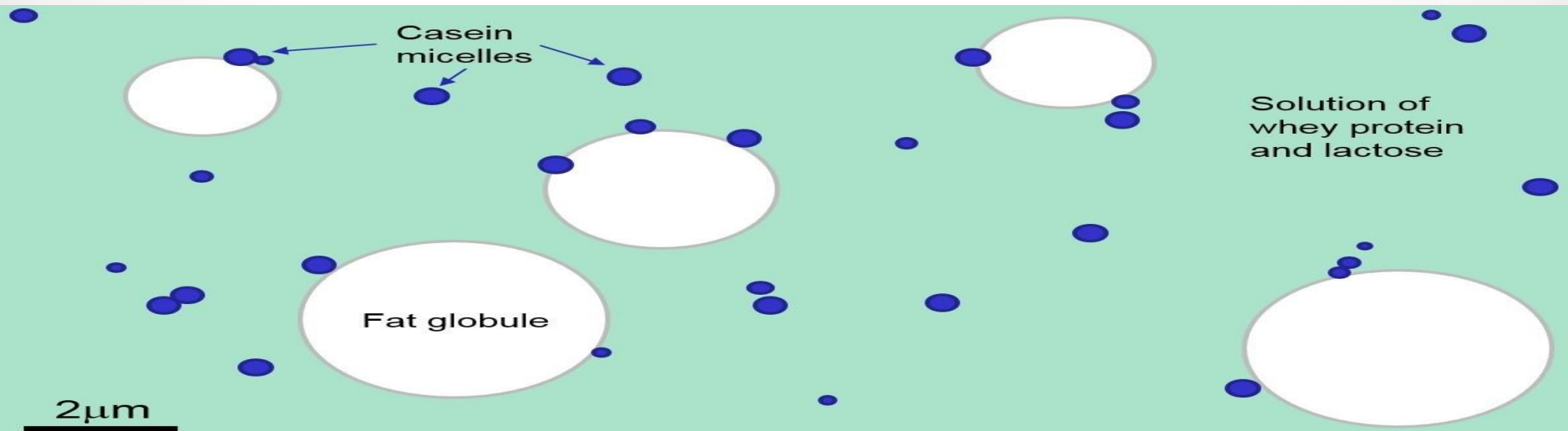


β -Casein



κ -Casein

Milk Protein



Introduction

- 1. Two-thirds of all cells consist of proteins and they have functional and structural roles in human body such as catalyst (enzymes), regulator (hormones), protection (Immunoglobulins), carrier proteins (haemoglobin, lactoferrin, etc.) and as structural proteins (collagens).**
- 2. Milk Protein is widely consumed human food infants and children.**
- 3. Major two groups of milk protein is Casein and Whey Protein.**
- 4. Casein is unique due to their characteristic physical properties which are different from globular proteins.**
- 5. Proteins are defined as high molecular weight polymers of α - amino acids that are formed by living organisms. All these amino acids have L-configuration except glycine.**
- 6. The primary structure of proteins consists of a polypeptide chain of amino acids residues joined through peptide bonds.**

Classification and Distribution of Milk Proteins:

Proteins in milk are classified on the basis of their fractionations and their behaviour during electrophoresis, difference in their solubility in various solutions, difference in their sedimentation rate etc.

a. Caseins (24-28 g/L)

α_{s1} -Caseins (12-15 g/L)

α_{s2} caseins (3-4 g/L)

β -Casein (9-11 g/L)

κ -Caseins (2-4 g/L)

b. Whey proteins (5-7 g/L)

α -Lactalbumins (0.6-1.7g/L)

β -Lactoglobulins (2-4 g/L)

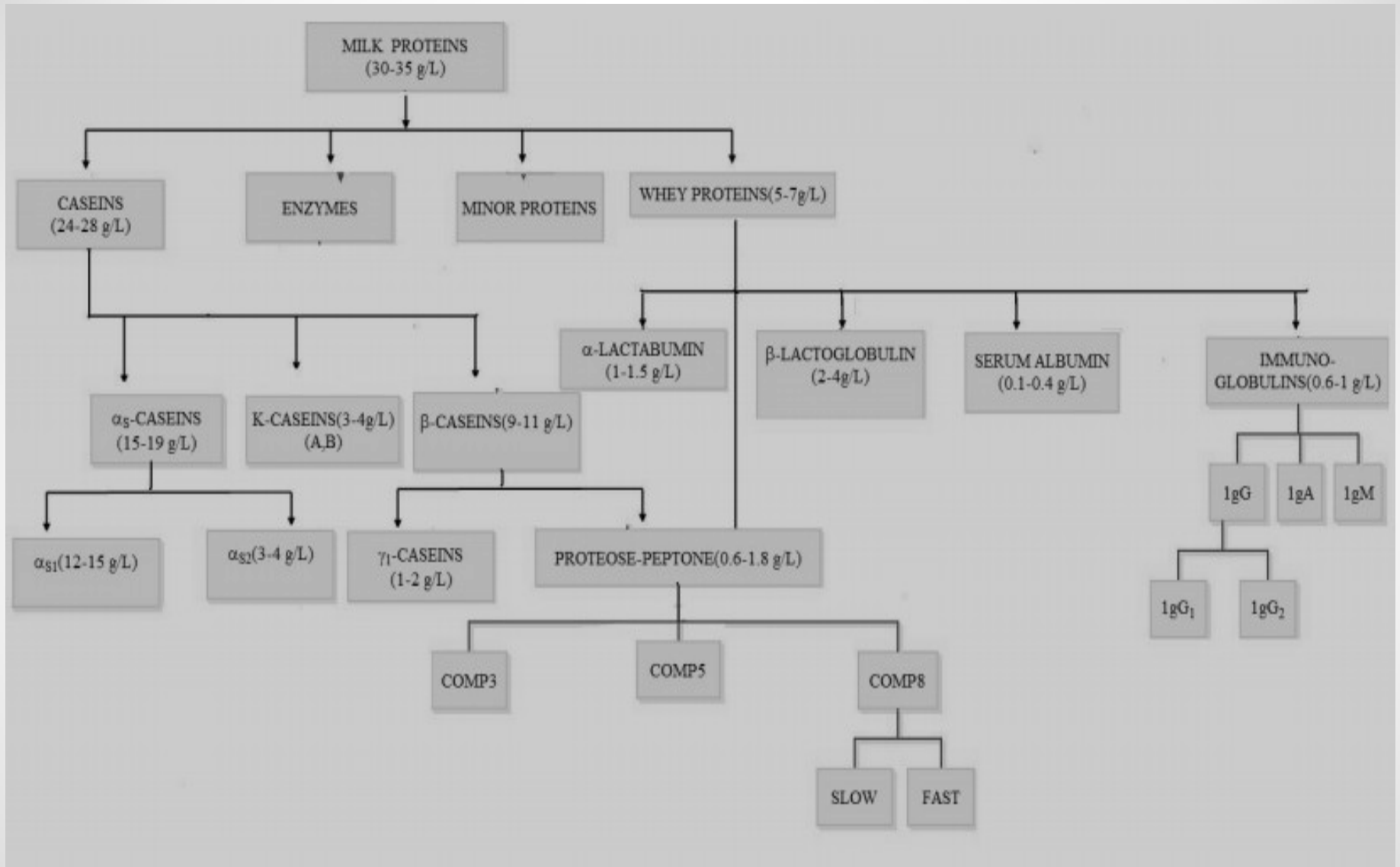
Bovine serum albumin (0.2-0.4 g/L)

Immunoglobulins (0.5-1.8 g/L)

Immunoglobulins

Distribution of Milk Proteins

- 1. Proteins in milk are chiefly the caseins, whey proteins and some minor proteins.**
- 2. Since enzymes are protein in nature they are also grouped along with other proteins. Caseins being the major fraction of the entire milk proteins are present in micellar form while the whey proteins being considered as soluble proteins.**
- 3. Enzymes play a significant role in the raw milk storage and processing of the milk and also found to have industrial importance.**
- 4. Minor proteins include vitamin binding protein, lactoferrin, metalli – protein, MFGM protein, ceruloplasmin etc.**



Casein

- 1. Low solubility at pH 4.6 and at this pH, all of the caseins except some of the proteolytic derivatives precipitate. As the solubility of caseins is much less and the whey proteins are having better solubility than casein the separation of the casein has become possible by lowering the pH value to 4.6.**
- 2. The ion exchange chromatography using DEAE-cellulose or hydroxyapatite columns would give satisfactory fractions. However, it is necessary to control the interaction of casein molecules.**
- 3. The unique feature of the caseins is their ester-bound phosphate. All of the casein polypeptide chains have at least one such group per molecule; whereas, none of the whey proteins have any ester bound phosphate.**
- 4. The α_1 and β -caseins contain no cysteine residues, while α_2 and κ -caseins each have two cysteine residues.**
- 5. Proline contents of caseins are rather high (α_1 -, α_2 -, β -, and κ -caseins contain 17, 5, 17 and 12 mol%, respectively).**

6. They have short lengths of α -helix or β -sheet structure in them. Their ionizable groups of the amino acids are accessible to titration and are also involved in several other side chains to reaction.
7. Denaturing agents and heating seems to have no effect on the secondary structure of these proteins. Thus their conformation appears to be much like that of denatured globular proteins.
8. As the protein is having large proportion of proline residues, closely packed orderly secondary conformation is being prevented in this protein molecule.
9. The four caseins differ greatly from each other in charge distribution and the tendency to aggregate in the absence and presence of Ca^{2+} ions.

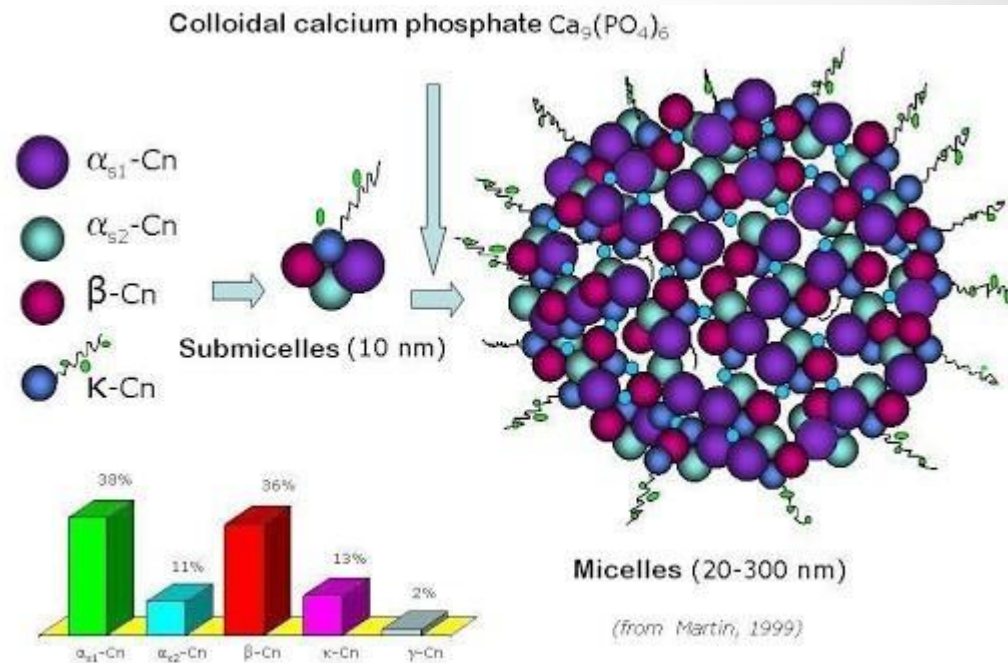
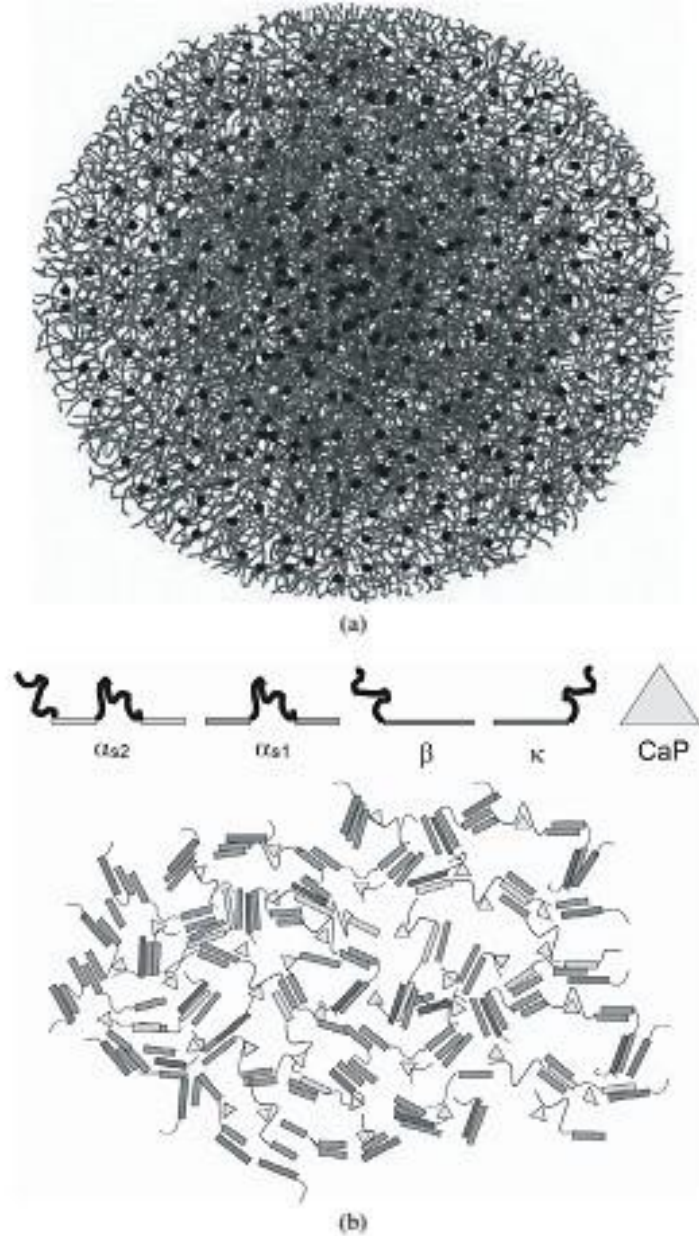
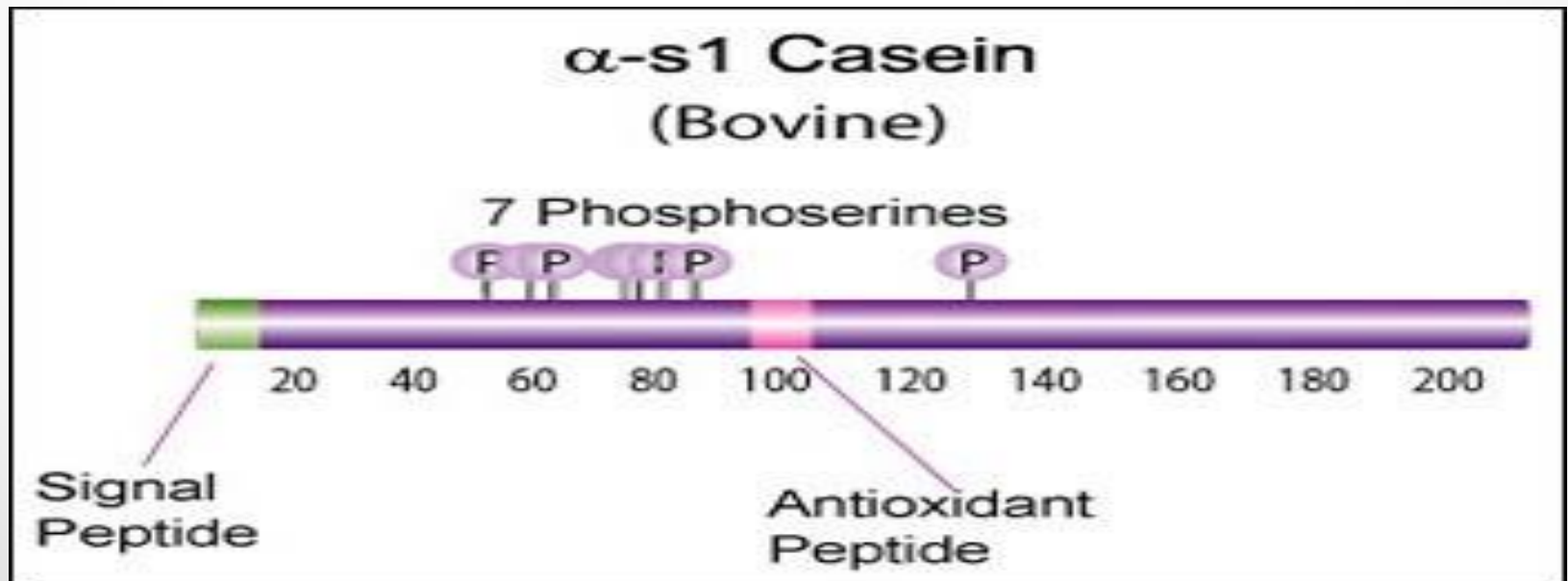


Figure 2. (a) The nanocluster model for casein micelles. Casein monomers are thread-like, while the dark circles represent calcium phosphate nanoclusters; reprinted from [12] with permission of Springer, NL. (b) The model of Horne – dual bonding model of casein micelles using casein monomers as indicated. Protein-protein interactions occur between hydrophobic regions (rectangular bars) while the protein hydrophilic regions (loops) bind to calcium phosphate clusters (triangles). κ -Casein is monomeric and on the surface; reprinted with permission from Elsevier.

α 1-casein

1. The polypeptide chain of α 1-casein consists of two predominantly hydrophobic regions (residues 1-44, and 90-199) and a highly charged polar zone (residues 45-89).
2. All but one of the phosphate groups is in the 45-89 residues segment, and prolines are distributed at intervals in the hydrophobic segments. Thus, this protein can be visualized as a rather loose flexible polypeptide chain.
3. Self-association of α 1-casein depends markedly on its concentration and on the pH, ionic strength, and kind of ion in the medium, but it is relatively independent of temperature.

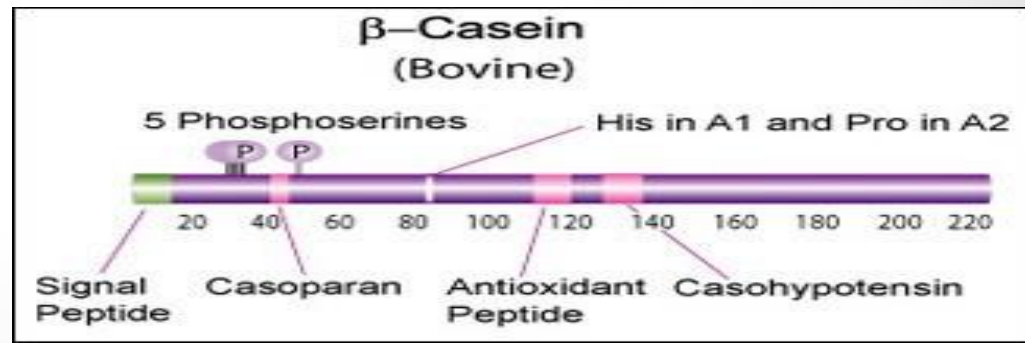


α 2-casein

1. α 2-casein has a remarkable dipolar structure with a concentration of negative charges near the N-terminus and positive charges near the C-terminus.
2. Its properties have not been investigated as but more thoroughly as those of the other caseins, certainly it binds Ca strongly and is even sensitive to precipitation by Ca^{2+} than α 1-casein.
3. It self-associates at neutral pH in the absence by Ca^{2+} and the association depends markedly on ionic strength and is at a maximum at an ionic strength of about 0.2.

β -casein

1. β -casein has a strong negatively charged N-terminal portion.
2. The net charge of the 21 -residue N-terminal sequence is 12 at pH 6.6, and the rest of the chain has virtually no net charge.
3. The large number of Pro residues effectively precludes extended helix formation. Thus, the β casein molecule is somewhat like that of an anionic detergent with a negatively charged head and an uncharged essentially hydrophobic tail.
4. The outstanding characteristics of the association of β -casein in both the absence and the presence of Ca^{2+} are its strong dependence on temperature.
5. In the absence of Ca^{2+} , only monomer is present at 4°C , but large polymers (20-24 monomers) are formed at room temperature.



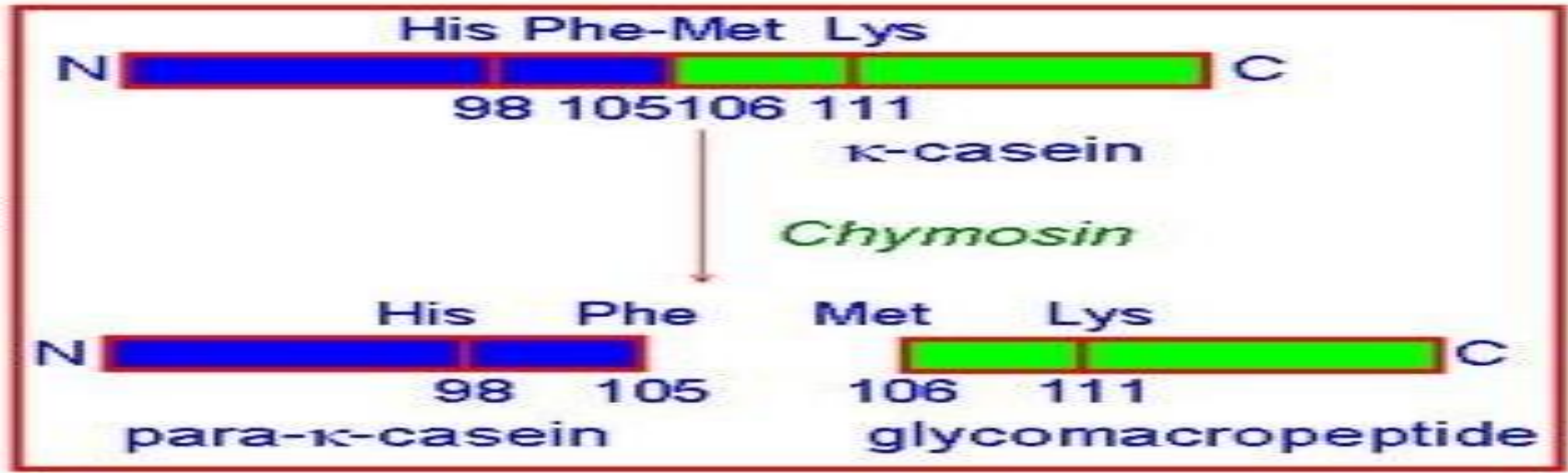
γ -casein

- 1. Group of caseins designated as γ -caseins have been known for some time to correspond to C terminal portions of the β -casein sequence. These are formed by cleavage of β -casein at positions 28/29, 105/106, and 107/108 by the enzyme plasmin.**
- 2. The fragments 29-209, 106-209, and 108-209 constitute the γ -caseins. The smaller fragments resulting from the cleavage appear in the whey when casein is precipitated by acid and constitute part of what has long been designated as the proteose-peptone fraction of the whey. Thus, fragments 1-105 and 1-107 were called as whey component 5, fragment 1-28 is whey component 8-fast, and fragments 29-105 and 29-107 were named as whey component 8-slow.**

κ -casein

1. About one-third of the κ casein molecules are carbohydrate- free and contain only one phosphate group (SerP-149).
2. They have varying numbers of N-acetylneuramic acid (NANA) residues and one, at least, appears to have a second phosphate (SerP-127).
3. Three different glycosyl oligomers linked to Thr-133 have been identified.
4. The N-terminal residue of κ -casein is glutamic acid. In the isolated protein it is present as the cyclic derivative pyroglutamic acid.
5. κ -casein consists of a mixture of polymers probably held together by intermolecular disulfide bonds; these polymers range in molecular weight from about 60,000 (trimers) to more than 150,000.

- 6. κ -casein is rapidly hydrolyzed at the Phe (105)-Met (106) bond by the enzyme chymosin (EC 3.4.23.4) yielding N-terminal fragment called para- κ casein, which contains the two cysteine residues, a C-terminal fragment of 64 residues called the glycomacropeptide, containing all of the carbohydrate and phosphate groups. κ -casein binds about 2 moles Ca^{2+} per mole of protein at neutral pH but differs markedly from the other caseins in its solubility.
- 7. The naturally occurring mixture of bovine κ -casein variants polymerizes through -S-S- linkages to subunits containing three to eight monomers. These further polymerize by non covalent association to particles of about 6,50,000 D. This polymerization is insensitive to concentration of Ca^{2+} and to temperature.



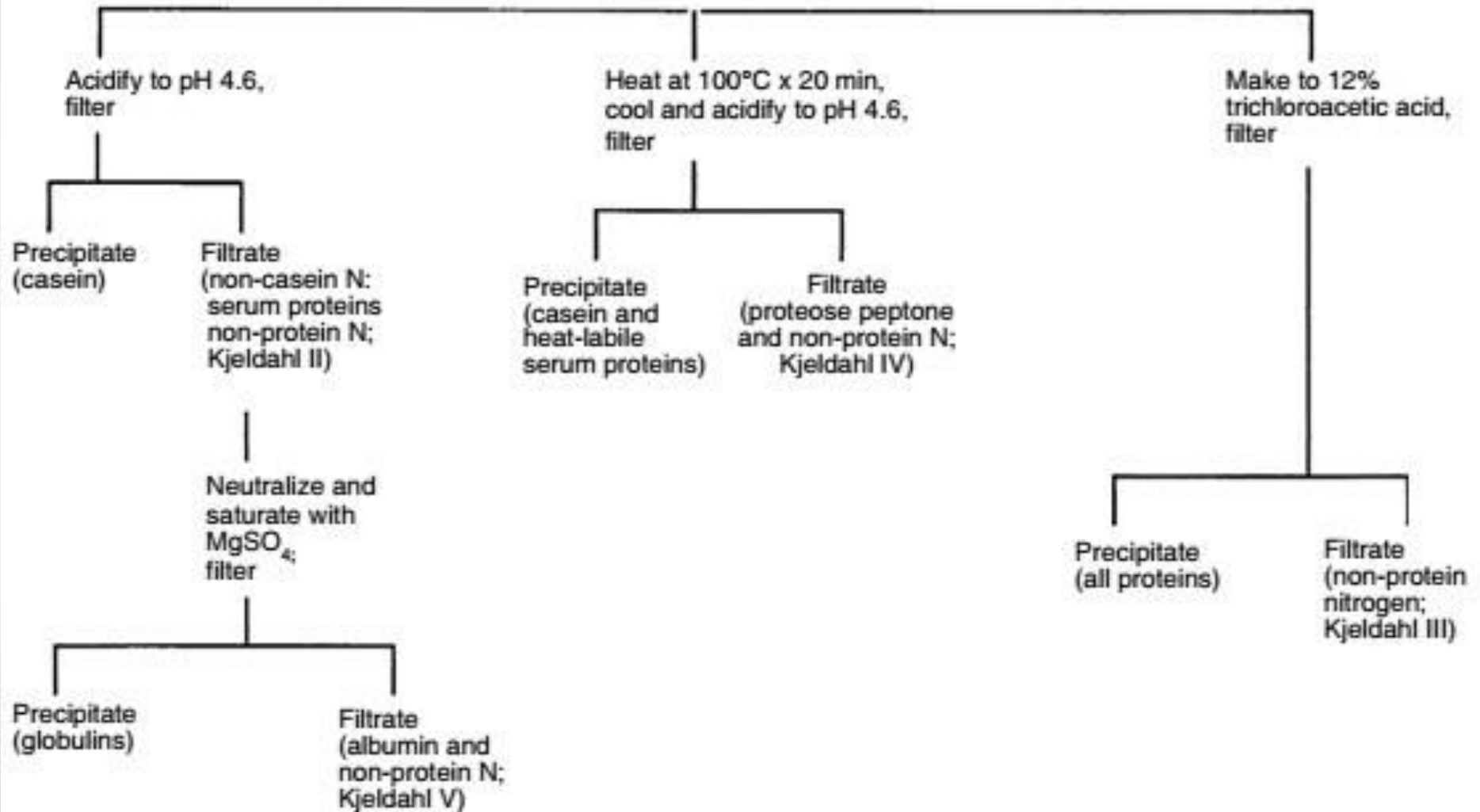
Milk Fractionation:

The behaviour of individual milk proteins and their concentration will be known when they are fractionated from milk.

Rowland method of fractionation

1. Rowland observed that when milk was heated to 95 C for 10 min, 80% of the nitrogenous compounds in whey were denatured and co-precipitated with the casein when the pH of the heated milk was adjusted subsequently to 4.6.
2. He considered that the heat-denaturable whey proteins represented the lactoglobulin and lactalbumin fractions and designated the remaining 20% 'proteose-peptone'.
3. The proteose peptone fraction, which is quite heterogenous is precipitated by 12% trichloroacetic acid (TCA) but some nitrogenous compounds remain soluble in 12% TCA and are designated as non-protein nitrogen.

Skimmilk (Kjeldahl I)



Structure of Casein Micelle:

- 1. Casein in cow milk forms intricate particles which are recognized as casein micelles.**
- 2. Highly insoluble material (calcium phosphate) has to be carried without disturbing either stability or increase in its size.**
- 3. The whole casein will form aggregate when it is in solution at a concentration, pH, and ionic strength as in milk and low Ca^{2+} activity.**
- 4. The micelles contain about 10-100 casein molecules.**
- 5. The aggregates like globular proteins will have a dense hydrophobic core in which most hydrophobic parts of the casein molecules are buried and a more loosely packed, hydrophilic outer layer containing most of the acidic (carboxylic and phosphoric) and some of the basic groups.**

6. Each of these small aggregates of the whole casein, usually called submicelles contains different casein molecules. The relative proportion of α s1: α s2: β and κ in casein micelle is 3: 0.8: 3:1 respectively.

7. Moreover, κ -casein probably exists in milk as an oligomer containing several molecules and held together by covalent bonds (S-S linkages).

8. Consequently there may be essentially two types of submicelles with and without κ -casein.

9. Earlier casein micelle models described were:

- 1) Core-coat model
- 2) Internal structure model
- 3) Sub-unit model

α -Lactalbumin:

This family of proteins consists of a major component and several minor components. Three genetic variants of α -lactalbumin have been identified.

Two genetic variants, A and B, of this protein exist. They differ by a single substitution, A having Gln and B having Arg at position 10.

In the milk of European breeds and yaks, only B variant is observed while both A and B variants occur in the milk of Indian cattle.

Some minor forms of bovine α -lactalbumin are revealed by

electrophoresis. Some of these contain covalently bound carbohydrate groups; the major component of bovine α -

lactalbumin is devoid of carbohydrate.

Other minor components have fewer amide groups than the major ones, and one minor α -lactalbumin has three instead of four disulfides. In total, the major components do not account for more than 10% of the α -lactalbumin.

The complete primary structure of the major α -lactalbumin has been determined. The B variant consists of 123 amino acid residues with a calculated molecular weight of 14,178 and the A variants differ from it only in having Gln instead of Arg at position 10.

The amino acid sequence of α -lactalbumin is similar to that of lysozyme. Indeed, bovine α -lactalbumin B and chicken egg white lysozyme have identical amino acid residues at 49 positions, and the four disulfide groups are located identically (positions between 6 and 120; 28 and 111; 61 and 77; and 73 and 91, respectively) in α -lactalbumin.

The biological activity of α -lactalbumin is its interaction with galactosyltransferase to promote the transfer of galactose from uridine diphosphate galactose (UDP-galactose) to glucose to form lactose. α -lactalbumin binds two atoms of Ca^{2+} very firmly. Removal of the bound Ca with ethylenediamine tetraacetic acid renders α -lactalbumin more susceptible to denaturation by heat or by addition of guanidine.

β -Lactoglobulin:

Aschaffenburg and Drewry (1957) demonstrated that there are two components of β -lactoglobulin in the electrophoretic pattern of this protein in the western cattle.

However, two more variants have also been identified by other workers. These genetic variants differ in their electrophoretic mobilities in starch or polyacrylamide gel in the ascending order as $A > B > C > D$.

Bovine β -lactoglobulin B consists of 162 amino acid residues. Their calculated molecular weight for monomer is 18,227 and dimer is 36000 respectively. The dimer contains five cysteine residues per mole, of which four are involved in disulfide linkages.

Location of one disulphide bond always occurs between Cys residues at 66 to 160 positions and the other link is between 106 and 119 or 121.

The single free thiol appears to be equally distributed between Cys 119 and Cys 121.

The existence of this thiol group is of great importance for changes occurring in milk during heating, as it is involved in reactions with other proteins, notably κ -casein and α -lactalbumin.

Considerable portions of the sequence of β -lactoglobulin exist in the α -helix and β -sheet structures. Regions that are most likely helical are residues 21-37, 51-63, 127-143 and 154-159 respectively. β -sheet structures are likely in 2-19, 39-43, 76-88, 91-99 and 101-107.

β -lactoglobulin exists naturally as a dimer of two monomeric subunits which is covalently linked. When more than one genetic variant is present, hybrid dimers are formed.

Dissociation to the monomer occurs below pH 3.4.

β -lactoglobulin A associates to form an octamer at pH 4.5 and low temperature. The B variant (predominant in Western cattle) octamerizes to a smaller extent.

Bovine Serum Albumin:

This is a major component of blood serum and synthesized in liver and gains entrance to milk through the secretory cells, but it comprises only about 1.2% of the total milk protein.

The protein as isolated from bovine milk could not be differentiated from that isolated from bovine blood by methods available in 1950. Since that time it has been assumed to be identical to that in blood.

The protein isolated from blood consists of a single polypeptide chain of 582 amino acid residues. Its tertiary structure reveals three equal-sized globular domains.

It has one free thiol and 17 disulfide linkages, which neatly hold the protein in a multi loop structure.

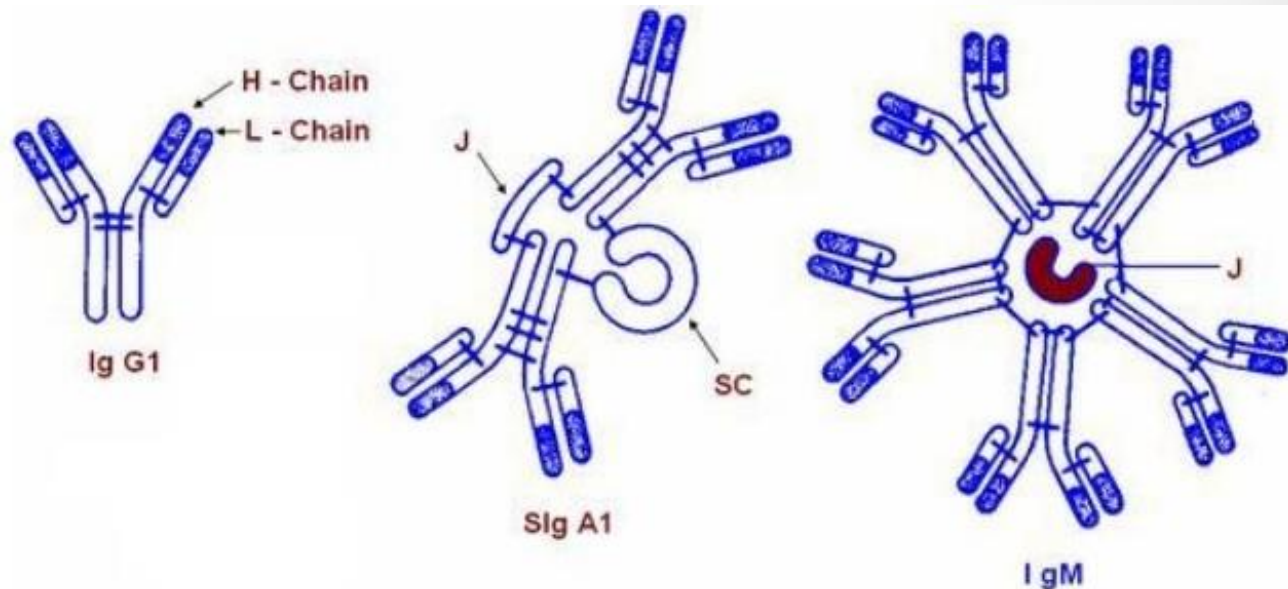
There is no specific role of this protein in the function of mammary gland.

Immunoglobulins:

- These are antibodies synthesized in response to stimulation by macromolecular antigens foreign to the animal.
- They are polymers with two kinds of polypeptide chains, light chain (L) of MW 22,400, and heavy chain (H). The latter are of several types, including γ (MW 52,000), α (MW 52,000-56,000) and μ (MW 69,000).
- Each of the L and H chains consists of a relatively constant and highly variable sequence and appears to be coded for by two genes. IgG1 and IgG2 are each polymers of two light chains and two heavy chains of the γ type (γ_1 and γ_2). The chains are joined by disulfide linkages to form two antibody sites, each consisting of the variable portion of an H and L chain.
- IgG1 and IgG2 have about 2.9% bound carbohydrate and MW of about 1,50,000. They differ slightly in electrophoretic mobility.
- IgA and IgM immunoglobulin likewise have the basic structure of two H and two L chains joined by disulfide bridges.

In IgA, the H chains are of α type, and in IgM they are of μ type. IgA, is secreted as a dimer of two of the basic four-chain units joined by a polypeptide of MW about 25,000 called J-component, and associated with another called secretory component, SC. This complex is called secretory IgA (SIgA) and has a MW of about 3,85,000. The secretory component is a protein of MW about 75,000, consisting of a single polypeptide chain with number of internal disulphide salt bridges. The carbohydrate content is high consists of N-acetylgalactosamine, D-glucose, D-mannose, L-fucose and N-acetylneuraminic acid. These sugars are bound to the SIgA.

IgM consists of pentamer of the basic four chain units joined by J component and has mol: weight of 9,00,000 and carbohydrate content of 11-12%.



Proteose-Peptide:

1. Rowland observed that if milk is heated, about 80% of the whey proteins consisting mainly of α -lactalbumin and β -lactoglobulins precipitate with the casein by acidification to pH 4.6.
2. The remaining 20% is a separate protein to which he applied the name proteose-peptide. Proteose and peptides are the polymers of amino acids which are of lower molecular weight than proteins.
3. They are usually not heat denaturable and hence it was easy for Rowland to analyze that the proteins of milk. The fraction may consist in part of native proteins and in part of breakdown products resulting from heat treatment.
4. PP fractions 5, 8 slow and 8 fast have little or no technological significance, proteose peptide 3 (PP3) has several interesting technological functionalities. PP3 is a heat-stable phosphoglycoprotein that was first identified in the proteose-peptide (heat-stable, acid-soluble) fraction of milk.
5. PP3 can prevent contact between milk lipase and its substrates, thus preventing spontaneous lipolysis and its emulsifying properties have also been evaluated in dairy products such as ice cream.

Non-protein Nitrogenous compounds:

- 1. Addition 12% TCA to milk would result in the of precipitation of all the caseins, α -lactalbumin, and β - lactoglobulins leaving the non protein nitrogenous compounds in the filtrate.**
- 2. The major compounds identified from this are uric acid, creatinine, orotic acid, phenylacetylglutamine etc.**
- 3. The compounds present in the urine of dairy animals have a remarkable similarity between those present in non- protein nitrogen fractions of milk.**
- 4. The compounds or substances present in the urine of dairy animals are the resultant waste metabolites of dairy animal body. It is apparent that the bulk of these waste metabolites in the urine of dairy animals originate from the blood and hence this appearance and levels in milk or urine are due to the protein metabolism of the animals.**

5. Intake of feed by the animals is directly proportional to the presence of these compounds or substances in milk.
6. NPN in milk varies from season to season and has no biological value as protein. It cannot be utilized by the body as a substitute of protein nor can it increase the cheese yield.
7. Pasteurization by itself has no effect. However pasteurization with homogenization causes an increase in the non protein and amino nitrogen content.

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

Other Whey Proteins: Lactoferrin and Transferrin

- These are the two iron-binding proteins are found in milk. One of them, transferrins (Tf), is a common blood plasmaprotein; the other, lactoferrin (Lf), is secreted not only by mammary glands but also by lacrymal, bronchial, and salivary glands.
- Both Tf and Lf appear to be large single chain polypeptides of 600-700 amino acid residues.
- In both proteins about 4 mol % of the residues are Cys, and both have covalently linked carbohydrate consisting of N- acetylglucosamine, mannose, galactose, and N-acetylneuraminic acid.
- All transferrins and lactoferrins appear to bind 2 mol of Fe^{3+} per mole.
- Tf and Lf differ markedly different from each other in amino acid composition and in electrophoretic mobility. They can be detected readily in electrophoretic patterns by auto radiography with ^{59}Fe .

- **Electrophoretic patterns of milk and blood preparations from individual animals reveal the occurrence of genetic variants of both proteins.**
- **No immunological cross-reaction between Tf and Lf has been demonstrated even when both are from a single species.**
- **Amino acid analyses and partial sequences of human Lf and Tf indicate some degree of homology between the two and some internal homology of peptide segments within each.**
- **The concentrations and ratios of Tf and Lf in milk vary greatly among species and with stage of lactation. The concentration of Lf in colostrums is about 1250 mg/kg in mid-lactation, the concentration falls to less than 100 mg/kg.**
- **Concentrations of Tf in milk have not been determined accurately but may be similar to Lf.**
- **Lactoferrin is an inhibitor of bacteria because it deprives them of iron. The concentration of Lf in bovine milk is so low, however, that it does not exert any significant antibacterial effect.**