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Casein Proteins: Structural and Functional Aspects

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Abstract

Mammalian milk is a complex fluid mixture of various proteins, minerals, and lipids, which play an important role in providing nutrition and immunity to the newborn. Casein proteins, which form about 80% of the bovine milk proteins, form large colloidal particles with calcium phosphate to form casein micelles, which for many years have been an important subject of interest. Casein micelles are composed of four main types of proteins: α_{S1}-casein, α_{S2}-casein, β-casein, and k-casein. These constituent casein proteins lack well-defined secondary and tertiary structure due to large amount of propyl residues. These micelles are being extensively studied because of their importance in functional behavior of milk and various milk products. However, the exact structure and nature of these casein micelles are still under debate. These different casein proteins possess different functional properties due to their primary amino acid sequence.

Keywords: Milk, Casein, Phosphoproteins, calcium

1. Introduction

It is now widely known that milk is a complex biological fluid secreted by mammals whose most important biological function is to supply nutrients for the nourishment of the off-spring. The term "micelle" has been applied to the dispersed phase of milk, that is the casein-protein complex. Casein protein component of milk is made up of different proteins, which possess different functions despite having no well-defined secondary and tertiary structure. These proteins, which include α_{S1}-, α_{S2}-, β-, and k-casein, have a primary amino acid sequence different from each other and occupy different positions in micelle and perform specific functions. Some of the proteins are involved in calcium phosphate transport while others in

stability of other caseins and micelle. The structure and properties of casein micelle as a whole and individual casein proteins, which constitute the micelle, are discussed.

2. Milk proteins and casein micelle

Milk is a complex biological fluid with high content of proteins, minerals, and lipids secreted by mammals to supply nutrition and to provide immunological protection to the newborn. The differences in the metabolic processes of the lactating mother and the nutritive requirements of the newborn are thought to be responsible for the interspecies differences in the composition of milk [1]. The main function of milk is to provide essential amino acids and minerals that are vital for the development and therefore function of muscular and other tissues in new born mammals. It also includes active proteins providing antibodies, metal and vitamin-binding proteins, and several protein hormones [2]. Milk proteins coagulate very rapidly in the stomach of newborn as they are structurally built in a way that they form large complexes with calcium phosphate. Normal bovine milk contains almost 3.2–3.7% protein which varies in composition and concentration during different stages of lactation [3, 4]. Milk proteins are divided into two classes and are no more thought to be a homogeneous protein [2]. Caseins constitute about 75–80% of total protein and precipitate at pH 4.6 at 30°C. The remaining fraction, serum or whey protein, is soluble under similar conditions [5]. The rest of proteins found in milk are trace fractions of glycoprotein [6]. Casein proteins and calcium phosphate form large colloidal particles called casein micelles, which have been the subject of interest for many years [7]. The main function of the casein micelle is to provide fluidity to casein molecules and solubilize phosphate and calcium. There is a very large flow of calcium through the mammary epithelial tissue, and despite this, there is rarely any formation of calcium stones in the mammary gland. It has been suggested that the calcification of the mammary gland is prevented by the formation of casein-micelle complex with calcium phosphate. The primary amino acid sequence of casein proteins and their conformation in solution are therefore thought to prevent calcification of the mammary gland in addition to providing nutrition [8]. In addition to their biological role, which is to provide nutrition, caseins are also studied for their role in human health and other malfunctions such as stone-forming diseases in bovine animals [9–12].

The casein-micelle structure is being studied extensively because of its importance in the functional behavior of milk and some milk products [5]. However, the exact structure of casein and its micelles is still under debate. Various physical and biochemical studies of these colloids have mainly focused on the properties, size of the colloids, protein composition, micelle reconstitution, etc. Due to the large size of the casein-micelles, which interfere with absolute structure determination, different models have been proposed. Models can be classified into three categories: coat–core model, subunit or submicelle model, and internal structure model. Waugh and Nobel in 1965, Payens in 1966, Parry and Carroll in 1969, and Paquin and others in 1987 proposed coat-core models. Coat-core model dictates that micelle is an aggregate of caseins with outer and interior of micelle having different composition, and there is an

inaccuracy in the identification of inner part of the structure [13–16]. This actually contains two diametrically opposite theories. Waugh and Nobel in 1965 were the first who proposed a model which fits in this class which is based primarily upon the solubilities of various caseins in Ca^{2+} solutions. According to them, α_{S1}- and k-caseins form low weight ratio complexes in the absence of calcium. Monomers of α_{S1}- or β-caseins with charged phosphate loop form caseinate core due to addition of calcium ions. The α_{S1}- or -β-caseins in their monomeric form with charged phosphate loops form limiting size aggregates/caseinate core. The formation of low weight α_{S1}-k-complex monolayer leads to the prevention of caseinate precipitation. The k-casein monomers spread out entirely on the surface of coat/complex formed, and therefore, its amount dictate the size of casein micelle. This model explains the lyophilic nature of the colloidal casein complex and also the ready accessibility of k-casein to chymosin and therefore is quite appealing [17]. According to Payens (1966) model based on his experimental data on the association of caseins, the densely folded α_{S1}-caseins remain adhered to loose network of β-caseins to form micelle core. Unlike the Waugh and Nobel models, colloidal calcium phosphate is present both on the outer surface and in the inner side of the micelle, while k-casein is confined to the surface of the micelle [7]. In 1969, Parry and Carroll used electron microscopy to locate k-casein on the surface of micelle as proposed by Waugh. They suggested that k-casein is present at the interior and acts as a nucleating agent to which calcium-insoluble caseins might cluster and gets stabilized the colloidal calcium phosphate. They used ferritin-labeled anti-k-casein antibodies to localize kappa casein at the outer surface of casein micelles. They found very little or no concentration of k-casein protein on the outer surface of the casein micelles as was suggested by previous workers. According to this model of casein micelle, the surface of the micelle comprises α_{S1}- and β-caseins with some colloidal calcium phosphate [18]. Paquin et al. in 1987 proposed a model based on results obtained from experiments using gel chromatography of EDTA-dissociated casein micelles for identifying two protein fractions by monolayer methods. This model describes the micelle core as a scaffold of colloidal calcium phosphate and α_{S1}-caseins, while β-caseins are held by hydrophobic interactions. These models predict a precise distribution of k-casein and are based upon nucleation around a core which is k-casein in case of Parry and Carroll and $\alpha S1$, β-calcium caseinate in case of Waugh [7].

The submicelle models that were proposed by Shimmin and Hill (1964), Morr (1967), Slattery and Evard (1973), Schmidt (1980), Walstra (1984), and Ono and Obata (1989) considered that casein micelles are composed of uniform subunits that are roughly spherical in shape [19–23]. Shimmin and Hill (1964) were the first who postulated a submicellar structure for the casein micelle [24]. They used electron microscopy to study the ultrathin cross sections of embedded casein micelles and measured a diameter of 10 nm for the submicelles [24]. Another model proposed by Morr (1967) which was based on results obtained from study of oxalate and urea treatment on the disruption of casein micelles and proposed that α_{S1}-, β-, and k-monomers formed small uniform submicelles. These casein micelles are composed of numerous, loosely packed, calcium caseinate complex units, joined in association by a combination of calcium and colloidal calcium phosphate and citrate linkages between casein phosphoserine and carboxyl groups. Hydrophobic bonding and calcium caseinate bridges stabilize the submicelles, while colloidal calcium phosphate helps to aggregate the submicelles into micellar structure [19]. Each of these calcium caseinate complex units is probably composed of an inner

core consisting of a α_{S1}- and β-casein, surrounded by an outer layer rich in α_{S1}- and k-casein, as suggested by Waugh and noble [14, 25]. According to Morr, these submicelles have a diameter of ∼30 nm studied by using sedimentation velocity which is somewhat larger than that postulated by Shimmin and Hill. Additional β-casein could become associated with the outer surfaces of the micelle under appropriate conditions which favor conversion of soluble casein (mainly β-casein) to micellar casein. A model was proposed for the native casein micelle which consists of numerous loosely packed calcium caseinate complex units joined in association by a combination of calcium and colloidal calcium phosphate–citrate linkages. The colloidal calcium phosphate–citrate is considered to be distributed throughout the micelle rather than as a layer on its outer surface. α_{S1}-, α_{S2}-, and β-casein precipitate when calcium binds to their phosphoserine residues. k-casein at the other end is not only calcium insoluble, but it also interacts with other calcium-sensitive caseins and stabilizes them thereby initiates the formation of the stable colloidal state. Various enzymatic, immunological, and chemical techniques usually recognized that while majority of the k-casein must reside on the outer surface of the casein micelles, other caseins might also occur there as well [26, 27]. k-casein is thought to be predominantly present on the outer surface of the casein micelle as shown by almost all researchers working in this field till date. Various methods for disruption of casein micelles have been used by several other researchers to study the nature of submicelle. Carroll et al. (1971) used urea, EDTA, sodium fluoride, and sodium lauryl sulfate for the disruption of micelles and found particles ∼8 to 12 nm in diameter [28]. Submicelles of 10 nm diameter were also found by Schmidt and Buchheim (1970) after they dialyzed milk free calcium in cold and using high pressure to disrupt casein micelles [29, 30]. These results were confirmed by Buchheim and Welsch in 1973. Pepper and Farrell (1982) used gel chromatography to study interaction of concentration-dependent interactions of EDTA dissociated whole-casein micelles. It was found that with increasing protein concentration at 37°C and pH 6.6, the individual components of casein formed polymers which approached a molecular radius of ∼9.5 nm [31]. These submicelles were thought to be formed by the interaction of SH-k-casein monomers with those of αS- and β-caseins as seen by analyzing concentration elution profiles. Carroll et al. (1970) and Farrell and Thompson (1971) also observed particles of ∼10-nm diameter in the Golgi vacuoles of lactating rat mammary gland and therefore supported the hypothesis of Shimmin and Hill (1964) [28, 32]. Another model for casein micelle structure is based on the results of various experiments on the effect of calcium on the sedimentation behavior of those particles which are formed in mixtures of caseins was proposed by Slattery and Evard in 1973. This model based upon casein interactions combines the best features of most casein micelle models. According to this model, submicelles which are rich in k-casein are found predominantly on the outer surface of the casein micelle, while those poor in k-casein content are internalized. They suggested that casein monomers interact to form submicelles of variable composition depending upon their casein content. This model also suggests an inverse relationship between k-casein content and micelle size. This model predicts large casein micelles which are poor in k-casein content, k-casein will occupy position on surface, while in smaller micelles which are rich in k-casein, k-casein is uniformly distributed [22]. Walstra (1984) proposed the submicelle model for casein which is the most accepted model for casein. According to this model, spherical subunits or submicelles are the building blocks

of casein micelles. Each submicelle is variable in composition with 20–25 casein molecules per submicelle, and the diameter of submicelle is 12–15 nm. Hydrophobic interactions between the constituent proteins and the calcium phosphate linkages keep the submicelle together. According to this model, there are two types of submicelles one consisting of αS- and β-caseins and another αS- and k-caseins, the former is has hydrophobic regions buried in the center while latter is more hydrophilic because of the presence of sugar residues on k-caseins. Further aggregation of submicelles is avoided by the steric and electrostatic repulsions by the hydrophilic part of the C-terminal end of k-casein located near outside of micelle, protruding from the micelle surface as a hairy layer [23]. Carroll and Farrell in 1983 also found that the location of k-casein is indeed related to casein micelle size using ferritin-labeled double-antibody technique coupled with electron microscopy [33]. These results confirm the inverse relationship between micelle size and k-casein content and also that larger casein micelles contain higher polymers of k-casein, indicating that k-k interactions are greater in k-poor micelles. Since according to this model, k-casein is not totally precisely localized in the micelles this model therefore contradicts with models proposed by Parry, Waugh, Garnier, and Ribadeau-Dumas and supports the more flexible model of Slattery and Evard [22, 25, 34].

The internal structure models, which are the last models, were proposed by Rose (1969), Garnier and Ribadeau-Dumas (1970), Holt (1992), and Horne (1998) indicate the manner in which different caseins aggregate [34–37]. The internal structure model of casein micelle is based upon the properties of isolated protein components which are involved in the formation of internal structure of the micelle. Rose (1969) was the first to propose internal structure model by using the endothermic polymerization of β-casein as the basis for his casein micelle model. According to this model, α_{S1}-monomers attach to chain like polymers of β-casein which are self-associated from β-casein monomers. k-casein interacts with α_{S1}-monomers. The β-casein is directed inward, while k-casein is directed outwards and a small amount of k-casein is placed in an internal position as these two segments associate. Colloidal calcium phosphate is incorporated as a stabilizing during the formation of micelle. The occurrence of some overall stoichiometry of the various casein components and the role of colloidal calcium phosphate in stabilizing micelle make this model appealing [37]. However, synthetic micelles can be formed from simple k- and α_{S1}-casein complexes in the complete absence of β-casein which makes β-casein as the basis of micelle formation questionable. Waugh et al. (1970) have also shown that the α_{S1}- and β-caseins tend to form mixed polymers randomly and β-casein is structure less in solution. It also forms micellar-like complexes rather than linear polymers [38]. Garnier and Ribadeau-Dumas (1970) who proposed another model emphasize on k-casein as the foundation of micelle structure. According to this model, three chains of α_{S1}- and β-casein are linked to the trimers of k-casein which radiate from the k-casein node which is present as a Y-like structure. There is a formation of loosely packed network when these α_{S1}- and β-caseins connect to other k-nodes. This model places steric restraints upon k-casein which posses few secondary structures. This model provides demonstrated porosity and explains a uniform distribution of k-casein regardless of micelle size. The model assigns no role to calcium caseinate interactions and ignores the role of colloidal calcium phosphate involvement in stabilization of the micelle. Although the submicelle casein model proposed by Walstra in 1999 has been widely accepted, there are two alternative models proposed by Holt in 1992 and

Horne in 1998 which fall into internal structure model [6, 35, 36]. According to model proposed by Holt, the casein micelle forms a tangled web of flexible casein networks forming a gel-like structure with C-terminal region of k-casein extending to form a hairy layer and microgranules of colloidal calcium phosphate at center. The surface location of k-casein and the cementing role of colloidal calcium phosphate are the two main features of this model. The caseins micelles according to this model are stabilized by two main factors one of which is steric stabilization by protruding k-casein layer hairs and another is by surface potential of approximately -20mV at pH 6.7. In 1998, Horne proposed dual bonding model which suggests that it is a balance between electrostatic repulsions and attractive hydrophobic interactions which held the proteins in casein micelles together. According to this model, hydrophobic interaction is the driving force for the formation of casein micelles and electrostatic repulsions are responsible for limiting the growth of polymers [36]. α_{S1}- and β-caseins self-associate by hydrophobic interactions as a result of formation of train–loop–train and tail–train like structures, respectively, upon adsorption at hydrophobic interfaces. There occurs a reduction in electrostatic repulsion because of colloidal calcium phosphate which form linkages between casein micelles and neutralizing agents of the negative charge of phosphoserine residues which makes the hydrophobic interaction between caseins a dominant force for the association of proteins. The lack of phosphoserine cluster to bind calcium in k-casein makes it to interact hydrophobically and act as a propagation terminator.

3. Forces responsible for the stability of the casein micelle

Linderstrom-Lang in 1929 postulated that mixture of calcium-insoluble proteins stabilized by calcium-soluble protein form the colloidal milk complex [39]. The calcium-soluble protein would be readily split by chymosin which leads to its coagulation due to destabilization of colloid. Such fractions exist as α_{S1}-, α_{S2}-, and β-caseins which are insoluble in calcium and k-casein which is soluble in presence of calcium and is split readily by chymosin. Sedimentation velocity experiments performed by Waugh et al., in 1971, demonstrated that α_{S1}- and k-casein complexes can be reformed from already isolated fractions [40]. A brief summary of the various types of bonding forces responsible for the stabilization of protein structure will be discussed.

3.1. Hydrophobic interactions

There is presence of large number of hydrophobic residues clustered together in α_{S1}-, β-, and k-casein as found by amino acid sequence analysis of these proteins. Since these are among the most hydrophobic proteins, role of hydrophobic bonding in the stabilization of casein cannot be ignored. The ability of β-casein to self-associate was reduced after removal of isoleucine and valine at C-terminal end of protein which normally self-associate in the absence of calcium [41]. Additionally, the ability of β-casein to form polymers was destroyed completely after removal of 20 amino acids at C-terminal which are mainly hydrophobic in nature [41]. Various investigators have found that α_{S1}-, β- and k-caseins diffuse out of the micelle at low temperature due to decrease in hydrophobic interactions [42–44]. The micelles containing rare α_{S1}-A genetic variant which possesses similar physical and solubility properties like that

of β-casein is also less stable in cold. On the basis of light scattering and electron microscopy, it has been found that increased pressure disrupts casein micelle structure which also acts primarily on hydrophobic interactions [42–46]. The dependence of hydrophobic interactions on temperature and pressure also explains the resistance of skim milk to withstand higher temperature which is otherwise destabilized at extremely low temperatures.

3.2. Electrostatic interactions

There are many potential sites for strong ion bonding in apolar environment that might play a role in the stabilization of casein micelles. It is not possible to exactly assess the role of various inter- and intramolecular ionic bonds present in αs-, β-, and k-casein in stabilization of casein micelle structure. The ability of k-casein to stabilize the α_{S1}-casein is abolished when there is carbamylation of lysine residues in k-casein which further demonstrate that ionic interactions play a role in the casein micelle structure [47]. Modification of arginine side chains also affects the casein micelle stability and chymosin-induced coagulation [48].

3.3. Hydrogen bonding

The α-helical and β-pleated structures in many globular and fibrous structures are stabilized by hydrogen bonding along the polypeptide chain. Since casein proteins posses very little secondary structure and 72–76% of protein exists in aperiodic form, the degree of stabilization by α-helix and β-structure is very low [49, 50]. Hydrogen bonds between the various components of casein during the formation of highly aggregated casein micelle are possible but in case of ionizable side chains of monomeric proteins which are accessible to water, their contribution to the stability of these monomeric proteins is very less. For the formation of a residue-residue hydrogen bond in case of these monomeric proteins, there must be breakage of water-residue hydrogen bond which has already formed. During the interaction of two subunits of a protein, there are chances of formation of hydrogen bonds between individual monomers as the surface groups are no longer fully hydrated. Hydrogen bond may exist during the formation of aggregated casein micelles and self-association of α_{S1}-casein.

3.4. Disulfide bonds

Disulfide bonds between cysteine residues during folding of pleated sheet structures, helical segments, and unordered structures leads to the formation of tertiary structure. Both α_{S2}- and k-casein contain cysteine but the degree of disulfide cross-linkages which are normally present in the casein micelle is controversial [51–53]. It has been reported by many investigators that disulfide cross-linkages contribute to the overall stability of the casein micelle but they are not the driving force for the formation of casein micelle. Slattery in 1978 found that larger micelles have higher molecular weight disulfide-bonded polymers of k-casein. These k-casein molecules are thought to be contiguous with each other and form disulfide-linked aggregates which compose the casein micelle structure [54].

4. Casein proteins as internally disordered calcium-binding phosphoproteins

Casein proteins are phosphoproteins which comprise approximately 80% of the total protein present in bovine milk [55]. They were defined as phosphoproteins which precipitate from raw milk upon acidification at pH 4.6 at 20°C [56]. Casein proteins belong to one of the larger family of secretory calcium-binding phosphoproteins as has been found by the analysis of structure of human genome. Casein proteins provide one of the best example of intrinsically disordered or natively disordered or natively unfolded proteins [57]. The previous assumption that only those proteins which possess a well-defined folded conformation is able to perform a specific biological function is not valid in case of many intrinsically disordered proteins as they have specific biological functions even in their unfolded state [58]. Furthermore, it has also been found that in case of many of the eukaryotic proteins involved mainly in signaling pathways, there is presence of regions with disordered backbone conformations. The presence of disordered region in a protein involved in signaling provides larger surface area for interactions with other proteins. This property can therefore also help these proteins to interact with multiple proteins or target molecules at one time [59]. All of the casein proteins possess very little secondary and tertiary structure but are still able to perform their function in their disordered state. Caseins belong to the scavenger family of secretory calcium-binding phosphoproteins as they are involved in trapping of calcium phosphate. The intrinsic disorder of these proteins not only help in forming a thermodynamically stable complex with calcium phosphate but also allow these proteins to form a more tightly packed complex than a globular structure [58]. These casein proteins are post-translationally phosphorylated at seryl and very less frequently at threonyl residues which is one of their unique characteristic. k-casein contains only one or two phosphoseryl residues and is only casein which is glycosylated [53]. These calcium-sensitive caseins are not only able to bind to calcium phosphate crystal surface but are also able to form calcium phosphate nanoclusters which are thermodynamically stable chemical complexes by sequestering amorphous calcium phosphate. Calcium phosphate sequestration also depends upon the formation of phosphate centers in the primary sequence by clustering of phosphorylated residues [60].

5. Properties and functions of different protein components of casein

Earlier principle protein of bovine milk was considered to be homogenous protein casein. Later on it was found that casein proteins are heterogeneous and are composed of distinct fractions like α-, β-, and k-casein [61]. Casein in milk in its native state exists as large associate of macromolecules in colloidal dispersion with a mass of $\sim 10^8$ Da and ~ 200 nm in size [62]. The major protein of the casein complex is α_{S1}-casein which almost 38% followed by β-casein 36%, k-casein 13%, and α_{S2}-casein 10% [63]. Proline which is known to disrupt alpha-helical and β-structures is present in higher amount in α_{S1}-casein. It has been found that 70% of α_{S1}-casein is in unordered form with only a small amount of α-helical and β-structure. α_{S1}-Casein plays an important role in the ability of milk to transport calcium phosphate. It has also been found

that one of its antioxidant peptide has 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. All casein proteins in their native states do not possess a well-defined tertiary or secondary structure [49]. Casein is classified as intrinsically disordered proteins implying that the protein is functional in its unstructured form. Further, it has been found by various investigators that α_{S1}- and β-casein possess chaperonic activity and are responsible for the stabilization of micelle by preventing aggregation of α_{S2}- and k-casein, respectively. Various investigators have also found that αs-casein prevents the stress-induced aggregation of various proteins like bovine serum albumin, whey proteins, β-lactoglobulin, carbonic anhydrase, and alcohol dehydrogenase by forming soluble, high molecular weight complexes [64]. The self-association of α_{S1}-casein monomers in aqueous solution is attributed to the high degree of hydrophobicity and small amount of structural content [38, 45]. There are ~43% hydrophobic amino acids in case of α_{S1}-casein, ~33% in case of α_{S2}-casein, ~52% in β-casein, and ~43% in k-casein. Under the normal pH, ionic strength, and temperature of milk, the major protein components of milk are insoluble. The second most abundant milk protein is β-casein with five phosphoserine residues and a molecular weight of 23,980 [65]. All the protein's net charge, phosphoserine content, and α-helical residues are restricted to the first 40 amino acid residues present at N-terminal portion of β-casein, while C-terminal contains many apolar residues responsible for its high hydrophobicity [49]. β-Casein possesses very little secondary structure and is present as a random coil in aqueous solution. The lack of secondary structure is due to the evenly distributed proline found during complete amino acid sequence of β-casein [66]. β-Casein plays an important role in determining the surface property of casein micelle. One of its peptide acts as a macrophage activator thereby increase the phagocytic activity of macrophages and their peroxide release. β-casein also possesses an antioxidant peptide which has antioxidant activity. The last casein sequenced was α_{S2}-casein which possesses most unique primary structure of all the caseins with a molecular weight of 25,150 [67]. This protein has two cysteine residues with no known carbohydrate. α_{S2}-Casein exists as a dimer or may have some intrachain disulfide. α_{S2}-Casein is least susceptible to aggregation because of alternating negatively charged and hydrophobic areas [68]. It also plays important role in the transport of calcium phosphate. Its anti-microbial peptide casocidin-I has the ability to inhibit growth of E. coli and other bacteria. k-casein, which is soluble over a very broad range of calcium ion concentrations unlike other forms of caseins like α_{S1}-, α_{S2}-, and β-casein, is the fourth major component of the milk-protein complex [69]. Calcium solubility of k-casein has led workers to assign to it the role of casein micelle stabilization whose other components are insoluble in calcium. It stabilizes micelle formation thereby prevent precipitation of casein in milk. Casoxins peptide possesses opoid antagonist, whereas casoplatelin inhibits platelet aggregation. The molecular weight of reduced k-casein is about 19,000 [51]. It exists as a high molecular weight mixtures of polymers. It is the only major component of casein which possesses carbohydrates bound to the highly soluble macropeptide portion formed after chymosin hydrolysis. There are only one or two phosphate residues per k-casein casein monomer which makes it soluble in calcium [70].

There are several genetic variants of casein components with variable numbers of phosphoseryl residues especially in case of α_{S2}-casein which exhibits a large variability in the extent of phosphorylation [71]. Another unique feature of caseins is the large amount of propyl

residues especially in case of β-casein which greatly affect the secondary and tertiary structure of caseins [53]. In addition, all casein proteins possess different hydrophilic and hydrophobic regions along the protein chain [46]. αS-Caseins are the major casein proteins with 8–12 seryl phosphate groups, while β-casein contains about 5 phosphoserine residues and is more hydrophobic than αS-caseins and k-casein [49]. Highly phosphorylated αS-caseins and β-caseins are very sensitive to the of calcium salt concentration, that is, these proteins precipitate in presence of high Ca^{2+} ions [40, 72]. Unlike other types of caseins, k-caseins are glycoproteins [56, 71] with only one phosphoserine group. This makes them stable in the presence of Ca^{2+} ions thereby playing an important role in protecting other caseins which are calcium sensitive from precipitation and makes casein micelle stable [69]. Casein is insensitive to heat, and it is only temperature above 120°C that causes the casein proteins to become insoluble, whereas it is sensitive to pH and will precipitate at its isoelectric pH [73]. The individual families of casein proteins were identified by alkaline urea gel electrophoresis. Each of the four different caseins may have a variety of numbers of phosphate groups attached through their serine or threonine residues. In terms of the extent of phosphorylation, α_{S1}-casein may have 8 or 9, α_{S2}-casein 10–13, β-casein may have 4 and k-casein, 1–3. α_{S1}- and β-Casein contain no disulfide bonds, and α_{S2}- and k-casein contain two cysteine residues which form inter- or intramolecular disulfide bonds [74, 75]. α_{S2}-Casein exists as a dimer, and k-casein can exist from dimer to decamer depending upon the pattern of intermolecular disulfide bonding [68]. There are 10 different molecular forms of k-casein on the basis of degree of glycosylation and is the only casein which is glycosylated [56, 70, 71]. Another source of variability in caseins is genetic polymorphism. α_{S1}-Casein has been shown to be present in bovine milk as α_{S1}-casein A-D [71]. Caseins are structurally classified as natively or intrinsically disordered proteins which is different from random coil conformation found in some globular proteins [76, 77]. Due to the lack of well-defined structure, crystallization of casein proteins to provide a three-dimensional crystal structure is not possible, but at the other end, this lack of structure helps to facilitate proteolysis and therefore ready absorption of amino acids and small peptides in the intestine [2, 78]. Caseins proteins possess very little three dimensional structure but possess some secondary structure [79]. The high number of proline residues which distort protein folding into α-helices and β-sheets is responsible for inhibition of higher proportions of secondary and tertiary structure. Casein proteins contain 32–42% non-polar amino acids which makes them highly hydrophobic but due to the presence of high number of phosphate and sulfur-containing amino acids and carbohydrates in case of k-casein, they are quite soluble in aqueous solvents [2]. Casein proteins are homologous in all the species as has been found by various protein and gene sequencing studies [80]. The proportion of various caseins varies widely. All species form colloidal casein micelles for the transport of calcium and phosphate. Casein micelles of most species appear quite similar at the ultra structural level. Despite the variations in casein components, the α_{S1}- and α_{S2}-caseins are calcium sensitive, whereas β- and k-casein are not sensitive to calcium. Casein proteins are important nutritionally because of their high phosphate content due to which they bind significant quantities of calcium and also are rich in lysine which is an essential amino acid in humans. α_{S1}- and α_{S2}-casein possess 14 and 24 lysines, respectively [2]. Each of the caseins possesses significant variability due to extent of their post-translational modification, disulfide bonding, genetic polymorphism [81]. The

properties of milk and various milk products mainly depend on proteins present in it and to some extent on other components like salts, fat, and lactose. Caseins which possess an extraordinary high heat stability make the milk and other milk products highly stable even at higher temperature [61].

6. Conclusion

Mammalian milk contains casein micelles that help to provide adequate nutrients to the neonate and at the same time prevent any risk of pathological calcification or amyloidosis. Interestingly, all caseins exhibit a disordered conformation and many have chaperonic activity (α_{S1}- and β-casein) which might be an attribute that help these casein proteins to self-associate and assembly into functional micelle. The structural disorderness and the chaperonic property would have been evolutionarily selected to make these molecules ideally suitable to thrive under various environmental insults since the milk is secretory product. It might be possible that that in addition to casein, many other milk proteins may also have chaperonic function. Identifying chaperonic function of other proteins present in milk will have many industrial and clinical insights.

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