

Models for Casein Micelle Formation

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Abstract

The synthesis and secretion of milk involves the formation of a distinctive disaccharide, lactose, and two quite unique biological structures, the fat globule and the casein protein-complex (the casein micelle). The mechanism of biosynthesis of lactose and the origin of the fat globule are beginning to be understood, but the precise mechanism of bio-assembly of the casein micelle has not been fully elucidated. The physical and chemical properties of the major micelle

components α_{s1} -, β -, and κ -casein are briefly reviewed, as well as the formation of synthetic micelles in the presence of calcium ions by native and dephosphorylated caseins. Several conflicting models for the casein micelle have been proposed based upon these properties. In order to determine which of these models might be applicable to casein micelle formation, correlations between the chemical and physical properties of the caseins and the actual events which occur in vivo in casein bio-assembly have been attempted. It is proposed that phosphorylation of caseins occurs in the Golgi apparatus of lactating mammary cells and that, subsequently, casein micelle formation oc-

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curs in Golgi vacuoles through the condensation of preformed subunits. These observations lend support to a model for the formation of the casein micelle from protein subunits of about 100 angstroms in diameter.

Introduction

Mammals constitute one of the dominant forms of animal life today. The evolutionary success of this class of animals may be due, in part, to their ability to provide well-balanced nutritional food for their young. The synthesis and secretion of milk involves the formation of a distinctive disaccharide, lactose, and two quite unique biological structures, the fat globule and the casein protein complex (the casein micelle). These three components, secreted in a fluid rich in minerals, provide an excellent food for the nourishment of the young. The mechanism of the biosynthesis of lactose and the origin of the fat globule are beginning to be understood, but the precise mechanism of formation of the casein micelle has not been fully elucidated. Various models for the casein micelle have been proposed based upon the chemical and physical properties of the isolated caseins, but the number of conflicting proposals drawn from coherent research illustrates the complexity of the problem. In our laboratory we recently have begun to correlate the known chemical and physical properties of the caseins with the actual events which occur *in vivo* in casein micelle formation, and this paper is an outgrowth of these reflections.

Components of the Casein Protein Complex

Linderström-Lang (24) and Mellander (26) in the 1930's demonstrated the heterogeneity of bovine casein. The latter termed the electrophoretically-distinct fractions α -, β -, and γ -caseins; but it was not until 1956 that Waugh and Von Hippel (54) discovered that the α -casein fraction was a mixture of α_{s1} -casein and κ -casein. Genetic variants of all the milk proteins have been reported. Farrell and Thompson (14) have reviewed the occurrence in various breeds and the possible biological significance of milk protein genetic polymorphism. From all the work on the isolation and characterization of casein has come the description of the three major components of the casein protein complex, namely α_{s1} -, β -, and κ -casein.

The major protein of cow's milk, α_{s1} -casein, is a single chain polypeptide of known sequence containing 199 amino acid residues and

having a molecular weight of 23,600 Daltons (27). The α_{s1} -molecule contains eight phosphate residues, seven of which occur in a cluster between residues 43 and 80. This highly acidic segment also contains 12 carboxyl residues and accounts for almost all of the molecule's net negative charge. The charge frequency, net charge, and hydrophobicity for various segments of the α_{s1} -casein molecule are in Table 1. The average hydrophobicity in Table 1 was calculated by the method of Bigelow (4) and measures the apolarity of a segment of the protein molecule. The data in Table 1 indicate a noncoincidence of high charge frequency and apolarity in the segments shown. The proline content of α_{s1} -casein is high (27), and these residues appear evenly distributed. Proline residues are known to disrupt helical and beta structures; thus, the sequence data confirm the hypothesis drawn from physical-chemical data, that the α_{s1} -molecule exhibits little recognizable secondary structure (18). The high degree of hydrophobicity exhibited by the C-terminal half of the molecule (residues 100 to 199) is probably responsible for the pronounced tendency to self-association of the α_{s1} -casein monomer in aqueous solution (45, 48, 55).

The second most abundant milk protein, β -casein, is also a single chain polypeptide with five phosphoserine residues and a molecular weight of 24,500 Daltons (39). The complete sequence of β -casein (39) is known. Like α_{s1} -casein, its high proline content is evenly distributed, explaining, in part, why this molecule also lacks any secondary structure (18, 32). Table 2 gives the charge frequency, hydrophobicity, and net charge for various segments of β -casein. The N-terminal portion of the β -casein molecule (residues 1 to 43) contains essen-

TABLE 1. Profile of the α_{s1} -casein molecule derived from its primary structure^a.

Residues considered	Net charge ^b	Charge frequency ^{b,c}	Average hydrophobicity ^c
1 → 40	+ 3	.25	1,340
41 → 80	-22½	.75	641
81 → 120	0	.35	1,310
121 → 160	- 1	.23	1,264
161 → 199	- 2½	.14	1,164

^a Adapted from Mercier et al. (27).

^b Some error as to assignment of these values may exist since the exact placement of all amides is not known. Serine phosphate = -2, histidine = +½.

^c Calculated as described by Bigelow (4).

TABLE 2. Profile of the β -casein molecule derived from its primary structure^a.

Residues considered	Net charge ^b	Charge frequency ^{b,c}	Average hydrophobicity ^c
1 → 43	-16	.65	783
44 → 92	-3½	.13	1,429
93 → 135	+2	.23	1,173
136 → 177	+3	.07	1,467
178 → 209	+2	.06	1,738

^a Derived from the data of Ribadeau Dumas et al. (39).

^b Some error as to assignment of these values may exist since the exact placement of all amides is not known. Serine phosphate = -2, histidine = +½.

^c Calculated as described by Bigelow (4).

tially all of the protein's net negative charge while the C-terminal half of the molecule (residues 136 to 209) contains many apolar residues (as demonstrated by its high hydrophobicity). This concentration of negative charge on one end and of apolarity on the other end indicates that β -casein is more soap-like than α_{s1} -casein. The temperature dependence of the self-association is well documented (34, 47, 55) and follows classically the theory of hydrophobic interactions as detailed by Kauzmann (23).

The third major component of the milk protein complex, κ -casein, is the least characterized fraction with regard to its primary structure; however, substantial work is in progress (19, 20, 28). In addition, the association properties of κ -casein are not well characterized. Compositionally, κ -casein is the only major component of the casein complex containing cystine (or possibly cysteine); however, data regarding the native state of these sulfhydryls (2, 20, 57) appear to conflict and, hence, the degree of disulfide bonding which occurs is uncertain. Woychik et al. (58) reported the reduced molecular weight of κ -casein in 5 M guanidine hydrochloride was around 17,000 Daltons. κ -Casein contains one phosphate residue per monomer and is the only carbohydrate-containing casein (28, 31). All of the carbohydrate associated with the κ -casein is bound to the macropeptide (19, 20, 28, 54), which is the highly soluble C-terminal portion of κ -casein cleaved in the primary phase of rennin hydrolysis.

Calcium Interactions with the Caseins

In 1929 Linderström-Lang (24), as a result of his studies on casein, postulated that the col-

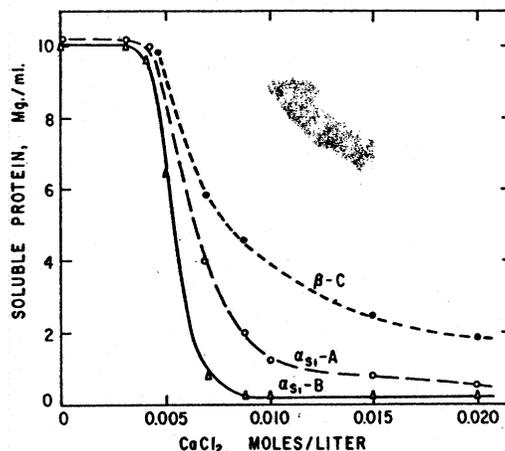


FIG. 1. Solubility at 37 C of the calcium salts of α_{s1} -caseins A and B and β -casein C as a function of increasing CaCl_2 concentration. Solutions buffered at pH 7.0, .01 M imidazole-HCl, from Thompson et al. (52).

loidal milk protein complex should be composed of a mixture of calcium insoluble proteins stabilized by a calcium soluble protein. The latter protein would be readily split by the enzyme rennin, destabilizing the colloid and allowing coagulation to occur. The results accumulated in the last 15 yr confirm this hypothesis. The total calcium concentration of milk has been estimated at 28 to 30 mM (12, 13, 56). However, the α_{s1} - and β -caseins, which account for up to 80% of the casein complex, are insoluble at Ca^{2+} concentrations of 5 to 10 mM (Fig. 1). Thus, the majority of the casein forms an insoluble curd under the conditions of pH, ionic strength, Ca^{2+} concentration, and temperature which normally occur in milk. On the other hand, κ -casein is completely soluble under similar conditions. At various κ/α_{s1} -casein ratios, the κ -casein does stabilize the α_{s1} -casein and form a colloidal complex (Fig. 2). These synthetic casein micelles (52) do not exhibit the properties of native casein complexes. κ -Casein also is the fraction attacked by rennin (22, 54); hence, it would appear, as Linderström-Lang had postulated, that κ -casein is the key to the stability of the casein protein-complex.

The role of the phosphate residues of the caseins in calcium binding and subsequent precipitation has been investigated by the study of enzymatically dephosphorylated α_{s1} -casein (6, 36). Bingham et al. (6) demonstrated that dephosphorylated α_{s1} -casein was still precipitated by calcium ions but was stabilized by the addition of κ -casein (Fig. 3). They calcu-

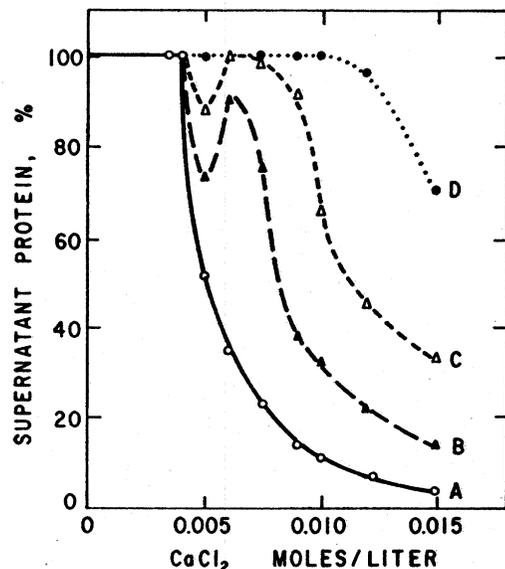


Fig. 2. Supernatant protein at 37 C resulting from the increment addition of CaCl_2 to A, α_{s1} -casein B, no κ -casein; B, α_{s1} -B + κ -casein, 40:1; C, α_{s1} -B + κ -casein, 20:1; and D, α_{s1} -B + κ -casein, 10:1. Solutions buffered at pH 7.0, .01 M imidazole-HCl. Initial protein = 4 mg/ml, from Thompson et al. (52).

lated that two nonphosphate calcium-binding sites occur in α_{s1} -casein and postulated that it is the binding to these sites which induces precipitation of the dephosphorylated casein. The investigation of the κ -casein stabilized, dephosphorylated α_{s1} -casein by electron microscopy (6) showed larger but fewer micelle-like structures. In milks containing α_{s1} -casein A (a rare genetic type) such large micelles (51, 52) are poorly solvated and less stable than normal micelles. Thus, the formation of micelle-like structures is not totally dependent upon the formation of calcium-phosphate bonds between caseins; however, the resulting synthetic micelles are less stable than synthetic phosphorylated complexes, which in turn are less stable than native casein micelles.

The large number of charged groups of the casein monomers (Tables 1 and 2) suggests that in the formation of a casein micelle from thousands of monomers, not all of the ionic groups of every casein monomer can occupy a surface position. This would indicate either that much energy is needed to bury these groups or that the structure is porous and available to the solvent, water. The latter proposition is borne out by the experimental evidence. Ribadeau Dumas and Garnier (40) noted that carboxypeptidase A is able to re-

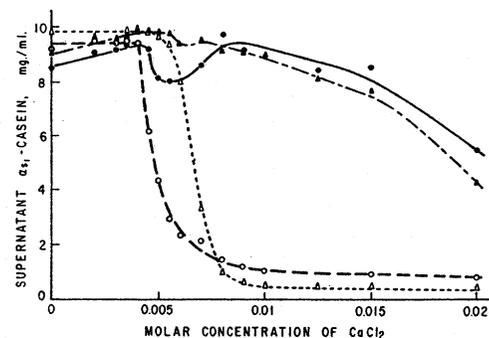


Fig. 3. Effect of κ -casein on the solubility of α_{s1} -casein at 37 C as a function of increasing CaCl_2 concentration. The solutions contained .01 M imidazole-HCl buffer (pH 7.0) and, initially, 10 mg/ml of α_{s1} -casein (native or dephosphorylated) with or without κ -casein (1.25 mg/ml). (\blacktriangle) α_{s1} - κ -casein; (\bullet) dephosphorylated α_{s1} - κ -casein; (Δ) α_{s1} -casein, no κ -casein; (\circ) dephosphorylated α_{s1} -casein, no κ -casein, from Bingham et al. (6).

move, quantitatively, the carboxyl-terminal residues from the α_{s1} -, β -, and κ -caseins of native micelles, demonstrating that this enzyme (M 40,000) is able to penetrate the center of the casein micelle. Thompson et al. (51, 52) have shown that the casein micelle is a highly solvated structure with an average of 1.90 g of water bound/g of protein. They also noted (Fig. 4) a strong positive correlation between the degree of solvation and heat stability (51). The degree of solvation of the micelle and, hence, the heat stability of the milk hinge upon a variety of factors (37, 41, 51) including the

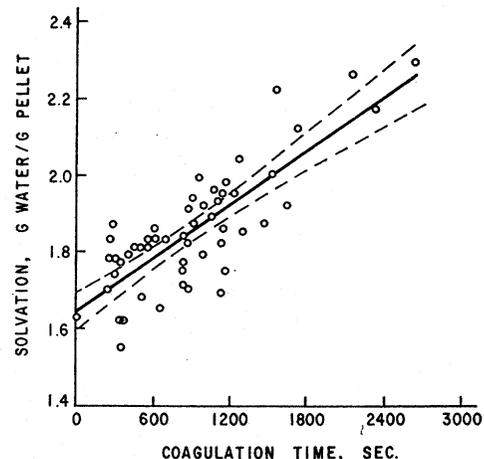


Fig. 4. Plot of the solvation of the casein pellets (g water/g pellet) versus heat coagulation time in seconds. Dotted lines represent 95% confidence limits from Thompson et al. (51).

calcium:phosphate ratio. Increases in the calcium content of milk cause decreased heat stability (37, 41) possibly by altering the degree of solvation of the casein micelle. Environments which tend to decrease solvent interaction lower the stability of the micelle, which in turn destabilizes the milk.

As cited above, the total calcium concentration of skim milk has been estimated to be 30 mM, but the calcium ion concentration of milk serum prepared by ultrafiltration or centrifugation of skim milk is only ~ 2.9 mM (7, 12). Therefore, greater than 90% of the calcium of skim milk is in some way bound to the casein micelles. Thus, two distinct forms of ions are associated with the casein micelle—an outer system, readily removed, perhaps in the form of a charged double layer (7, 12), and an inner system resistant to removal and often termed as colloidal calcium phosphate. As noted above, the casein micelle is a highly porous, well-solvated system, and the occlusion of ions within this network is not unexpected;

however, some actual complex formation between the colloidal calcium phosphate and the casein cannot be ruled out. Termine and Posner (50) studied the *in vitro* formation of calcium phosphate at pH 7.4 and concluded that an amorphous calcium phosphate phase (with a Ca/PO_4 molar ratio of 1.5) formed before the transition to crystalline apatite. In a subsequent study (49), casein, as well as Mg^{2+} ions, enhanced the stability of the amorphous calcium phosphate and retarded the amorphous \rightarrow crystalline transition. Therefore, under these conditions (i.e., the presence of Mg^{2+} and casein) the formation of an amorphous calcium phosphate-caseinate complex should be favored in milk. The exact nature of this complex (or occlusion) is as yet undetermined though its role in casein micelle stabilization is well documented (25, 30, 38, 42).

Casein Micelle Models

Over the years we have gained much information about the nature of the individual ca-

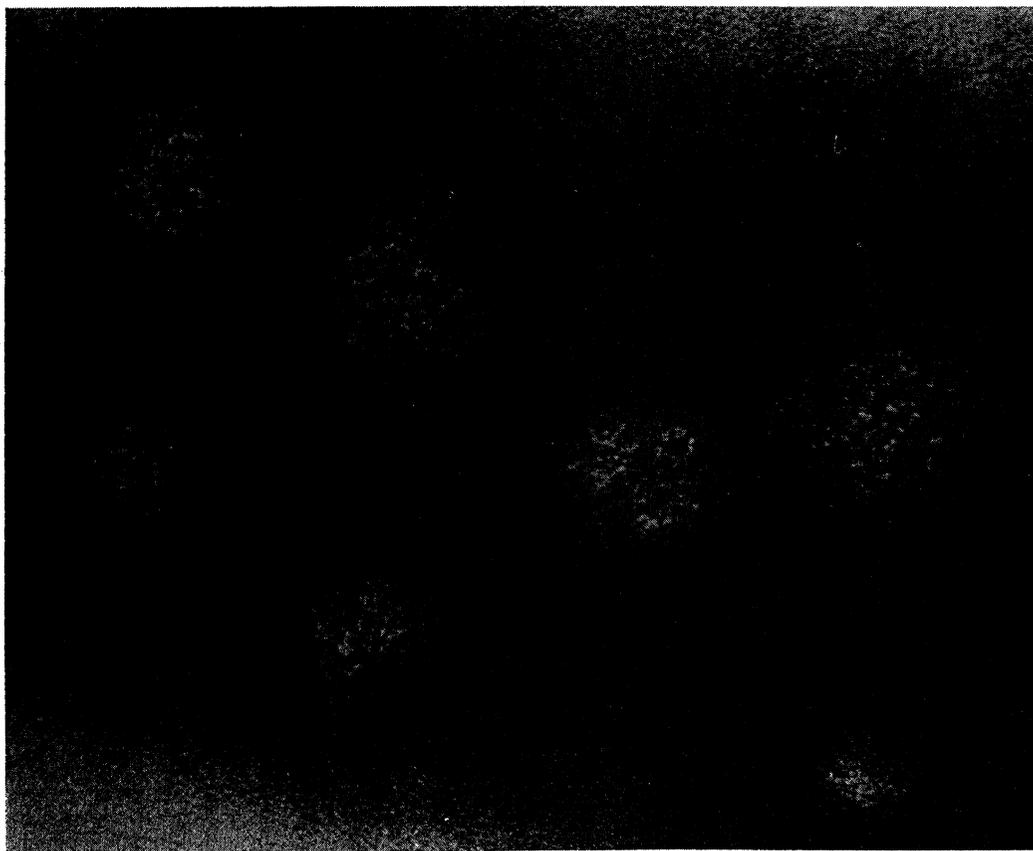


FIG. 5a. Casein micelles of bovine skim milk. Fixed in 1% glutaraldehyde and negatively stained with phosphotungstic acid. (Photo courtesy of R. J. Carroll.)

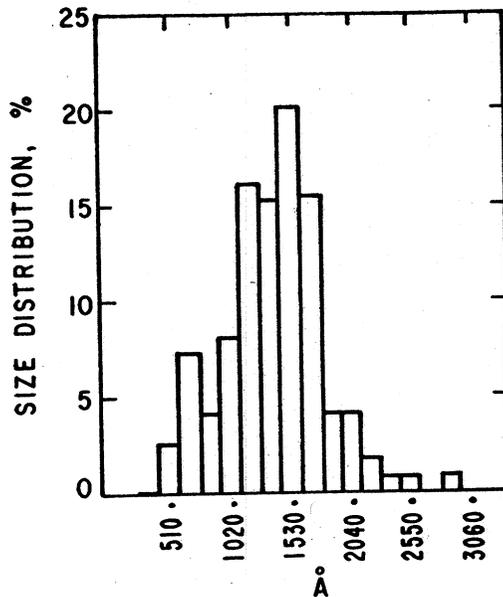


FIG. 5b. Determination of the size distribution of glutaraldehyde-fixed casein micelles from skim milk as described by Carroll et al. (11).

seins components, yet the mechanism of formation and the structure of the casein protein-complex have proven to be enigmatic. Electron micrographs (11) of casein micelles show them to be roughly spherical, with an average diameter of ~ 1400 angstroms (Fig. 5a, b). Such a structure would easily accommodate 25,000 casein monomers, have the required porosity to solvent, contain the colloidal calcium phosphate, and have a molecular weight of 10^8 to 10^9 . Thus, while we know that the α_{s1} -, β -, and κ -caseins do interact in the presence of Ca^{2+} , the molecular details of the complex remain unresolved. Several models for casein micelle structure, which explain many of the observed features of the casein protein-complex, have been presented.

The model for casein micelle (Fig. 6a) formation proposed by Waugh and his coworkers (43, 55) describes the formation of core polymers of α_{s1} - and β -caseins upon the addition of calcium ions. In the absence of κ -casein, these core polymers achieve a limiting size, then agglutinate and precipitate from solution. When κ -casein is present, colloidal stabilization occurs through the formation of a surface coat of κ -casein. This model predicts that all of the κ -casein will occur on the surface of the micelle. Parry and Carroll (33) found little concentration of κ -casein on the surface of the casein micelle by the use of electron micro-

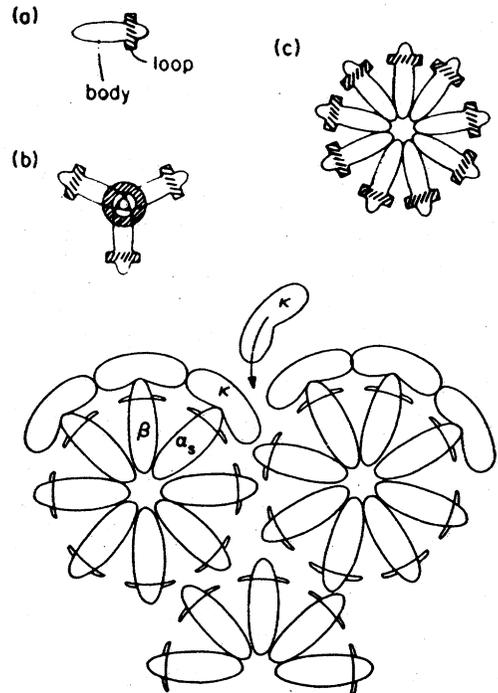


FIG. 6a. Waugh's proposed model for the casein micelle: (a) monomer model of α_{s1} - or β -casein with charged loop, (b) a tetramer of α_{s1} -casein monomers, (c) planar model of a core polymer of α_{s1} - and β -caseins. The lower portion shows how κ -casein might coat core polymers, adapted from Rose (43).

scopy and specific antibody tagging. Based on these results and the size of isolated κ -casein samples, Parry (Fig. 6b) concluded that κ -casein might serve as a point of nucleation about which the calcium insoluble caseinates might cluster and be stabilized. Thus, the models of Parry and Waugh both predict a non-uniform distribution of κ -casein and are based

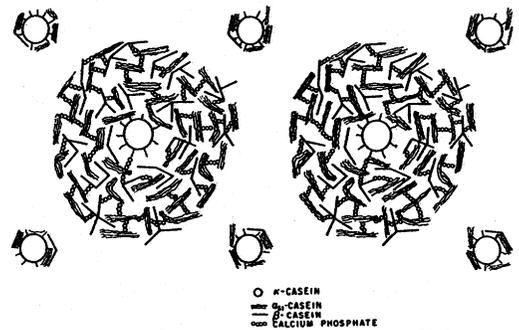
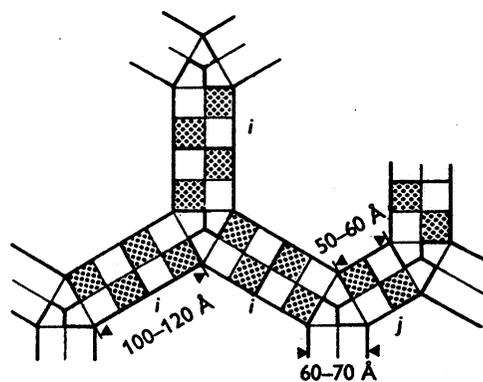


FIG. 6b. Casein micelle model proposed by Parry and Carroll (33) depicting the location of κ -casein in the micelle.



(f)

□ α_{s1} casein
 ▨ β casein
 ▽ κ casein

FIG. 7a. Structure of the repeating unit of the casein micelle adapted from Garnier and Ribadeau Dumas (16).

upon nucleation about a core (Parry's core = κ -casein, Waugh's core = α_{s1} , β -calcium caseinate). Ashoor et al. (1) have demonstrated recently that papain, which had been cross-linked by glutaraldehyde into a large insoluble polymer, caused proteolysis of all three major components of isolated casein micelles. The α_{s1} , β -, and κ -caseins were all cleaved proportionately by the enzyme super polymer. Therefore, all three components must occupy surface positions on the micelle in relatively the same proportions they occur in milk. This result would seem to rule out any preferential localization of κ -casein.

Garnier and Ribadeau Dumas (16) have proposed a model for the casein micelle, which places a good deal of emphasis on κ -casein as the keystone of micelle structure. Trimers of κ -casein are linked to three chains of α_{s1} - and β -casein which radiate from the κ -casein node (a Y-like structure) as shown in Fig. 7a. These chains of α_{s1} - and β -casein may connect with other κ -nodes to form a loosely packed network which they have shown to be required from their carboxypeptidase-A experiments (40). Rose, on the other hand, used the known endothermic polymerization of β -casein as the basis for his micelle model (43). In this model, β -casein monomers begin to self-associate into chain-like polymers to which α_{s1} -monomers become attached (Fig. 7b), and κ -casein, in turn, interacts with the α_{s1} -monomers. The β -casein of the thread is directed in-

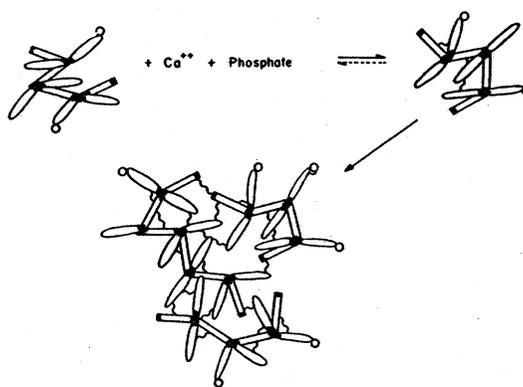


FIG. 7b. Schematic representation of the formation of a small casein micelle. The rods represent β -casein, the more elliptical rods represent α_{s1} -casein, and the S-shaped lines depict apatite chain formation. The circles represent κ -casein, adapted from Rose (43).

ward, the κ - outward. Both of these models rely somewhat heavily on the ability of the casein trimers, or polymers, to maintain structural integrity during micelle formation. Since caseins contain little or no classical protein structure and the nature of the disulfide bonds in κ -casein has not been clearly established (2, 20, 57), these models may not be totally valid. Rose's model, however, predicts that as the micelle is formed, colloidal calcium phosphate is incorporated into the network as a stabilizing agent; his model also accounts for the occurrence of some overall stoichiometry of the various casein components.

The final models are those which propose a subunit structure for the casein micelle. Shimmin and Hill (46) first postulated such a model based upon their study of ultra-thin cross sections of embedded casein micelles by electron microscopy. They predicted a diameter of 100 angstroms for the subunits of the casein micelle. Subsequently, Morr (29) studied the disruption of casein micelles and pro-

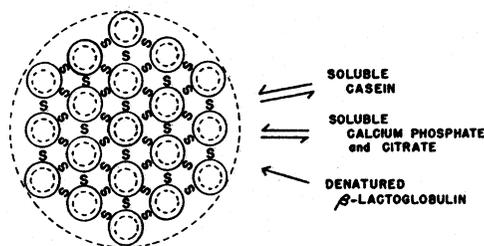


FIG. 8. Structure of the casein micelle after Morr (29). The S-shaped lines represent calcium phosphate linkages between small spherical complexes of the α_{s1} -, β -, and κ -caseins.

posed that the α_{s1} -, β -, and κ -monomers were aggregated by calcium into small subunits in much the same fashion as Waugh (55) had proposed for the entire micelle. Morr's subunits, as estimated by sedimentation velocity, have a diameter of ~ 300 angstroms. The subunits, in turn, are aggregated into micellar structure by colloidal calcium phosphate. Morr's model is in Fig. 8. The average subunit size, as postulated by Morr, is somewhat larger than that of Shimmin and Hill.

These casein micelle models have both strong points and weaknesses and have been extensively reviewed elsewhere (15).

Observations on Micelle Bio-Assembly

All of the models, briefly described above, have been drawn from evidence accumulated on the isolated milk proteins. In order to gain a fresh insight into the problem of casein micelle structure, it was decided to re-examine the actual bio-assembly of the casein micelle. The epithelial cells of lactating mammary gland possess a well-defined rough endoplasmic reticulum and a well-developed Golgi apparatus. It is in the former that casein polypeptide synthesis occurs (3) and in the latter where micelle bio-assembly takes place (10, 17). Examination of lactating rat mammary tissue, by electron microscopy, revealed that many small particles of ~ 100 angstroms diameter occurred in the Golgi vacuoles (10, 14). These particles were rather uniform in size and in many instances appeared to form chain-like structures. Those vacuoles, which were thought to contain micelles in the process of formation, occurred near the dictyosomes and the endoplasmic reticulum (Fig. 9a). Golgi vacuoles, nearer the apical end of the cell, contained more completely formed casein micelles (Fig. 9b). Based on these observations and the evidence of Turkington and Topper (53), which indicated that a lag occurred between peptide synthesis and phosphorylation, several hypotheses on the mechanism of micelle bio-assembly were made:

- 1) After protein synthesis on the ribosomes of the rough endoplasmic reticulum, the casein polypeptides may interact to form subunits composed of several monomers.
- 2) Upon reaching the Golgi apparatus these subunits would be phosphorylated to form the electron dense 100 angstrom particles observed in early stages of micelle assembly.
- 3) Addition of Ca^{2+} then should initiate polymerization of these particles into strands and/or partially condensed micelles.
- 4) Deposition of colloidal calcium phos-

phate then would yield the mature casein micelle in the Golgi vacuole.

- 5) Secretion of the fully formed micelle occurs.

The results of recent experiments have tended to support these hypotheses. Carroll et al. (9) studied casein micelle disruption by dissociating reagents such as NaF, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), and urea. In the case of NaF and urea, partial breakdown of the casein micelles occurred yielding thread-like material, as well as subunits with diameters of 120 to 140 angstroms. The dissociating agents, EDTA and SDS, yielded more totally disrupted micelles and smaller particles (70 to 80 angstroms). Pepper (35) has studied α_{s1} - κ -casein interactions and first cycle (Ca^{2+} free) caseins by gel filtration. He determined that the first cycle casein had a Stokes radius of 50 angstroms and these particles contained a rather even distribution of α_{s1} -, β -, and κ -casein. These experiments serve to point out that a subunit structure may exist in the casein micelle.

Recently, Bingham et al. (5) studied the subcellular distribution of the ATP:casein kinase of lactating mammary gland. They found (Table 3) that the Golgi fraction exhibits the highest specific activity toward dephosphorylated casein. Hence, phosphorylation of a pool of unphosphorylated casein, as suggested by Turkington and Topper (53), may occur in mammary Golgi apparatus. In an earlier paper Bingham et al. (6) have suggested that while the dephosphorylated α_{s1} - and κ -caseins may interact, few native micelles are formed by these interaction products. This would indicate that total phosphorylation of the caseins by the kinase of the Golgi apparatus should occur before micelle assembly begins.

Many questions remain unanswered in the mechanism of bio-assembly of the casein micelle, not the least of which are the mechanism of Ca^{2+} addition and the nature of the micelle subunits. Total micellar casein exhibits an overall ratio of 3 α_{s1} :2 β :1 κ -casein. The apparent uniformity of first cycle (Ca^{2+} free) casein and the subunits of the Golgi vacuoles would argue in favor of some consistent stoichiometry, but there exists the reported correlation between micelle size and κ -casein content (42, 44, 55) which would argue against uniform subunit composition. Thus, the existence of some type of subunit structure appears certain, but the question to be decided now is the nature of these subunits.

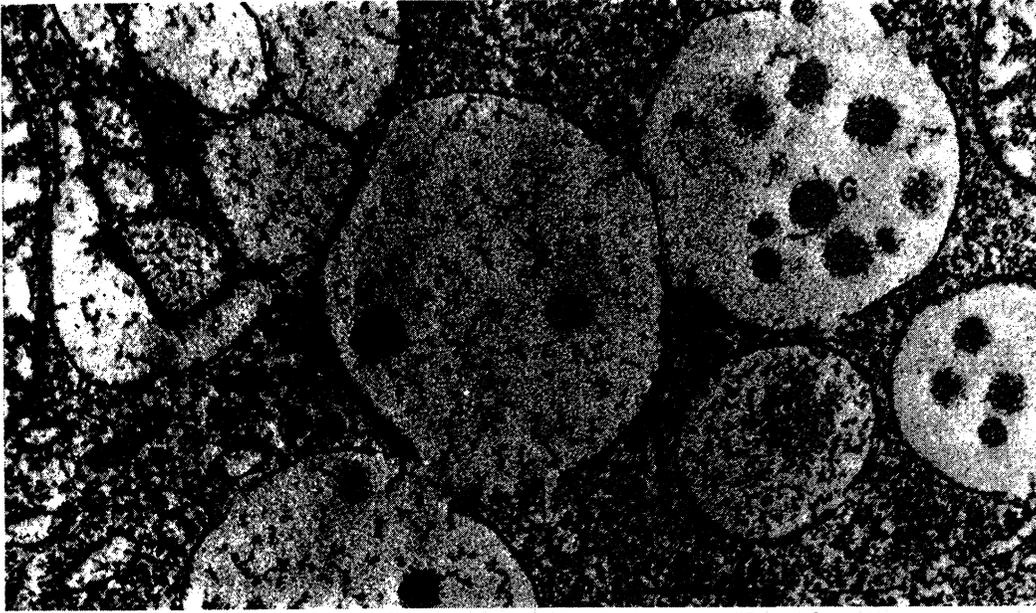


FIG. 9a. Formation of casein micelles (CM) within Golgi vacuoles (G) of lactating rat mammary gland. Initially, thread-like structures with some degree of periodicity appear, then more compact micelles seem to occur. Sections of the gland were fixed in buffered OsO_4 , Epon embedded, and stained with uranyl acetate and lead citrate, from Carroll et al. (10).



FIG. 9b. A Golgi vacuole about to discharge its contents into the alveolar lumen. The Golgi vacuole shown appears to impinge upon the plasma membrane. A casein micelle is already present in the lumen, from Carroll et al. (10).

TABLE 3. Subcellular distribution of protein kinase from lactating mammary gland^a.

Fraction	Protein kinase activity ^b		
	α_{s1} -casein	Dephosphorylated	
		α_{s1} -casein	Lactose synthetase ^b
	($\mu\mu$ moles/mg protein/20 min)	(cpm/ μ g protein/30 min)	
Total homogenate	.16	.20	33.0
Golgi apparatus	5.44	24.02	1,101.0
Nuclei	.54	.48	38.4
Mitochondria	.54	1.10	91.2
Microsome	1.72	1.80	137.4
Cytoplasm	.50	.22	2.4

^a Taken from data of Bingham et al. (5).

^b These results are representative of individual experiments with three rats.

From the biosynthetic point of view, the build-up of the micelle from subunits is quite attractive, as it brings the casein components into the region of assembly with minimal interactions. Addition of calcium ions could initiate the polymerization of casein subunits, and condensation into micellar spheres could occur by the deposition of colloidal calcium phosphate; but these remain only hypotheses. The biological function of the micelle is efficient nutrition which need not require a high degree of ordered structure. This is in contrast to tobacco mosaic virus where the structured RNA core (8) plays a vital role in directing correctly the assembly of the virus particle from its preformed subunits. In the case of the micelle, only amorphous apatite could serve this function. The formation of the casein micelle does provide an effective mechanism for the secretion of a highly concentrated solution of protein. The elevated viscosities of solutions of nonmicellar caseins and colloidal-calcium phosphate-free milks (25, 38) illustrate this point. In addition, while milk is secreted as a fluid, the casein micelle is readily destabilized by the action of acid and rennin in the stomach. The resulting curd provides a longer and more efficient digestion time. Thus, while we have some understanding of the interactions which yield the casein micelle, much research is needed to achieve a more precise model for casein micelle formation.

References

- (1) Ashoor, S. H., R. A. Sair, N. F. Olson, and T. Richardson. 1971. Use of a papain superpolymer to elucidate the structure of bovine casein micelles. *Biochim. Biophys. Acta* 229:423.
- (2) Beeby, R. 1964. The presence of sulfhydryl groups in κ -casein. *Biochim. Biophys. Acta* 82:418.
- (3) Beitz, D. C., H. W. Mohrenweiser, J. W. Thomas, and W. A. Wood. 1969. Synthesis of milk proteins in a cell-free system isolated from lactating bovine mammary tissue. *Arch. Biochem. Biophys.* 132:210.
- (4) Bigelow, C. C. 1967. On the average hydrophobicity of proteins and the relation between it and protein structure. *J. Theoret. Biol.* 16:187.
- (5) Bingham, E. W., H. M. Farrell, Jr., and J. J. Basch. 1972. Phosphorylation of casein: Role of the Golgi apparatus. *J. Biol. Chem.* 247:8193.
- (6) Bingham, E. W., H. M. Farrell, Jr., and R. J. Carroll. 1972. Properties of dephosphorylated α_{s1} -casein. Precipitation by calcium ions and micelle formation. *Biochem.* 11:2450.
- (7) Boulet, M., A. Yang, and R. R. Reil. 1970. Examination of the mineral composition of the micelle of milk by gel filtration. *Can. J. Biochem.* 48:816.
- (8) Butler, P. J. G. 1971. Assembly of the tobacco mosaic virus particle. *Nature* 233:25.
- (9) Carroll, R. J., H. M. Farrell, Jr., and M. P. Thompson. 1971. Electron microscopy of the casein micelle—forces contributing to its integrity. *J. Dairy Sci.* 54:752. (Abstr.)
- (10) Carroll, R. J., M. P. Thompson, and H. M. Farrell, Jr. 1970. Formation and structure of casein micelles in lactating mammary tissue. 28th Annu. Proc. EMSA p. 150.
- (11) Carroll, R. J., M. P. Thompson, and G. C. Nutting. 1968. Glutaraldehyde fixation of casein micelles for electron microscopy. *J. Dairy Sci.* 51:1903.
- (12) Davies, D. T., and J. C. D. White. 1960. The use of ultrafiltration and dialysis in isolating the aqueous phase of milk and in determining the partition of milk constituents between the aqueous and disperse phases. *J. Dairy Res.* 27:171.
- (13) Demott, B. J. 1968. Ionic calcium in milk and whey. *J. Dairy Sci.* 51:1008.
- (14) Farrell, H. M., Jr., and M. P. Thompson. 1971. Biological significance of milk protein polymorphism. *J. Dairy Sci.* 54:1219.
- (15) Farrell, H. M., Jr., and M. P. Thompson.

1973. Physical equilibria:proteins. Fundamentals of dairy chemistry, 2nd edition. B. Webb, ed. Avi Publishing Co., Westport, Connecticut.
- (16) Garnier, J., and B. Ribadeau Dumas. 1970. Structure of the casein micelle: a proposed model. *J. Dairy Res.* 37:493.
- (17) Heald, C. W., and R. G. Saacke. 1972. Cytological comparison of milk protein synthesis of rat mammary tissue in vivo and in vitro. *J. Dairy Sci.* 55:621.
- (18) Herskovits, T. T. 1966. On the conformation of caseins. Optical rotatory properties. *Biochem.* 5:1018.
- (19) Hill, R. J., and R. G. Wake. 1969. Amphiphile nature of κ -casein as the basis for its micelle stabilizing property. *Nature* 221:635.
- (20) Jolles, J., P. Jolles, and C. Alais. 1969. Present knowledge concerning the amino acid sequence of cow κ -casein. *Nature* 222:668.
- (21) Jolles, P., C. Alais, and J. Jolles. 1962. Amino acid composition of κ -casein and terminal amino acids of κ - and para κ -casein. *Arch. Biochem. Biophys.* 98:56.
- (22) Kalan, E. B., and J. H. Woychik. 1965. Action of rennin on κ -casein, the amino acid compositions of the para- κ -casein, and glycomacropeptide fractions. *J. Dairy Sci.* 48:1423.
- (23) Kauzmann, W. 1959. Some factors in the interpretation of protein denaturation. *Advances Protein Chem.* 14:1.
- (24) Linderstrøm-Lang, K. 1929. Casein. III. The fractionation of casein. *Compt. Rend. Trav. Lab. Carlsberg* 17(9):116.
- (25) McGann, T. C. A., and G. T. Pyne. 1960. The colloidal phosphate of milk. III. Nature of its association with casein. *J. Dairy Res.* 27:403.
- (26) Mellander, O. 1939. Electrophoretic studies of casein. *Biochem. Z.* 300:240.
- (27) Mercier, J. C., F. Grosclaude, and B. Ribadeau Dumas. 1971. Structure primaire de la casein α_{s1} -bovine. Séquence complète. *Europe. J. Biochem.* 23:41.
- (28) Mercier, J. C., J. Uro, B. Ribadeau Dumas, and F. Grosclaude. 1972. Structure primaire du caseinomacropeptide de la casein κ -B₁ bovine. *Europe. J. Biochem.* 27:535.
- (29) Morr, C. V. 1967. Effect of oxalate and urea on the ultracentrifugation properties of raw and heated skim milk micelles. *J. Dairy Sci.* 50:1744.
- (30) Morr, C. V., R. V. Josephson, R. Jenness, and P. B. Manning. 1971. Composition and properties of submicellar casein complexes in colloidal calcium phosphate-free skim-milk. *J. Dairy Sci.* 54:1555.
- (31) Nitschmann, Hs., and R. Henzi. 1959. Das lab und seine wirkung auf das casein der milch. XIII. Untersuchung der bei der labung in freiheit gesetzten peptide. *Helv. Chim. Acta* 42:1985.
- (32) Noelken, M., and M. Reibstein. 1968. Conformation of β -casein B. *Arch. Biochem. Biophys.* 123:397.
- (33) Parry, R. M., Jr., and R. J. Carroll. 1969. Location of κ -casein in milk micelles. *Biochim. Biophys. Acta* 194:138.
- (34) Payens, T. A. J., J. A. Brinkhuis, and B. W. Van Markwijk. 1969. Self-association in non ideal systems. Combined light scattering and sedimentation measurements in β -casein solutions. *Biochim. Biophys. Acta* 175:434.
- (35) Pepper, L. 1972. Casein interactions as studied by gel chromatography and ultracentrifugation. *Biochim. Biophys. Acta* 278:147.
- (36) Pepper, L., and M. P. Thompson. 1963. Dephosphorylation of α_s - and κ -caseins and its effect on micelle stability in the κ - α_s -casein system. *J. Dairy Sci.* 46:764.
- (37) Puri, B. R., K. Arora, and K. K. Toteja. 1969. Studies in stability of protein dispersions in milk: V. Effect of altering calcium content, replacing calcium by other cations in caseinate complex and other factors on heat stability of milk. *Indian J. Dairy Sci.* 22:85.
- (38) Pyne, G. T., and T. C. A. McGann. 1960. The colloidal phosphate of milk. II. Influence of citrate. *J. Dairy Res.* 27:9.
- (39) Ribadeau Dumas, B., G. Brignon, F. Grosclaude, and J. C. Mercier. 1972. Structure primaire de la casein β -bovine. *Europe. J. Biochem.* 25:505.
- (40) Ribadeau Dumas, B., and J. Garnier. 1970. Structure of casein micelle. The accessibility of the subunits to various reagents. *J. Dairy Res.* 37:269.
- (41) Rose, D. 1961. Variations in the heat stability and composition of milk from individual cows during lactation. *J. Dairy Sci.* 44:430.
- (42) Rose, D. 1968. Relation between micellar and serum casein in bovine milk. *J. Dairy Sci.* 51:1897.
- (43) Rose, D. 1969. A proposed model of micelle structure in bovine milk. *Dairy Sci. Abstr.* 31:171.
- (44) Rose, D., D. T. Davies, and M. Yaguchi. 1969. Quantitative determination of the major components of casein mixtures by column chromatography on DEAE-cellulose. *J. Dairy Sci.* 52:8.
- (45) Schmidt, D. G. 1970. The association of α_{s1} -casein B at pH 6.6. *Biochim. Biophys. Acta* 207:130.
- (46) Shimmin, P. D., and R. D. Hill. 1964. An electron microscope study of the internal structure of casein micelles. *J. Dairy Res.* 31:121.
- (47) Sullivan, R. A., M. M. Fitzpatrick, E. K. Stanton, R. Annino, G. Kissel, and F. Palermi. 1955. The influence of temperature and electrolytes upon the apparent size and shape of α - and β -casein. *Arch.*

- Biochem. Biophys. 55:455.
- (48) Swaisgood, H. E., and S. N. Timasheff. 1968. Association of α_1 -casein C in the alkaline pH range. Arch. Biochem. Biophys. 125:344.
- (49) Termine, J. D., R. A. Peckauskas, and A. S. Posner. 1970. Calcium phosphate formation *in vitro*. II. Effects of environment on amorphous-crystalline transformation. Arch. Biochem. Biophys. 140:318.
- (50) Termine, J. D., and A. S. Posner. 1970. Calcium phosphate formation *in vitro*. I. Factors affecting initial phase separation. Arch. Biochem. Biophys. 140:307.
- (51) Thompson, M. P., R. T. Boswell, V. Martin, R. Jenness, and C. A. Kiddy. 1969. Casein-pellet-solvation and heat stability of individual cow's milk. J. Dairy Sci. 52:796.
- (52) Thompson, M. P., W. G. Gordon, R. T. Boswell, and H. M. Farrell, Jr. 1969. Solubility, solvation, and stabilization of α_{s1} - and β -caseins. J. Dairy Sci. 52:1166.
- (53) Turkington, R. W., and Y. J. Topper. 1966. Casein biosynthesis: Evidence for phosphorylation of precursor proteins. Biochim. Biophys. Acta 127:366.
- (54) Waugh, D. F., and P. H. Von Hippel. 1956. κ -Casein and the stabilization of casein micelles. J. Amer. Chem. Soc. 78:4576.
- (55) Waugh, D. F., L. K. Creamer, C. W. Slatery, and G. W. Dresdner. 1970. Core polymers of casein micelles. Biochemistry 9:786.
- (56) White, J. C. D., and D. T. Davies. 1958. The relation between the chemical composition of milk and the stability of the caseinate complex. I. General introduction, description of samples, methods and chemical composition of samples. J. Dairy Res. 25:236.
- (57) Woychik, J. H. 1965. Preparation and properties of reduced κ -casein. Arch. Biochem. Biophys. 109:542.
- (58) Woychik, J. H., E. B. Kalan, and M. E. Noelken. 1966. Chromatographic isolation and partial characterization of reduced κ -casein components. Biochemistry 5:2276.