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Review

The casein micelle: Historical aspects, current concepts and significance

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A B S T R A C T

The caseins, a group of unique milk-specific acid-insoluble phosphoproteins, represent ≈80% of the total protein in the milk of cattle and other commercial dairy species. Owing to their commercial importance, the caseins have been studied very extensively and are probably the best characterized food protein system. It has been recognized since the work of Schübler in 1818 that the caseins exist in milk as large particles suspended in the aqueous phase (milk serum). Initially, the casein particles were usually referred to as "calcium caseinate–calcium phosphate particles". The term "casein micelle" was introduced in 1921 and the two terms were used interchangeably for several years but since about 1960, the latter term has been used exclusively. It has been suggested that the calcium caseinate–phosphate particles are not true micelles. The term "micelle" was introduced by Nägeli and Schwendener [Nägeli, C.W., & Schwendener, W. (1877). *Das Mikroskop: Theorie und Anwendung Desselben* (2nd ed.). Leipzig: W. Engelmann] for microparticles of cellulose in plant cells visible in the light microscope; later, it was used for various other types of aggregates. Owing to the importance of the casein micelles for many of the physico-chemical properties of milk and dairy products, their structure and properties and the effects of compositional and processing factors thereon have been studied extensively. Since the discovery of the micelle-stabilizing protein, κ-casein, in 1956, several models of the casein micelle have been proposed and refined. This review will focus on the following aspects: introduction and use of the term "micelle", early views on the stability of casein in milk, introduction of the term "casein micelle" for the calcium phosphate–calcium caseinate particles in milk, and the structure and stability of casein micelles.

Contents

1. The caseins

The caseins, a group of unique milk-specific proteins, represent ≈80% of the total protein in the milk of cattle and other commercial dairying species. Owing to their commercial importance, the caseins have been studied extensively and are probably the best characterized food protein system (see Fox & McSweeney, 2003). Research on casein dates from Berzelius (1814), Schübler (1818) and Braconnet (1830); the early work was reviewed by Kastle and Roberts (1909), Beau and Bourgain (1926) and Beau (1932a).

Casein is usually prepared by isoelectric precipitation at pH 4.6, a method developed by Hammersten (1883) and improved by van Slyke and Barker (1918). As early as 1880, Danilewsky and

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Radenhausen reported that isoelectric casein is heterogeneous but Hammersten (1883, 1885) concluded that properly prepared isoelectric casein is homogeneous. Based on differences in solubility in aqueous systems, several authors (e.g., Linderstrøm-Lang, 1925, 1929; Linderstrøm-Lang & Kodama (1925); Osborne & Wakeman, 1918), suggested that isoelectric casein is heterogeneous, which was confirmed by the electrophoretic studies of Mellander (1939), who showed that isoelectric casein is a mixture of three proteins, which he called α-, β- and γ-caseins in order of decreasing mobility on free boundary electrophoresis. These proteins were fractionated by Warner (1944) and by Hipp, Groves, Custer, and McMeekin (1952). Waugh and von Hippel (1956) used $CaCl_2$ to resolve casein into insoluble and soluble fractions. The former, which represented ~85% of total casein, contained α_s- and β-caseins, while the soluble fraction contained a previously unknown protein, which Waugh and von Hippel (1956) called κ-casein. From the point of view of the stability and properties of the casein micelle, κ-casein is its most important component. α_s-Casein is a mixture of two proteins, α_{s1}-casein and α_{s2}-casein (Annan & Manson, 1969). The four caseins exhibit microheterogeneity arising from differences in the degree of phosphorylation or glycosylation (κ-) or genetic polymorphism. γ-Caseins represent C-terminal segments of β-casein while the corresponding N-terminal parts are proteose peptones 5, 8_{slow} and 8_{fast}.

In milk, the caseins exist as large colloidal particles, 50–600 nm in diameter (mean $\approx$150 nm), called "casein micelles". Since many of the technologically important properties of milk, e.g., its white colour, stability to heat or ethanol and coagulation by rennet, are due to the properties of the casein micelles, there has been an economic and technological incentive to characterize their properties and elucidate their structure. An extensive literature on these subjects has accumulated and has been reviewed at regular intervals (e.g., de Kruif, 1998, 1999; de Kruif & Holt, 2003; Farrell, 1973; Farrell & Thompson, 1974; Fox, 2003; Fox & Kelly, 2004; Garnier, 1973; Garnier & Ribadeau-Dumas, 1970; Holt, 1992, 1994; Holt & Horne, 1996; Horne, 1998, 2002; McMahon & Brown, 1984; McMahon & McManus, 1998; Morr, 1967; Payens, 1966, 1979, 1982; Rollema, 1992; Rose, 1969; Ruettimann & Ladisch, 1987; Schmidt, 1980, 1982; Slattery, 1976; Slattery & Evard, 1973; Tuinier & de Kruif, 2002; Visser, 1992; Walstra, 1990, 1999; Walstra, Geurts, Noomen, Jellma, & van Boekel, 1999; Walstra & Jenness, 1984; Waugh, 1971).

According to Dickinson (1992), the casein particles in milk are not true micelles because they are irreversibly formed over normal time-scales; a solution of pure β-casein forms reversible aggregates at concentrations above the critical micelle concentration in a way that resembles classical surfactant micelles. Dickinson (1992) urged that, to avoid confusion, the term "casein micelle" should never be shortened to "micelle"; presumably, he considered "soap micelles" to be the "standard/typical micelle". However, as discussed below, this is a rather late definition of "micelle" and in any case, micellar caseins are in equilibrium with their "monomeric" counterparts in the milk serum (Waugh, 1958). Micelle formation and equilibria were studied in depth by Waugh and collaborators (see Waugh, 1961, 1971). The term "casein micelle" was not generally used until about 1960, mainly through the influence of Waugh, who used it without explanation. In fact, when and why the term "casein micelle" was introduced does not seem to have been explored. In this review, the general use and meaning of the term "micelle" will be considered initially, followed by a discussion on when and why the term "casein micelle" came into use, and finally a summary of the current views on the structure and properties of the casein micelle.

2. Micelles

Although the word "micelle" is now generally used to describe aggregates of amphipathic molecules, classically soaps, it has been used for at least five, largely unrelated, types of particles. The word "micelle" or "micella" (diminutive of the Latin, *mica*, meaning crumb, morsel) was coined by Nägeli and Schwendener (1877) to describe molecular aggregates or crystalline particles of cellulose, which they considered to be the building blocks of plant cells. The quasi-crystalline regions of cellulose are still referred to as "micelles". Nägeli, who was active in the debate on evolution and spontaneous generation, considered micelles to be the first stage in the transition of non-living molecules to living protoplasm; he developed a micellar theory of life (Nägeli, 1884; see also Strick, 2000).

Felix d'Herelle, who discovered bacterial viruses (bacteriophage) in 1915, also developed a micellar theory of life, in which the term "micelle" was used for colloidal particles, including bacteriophage, which he thought represented the ultimate units of living things (d'Herelle, 1924; see also Summers, 1999). d'Herelle's concept was, in principle, similar to that of Nägeli; both considered micelles to be aggregates of macromolecules (d'Herelle cited the work and ideas of Nägeli). d'Herelle described a micella as the smallest particle of protein matter and consequently the smallest possible particle of living substance; he proposed that living matter is an "*assemblage of micellae*" and that "*the protoplasmic micella should be to the cell what the cell is to the whole organism*". d'Herelle considered protoplasmic micellae to be polymers of an acid amine (i.e., a protein), a cyclic base (i.e., a nucleic acid), a lipid (i.e., a fat globule) or various carbohydrates (i.e., a polysaccharide). Henderson and Henderson (1920) defined a micelle (micella) as an ultra supra-molecular unit of a cell.

In the early years of the 20th century, the term "micelle" was also used to describe aggregates of inorganic molecules, e.g., hydrated ferric oxide (see Duclaux, 1920; Zsigmondy, 1921).

Today, physical chemists use the term "micelle" to describe aggregates of amphipathic molecules (e.g., surfactants, detergents, soaps) that are in dynamic equilibrium with surfactant monomers (Dickinson, 1992; Hunter, 1993). These classical micelles were first described by McBain (1913) and since then have been studied extensively. In modern textbooks on colloidal or physical chemistry or biochemistry, the term "micelle" is used exclusively for soap-like micelles without reference to the older and more general meaning of the word, which is not even acknowledged or referenced (see Dickinson, 1992; Hunter, 1993; Patterson, 1987).

Williams and Williams (1973) wrote "*The term "particle" is a general one and can be applied to any species in or near the size range* 1–1000 nm. *Some oil droplets, soap particles, and metal oxide particles fall within these size dimensions and give rise to systems traditionally referred to as colloidal dispersions. The particles in these systems (colloidal dispersions) are frequently called* **micelles** *as opposed to the term macromolecules, which is reserved for individual large molecules or a very low-order polymer of giant molecules*".

Dictionaries (e.g., *The Oxford Dictionary*, 1989 and *Tresor de la Langue Francaise*, 1985) credit Nägeli and Schwendener (1877) with introducing the term "micelle", of which they give several examples, including casein micelles.

3. Caseinate particles in milk*:* early studies

It has been known since the work of Schubler (1818) that casein is "merely suspended" in milk (see Palmer & Richardson, 1925). Sheldon (1880) gave the following interesting description of casein: "*Casein is a member of the albumin group, about which so little is known .... Casein is not in solution in the milk, but is swelled* *up by its absorption of water into a kind of very thin jelly. This may be proved by putting milk into a dialysator, when the casein does not pass the membrane, as it would if it were dissolved*''. Duclaux (1887) referred to casein as a colloid, probably being the first to do so. Johnson (1868) makes the following interesting comment: ''*When casein is separated from milk by rennet, as in making cheese, it carries with it a considerable portion of the phosphates and other salts of the milk; these salts are not found in the casein precipitated by acids, being held in solution by the latter*''. By the end of the 19th century, it was recognized that the casein in milk exists as large colloidal particles that contain calcium phosphate and are retained by Pasteur-Chamberland porcelain filters (see Kastle & Roberts, 1909).

During the early years of the 20th century, a number of studies were reported on the ''colloidal chemistry of milk'' (see Wiegner, 1914a, 1914b), in which the term ''casein particles'' or ''large casein particles'' was used to describe the physical state of casein. Wiegner (1914b) showed that the particles dissociated if the calcium therein was replaced by sodium. The stability of these particles, especially how they are destabilized during the rennet-induced coagulation of milk, attracted much attention. It was suggested by Alexander (1910, 1912) and Alexander and Bullowa (1910) that the casein particles are an irreversible, unstable colloid, stabilized by a reversible, stable, protective colloid (*Schutzkolloid*), which he proposed is ''lactalbumen'', i.e., whey proteins, and which is destroyed by rennet, leading to the coagulation of the casein particles. He suggested that the poor renneting properties of human and ass's milk are due to the low level of casein and the high level of lactalbumen in those milks.

The term *Schutzkolloid* (protective colloid) was coined by Zsigmondy (1909) to describe the ability of biopolymers to prevent the coagulation of a gold sol by electrolytes. The efficacy of biopolymers was expressed as the ''gold number'', defined as the amount (mg) of biopolymer that when added to 10 cm^3 of a solution of a gold salt, just prevents its coagulation on addition of 1 cm^3 of a 10% NaCl solution (Zsigmondy, 1901).

Destruction of the protective colloid by rennet, essentially the view of Alexander (1910), was also considered by Schryver (1913) and Clayton (1918) to be responsible for the coagulation of milk by rennet. Wright (1924) proposed that the coagulation of milk by rennet is due to an unspecified change in the ''colloidal condition'' of the calcium salt of caseinogen. Early work on the rennet coagulation of milk was reviewed by Palmer and Richardson (1925), who dismissed the protective colloid idea and proposed that the rennet coagulation of milk is due to the ''*precipitation of micellae by cations in the presence of a suspensoid which is peptidised by the precipitating ion*''. The word ''micellae'' was used only once in that paper and no explanation of the term is given; usually, the term ''calcium caseinate–calcium phosphate complex'', which was regarded as an irreversible colloid (suspensoid), was used.

4. Introduction and early use of the term ''casein micelle''

Duclaux (1920) used the term ''micelle'' in relation to various macromolecules, including casein; he acknowledged that this term had been introduced by Nägeli. However, as far as we can ascertain, the first author to use the term ''casein micelle'' was Beau (1921) who used the term ''*lactéine*'' for all milk proteins in their native state, which he considered to be aggregates comprised of caseins and whey proteins and which he called ''*micelles*''. Beau's concept of the casein micelle was probably based on the ''protective colloid'' model (Alexander, 1910, 1912; Alexander & Bullowa, 1910). Beau referred to the work of Duclaux (1920) and was probably familiar with the then current views on association colloids (micelles) formed by the association of inorganic molecules (Duclaux, 1920; Zsigmondy, 1901, 1909) or amphipathic molecules (McBain, 1913).

Although most of the amino acids had been isolated by 1900, views on the structure of proteins were very unclear until after 1930 (see Edsall, 1962; Tanford & Reynolds, 2001). It was not possible to determine the molecular mass of proteins until the analytical ultracentrifuge was developed in 1925 by Svedberg and Fahraeus and there was a widely held view that proteins did not have a clearly defined structure, as did small organic or inorganic molecules, but were random aggregates of low molecular mass polypeptides. Beau (1921), who would not have known the size of casein molecules, may have assumed that the casein micelles were actually casein molecules; unfortunately, he did not explain his choice of word and the article is poorly referenced.

The term ''micelle'' was used extensively by Porcher (1923) in his review on the chemistry of milk constituents but he did not explain why he used this term and the article was not referenced. In their review of the rennet coagulation of milk, Palmer and Richardson (1925) usually used the terms ''colloidal calcium caseinate'', ''calcium caseinate'' or ''calcium caseinate–calcium phosphate'' to describe the casein system in milk but used the term ''micellae'' once, without explanation, and did not refer to Beau (1921) or Porcher (1923). It is not clear what Beau (1921), Porcher (1923) or Palmer and Richardson (1925) meant by ''a micelle'' and none of them cited Nägeli and Schwendener (1877), Zsigmondy (1901), McBain (1913) or d'Herelle (1924).

In a paper on the rennet coagulation of milk, Marui (1926) used the term ''casein micelle'' freely but without explanation; the paper is sparsely referenced and Beau (1921), Porcher (1923) and Palmer and Richardson (1925) were not cited. Marui (1926) used the term ''*Schutzkolloid*'' (introduced by Zsigmondy, 1909) in relation to casein micelle stability and rennet-induced coagulation. Linderstrøm-Lang (1929) also used the term Schutzcolloid in relation to the casein system although he did not use the term ''casein micelle''. In their studies on the rennet-induced coagulation of milk, Richardson and Palmer (1929) and Hankinson and Palmer (1943) freely used the terms ''micellae'', ''caseinate micellae'', ''calcium caseinate micellae'', ''casein/caseinate micelles'' and even ''rennin micellae''. Hankinson and Briggs (1941) used the term ''micelle'' to describe the protein particles in sodium caseinate. Clearly, at this time, the term ''micelle'' was being used rather indiscriminately to describe proteins/polypeptides which were sufficiently large to behave as a colloid.

The casein system, including the colloidal aspects, was described in considerable detail by Beau and Bourgain (1926) and Beau (1932a, 1932b, 1941). In a book on the colloidal aspects and rennet-induced coagulation of milk, Porcher (1929) used the term ''micelle'' widely; this book was serialized in *Le Lait*, volumes 9, 10 and 11 (1929, 1930, 1931). The term ''micelle'' was also used by Piettre (1931) and Brigando (1933), although they usually used the term ''colloidal calcium caseinate–calcium phosphate'' or a variant thereof and did not refer to Beau (1921), Porcher (1923) or Palmer and Richardson (1925).

Interestingly, most of the early papers in which the term ''casein micelle'' was used involved attempts to explain the rennet-induced coagulation of milk, and, with the exceptions of Marui and Palmer, were by French authors, suggesting a common origin, perhaps Duclaux or d'Herelle, both of whom worked at the Pasteur Institute in Paris.

Sørensen (1930) compiled an extensive review on the formation of aggregates (bundles, micelles) of macromolecules. He cited the work of Meyer (1930) on the formation of cellulose micelles and described his own work on the formation of micelles from various soluble proteins, including casein. In his view, micelles are held together by secondary (associating) forces rather than covalent bonds. Sørensen (1930) proposed that the term "component system" rather than "micelle" should be used to describe aggregates of macromolecules. He argued that Nägeli's "micelles" corresponded to his "components", i.e., single molecule-like substances; to quote: *Hence to Nägeli the conception micelles is a unity of the same kind as the molecule conception in the case of simple substances, and in the colloid-chemical literature where it is frequently made use of especially by French investigators, the conception micelle is used in the same sense. We need only refer to J. Duclaux (Les Colloides, Gauthier-Villar, Paris, 1929), who writes "La micelle sera pour le colloïde l'analogue de ce le molécule chimique ordinaire est pour le cristalloïde*". This seems to imply that the term "micelle" was a French concept. Although he cited the work of McBain briefly, Sørensen (1930) did not acknowledge that McBain used the term "micelle". Sørensen (1930) considered casein, which had just been shown to be heterogeneous, to be a particularly interesting system. However, his discussion on casein related to solutions of the protein in dilute acid or alkali which he considered to exist as micelles. Surprisingly, Sørensen (1930) did not discuss the natural colloidal casein particles and did not reference any of the earlier studies in which the term "micelle" was used or Alexander (1910), Alexander and Bullowa (1910), Wiegner (1914a, 1914b) or Clayton (1918) in which the colloidal aspects of casein were discussed.

The term "micelle" was not used in early textbooks on Dairy Science/Chemistry, e.g., Grimmer (1926), Tague (1926), Beau (1932a), Sutermeister (1927), Associates of Rogers (1928), Rahn and Sharp (1928), Davies (1936, 1939), Ling (1946) or Davis and MacDonald (1953), or in the comprehensive review on milk proteins by McMeekin and Polis (1949). In the second edition of *Fundamentals of Dairy Science* (Associates of Rogers, 1935), the term "casein micelle" was not used in the chapters on *Milk Proteins* or *Physical Equilibria of Milk* but was used to a very limited extent by L.S. Palmer in his article on the *Rennet Coagulation of Milk*. Sutermeister and Browne (1939) included several references to the "casein micelle".

The analytical ultracentrifuge made it possible to study the polydispersity of the casein particles in milk and model systems (see Pedersen, 1936; Svedberg, Carpenter, & Carpenter, 1930a, 1930b) but the term "casein micelle" was not used by these investigators. There was quite an amount of research on the polydispersity, composition and properties of casein particles during the period 1930–1950 (see Lindqvist, 1963; Pyne, 1955) but the term "casein micelle" was very rarely used, an exception being Eilers (1947), who used it occasionally.

The term "casein micelle" became fairly widely used in the 1950s. Pyne (1953, 1955) usually used the term "caseinate–phosphate complex" but used "caseinate micelle" occasionally; he did not explain his choice of the term "micelle" and cited none of the earlier authors who had used the term. Berridge (1954) usually referred to the caseinate particles as "micelles" and stated that: "*it has been known for a considerable time that the casein in milk exists in the form of micelles with calcium phosphate*". He cited several references (Hostettler & Imhof, 1951; Nitschmann, 1949; Ter Horst, 1947) to support this or similar statements but none of these authors referred to the calcium caseinate–calcium phosphate particles as micelles.

In a brief description of the milk protein system, Fox and Foster (1957) made the following interesting but unreferenced comment: "*Casein does not occur in milk in true solution, but rather in the form of large aggregates or micelles of indefinite size, ranging up to 2000 Å or more in diameter and averaging perhaps 1000 Å*". In their widely acclaimed text-book, Jenness and Patton (1959) acknowledged the term "micelle" but decided to use the term "calcium caseinate–phosphate complex".

Early investigators (e.g., Hostettler & Imhof, 1951; Knoop & Wortmann, 1960; Nitschmann, 1949) who applied electron microscopy to milk, used the term *Calciumcaseinteilchen* exclusively. Probably the first electron microscopy study in which the term "casein micelle" was used is that of Barbaro and Calapuj (1958); Shimmin and Hill (1964) used the term extensively.

Thus, prior to 1955, few authors other than the French, consistently used the term "casein micelle". The term was not used as a sub-head in the index of *Dairy Science Abstracts*, first published in 1939, until 1955, when von Hippel and Waugh (1955) was cited.

5. κ-Casein and the casein micelle

von Hippel and Waugh (1955) studied the influence of temperature, pH and protein concentration on the equilibrium between casein monomers and polymers (aggregates, micelles). They concluded that "*Clearly, there exists in each case a balance between attractive and repulsive forces dependent on the physical conditions. These polymerizations bear a marked resemblance to those responsible for the formation of soap micelles, as described and analysed by Debye*". None of the previously cited articles in which the term casein micelle was used was cited nor was the work of Nägeli or d'Herelle.

Waugh had worked on the association of proteins during the 1940s and published a paper (Langmuir & Waugh, 1940) on the surface activity of proteins which contained the following interesting comment "*The spreading of a water-soluble protein to give an insoluble monolayer depends on the presence of hydrophobic side chains in some of the amino acids of the protein. In aqueous solutions of the globular proteins these hydrophobic groups must be inaccessible, probably being enclosed within cage-like protein molecules having hydrophilic surfaces; a structure like that of the micelles in solutions of soap and other detergents in which hydrocarbon chains are packed into the interior of spherical micelles whose surfaces contain all the polar groups*". Waugh (1946) also used the term "micelle" to describe aggregates of heat-denatured insulin (alternative terms used were floccules, spherites, fibrils and micelles). He published a very extensive review (Waugh, 1954) on the non-covalent forces involved in protein association and aggregation, in which the term "micelle" was used widely; he described some work on β-lactoglobulin but made no reference to the caseins. He had also worked on the membrane of erythrocytes and on blood clotting.

Waugh and von Hippel (1956) isolated κ-casein and revolutionized ideas on the structure of the casein micelle. κ-Casein, a relatively minor component of the casein system (12–15% of whole casein), is soluble in the presence of Ca^{2+} whereas the remaining 85% of casein are precipitated by Ca^{2+}; κ-casein can stabilize up to 10 times its weight of the Ca-sensitive caseins via the formation of micelles, thus functioning as the *Schutzcolloid* proposed 50 years earlier. This discovery led to a series of publications by Waugh and collaborators on the mechanism of stabilization and the structure of the casein micelle, which was reviewed by Waugh (1971).

6. Relationship between calcium phosphate and calcium caseinate

As discussed previously, it has been known since the end of the 19th century that the colloidal caseinate particles contain calcium phosphate. The relationship between the colloidal casein particles and calcium phosphate, now referred to as colloidal calcium phosphate (CCP), has been the subject of numerous investigations

Table 1
Average characteristics of casein micelles

Characteristic	Value
Diameter	120 nm (range: 50–500 nm)
Surface area	8×10^{-10} cm^2
Volume	2.1×10^{-15} cm^3
Density (hydrated)	1.0632 g cm^{-3}
Mass	2.2×10^{-15} g
Water content	63%
Hydration	3.7 g H_2O g^{-1} protein
Voluminosity	44 cm^3 g^{-1}
Molecular mass (hydrated)	1.3×10^9 Da
Molecular mass (dehydrated)	5×10^8 Da
No. of peptide chains	5×10^3
No. of particles per mL milk	10^{14}–10^{16}
Surface of micelles per mL milk	5×10^4 cm^3
Mean free distance	240 nm

during the past 100 years but the matter has not yet been fully resolved. The principal issues investigated include: the composition of CCP; the nature of the association between CCP and casein; and the effect of CCP on casein micelle stability and size. These topics are critical to the structure and properties of the micelles but are outside the scope of this review. The reader is referred to Pyne and McGann (1960), McGann and Pyne (1960), Pyne (1962). Schmidt (1982), McGann, Buchheim, Kearney, and Richardson (1983) and Holt (1985, 1997, 1998).

7. Structure of the casein micelle

The general properties of casein micelles are now well established and are described in the reviews cited earlier. The key features are summarized in Table 1. The mechanism by which the casein particles (micelles) in milk are stabilized and destabilized by the action of various agents and conditions have attracted much attention during the 20th century and there is a vast volume of information in the literature. One of the important topics investigated has been elucidation of the structure of the micelle.

The development of a realistic model of casein micelle structure became possible only after the isolation and characterization of κ-casein (Waugh & von Hippel, 1956). The first model of the casein micelle was published by Waugh (1958). This was followed by a series of papers on the formation and properties of artificial casein micelles, e.g., Waugh (1961), Noble and Waugh (1965), Waugh and Noble (1965) and Waugh, Creamer, and Slattery (1970). These studies were reviewed by Waugh (1971), who elaborated on a model for the structure of casein sub-micelles and explained for his choice of the term "micelle" to describe the calcium phosphate–calcium caseinate particles in milk. To quote: "*A considerable amount of space could be devoted to the question of the meaning of the word 'micelle'. In most cases, micelle has been used to designate what obviously are colloidal association products. However, the designation has most frequently been used before even a general understanding of the structure was available. It is not surprising, at this time, that different micelle types have different structures: the cellulose micelles of von Nägeli* (see Nägeli & Schwendener, 1877) *are different in structure from soap micelles* (Shinoda, Nakagawa, Tamamushi, & Isemura, 1963) *and from the casein micelle. The micelles of milk are customarily defined as the colloidal association products of the caseins. To write them as colloidal particles is permissible on the basis that they are large with respect to the constituent monomers but stable with respect to each other and to the earth's gravitational field. What might be anticipated, from the properties of other systems, as permissible plans for the structure of the casein micelle? Micelles are expected to fall into one or more of the categories of (a) single-phase particles, (b) large chemical compounds or (c) structures having a composition which changes in going from the surface to the center*". He described the forces involved in stabilizing colloidal particles and included some examples, including soap micelles, bacteriophage and complex enzyme systems. He continued: *In the casein micelle system, as will be seen, the micelle state may be the lowest free-energy state of the system. Of particular interest will be micelle structure and the mechanisms which operate in determining micelle size*. He proceeded to present a detailed discussion on his views on the structure and properties of the casein micelle, based mainly on the work of his group on model systems prepared, mainly, from α_s- and κ-caseins and $CaCl_2$. The review is copiously referenced but, rather surprisingly, he cited none of the early workers who had used the term "casein micelle".

Since the pioneering work of Waugh (1958), there has been continuous work on the properties and structure of the casein micelle, and structural models have been refined progressively. While views on the detailed structure of the casein micelle are not unanimous, its general properties are widely accepted. Progress can be followed through the numerous reviews cited earlier and has been summarized by Fox and Kelly (2004). Some key features and characteristics to be considered are as follows.

κ-Casein, which is soluble at the calcium concentration in milk and which is about 12% of total casein, can stabilize about 10 times its mass of Ca-sensitive caseins (α_{s1}-, α_{s2}- and β-). The most obvious organization which would permit this is a core of Ca-sensitive caseins surrounded by a layer of κ-casein, analogous to the stabilization of lipids by an emulsifier. κ-Casein is readily hydrolysed by chymosin (~35 kDa) and reacts via sulphydryl–disulphide interaction with β-lactoglobulin (dimeric, ~36 kDa) on heating milk. Both of these reactions suggest either that κ-casein is exposed on the surface of the micelles or that the micelles are very porous so that large molecules can diffuse readily through them.

However, milk is not coagulated by immobilized rennets, suggesting that the κ-casein is not very exposed. Super-polymerized aminopeptidase, which cannot diffuse into the micelle, releases the N-terminal residue of all four caseins, suggesting that the surface of the micelles is not covered exclusively with κ-casein and that some of all four caseins is located on the surface.

On removal of colloidal calcium phosphate (by acidification-dialysis or a calcium chelator), the micelles disperse into particles of $\sim 0.5 \times 10^6$ Da, suggesting that CCP plays an integrating role in the micelles. However, the micelles are also dispersed by urea, SDS, high pH or ethanol (>35%, >70 °C), indicating that hydrogen bonds, hydrophobic and electrostatic interactions are also involved in micelle integrity. Up to 50% of β-casein, the most hydrophobic of the caseins, dissociates reversibly from the micelles on cooling, indicating the importance of hydrophobic interactions and suggesting that the micelle is sufficiently porous to allow the β-casein to diffuse out of the micelle; on rewarming, the β-casein appears to form a fuzzy layer on the surface.

Electron microscopy shows that the micelles have an uneven, raspberry-like appearance which was interpreted to mean that the micelles are built up from sub-micelles, with a core of Ca-sensitive caseins and a κ-casein-rich coat. This model underwent several refinements, e.g., whether the κ-casein coat was uniform or in patches, whether all sub-micelles have a layer of κ-casein or whether there are κ-casein-rich and κ-casein-deficient sub-micelles, with the latter concentrated in the core of the micelle and the former concentrated at the surface, giving the micelle a κ-casein-rich surface. Most, but not all, authors proposed that the sub-micelles are held together by microcrystals of CCP.

However, recent electron microscopy studies using improved microscopes have failed to confirm the presence of sub-micelles; in one study (Dalgleish, Spagnuolo, & Goff, 2004), the irregularities were considered to be microtubules. Three alternatives to the sub-micelle models (Holt, 1994; Horne, 2002; Visser, 1992), depict the micelle as being made up of casein molecules linked together by CCP microcrystals and hydrophobic bonds but differ in detail. Further refinement of these models can be expected, especially as electron microscopes are improved.

8. Stability of casein micelles

The colloidal stability of milk is, in many cases, its most important physico-chemical aspect and, consequently, has attracted much attention during the past century. In good quality milk, the casein micelles are stable to all processes to which it is normally subjected:

- *Concentration by evaporation or ultrafiltration*: However, stability decreases with the degree of concentration, due mainly to the closer packing of casein micelles, an increase in [Ca^{2+}] and a decrease in pH due to the precipitation of CaH_2PO_4 and $CaHPO_4$ as $Ca_3(PO_4)_2$ accompanied by the release of H^+.
- *Dehydration*: In the absence of heat-induced changes, the micelles in milk powder reconstitute readily and their properties are changed little.
- *Freezing*: Freezing per se has little, if any, effect on the casein micelles but slow freezing and storage at a temperature in the range -10 to $-20\,^{\circ}C$ cause destabilization due an increase in [Ca^{2+}] and a decrease in pH, due to the precipitation of $Ca_3(PO_4)_2$; these effects are exacerbated by the crystallization of lactose.
- *Homogenization*: Normal homogenization of milk, i.e., up to 20 MPa, has little or no effect on the casein micelles, but high-pressure homogenization (>200 MPa) or high-pressure treatment >200 MPa cause some dissociation.
- *Heat treatment*: HTST pasteurization ($72\,^{\circ}C \times 15$ s) has little or no effect on the casein micelles but heating at a higher temperature causes denaturation of the whey proteins and their interaction with the casein micelles via sulphydryl–disulphide interactions, especially between β-lactoglobulin and κ-casein. This change affects many properties of the micelles, e.g., heat stability and rennet coagulation properties. Severe heating, especially of concentrated milk, causes Maillard browning, a decrease in pH, dissociation of κ-casein from the micelles, and, eventually, coagulation.

The micelles are destabilized by a number of factors, some of which are industrially important:

- hydrolysis of the κ-casein by selected proteinases (rennets), which is exploited in the manufacture of most cheese varieties;
- acidification to about pH 4.6, which is exploited in the manufacture of some cheeses, fermented milks and functional caseinate products;
- ethanol (or other alcohol);
- anionic detergents, e.g., SDS;
- high pressure.

The micelles are dispersed by dissolving the colloidal calcium phosphate (by acidification-dialysis or calcium chelator), at a high pH, by about 35% ethanol $>70\,^{\circ}C$, by >4 M urea or by SDS.

9. Significance of the casein micelle

The casein in the milk of all species studied exists as micelles, as indicated by the fact that they are more or less white due to light scattering, for which the micelles are mainly responsible. The widespread or universal distribution of micelles in milk, suggests that they have some physiological or nutritional significance over the nutritional value of the proteins per se. Two benefits are apparent:

1. Calcium and phosphate are required for the development of teeth and bone and growth rate is positively correlated with the concentrations of Ca and Pi. However, calcium phosphate has low solubility at the pH of milk with which it is supersaturated; therefore, calcium phosphate would be expected to precipitate in the mammary gland, with the formation of etopic stones that would block the ducts of the gland, resulting in the death of the organ and perhaps of the animal. By forming micelles, casein maintains the excess calcium phosphate in a colloidally stable state. Thus, the casein micelles may be regarded as a device by which to enable the secretion of milk with a high concentration of calcium phosphate in a "soluble" form.
2. Presumably, the micelles are designed to be coagulated in the stomach of the neonate by chymosin, a proteinase designed for this function. Coagulation delays the entry of milk constituents into the small intestine, thereby improving digestibility. Furthermore, the coagulum acts as a buffer to facilitate nursing at intervals that may be very long (24 h) for some species, e.g., the hare.

The stability of casein micelles is critical for the technology of most dairy products. However, these aspects are incidental and not the reason why casein micelles evolved.

10. Is the "casein micelle" a "true micelle"?

Today, the term "micelle" is normally used in physical chemistry and biochemistry for aggregates of relatively small amphipathic molecules, such as soaps and bile salts—this is the context introduced by McBain (1913). Such micelles can dissociate rapidly and reversibly to monomers on dilution or by changing other environmental conditions. Casein micelles do not fall into this category, although von Hippel and Waugh (1955) clearly considered that the casein micelles in milk are in equilibrium with casein monomers.

The term "micelle" was introduced by Nägeli and Schwenderener (1877) for aggregates of cellulose or starch which, clearly, are not amphipathic molecules. Nearly 50 years later, the term was used by d'Herelle (1924) for bacteriophage which does not dissociate. The casein monomers are clearly amphipathic molecules (Swaisgood, 2003) and the structure of the casein micelle appears to be intermediate between those of the original "biological micelles" and the later "soap micelles". Based on the literal meaning of micelle (small particle, crumb), there seems to be no good reason for not using the term "casein micelle" to describe the calcium phosphate–calcium caseinate particles in milk. The term has been used widely for about 50 years and to a more limited extent for about 35 years before that. The term appears to have been introduced by Beau (1921) and to have been used mainly by French scientists during the period 1921–1932. Usage of the term then declined, although it was used occasionally as an alternative for calcium caseinate–calcium phosphate particles by Eilers (1947), Pyne (1953, 1955) and Berridge

(1954). The term has been used universally since about 1960 owing mainly to the work of Waugh and co-workers.

The recommendation of Dickinson (1992) that, in relation to the nomenclature of the colloidal calcium phosphate–calcium caseinate particles in milk, the term "micelle" should always be qualified as "casein micelle", in the interest of precision, seems to be good advice. However, equally, in the interests of precision, aggregates of soap molecules should be referred to as "soap micelles". The meaning of the term "casein micelle" is clearly understood in Dairy Chemistry and cognate areas and its use is more convenient than alternatives, e.g., "calcium caseinate–calcium phosphate particles".

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