Extended abstract

The casein micelle and its reactivity

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Casein micelles are the basis of many new and traditional dairy products, and in all of these products the aggregation properties of the casein micelles are of primary importance. Either we seek to avoid the aggregation of the milks (as in long-storage liquid products) or we wish to promote aggregation and structure formation (as in cheese and yogurt manufacture). In either case, therefore, we need to be able to understand and control the surface of the casein micelle, because the stability of the particle is defined by the structure and properties of what is on its surface. To some extent, the interior of the particle is not relevant to its gelation properties (that is, the coming together of particles to form a network), although it may be very important in defining the properties of the gel once it is formed.

Over the last 30–40 years, many models of the casein micelle have been proposed, and it must be admitted that a consensus has not been achieved. It is only necessary to compare two recently-published reviews [3, 5] to see how far apart the points of view can be, especially as regards the interior structure of the casein micelle. The aspect that seems to be generally accepted is that the κ-casein of the micelle is located mainly, if not completely, on the micellar surface. This surface location permits the macropeptide of the κ-casein to protrude into the surrounding serum, to give a sterically-stabilizing "hairy layer" to the particles. This layer is also negatively charged, although it has been calculated that the charge alone would not be sufficient to prevent close approach of the casein micelles. Breakdown or removal of the hairy layer by acidification or renneting removes its stabilizing influence and the casein micelles coagulate. Much evidence has been collected on the change of hydrodynamic size of casein micelles during acidification or renneting, consistent with the existence of this hairy layer.

It should be noted, however, that this hairy layer need not be intact, in the sense that it is not necessary for the casein micelles to have a complete "skin" of κ-casein [2]. Other caseins can be present on the micellar surface. All that is necessary is that there should be sufficient steric stabilization to prevent the casein micelles from coming too close. In a sense, native bovine casein micelles are "over-stabilized" because we know that it is possible to remove a sizeable proportion of the micellar κ-casein (by, for example, treatment with rennet) without causing the milk to coagulate.

How then can we modify the properties of the surfaces of the casein micelles so as to control their properties? It is surprising that relatively little has been done in this direction, except for a rather "all-or-nothing" approach. That is, there are many traditional cheeses that depend on the complete removal of the hairy layer, and there are acid products that

depend on its collapse, but there are many fewer products where the stabilizing influence of the κ-casein layer is partially modified. However, a combination of mild renneting and acidification can give much stronger acid gels than can be obtained from either process alone [6, 7]. There is possibly more scope for the manipulation of casein micelles than is in fact achieved at the present time.

The most significant modification of the casein micellar surface that is routinely (if inadvertently) practised, is caused by heat treatments. Heat-treatment of milks at 75 °C or above cause denaturation of the whey proteins, and these react with themselves and with κ-casein. Depending on the pH at which the milk is heated, the whey protein complexes either remain on the casein micelles (at pH around 6.3) or mainly dissociate into solution (at pH > 6.7) to form "soluble complexes" containing a few tens of protein molecules, and with diameters of about 30–50 nm [4, 8]. Thus, the result of heating is to partially remove the κ-casein of the micellar surface, but also to produce casein micelles with a "new" surface, made of denatured whey proteins. This alters the reactivity of the casein micelles, by reducing their susceptibility to rennet (even though some of the κ-casein has been removed into the serum) and making them difficult to coagulate. On the other hand, the presence of the serum protein patches on the micellar surface has a very beneficial effect on the strength of the acid gel produced from heated milk; this is further enhanced by the binding of the soluble complexes to the micelles as acidification proceeds, to form bridges between them [1]. Thus in an acid gel from heated milk, the points of contact between the casein micelles are probably not the caseins but the whey proteins that are bound to the micelles. This may explain the increased strength of the gel.

We know that casein micelles form gels, and that the strengths of the gels are dependent on the number and strengths of the inter-micellar junctions. There has been no detailed consideration of the actual way in which casein micelles touch and interact when they form gels. Especially, it is important to understand the extent to which casein micelles retain their identity in a gel; whether they remain as individual entities or whether they fuse together. It is in this stage of the gelation that the structure and stability of the micellar interior becomes important. We can qualitatively imagine that acid gels from unheated milk are weak because the κ-casein of the micellar surface is still intact; the CMP layer is no longer "hairy" because it has collapsed, but it is still present. Therefore, its hydrophilic character is maintained and it will prevent close contact of the micellar surfaces. On the other hand, renneted micelles will have points of contact between what may be termed the inner surface of the casein micelle. These contact areas almost certainly contain para-κ-casein but it is probable that other caseins are involved; the contact is therefore via hydrophobic interactions, probably accompanied by some bridging via calcium ions. Finally, in heated milk that has been acidified, the contact points between the casein micelles are not even dependent on the caseins (apart from κ-casein) because the linking may be between denatured whey proteins. Very little work seems to have been done on defining the structures of these different contact types and to what extent they can be modified.

There is of course another type of destabilization of milk that involves physical, rather than chemical, effects. Addition of polysaccharides to milk can lead to destabilization by a process of depletion flocculation, leading to phase separation at long times. While this does not in itself alter the structure or stability of the casein micelles, it is undesirable in long-life products. The phenomenon can be altered by introducing polysaccharides such as κ-carrageenan that actively bind to casein micelles. This produces a weak network of casein/carrageenan that resists the tendency for phase separation. Similarly, casein micelles in acidified milks can be stabilized by the binding of pectin, as is done in the

manufacture of drinking yogurts. Stabilization is produced by the formation of a layer of pectin around the casein micelles, to give steric and perhaps electrostatic stabilization to the particles.

We may ask how these behaviour patterns are explained by the existing models, and what refinements in the models are needed to allow predictions of aggregation and gel properties to be made. It has to be admitted that for this there is not an adequate model of the structure. Only the behaviour of native unheated micelles can be modelled at all on the basis of the extended CMP layer, and that only partially. Much more knowledge is required of the internal strength of the casein micelles and of their points of interaction before the properties of gels can be described from first principles.

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