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The rennet coagulation mechanism of skim milk as observed by transmission diffusing wave spectroscopy

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Abstract

The technique of forward-scattering diffusing wave spectroscopy has been used to study the rennet-induced gelation of skim milk. The results allow the comparison of a colloidal suspension at a realistic concentration ($\Phi \sim 10\%$) compared with well-established measurements made on highly-diluted milk samples. It is shown that the partially renneted casein micelles do not begin to approach one another until the extent of breakdown of κ-casein has reached about 70%; above this point, they interact increasingly strongly with the extent of proteolysis. This interaction initially restricts the diffusive motion of the particles rather than causing true aggregation. Only after more extensive removal of the protective κ-casein does true aggregation occur, with the appearance of a space-filling gel (defined by rheology as having a value of $\tan\delta < 1$). The results show in greater detail than hitherto the progress of interactions between the particles in a system where the steric stabilization is progressively destroyed, and suggest that the renneting of milk at its normal concentration cannot be described simply by reactions between freely-diffusing particles.

Keywords: Casein micelles; Diffusing wave spectroscopy; Renneting; Milk coagulation

1. Introduction

Rennet coagulation of milk is a very important aspect of the dairy industry, and has been intensively studied over many years. The process results from the destabilization of colloidal particles (the casein micelles) that are originally sterically stabilized, but whose surface is progressively destroyed by the action of a proteolytic enzyme (chymosin). The casein micelles themselves are particles, with diameters between 80 and 300 nm, composed of many thousands of molecules of the casein proteins bound together by calcium phosphate [1]. Stability of these colloidal particles is provided by a "hairy" surface layer of the protein κ-casein, part of which protrudes into the aqueous phase of the milk and provides steric and possibly electrostatic stabilization of the particles [2,3]. It is this layer of κ-casein that is destroyed by the chymosin, which specifically cleaves the protein at the Phe_{105}–Met_{106} peptide bond, allowing the stabilizing caseinomacropeptide (CMP, κ-casein peptide 106–169) to diffuse away from the micellar surface [4]. It is believed that in unheated milk all of the κ-casein is accessible to the enzyme, so that each molecule of the protein has an equal chance to be cleaved by chymosin [5]. Decreases in turbidity, viscosity, and apparent radius of the micelles have been observed during the early stages of the renneting process, and have been attributed to the removal of the hairy layer by the enzyme action [2,6–12].

The cleavage of the κ-casein results in a progressive reduction in the steric protection provided by the CMP, and simultaneously reduces the charge on the micellar surface, as estimated by the ζ-potential [11,13]. Nevertheless, extensive proteolysis of the κ-casein (at least 80–90% release of CMP from different studies) has to occur before the casein micelles can start to aggregate [5,14]. Several authors have attempted to describe the loss of the stability of the micelles by a combination of DLVO and steric stabilization mechanisms [15–17]. Most recently, de Kruif and Zhulina [3] have explained the renneting mechanism by considering κ-casein as a 'salted polymer brush' on the surface of the casein micelle. The stability of this hairy layer depends on the solvent quality, chain density and the charge

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density along the polyelectrolyte chain. When it is in an extended form, the long range electrostatic and steric interactions between casein micelles dominate over short range van der Waals interactions. When the brush is removed by renneting, closer approach of the particles is possible and van der Waals interactions induce flocculation [18]. However, this model does not fully predict the experimentally-observed changes in the viscosity during renneting [19].

Once the breakdown of the κ-casein has reached the critical level (about 80–90%), the casein micelles begin to aggregate and eventually form a gel [14,20]. This aggregation has been suggested to occur by a Smoluchowski process, that is, by a process of random collision, so that there is no preference for aggregates of specific sizes to be formed or to react. Without at that time specifying a cause for the aggregation, Dalgleish [21] suggested a general model treatment for rennet coagulation incorporating the enzyme kinetics, the Smoluchowski aggregation mechanism, and a critical level of κ-casein breakdown required for an individual micelle to aggregate. This was later extended to explain the need for a critical breakdown of κ-casein on the basis of a simple geometric model of steric stabilization [16]. The model showed that aggregation could only occur when large bare patches lacking CMP had been produced on the micellar surface, allowing the inner parts of the casein micellar surfaces to come into contact. This can only happen when extensive renneting has occurred.

A drawback in most of the experiments leading to models of rennet coagulation is that they were performed on milks that were highly diluted to allow measurements of turbidity or light scattering. This dilution perhaps forces the casein micelles to react by a Smoluchowski mechanism, because their volume fraction in such systems is very low (typically about 0.005%) and they must diffuse long distances before finding another particle with which to interact. On the other hand, in skim milk the casein micelles have a volume fraction of about 10% and the average separation between the particle surfaces is about 0.75 of the particle diameter. It is therefore arguable whether a classical diffusive mechanism is the appropriate model to use for the aggregation of the casein micelles in milk. We have tried to investigate the gelation by using the technique of diffusing wave spectroscopy (DWS), which is a light scattering technique that is capable of making measurements in turbid suspensions, such as milk, without the necessity of diluting them. The technique has been used in several studies on milk over recent years, although much of the work has focused on the aggregation caused by acidification [22–27] rather than by renneting [12,28]. We wished to define whether the technique would allow us some further insight into the mechanism of the reaction.

2. Experimental

2.1. Skimmed milk

Fresh whole milk was obtained from Elora Dairy Research (Elora, ON, Canada), and sodium azide was immediately added at a concentration of 0.02% (w/v) to act as a bacteriostatic agent. The milk was skimmed by centrifugation at 6000 rpm for 20 min at 4 °C using a Beckman J2-21 centrifuge and JA-10 rotor (Beckman Coulter, Mississauga, ON, Canada) and was then filtered three times through Whatman glass fiber filters (Fisher Scientific, Whitby, ON, Canada). The milk was then kept at refrigeration temperature until it was used.

2.2. Rennet treatment of the milk

The rennet used was Chymostar Double Strength (Rhodia, Cranbury, USA). Three different dilutions (designated as 1×, 2×, and 4×) were used, which were two-stage dilutions of the original rennet. First, volumes of 50, 100, or 200 µl of the full-strength rennet were diluted to 2 ml with Milli-Q water. Aliquots (100 µl) of these solutions were then added to a further 900 µl of Milli-Q water. These diluted rennet solutions were then added to the milk samples at a ratio of 7 µl per 1 ml milk. The rennet preparations were all added to milk within 15 min after dilution. These rennet concentrations are very low when compared with the concentration used in the cheese making industry, but this was done to allow maximum time for measurement by the DWS.

2.3. Diffusing wave spectroscopy (DWS)

Transmission DWS was used to monitor the changes during renneting coagulation. The light source was a solid state diode pumped Nd:YAG laser type of wavelength 532 nm and power of 100 mW (Coherent, Santa Clara, CA, USA), and the scattered light was collected by a single fiber optic that was then bifurcated and fed to two matched photomultipliers (HC120-03, Hamamatsu, Loveland, OH, USA) and a correlator (FLEX2K-12 × 2, Bridgewater, NJ, USA). The equipment is based on a design used in other laboratories (32). A volume of 1.5 ml of undiluted milk was put into a 5 mm path length optical glass cuvette (Hellma Canada Limited, Concord, Canada) and the temperature of the equipment was equilibrated to 30 °C prior to rennet addition and maintained at this temperature with water from a circulating bath. Standard latex spheres of 269 nm diameter (Portland Duke Scientific, Palo Alto, CA, USA) were used to calibrate the laser intensity daily and analysis was done with adjustment for the laser intensity on the day of analysis to count for intensity fluctuation [28]. The calibration of the laser allowed the subsequent measurement of the photon transport mean free path (l^*) in the renneted milks. This parameter is a function of the concentration of the scattering particles, their radius and refractive index, and the two light scattering parameters $F(q)$ (form factor) and $S(q)$ (structure factor) [29]. These arise from interference of scattered photons within the particles themselves and those scattered within assemblages of particles (q is the scattering vector $4\pi n/\lambda \sin(\theta/2)$).

Correlation functions and intensity of the transmitted scattered light were measured at intervals of 5 min (295 s collecting followed by a 5 s break) during renneting experiments. From these and the measured values of l^* the characteristic decay time (relaxation time, τ) was calculated using the equations described previously [28,29], and this was used to calculate the apparent diffusion coefficient and apparent radius (assuming