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Heat-induced gelation of casein micelles in aqueous suspensions at different pH

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ABSTRACT

Heat-induced gelation of casein micelles in aqueous solution was investigated between pH 5.2 and pH 6.7 over a wide range of protein concentrations ($C = 25–160\,g\,L^{-1}$). For $C \geq 40\,g\,L^{-1}$ the casein micelles rapidly formed a self-supporting gel above a critical temperature (T_c). At $C = 160\,g\,L^{-1}$, T_c decreased from 90 °C at pH 6.5 to 30 °C at pH 5.4 and increased with decreasing protein concentration. Oscillatory shear measurements during heating showed that the elastic modulus (G_{el}) of the gels increased strongly with increasing protein concentration, but was insensitive to the pH and the heating temperature except close to T_c where G_{el} decreased sharply with decreasing temperature. The microstructure of the gels was observed by confocal scanning laser microscopy. Heat-induced gelation of casein micelles was compared with that of sodium caseinate solutions free of calcium phosphate.

1. Introduction

In milk, the main protein fraction (80% of total protein) is composed of four different types of casein (α_{s1}, α_{s2}, β and κ-casein) associated into a complex with colloidal calcium phosphate (CCP) called casein micelles [1–5]. Steric and electrostatic repulsion of a κ-casein brush at the surface of the micelles inhibits aggregation of casein micelles in aqueous solution [6,7]. CCP is present in the form of nanoclusters that also incorporates phosphoserine groups of the caseins. Reduction of the pH causes progressive dissolution of the CCP, which is complete at pH 5.2 [8–10]. Recently, we have shown using ^{31}P MAS NMR that solubilization of the CCP by decreasing the pH allows the phosphoserines in the casein micelles to become mobile [11].

In milk, the presence of CCP is necessary to maintain the integrity of the micelles. However, the micelles remain intact with approximately the same size down to pH ~5.1 where the net charge density of the caseins and thus the electrostatic repulsion is small, because hydrophobic and other physical interactions are sufficient to maintain their integrity [12–16]. Below pH 5.1, electrostatic and steric repulsion between the micelles is no longer sufficient to inhibit aggregation leading to precipitation or gelation [9,11,17,18].

In milk, the micelles aggregate when they are heated above 100 °C [19], but at lower temperatures they are stable. Nevertheless, preheating of milk above 70 °C has a major effect on the acid-induced gelation of casein micelles as it leads to gelation at higher pH and to stiffer gels and has been investigated extensively [18]. The effect is principally attributed to heat-induced denaturation and aggregation of the globular whey proteins that are present in the serum of milk. It was found that denatured whey proteins co-aggregate with κ-casein into complexes that are in part bound to the casein micelles [20]. Aggregation of micelles covered with whey proteins starts at a higher pH during acidification.

Recently, Koutina et al. [21,22] studied the effect of the temperature on acid induced gelation of casein micelles in milk and related it to solubilization of the CCP. They found that the critical pH below which the systems gelled increased with increasing temperature: pH 5.2, 5.4 and 5.6 for 40, 50 and 60 °C, respectively. The effect of was attributed to changes in the content of calcium and orthophosphate in the micelles with increasing temperature. Indeed, NMR studies showed that restructuring occurred of the complexes between calcium, orthophosphate and phosphoserines in the caseins in milk during heating [23].

Interestingly, it was found that aggregation and gelation of casein micelles could also be induced by increasing the temperature at a fixed reduced pH [24,25]. Contrary to the effect of heating milk and subsequently reducing the pH, this process has attracted little attention so far. The critical temperature above which aggregation of micelles occurred in skimmed milk was found to increase with increasing pH from 0 °C at pH 4.4 to 30 °C at pH 5.2 [24]. Vasbinder, et al. [25] investigated this phenomenon using diffusive wave spectroscopy to detect aggregation of the micelles. They observed that

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the critical temperature above which aggregation of the micelles occurred increased from about 25 °C at pH 5.0 to 50 °C at pH 5.4. Comparison between skimmed milk and whey protein free milk did not show major differences indicating that the presence of native whey proteins did not influence the onset of gelation of the casein micelles. In addition, it was found that the heating rate did not influence the critical gelation temperature. The authors pointed out that increasing the temperature at fixed pH was not equivalent to decreasing the pH at fixed temperature.

As far as we are aware, thermal gelation of casein micelles above pH 5.6 has not yet been reported, nor have the mechanical properties and the microstructure of heat-set casein micelle gel been investigated. The objective of the present study was to investigate the phenomenon of heat induced gelation of casein micelles in aqueous solution at fixed pH over a wide range of protein concentration (25–160 g L^{-1}) and pH (5.2–6.7). We have used native phosphocaseinate powder dispersions in order to obtained dispersion of casein micelles in water free from whey protein, lactose and serum minerals. It was shown that the size and structure of these casein micelles and their behaviour during acidification at room temperature is almost the same as that of caseins micelles in milk [9,26].

We will show that gels can be formed by casein micelles up to pH 6.5 when the solutions are heated at 90 °C. The stiffness of the heat-set gels was studied using oscillatory shear rheology and the microstructure was studied using confocal laser scanning microscopy (CLSM). We will compare the behaviour of casein micelles with that of sodium caseinate (NaCas) that is obtained if the CCP is removed. We believe that the detailed investigation of heat-induced gelation of pure casein micelles in water presented here can serve as a bench mark for the more complex behaviour of mixtures of casein micelles and denatured whey proteins.

2. Materials and methods

2.1. Materials

Casein micelles were provided in the form of native phosphocaseinate powder (NPCP) by INRA STLO in Rennes. NPCP was produced following a method developed by Pierre et al. [27] and Schuck et al. [28]. Briefly, skimmed milk was processed through cross-flow microfiltration to separate the casein micelles from the serum proteins. The micelles were washed with water in diafiltration mode and subsequently the solution was dried in low-temperature conditions through spray-drying. The powder contained 83% (w/w) protein (TNC Kjeldahl) and 8.7% (w/w) ashes including 2.6% (w/w) of calcium and 1.7% (w/w) of total phosphorus. NPCP has been used as a model for native casein micelles before and after dispersion in water the size and structure of NPCP and its behaviour during acidification is almost the same as that of caseins micelles in milk [9,26]. The sodium caseinate powder that was used for this study (Lactonat EN, Lactoprot, Kaltenkirchen, Germany) contained 90% (w/w) protein (TNC, Kjeldahl), 1.3% (w/w) sodium and 0.7% (w/w) phosphorus. The fraction of residual whey protein in either of the casein powders was at most a few wt%.

2.2. Sample preparation

The powder was dispersed in milliQ water containing 3 mM of sodium azide (NaN_3) to prevent bacterial proliferation. Fully dispersed suspensions of NPCP were obtained after heating the solution at 50 °C during 16 h. Alternatively, the micelles were dispersed using a homogenizer at room temperature. No difference in the behaviour of the micelles was observed. Static and dynamic light scattering measurements were done in the same manner as described by Pitkowski et al. [29]. The same results were obtained, which showed that the micelles were fully dispersed and not aggregated. About 10% of the protein did not sediment after centrifugation for 2 h at 5×10^4 g and was therefore not in the form of micelles in the powder. In the concentration range used here less than 5% of micelles disintegrated over a period of one day after preparation [30]. For NaCas, homogeneous solutions were obtained after heating at 80 °C during 30 min in the thermostat bath. The pH was adjusted at 20 °C under vigorous stirring by slow addition of aliquots of HCl or NaOH solutions (0.1–1 M). The protein concentration was determined by UV-absorbance at 280 nm (Varian Cary-50 Bio, Les Ulis, France) using an extinction coefficient of 0.81 L g^{-1} cm^{-1}.

2.3. Confocal Laser Scanning Microscopy (CLSM)

CLSM observations were made using a Leica TCS-SP2 (Leica Microsystems Heidelberg, Germany). A water immersion objective lens was used HCxPL APO 63x NA = 1.2 with theoretical resolution of 0.3 μm in the x–y plane. The proteins were labelled with the fluorochrome rhodamine B that physically adsorbed to the proteins, by adding a small amount of a concentrated rhodamine solution (5 ppm) to the micelle solutions. No effect of labeling on the structure was observed at this concentration of rhodamine. The samples were heated in sealed microscope slides in a thermostat bath for 15 min.

2.4. Rheology

Oscillatory measurements were performed with two stress-imposed rheometer (TA Instruments Rheolyst, ARG2 and AR2000) using a plate-plate geometry (40 mm, gap 1 mm). The temperature was controlled by a Peltier system and the geometry was covered with a mineral oil to avoid water evaporation. Measurements were done at a stress of 0.01 Pa which was within the linear response regime. The frequency was set at 0.1 Hz unless otherwise specified. Temperature ramps were done at a rate of 2 °C/min except close to the critical gel temperature ($T_c \pm 5$ °C) where they were done at a rate of 0.1 °C/min.

3. Results and discussion

3.1. Gelation of casein micelles

3.1.1. Sol-gel state diagram

A series of aqueous solutions of casein micelles was prepared at room temperature (20 °C) at four different concentrations between 25 g L^{-1} and 160 g L^{-1} and at different pH between pH 5.2 and pH 6.7. For pH ≤ 5.2, precipitation was observed at room temperature. The solutions were heated in a thermostat bath that was set at different temperatures during 15 min. After cooling to room temperature the samples were observed while tilting and were considered gels if they did not flow. It was found that gelation occurred above a critical temperature (T_c) that increased with increasing pH and decreasing protein concentration, see Fig. 1. At C = 160 g L^{-1}, T_c varied little between pH 5.4 and pH 5.8, but increased steeply with increasing pH at higher pH from about 30 °C at pH 5.8–90 °C at pH 6.5. We note that similar results were obtained in trials with a commercial casein micelle powder (Promilk 852B, Cremo/Ingrédia, Fribourg, Switzerland). These results show that heat induced gelation of pure casein micelle solutions in water occurs at higher pH than was previously realized [24,25].

Reducing the protein concentration to C = 100 g L^{-1} did not have much influence on T_c, but at C = 55 g L^{-1} the critical temperature was significantly higher. Decreasing the protein concentration