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Dissociation of native casein micelles induced by sodium caseinate

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ABSTRACT

The effect of addition of sodium caseinate (NaCas) to aqueous solutions of casein micelles was studied at pH 6.7. Addition of NaCas leads to reduction of the turbidity and to a decrease of the fraction of proteins that precipitates during ultracentrifugation. The calcium and orthophosphate concentrations in the supernatant were found to increase with increasing NaCas concentration. The results show that addition of NaCas causes release of colloidal calcium phosphate and dissociation of native caseine micelles. The process is controlled by the weight fraction of NaCas in the mixture and dissociation is complete when the fraction exceeds 75%. The efficacy of NaCas as chelating agent for casein micelles is compared with that of polyphosphate reported in the literature. The liberated calcium and orthophosphate are bound to the added NaCas, which leads to aggregation at higher casein concentrations.

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1. Introduction

Casein is the most abundant protein of milk and consists of mainly four different types: α_{s1}, α_{s2}, β and κ in different proportions (Morris, 2002). In milk, casein is associated into an approximately spherical particle with a radius of roughly 100 nm that is called casein micelle (Holt, 1992; Horne, 2006; Schmidt, 1982). Casein micelles contain colloidal calcium phosphate (CCP) in the form of nanoclusters (De Kruif & Holt, 2003; Holt, 1982; McMahon & Brown, 1984) to which the caseins are connected via phosphoserine (P-ser) units (Horne, 2006). CCP maintains the integrity of the casein micelles together with hydrophobic interactions and hydrogen bonds between caseins (Dalgleish, 2011; Farrell, Malin, Brown, & Qi, 2006; Holt, 1992, 1998).

At neutral pH, casein micelles dissociate after removal of the CCP by dialysis (Aoki, Yamada, Kako, & Imamura, 1988; Hansen et al., 1996; Stothart & Cebula, 1982), or addition of calcium-chelating agents like EDTA (Griffin, Lyster, & Price, 1988; Lin, Leong, Dewan, Bloomfield, & Morr, 1972; Marchin, Putaux, Pignon, & Léonil, 2007; Pitkowski, Nicolai, & Durand, 2007), citrate (Kaliappan & Lucey, 2011; de Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2011; Mizuno & Lucey, 2005), or polyphosphate (de Kort et al., 2011; Mizuno et al., 2005; Pitkowski et al., 2007). After removal of the CCP the casein molecules assemble into small particles with a radius of about 10 nm, that are sometimes called submicelles (Hansen et al., 1996; Panouillé, Durand, Nicolai, Larquet, & Boisset, 2005; Panouillé, Nicolai, & Durand, 2004; Stothart et al., 1982). A consequence of the dissociation of casein micelles is that the system scatters less light so that the turbidity decreases (Pitkowski et al., 2007).

Sodium caseinate (NaCas) is obtained by precipitation of casein micelles at pH 4.6 and washing, which removes the calcium phosphate, followed by addition of NaOH to resolubilize the proteins (Mulvihill & Fox, 1989). At neutral pH, NaCas forms particles with a radius of about 10 nm depending on the ionic strength (Alvarez, Risso, Canales, Pires, & Gatti, 2008; Chu, Zhou, Wu, & Farrell, 1995; Farrell et al., 1996; Farrer & Lips, 1999; HadjSadok, Pitkowski, Benyahia, Nicolai, & Moulai-Mostefa, 2008; Lucey, Srinivasan, Singh, & Munro, 2000). NaCas strongly binds calcium ions (Alvarez et al., 2008; Dalgleish & Parker, 1980; Dalgleish, Paterson, & Horne, 1981; Farrell Jr, Kumosinski, Pulaski, & Thompson, 1988; Parker & Dalgleish, 1981; Zittle & Dellamonica, 1958), which above a critical concentration of calcium ions leads to aggregation and decreased solubility (Guo, Campbell, Chen, Lenhoff, & Velev, 2003; Pitkowski, Nicolai, & Durand, 2009; Thomar, Durand, Benyahia, & Nicolai, 2012). NaCas may thus be considered as a calcium-chelating agent.

Here we address the question as to what extent addition of NaCas to aqueous solutions of casein micelles leads to release of CCP from the micelles and dissociation of the micelles. A second issue is how the redistributed calcium and phosphate influence the properties of the caseins in solution. These issues have to our

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knowledge not yet been addressed in the literature. They are not only interesting from a fundamental point of view, but are also important for applications in the dairy industry when NaCas is added to milk for processing (Gaygadzhiev, Massel, Alexander, & Corredig, 2012; Nair & Corredig, 2015).

2. Material and methods

2.1. Materials

Casein micelles in the form of native phosphocaseinate powder (NPCP) was provided by INRA-STLO, Rennes. NPCP was obtained by microfiltration of skimmed milk as reported in (Schuck et al., 1994). The batch used for this study was composed of 83% (w/w) of protein (TNC, Kjeldahl), and contained 2.6% (w/w) of calcium and 1.7% (w/w) of organic and inorganic phosphorus. The sodium caseinate (NaCas) powder used for this study (Lactonat EN, Lactoprot, Kaltenkirchen, Germany) contained 90% (w/w) protein (TNC, Kjeldahl), 1.3% (w/w) of sodium and 0.7% (w/w) of phosphorus.

2.2. Casein composition

The casein composition of the samples was obtained using reverse phase high pressure liquid chromatography (RP-HPLC). Fig. 1 shows the RP-HPLC chromatograms for the NaCas and the NPCP samples used for this study. The positions of the different caseins have been identified by comparing with purified caseins. The fractions of α_{s1}-, α_{s2}-, β-, and κ-casein in NaCas were 34, 3, 44, 9%, respectively. In addition, the sample contained 9% unidentified protein, most likely partially degraded casein. The fractions of α_{s1}-, α_{s2}-, β-, and κ-casein in NPCP were 24, 4, 36, 9%, respectively. This sample contained a relatively large fraction of unidentified c asein (27%).

2.3. Sample preparation

The powders were hydrated with milliQ water containing 3 mM of sodium azide (NaN_3), as bacteriostatic agent. Homogeneous dispersions of NPCP were obtained after keeping the system at 50 °C during 16 h, while homogeneous dispersions of NaCas were obtained after heating at 80 °C during 30 min. The pH was adjusted at 20 °C to 6.7 by addition of aliquots of HCl or NaOH solutions (1 M). The protein concentration was determined by absorption of UV-light with wavelength 280 nm (Varian Cary-50 Bio, Les Ulis, France) using an extinction coefficient of 0.81 L g^{-1} cm^{-1}. NaCas solutions contained a small amount of residual fat globules that were removed by centrifugation (5.10^4 *g*; 2 h). The loss of protein by this procedure was less than 5%. Mixtures were prepared by mixing the two stock solutions with known protein concentrations determined by UV-absorption and diluting with milliQ water to the required amount.

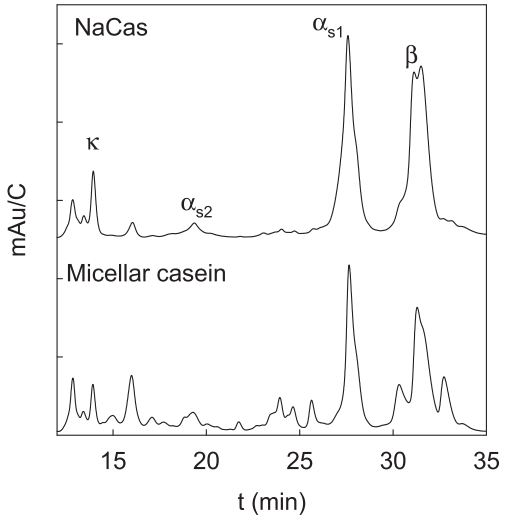

Fig. 1. RP-HPLC chromatograms of micellar casein at 15 g L^{-1} and NaCas at 77 g L^{-1}.

2.4. Determination of the fraction of sedimentable casein

Solutions were centrifugated at 5.10^4 *g* during 2 h at 20 °C using an ultracentrifuge (Beckman Coulter, Allegra 64R, Villepinte, France). We checked different centrifugation times and speeds and found that the conditions used here were largely sufficient to sediment all casein micelles, but none of the NaCas. Subsequently, the protein concentration of the supernatant was determined by UV spectroscopy from which the fraction of sedimentable casein was calculated.

2.5. Determination of the calcium content

The concentration of calcium was determined by flame spectroscopy at 422.7 nm (Varian AA240FS, Les Ulis, France). Before measurement the solutions were diluted in milliQ water to be within the calibration range that was prepared with known concentrations of $CaCl_2$ in milliQ water.

2.6. Determination of the phosphorus content

1 g of sample was dried at 100 °C during 5 h and at 500 °C until dry ashes were obtained. The ashes were suspended in 1 M HCl and diluted with water to 1 g L^{-1}. The dilute sample was dissolved in water containing sodium molybdate and ascorbic acid with final concentrations of 0.125 g L^{-1} sodium molybdate and 0.1 g L^{-1} ascorbic acid. Total phosphorus was determined by UV spectroscopy at 820 nm using a calibration curve that was made with potassium orthophosphate (KH_2PO_4) solutions containing sodium molybdate and ascorbic acid at final concentrations of 0.125 g L^{-1} and 0.1 g L^{-1}.

2.7. RP-HPLC

The different types of caseins were determined by using reverse phase high pressure liquid chromatography (Ultimate 3000, Dionex) with an analytical column C5 Jupiter (250 × 4.6 mm), 30 nm pore size, 5 μm particle size (Phenomenex, Torrance, USA), see (Miranda, Mahé, Leroux, & Martin, 2004; Visser, Slangen, & Rollema, 1991). Following a procedure described by (Miranda et al., 2004), the caseins were diluted at a concentration of at 0.4%(w/w) in 0.1 M bis-Tris buffer at pH 8.0, containing 8 M urea, 1.3% trisodium citrate and 0.3% DTT. After filtration through 0.45 μm pore size filters, 2 μL of the solution was injected. The mobile phase was composed of deionised water and trifluoroacetic acid with a gradient of acetonitrile. The UV absorption was detected at 214 nm.

2.8. Turbidity measurements

The turbidity (τ) was measured by spectrophotometry as a function of the wavelength (400–1100 nm) as reported by (Pitkowski et al., 2007). The spectrophotometer (Varian Cary-50 Bio, Les Ulis, France) was coupled with a thermostated bath. The temperature was probed in the samples.

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