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Hydration of casein micelles and caseinates: Implications for casein micelle structure

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ABSTRACT

By studying the hydration of casein micelles using a variety of techniques, a distinction could be made between water that appeared bound by the protein (~0.5 g g^{-1} protein), water associated with the κ-casein brush (~1.0 g g^{-1} protein) and water entrapped in the casein micelles (~1.8 g g^{-1} protein), yielding a total micellar hydration of ~3.3 g g^{-1} protein, in line with casein micelle voluminosity derived from intrinsic viscosity measurements. For caseinate particles, however, the main contributor to intrinsic viscosity was not protein hydration but the non-spherical particle shape. These non-spherical particles in caseinate are likely to be naturally present as primary casein particles (PCP) in casein micelles. PCP could be used to build casein micelles by controlled introduction of micellar salts. Based on the findings of this study, casein micelles could be described as a porous network of non-spherical PCP linked by calcium phosphate nanoclusters.

1. Introduction

Understanding the behaviour of water is of great industrial importance in protein-rich dairy products, such as cheese, concentrated milk products and in the manufacture of milk protein ingredients. Given the importance of efficient water removal during the evaporation and drying of ingredients, but also the prevention of moisture separation from products such as cheese and yoghurt during storage, understanding of the factors governing casein micelle hydration is important. A further aspect where such factors are important is the use of caseinate as an ingredient. In particular, sodium and potassium caseinate are often considered protein ingredients of choice for conferring viscosity to liquid products. A sodium caseinate suspension can be considered to be a particle suspension, with particles having molar masses of several hundred kDa (Farrell, Brown, & Malin, 2013; HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008; Lucey, Srinivasan, Singh, & Munro, 2000), rather than a solution of monomeric casein. As also for casein micelles, the contribution of water binding or other factors to caseinate viscosity are still largely unexplored.

The distribution of water throughout the micelle has also not been elucidated. Typical voluminosity values reported for casein micelles in milk are in the order of 3.5–4.5 mL g^{-1} (Kinsella, Fox, & Rockland, 1986; Walstra, Wouters, & Geurts, 2005) and it is agreed that the κ-casein brush on the surface is more strongly hydrated than the core of the micelle (Anema & Creamer, 1993; Dalgleish, 1998; Van Hooydonk, Boerrigter, & Hagedoorn, 1986). However, distribution of water in the core of the micelle remains a largely unexplored area. Although, traditionally, a rather homogeneous distribution of water and protein in the micelles has been assumed, more recent studies highlight an inhomogeneous water and protein distribution, involving protein- and water-rich domains (Bouchoux, Gésan-Guiziou, Pérez, & Cabane, 2010; Dalgleish, 2011; De Kruif, Huppertz, Urban, & Petukhov, 2012). In addition, the actual nature of the hydration of the casein micelle is another topic of consideration. Although the term 'water-binding' is widely used, the subjectivity of this term can also be an area of confusion as it relates to specific conditions of study applied to determine 'water-binding'. Since virtually all water can be removed when dried casein micelle ingredients are prepared, the term 'bound water' is highly conditional. Some water associated with casein micelles appears to be non-freezable and unavailable as a solvent or for reactions (Walstra et al., 2005), but a larger proportion appears readily available as a solvent and for reactions; this latter fraction

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cannot be removed by traditional fractionation techniques such as centrifugation or osmotic dehydration, and may be considered entrapped in the casein micelles. The studies described in this paper were carried out to provide new insights into casein hydration. It is important, however, that data on casein hydration are not considered as stand-alone data, but should be placed in the context of particle structure, i.e., of casein micelles or caseinate particles. Particularly for casein micelles, which in addition to caseins also contain calcium phosphate nanoclusters, the distribution of matter is crucial to elucidate substructures.

The casein micelle has been the subject of study from the aforementioned perspectives for decades, and many aspects of casein micelle structure have been elucidated. From a colloidal perspective, casein micelles can be considered as sterically stabilised association colloids. Electron microscopy suggests a near-spherical shape (Dalgleish, Spagnuolo, & Goff, 2004; McMahon & McManus, 1998), and light scattering suggests a hydrodynamic radius of 60–120 nm (De Kruif & Huppertz, 2012; Holt, 1975; Horne & Dalgleish, 1985). Polydispersity of the casein micelle distribution is small for milk from individual cows, but is considerably larger for bulk milk as a result of mixing of milk different casein micelle size distributions (De Kruif & Huppertz, 2012). Steric stabilisation of casein micelles is provided by a brush of (predominantly) disulphide-linked κ-casein molecules protruding from the surface of the micelle, acting as a polyelectrolyte brush (Dalgleish, 1998; De Kruif, 1999; Holt & Horne, 1996). The presence of calcium phosphate in the micelles has long been recognised as an essential contributor to casein micelle substructure (Holt, 1992; Schmidt, 1982; Walstra, 1990). Small-angle X-ray and neutron scattering studies have shown this micellar calcium phosphate (MCP) to be present predominantly in the form of calcium phosphate nanoclusters with a radius of 2.0–2.5 nm (De Kruif et al., 2012; Holt, De Kruif, Tuinier, & Timmins, 2003). Caseins with a centre of phosphorylation (i.e., at least three phosphorylated serine residues in close proximity) can adsorb onto the surface of the calcium phosphate nanoclusters and thereby limit growth (De Kruif & Holt, 2003). In most descriptive models for casein micelles, casein interactions result in further growth of the matrix to a casein micelle (Dalgleish, 2011; De Kruif & Holt, 2003; De Kruif et al., 2012; Holt, 1992; Horne, 1998), which typically contains hundreds of calcium phosphate nanoclusters spaced at ~18 nm (De Kruif et al., 2012; Holt et al., 2003). However, further elucidation of intra-micellar casein interactions is warranted, as is the distribution of caseins in the micelles and the distribution of water in the casein micelles.

In the studies described in this paper, hydration of caseins in casein micelles and caseinates was determined by different methods, the combination of which yielded considerable further insights into water distribution in the particles, and the relationship to solution properties, such as viscosity. The outcomes could also be placed in a self-consistent model for the substructure of a casein micelle and particles present in suspensions of sodium caseinate.

2. Materials and methods

2.1. Sample preparation

Pasteurised (72 °C for 15 s) skim milk was obtained from the NIZO pilot plant, whereas liquid micellar casein isolate (MCI) prior to evaporation and drying, containing ~18% dry matter and ~14% protein, of which ~90% was casein, was supplied by Friesland-Campina (Lochem, The Netherlands). Sodium caseinate (Na-caseinate) and calcium caseinate (Ca-caseinate) were supplied by DMV International (Veghel, The Netherlands). α-Lactalbumin and β-lactoglobulin (both >95% purity) were obtained from Davisco (Le Sueur, MN, USA). Milk permeate was prepared by filtration of skim milk over a 5 kDa membrane at 50 °C.

2.2. Determination of non-freezable water

Non-freezable water of milk protein suspension was determined by osmotic concentration followed by differential scanning calorimetry (DSC) analysis. For this purpose, suspensions of MCI at 14% (w/w) protein or Na-caseinate, Ca-caseinate, α-lactalbumin or β-lactoglobulin at 10% (w/w) protein content were used. Samples were dialysed against 2 × 25 volumes of milk permeate (for MCI), 10% Na-caseinate (for Na-caseinate), 10% Ca-caseinate (for Ca-caseinate) or 10 mM NaCl (for α-lactalbumin and β-lactoglobulin) containing 0, 10, 20, 30 or 40% polyethylene glycol (PEG, molecular mass 35 kDa; Sigma–Aldrich, St. Louis, MO, USA) for 48 h. Following dialysis, moisture content of the concentrated samples was determined by freeze-drying. In addition, concentrated samples were also analysed by DSC using a Q1000 DSC (TA Instruments, New Castle, DE, USA). For this purpose, samples were cooled from 20 to −80 °C at a rate of 1 °C min^{-1}, subsequently held at −80 °C for 1 h prior to increasing temperature at a rate of 1 °C min^{-1} to 20 °C. The melting enthalpy was calculated for samples, and linear plots of melting enthalpy as a function of dry matter content were extrapolated to the dry matter where melting enthalpy was zero. Residual water at this dry matter content was considered non-freezable and expressed as g water g^{-1} dry matter.

2.3. Determination of sample hydration by ultracentrifugation

For the determination of hydration by ultracentrifugation, pasteurised skim milk and suspensions of Na-caseinate (2.8%, w/w, dry matter) in 100 mM NaCl adjusted to pH 5.5–7.5 were centrifuged at 20,000, 40,000, 100,000, 200,000 or 400,000×*g* for 60 min or 400,000×*g* for 72 h at 5 or 20 °C. Subsequently, the serum phase and pellet were separated. The moisture content of the pellet was determined by freeze-drying and expressed as g water g^{-1} dry matter. Whole samples and supernatants were also analysed for protein composition by reverse phase-high performance liquid chromatography (RP-HPLC) as described by Hinz, Huppertz, and Kelly (2012).

2.4. Determination of dynamic and intrinsic viscosity

Kinematic viscosity (η_{kin}) and density (ρ) of samples were determined at 10–50 °C using a LOVIS 2000 M/ME rolling-ball viscometer (Anton-Paar, Graz, Austria) coupled to a DMA 5000 M density meter (Anton Paar) and an Xsample 122 autosampler (Anton Paar). Dynamic viscosity (η) was calculated as:

$$\eta = \eta_{kin} \times \rho \tag{1}$$

Relative viscosity (η_{rel}) of samples was calculated as the ratio of the dynamic viscosity of the sample and that of its serum phase:

$$\eta_{rel} = \frac{\eta_{sample}}{\eta_{serum}} \tag{2}$$

Intrinsic viscosity was determined from dynamic viscosity measurements, as outlined above, for dilution series (0.0–1.0% protein) of caseinates in 10, 25 or 100 mM NaCl or MCI in milk permeate. Intrinsic viscosity ([η]) is defined as the intercept of a plot of reduced viscosity (η_{red}) as a function of protein concentration (c),

$$\text{where :} \quad \eta_{red} = \frac{\eta_{sp}}{C} \tag{3}$$

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