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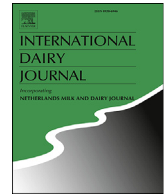

Characterisation of milk protein adsorption onto hydroxyapatite

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ABSTRACT

Adsorption behaviour of α_S-casein, β-casein, κ-casein, β-lactoglobulin and α-lactalbumin on hydroxyapatite (HA) was characterised by determination of adsorbed protein levels and surface charge of HA. Individually, the proteins were able to bind onto HA causing a decrease in the zeta-potential magnitude of the HA particles. The maximum amount of protein that could bind onto HA and the affinity of the proteins for HA were quantified using a Langmuir model, and were different between the different proteins. α_S-Casein and β-casein could bind to higher levels onto HA and had a higher affinity for HA, probably because of the presence of clusters of phosphoserine residues in their primary structures. β-Casein was also able to displace adsorbed β-lactoglobulin from the HA surface when added in a suspension of β-lactoglobulin-covered particles, probably because the affinity of the casein phosphoserine residues for HA was stronger than that of the carboxyl groups of the whey protein.

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1. Introduction

Hydroxyapatite (HA) particles are widely used in calcium-fortified UHT treated milks, where they are often described as chemically unreactive. This view is taken because HA is virtually insoluble at milk pH, so it can be added to milk and it does not cause stability issues induced by the interaction with milk proteins, unlike soluble calcium salts (Omoarukhe, On-Nom, Grandison, & Lewis, 2010). The disadvantage of insoluble salts such as HA is that they may precipitate during storage, leading to phase separation and sedimentation of a layer of calcium salt at the bottom of containers. However, it is well-known that HA interacts strongly with proteins in a wide range of biological applications, such as chromatography, nano-ceramics and bone implants or tooth enamel (Juriaanse, Booij, Arends, & Bosch, 1981; Kawasaki, 1991).

Despite its industrial relevance, there is a lack of literature on the interactions between HA and milk proteins. Only a few studies have reported the adsorption of milk proteins onto HA, and this is in relation to bone or dentistry topics. For example, Van Kemenade and de Bruyn (1989) showed that caseins were able to inhibit the growth of HA by binding to calcium, in the order κ-casein < β-casein < α_{S1}-casein. Devold et al. (2006) studied the in-vitro adsorption of milk proteins on tooth enamel and showed that, at neutral pH, caseins were preferentially bound to tooth enamel, forming protein pellicles. Reynolds and Wong (1983) showed that κ-casein, α_{S1}-casein, β-casein, β-lactoglobulin and α-lactalbumin all bound to HA and rendered the zeta-potential of HA more negative. This leads to the hypothesis that interactions may occur between milk proteins and HA particles of micrometre sizes (median particle size: 4.5 μm) when the HA particles are suspended in milk, resulting in the adsorption of the milk proteins onto the surface of the HA particles, and a change in the zeta-potential and colloidal properties of the particles.

The adsorption of milk proteins onto the surface of HA is likely to be governed mainly by specific electrostatic interactions, between the carboxyl groups of the proteins and the calcium ions in the HA crystal lattice (C-sites), but also between the phosphoserine residues of casein molecules and the HA calcium ions (Bernardi & Kawasaki, 1968; Gorbunoff & Timasheff, 1984a). Caseins are flexible linear proteins, whereas whey proteins are globular proteins. Their differences in structure and amino-acid sequences, their ability to self-associate or form oligomers, as well as the phosphorylated nature of the caseins are likely to cause different adsorption profiles for the two types of proteins (Gorbunoff & Timasheff, 1984b).

The work carried out with sodium caseinate, whey protein isolate (WPI) and skim milk in our previous reports (Tercinier, Ye, Anema, Singh, & Singh, 2013, 2014a, 2014b) demonstrated that

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the different main proteins contained in sodium caseinate (α_S-, β- and κ-casein) and WPI (β-lactoglobulin and α-lactalbumin) adsorbed to a different extent onto HA. In water, caseins in sodium caseinate adsorbed about two times more than the whey proteins in WPI. α_S-Casein (α_{S1}- + α_{S2}-) and β-casein were preferentially adsorbed compared with κ-casein but the relative proportions of adsorbed α_S-casein and β-casein seemed to vary, depending on the initial protein concentration of sodium caseinate, and on the ionic strength of the initial solution. In WPI, β-lactoglobulin was preferentially adsorbed compared with α-lactalbumin.

However, the different caseins in sodium caseinate and whey proteins in WPI are present as mixed proteins in the same solution and can interact with each other (HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008). This might affect the adsorption of the different proteins: the amount and type of proteins that can bind on to HA are likely to be different when using individual proteins from when using sodium caseinate or WPI (Dickinson, 2011). Therefore, to fully understand the mechanisms of adsorption of milk proteins onto HA, it is important to study the binding of purified isolated milk proteins onto HA, both individually and in mixtures containing equal amounts of each protein for easier comparison of the interaction levels of each protein with HA.

α_S-Casein, β-casein, κ-casein, β-lactoglobulin and α-lactalbumin have different structural and physico-chemical properties, they are likely to have different binding affinities for HA, or/and bind to different maximum amounts on HA (Nakanishi, Sakiyama, & Imamura, 2001; Wahlgren & Arnebrant, 1991). For example, the net charge of the proteins, the type of negatively charged residues and the distribution of the charged residues within the protein molecules, may all play an important role in the binding of the different milk proteins onto HA (Wang, Zhou, Hong, & Zhang, 2012). Studying the adsorption behaviour of the individual milk proteins onto HA in model protein solutions, and understanding the competitive mechanisms involved between the different milk proteins will provide further understanding of the interactions between milk proteins and HA.

The objective of this study was, therefore, to characterise the adsorption of the different types of caseins and whey proteins onto HA particles, individually and to relate the adsorption results to the physico-chemical characteristics of the proteins. In addition, this study also looked at the competitive adsorption between caseins and whey protein, by looking at competitive adsorption between β-casein and β-lactoglobulin. The ability of caseins to displace the adsorbed β-lactoglobulin from the HA surface or of β-lactoglobulin to displace the adsorbed caseins from the HA surface was also explored by adsorbing first one type of protein (casein or whey protein) onto HA, and then adding the other type of protein.

2. Materials and methods

2.1. Materials

Isolated proteins: Freeze-dried powders of α_S-casein (comprising a mix of α_{S1}- and α_{S2}-caseins), β-casein, κ-casein, β-lactoglobulin, α-lactalbumin, and lysosyme were purchased from Sigma–Aldrich (St. Louis, MO, USA). Food-grade HA powder was purchased from Budenheim (TCP 53–83, Budenheim, Germany. Note that even though the ingredient is called tricalcium phosphate (TCP), it is chemically made of hydroxyapatite $Ca_5(PO_4)_3(OH)$, as indicated by the specification sheet of the material). The particle size distribution was calculated based on a refractive index of 1.63 and an absorption factor of 0.001 and the median particle size $d_{(0.5)}$ was found to be 4.5 μm. The reported specific surface area of the powder, determined by the BET (Brunauer–Emmett–Teller) method, was 65 $m^2\ g^{-1}$. The particles are porous. All other chemicals used were of analytical grade and were obtained from Sigma–Aldrich unless specified otherwise.

2.2. HEPES buffers

Two 50 mM HEPES buffers (pH 7, two different ionic strengths) were prepared. A low ionic strength buffer (~7 mM) was prepared by mixing 11.92 g HEPES powder and 7.5 mL 1 M NaOH in ~900 mL of water. A 100 mM ionic strength buffer was prepared by mixing 11.92 g HEPES powder, 7.5 mL 1 M NaOH and 92.5 mL 1 M NaCl in ~800 mL of water. The pH was adjusted to pH 7 using 1 M NaOH and the solutions were made up to 1 L.

2.3. Adsorption of individual milk proteins

The adsorption of individual α_S-casein (α_{S1}-+ α_{S2}-), β-casein, κ-casein, β-lactoglobulin or α-lactalbumin on to HA was studied by adding 5% (w/w) HA particles to solutions containing different concentrations (0.1%–5%, w/w) of the different isolated protein. The protein powders were reconstituted in 50 mM HEPES buffers (pH 7, 7 mM or 100 mM ionic strength). The protein concentrations of the initial solutions were calculated based on the purity of the protein, determined by UV spectroscopy at 280 nm, using the following extinction coefficients: 0.46 $cm^2\ g^{-1}$ for β-casein, 1.05 $cm\ g^{-1}$ for α_s-casein, 1.02 $cm^2\ g^{-1}$ for κ-casein, 0.97 $cm^2\ g^{-1}$ for β-lactoglobulin and 2.01 $cm^2\ g^{-1}$ for α-lactalbumin (Swaisgood, 2003). The protein concentration of the supernatants obtained after centrifugation (3000 × g for 20 min at 20 °C) of the HA particles was measured by UV spectroscopy at 280 nm and the amount of adsorbed protein was determined by difference between the protein concentration of the initial solution and that of the adsorption supernatants.

The Langmuir model was fitted to the adsorption data, as described in Tercinier et al. (2013). The HA pellets, obtained after protein adsorption and centrifugation of the HA particles, were resuspended in the same HEPES buffer used for the adsorption experiment, and characterised for zeta-potential. All experiments were carried out at room temperature (~20 °C) and the determinations were carried out after 2 h after mixing the protein solutions and HA particles.

The Langmuir model is given by Eq. (1), as follows:

$$\frac{m_{abs}}{S} = q_m \times \frac{K[P]}{K[P]+1} \quad (1)$$

where m_{abs}/S is the amount of protein bound to the surface expressed in mg m^{-2}, [P] is the concentration of protein at equilibrium (g 100 g^{-1}), q_m is the maximum monolayer surface coverage and K (g 100 g^{-1}) is the Langmuir equilibrium constant.

2.4. Calculation of surface protein concentration

The amount of adsorbed protein (mg m^{-2}) was calculated as follows:

$$Ţ\ (\text{mg m}^{-2}) = [M_i \times (C_i/100 - C_{sup}/100)] \times 1000/(M_{HA} \times SA_{HA})$$

where M_i is the mass of the initial protein solution (g), C_i is the protein concentration of the initial protein solution (g $100^{-1}\ g^{-1}$), C_{sup} is the protein concentration of the supernatant (g $100^{-1}\ g^{-1}$) determined by one of the four methods mentioned above, M_{HA} is the initial mass of HA powder (g) and SA_{HA} is the specific surface area of the HA powder (65 $m^2\ g^{-1}$).

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