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Interactions between whey proteins and kaolinite surfaces

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Abstract

The nature of the interactions between whey proteins and kaolinite surfaces was investigated by adsorption–desorption experiments at room temperature, performed at the isoelectric point (IEP) of the proteins and at pH 7. It was found that kaolinite is a strong adsorbent for proteins, reaching the maximum adsorption capacity at the IEP of each protein. At pH 7.0, the retention capacity decreased considerably. The adsorption isotherms showed typical Langmuir characteristics. X-ray diffraction data for the protein–kaolinite complexes showed that protein molecules were not intercalated in the mineral structure, but immobilized at the external surfaces and the edges of the kaolinite. Fourier transform IR results indicate the absence of hydrogen bonding between kaolinite surfaces and the polypeptide chain. The adsorption patterns appear to be related to electrostatic interactions, although steric effects should be also considered.

Keywords: Minerals; Protein adsorption; Protein bonding; Texture

1. Introduction

Clays are known to be strong adsorbents of organic molecules, including proteins. Different research groups have studied the adsorption and binding of amino acids and proteins by these sedimentary minerals [1–11]. The adsorption of a protein to a surface is a complex process in which the structural stability of the protein, the surface properties of the sorbent, the ionic strength and the pH of the solution have an influence on the affinity of the protein for a given surface [12]. It was reported [13,14] that the proteins adsorbed on hydrophilic surfaces generally maintained their native structure to a higher degree than those adsorbed on hydrophobic surfaces. However, it seems that the proteins' native structure is reduced when the electrostatic interactions are increased [12,15,16].

Kaolinite, $Al_2Si_2O_5(OH)_4$, is a layered aluminosilicate. Each layer consists of one tetrahedral siloxane surface with very inert –Si–O–Si– links, and an octahedral gibbsite ($Al(OH)_3$) sheet [17]. The layers are bound together in the *c*-axis direction by hydrogen bonding between hydroxyl groups on the gibbsite basal plane and the oxygens of the silicon tetrahedral sheet. Kaolinite has different surface structures between base planes (001) and edge planes (110) and (010), which means that this mineral has a heterogeneous surface charge [18]. It is believed that the charge on the edges is due to the protonation/deprotonation of hydroxyl groups and depends on the pH of the solution, whereas kaolinite basal surfaces have a constant structural charge due to the isomorphous substitution of Si^{4+} by Al^{3+}. Recently, Johnson et al. [19] reported that the charge on the basal surface also has possible pH dependence. Different authors [20,21] have investigated the surface electric properties of kaolinite and found that the point of zero charge is in the neighbourhood of pH 7.3.

The milk industry produces a great amount of whey, which contains ~5.4 g of proteins per liter, mainly β-lactoglobulin (~50%) and α-lactalbumin (~22%). Whey-containing effluents produce bacteria, which contaminate the water, and solutions have to be found to prevent this

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important pollution problem. Furthermore, whey can be recovered for use in various industries, and it is likely that obtaining high quality protein rich products from food industry by-products will increase in the future.

The aim of this work was to investigate the extent and nature of binding of the whey proteins bovine serum albumin (BSA), β-lactoglobulin (B-LG) and α-lactalbumin (A-LA) by kaolinite. This clay mineral was chosen for this study, because the adsorption of proteins is expected to occur only in the external area of the kaolinite particles. With other clay minerals, e.g., bentonite or montmorillonite, adsorption on the particle internal surface is possible, thereby increasing the degree of complexity of the problem. It will be shown that the relative influence of electrostatic and hydrophobic interactions on adsorption plays an important role in kaolinite-to-protein binding.

2. Experimental

2.1. Materials

The kaolinite clay mineral used in the experiments was provided by Arcichamotas Mines in the North of Spain. The clay was treated with H_2O_2 (Panreac) to eliminate the organic phases present in the mineral, and then was washed thoroughly with distilled water using a liquid/solid ratio (L/S) of 50 ml g^{-1} kaolinite. The suspended solid was filtered, dried at 120 °C to eliminate physisorbed water, ground and sieved. All the adsorption experiments were performed with kaolinite particles <100 μm in size. The whey proteins used were all purchased from Sigma: A-LA, type III, calcium depleted, 85% purity; B-LG, ∼90% PAGE purity; and BSA, fraction V, 99% purity.

2.2. Techniques of characterization

The protein concentrations in the initial solutions and the final supernatant obtained after the adsorption experiments were measured with a UV spectrophotometer (PU 8625 UV/VIS Philips) at 280 nm. A previous filtration through 0.45 μm polyvinylidene difluoride membrane syringe filters (Acrodisc) is required to retain the fine particles.

The kaolinite composition was determined by elemental analysis with a Perkin–Elmer 2380 elemental analyzer. X-ray diffraction experiments were performed using a Philips X'Pert MPD Pro instrument, operating at 45 kV, 40 mA, using Kα copper radiation. The specific surface area of the kaolinite was obtained by N_2 adsorption–desorption at 77 K using a Micromeritics ASAP 2000 instrument. Macroporosity and total pore volume were analyzed by mercury intrusion porosimetry with a Micromeritics Poresizer 9320 instrument. The microscopic morphology of the kaolinite was studied using a Jeol 6100 scanning electron microscope, operating at 0.3–30 kV. The Fourier transform-IR (FT-IR) spectra of kaolinite, pure proteins and protein–kaolinite complexes were obtained at room temperature with a Perkin–Elmer PARAGON 1000, by the KBr pellet technique.

2.3. Adsorption experiments

The protein adsorption experiments were carried out in batch mode in 50 ml Pyrex glasses at room temperature. Suspension of kaolinite particles was achieved by means of magnetic agitation (400 rpm) for 6 h. Blank experiments (without protein analysis) were run in parallel under the same experimental conditions. Protein concentrations of 1 g l^{-1} and liquid/solid ratios from 5 to 35 ml g^{-1} were used in order to determine the maximum retention capacity. Using a minimum liquid/solid ratio of 25 ml g^{-1}, which was established as the most convenient value, the equilibrium isotherms for each protein were obtained, varying initial protein concentrations. The experiments were performed at two different pH values, close to the IEP of the proteins and at pH 7.0. The protein solutions were buffered using acetate buffer for pH 5.0 and 5.4, and Trizma®–HCl for pH 7.0.

The adsorption kinetics were determined using buffered solutions with protein concentrations of 2 g l^{-1} in the case of BSA and B-LG, and 0.7 g l^{-1} for A-LA, and a L/S ratio of 25 ml g^{-1}. All these experiments were performed at room temperature.

2.4. Desorption

After the adsorption experiments, the solutions were centrifuged for 10 min at 2000 rpm in a Kubota 6700 centrifuge, to separate the protein–kaolinite complex. The proteins were not desorbed by washing with distilled water. Therefore, other eluents were tested, such as 5% v/v polyethyleneglycol 400 and 6% w/v PEG 3350 (Fluka), 1 M sodium chloride (Panreac), 30% v/v ethanol (Panreac), and mixtures of ethanol with the anionic tensoactive, sodium dodecyl sulphate (SDS) (Panreac), with different proportions from 10 to 30% v/v ethanol and 0.5–2% w/v SDS. Using the L/S ratio of 25 ml g^{-1}, samples of supernatant were taken after a 2 h agitation period (at 400 rpm). After protein elution, the kaolinite was reused in new adsorption steps.

3. Results and discussion

3.1. Chemical and physical characterization

The kaolinite compositions determined by elemental analysis, with a Perkin–Elmer elemental analyzer are given in Table 1.

The diffractogram of the adsorbent (Fig. 1a) shows the diffraction lines characteristic of a high-purity kaolinite. Diffraction lines corresponding to other mineral phases are not observed. The X-ray diffractograms of kaolinite samples containing retained proteins (Fig. 1b) do not show a significant shift to higher values of the d-spacings for the