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Research paper

Structural, textural and protein adsorption properties of kaolinite and surface modified kaolinite adsorbents

R. Duarte-Silva [a], M.A. Villa-García [b,*], M. Rendueles [a], M. Díaz [a]

[a] Department of Chemical Engineering and Environmental Technology, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain
[b] Department of Organic and Inorganic Chemistry, University of Oviedo, Julian Clavería 8, 33006 Oviedo, Spain

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ABSTRACT

The structural, textural and protein adsorption properties of kaolinite from clay sedimentary deposits, metakaolinite obtained by thermal dehydroxylation of kaolinite, and the organic derivative prepared by reacting kaolinite with the silane coupling agent tert-butyldimethylchlorosilane, were studied. The retention capacities for the proteins α-lactalbumin (A-LA), bovine serum albumin (BSA) and β-lactoglobulin (B-LG) and the nature of the interactions responsible for protein binding were studied by adsorption experiments, performed at room temperature and pH 5.0. The protein adsorption capacity and the selectivity show a clear dependence on the chemical nature of the adsorbents surface and on the textural properties. Kaolinite behaves as a strong adsorbent for A-LA and BSA, and exhibits a very high affinity for B-LG. Metakaolinite shows good retention capacity for A-LA and B-LG, but does not retain significant amounts of BSA. The adsorption capacity of the organo–kaolinite hybrid considerably increases for BSA and A-LA. FTIR results indicate the absence of hydrogen bonding between the adsorbents surface and the polypeptides. The interactions responsible for protein binding are closely related to the hydrophilic or hydrophobic character of the adsorbent surface and the amino acid composition of the proteins, steric effects also should be considered for the adsorption patterns.

1. Introduction

The interactions of peptides and proteins with clay surfaces have been studied extensively in the past (Bujdák and Rode, 1997; Bujdák et al., 1996; Causserand et al., 1997; Ding and Henrichs, 2002; Fusi et al., 1989; Gupta et al., 1983; Quiquampoix et al., 1993, 1995; Rigou et al., 2006; Violante et al., 1995; Yu et al., 2000). Since clays are very abundant, their use as inorganic hosts is important not only from an economic perspective, but also for their unique physical and chemical properties, and the ease with which these materials can be modified and adjusted to new uses.

Kaolinite has a wide variety of applications in industry (Bergaya and Lagaly, 2001; Bergaya et al., 2000; Braggs et al., 2006). This mineral has two different basal cleavage faces. One face consists of a tetrahedral siloxane surface with inert –Si–O–Si– links; the other basal surface consists of an octahedral gibbsite ($Al(OH)_3$) sheet (Frost et al., 2002a, 2002b). Kaolinite has different surface structures between base planes (001) and edge planes (110) and (010). The charge on the edges is due to the protonation/deprotonation of hydroxyl groups and depends on the pH of the solution. The hydroxyl groups located at the edge planes are considered the major reactive sites of kaolinite surfaces (Rausell-Colom and Serratosa, 1987).

Kaolinite surface is hydrophilic but it can be rendered hydrophobic by reaction with organo–functional molecules (Dai and Huang, 1999). Organic derivatives of clays are generally obtained by using silane coupling agents (Dai and Huang, 1999; Ishida and Miller, 1985; Waddell et al., 1981). After surface modification, the organic groups can be attached to the clay by chemical bonding, adsorption and coating. Kaolinite surface can also be modified by thermal treatment. At temperatures higher than 450 °C dehydroxylation occurs to form metakaolinite ($Al_2O_3 \cdot 2SiO_2$), and at 650 °C dehydroxylation is by ca 90% complete (Grim, 1968).

The dairy industry generates many by-products with high protein contents. These residues can cause severe environmental contamination when they are not properly disposed, and it is necessary to find solutions to prevent this pollution problem. Furthermore, the recovered proteins can be used to obtain high quality protein rich food products. Protein recovery by adsorption on various types of supports is a commonly used technique; however, the use of clay minerals as adsorbents of protein molecules has received considerably less attention.

The adsorption/desorption of proteins on clay surfaces is a complex process controlled by different factors, such as the surface properties of the adsorbent, the structural stability of the proteins, the ionic strength and the pH of the adsorption/desorption experiments (Haynes and Norde, 1994). In a recent work we found that kaolinite showed a high adsorption capacity at the isoelectric point (IEP) of each protein (Barral et al., 2008). Moreover, there was a clear correlation between the adsorption patterns and the presence of hydrophobic or hydrophilic

* Corresponding author. Tel.: +34 985102976; fax: +34 985103446.
E-mail address: mavg@uniovi.es (M.A. Villa-García).

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groups in the polypeptide surface. In order to achieve a deeper insight on the nature of the interactions responsible for protein adsorption on clay surfaces and explain the retention patterns, adsorption experiments were run using as adsorbents kaolinite, metakaolinite obtained by thermal dehydroxylation of kaolinite, and the organic derivative obtained by reacting kaolinite with tert-butyldimethylchlorosilane. Three whey proteins were chosen for this study: BSA, B-LG and A-LA. Physico-chemical characterization of the adsorbents was carried out using different techniques: elemental chemical analysis, FTIR spectroscopy, X-ray diffraction (XRD), thermal gravimetric (TG) and differential thermal analysis (DTA), N_2 adsorption–desorption at 77 K, mercury intrusion porosimetry and scanning electronic microscopy (SEM).

Evidence of protein intercalation in kaolinite has not been reported yet. There was no significant expansion of the clay after protein binding, as shown by X-ray diffraction analysis. Kaolinite, a non swelling clay does not expand upon wetting, which prevents the exposure of internal surface, and adsorption of proteins is restricted to the external crystal surfaces and the edges of the clay (Fiorito et al., 2008; Venkateswerlu and Stotzky, 1992).

As will be shown, the protein retention patterns are controlled by the textural properties and the chemical nature of the adsorbent surface. Steric effects should be also considered, since experimental data show a correlation between the extent of adsorption and protein sizes.

2. Materials and methods

2.1. Materials

The kaolinite mineral used in the experiments (sample K) was provided by Arcichamotas Mines in the North of Spain (Asturias). The clay was treated with H_2O_2 (Panreac) to eliminate organic matter present in the mineral, and then was washed thoroughly with distilled water. The solid was filtered, dried at 120 °C, ground and sieved. All the experiments were performed with kaolinite particles 100–250 μm in size. Tert-butyldimethylchlorosilane (reagent grade), imidazole (ACS reagent) and dichloromethane (ACS reagent) were purchased from Sigma. The whey proteins used were also supplied by Sigma: A-LA, type III, calcium depleted, 85% purity; B-LG, aprox. 90% PAGE purity; and BSA, fraction V, 99% purity. The sodium acetate buffer was purchased from Panreac. Metakaolinite (sample MK) was obtained from calcination of kaolinite. The clay mineral was heated in a ceramic crucible with a heating rate of 10 °C/min up to 650 °C and then was kept at that temperature for 12 h. Between 450 and 650 °C kaolinite undergoes a strong endothermic dehydration reaction, resulting in the conversion to metakaolinite.

The organo–kaolinite composite (sample KR) was synthesized by reaction between tert-butyldimethylchlorosilane (TBSCl) and kaolinite in dichloromethane, in the presence of imidazole as a base catalyst. In a typical synthesis, 5 g of kaolinite was suspended in 50 mL of CH_2Cl_2 and 14 g of TBSCl and 6.5 g of imidazole were added, under vigorous stirring. The reaction mixture was sonicated for 48 h, the supernatant liquid was removed by decantation, the solid was thoroughly washed with dichloromethane to remove excess of organic species, and dried under vacuum to remove residual dichloromethane.

The imidazolium chloride–kaolinite complex (sample KI) was obtained by bubbling hydrogen chloride, obtained by reaction of NaCl with H_2SO_4, through a solution containing 6.5 g of imidazole in 50 mL of CH_2Cl_2. Five g of kaolinite was added to the imidazolium chloride solution, and the reaction mixture was treated under the conditions described above for the preparation of the organo–clay solid.

2.2. Methods

The chemical composition of the kaolinite was determined by X-ray fluorescence spectroscopy (XRF) using a Philips PW 2404 XRF spectrometer. Powder X-ray diffraction (XRD) patterns were obtained with a Philips X'Pert MPD Pro instrument-ray diffractometer, operating at 45 kV, 40 mA, using Cu Kα radiation. FTIR spectra of all the materials were obtained at room temperature with a Perkin-Elmer PARAGON 1000 spectrometer, by the KBr pellet technique. Thermal analysis, TG and DTA, were carried out in Metller TA-4000 TG-50 type thermobalance under dynamic heating conditions (10 °C/min heating rate) in a flowing nitrogen atmosphere of 99.999% purity, 50 mL/min. The samples were heated in alumina crucibles up to 1000 °C. Nitrogen adsorption–desorption isotherms at 77 °K were obtained with a Micromeritics ASAP 2020 instrument, using static adsorption procedures. Macroporosity and total pore volume were measured by mercury intrusion porosimetry with a Micromeritics Autopore IV instrument. The microscopic morphology of the samples was studied using a Jeol 6100 scanning electron microscope operating at 0.3–30 kV. Samples were prepared by sonicating the powdered sample in ethanol and then evaporating two droplets on carbonated copper grids.

Protein concentrations in the initial solutions and the final supernatant obtained after the adsorption, were measured with the UV/VIS spectrophotometer (Heλios UV/VIS Thermo Electron Corporation) at 750 nm, according to the Folin–Ciocalteu colorimetric method (Lowry et al., 1951). A previous filtration through 0.45 μm polyvinylidene difluoride membrane syringe filters (Acrodisc) is required to retain the fine particles.

2.3. Adsorption experiments

The protein adsorption experiments were carried out in batch mode. In all the experiments 0.5 g of the different samples was transferred to a 250 mL Erlenmeyer flask and suspended in 100 mL of protein solution buffered using a sodium acetate buffer (pH = 5.0). The suspensions were stirred at 25 °C (250 rpm) for 6 h in a New Brunswick Scientific Excella E25 incubator-shaker. Blank experiments were run in parallel under the same experimental conditions. Using a liquid/solid ratio of 200 mL g^{-1}, which was previously established as the most convenient value, adsorption isotherms on kaolinite for each protein were obtained by changing the initial protein concentrations. Adsorption data of each protein on kaolinite and samples MK, KR and KI were obtained under the conditions above described, using protein concentration of 2 g L^{-1}. Every protein adsorption experiment on the different adsorbents was repeated six times, being the values obtained very close, average values are shown in this work. Protein concentrations in the initial solutions and after adsorption were measured using the Folin–Ciocalteu colorimetric method (Lowry et al., 1951) with a UV/VIS spectrophotometer.

3. Results and discussion

3.1. Kaolinite chemical composition

The chemical composition of the kaolinite mineral determined by X-ray fluorescence spectroscopy (XRF) was: 50.25% SiO_2; 34.22% Al_2O_3; 0.94% TiO_2; 0.97% Fe_2O_3; 0.03% CaO; 0.16% MgO; 0.04% Na_2O; 1.14% K_2O; 12.25% H_2O.

3.2. Mineralogical composition

The XRD powder pattern for kaolinite (Fig. 1A) shows the diffraction lines characteristic of a high crystal order mineral, diffraction lines corresponding to quartz, mica and feldspars are also present in the diffractogram indicating the presence of these minerals as impurities. The values of the (001) d-spacing corresponding to kaolinite and the conditioned one were 7.14 Å and 7.17 Å, respectively. Conditioning did not expand the kaolinite layers, but slightly increased the defects of the original low defect kaolinite. The sample KR (Fig. 1B) has a lower crystal order due to the presence of adsorbed organic species, or due to the presence of some smaller clay particles formed during the treatment with tert-butyldimethylchlorosilane. The (001) d-spacing of