Colloids and Surfaces A: Physicochem. Eng. Aspects 403 (2012) 114–120

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Adsorption of cysteine on TiO_2 at different pH values: Surface complexes characterization by FTIR-ATR and Langmuir isotherms analysis

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ARTICLE INFO

Article history:
Received 23 November 2011
Received in revised form 29 March 2012
Accepted 30 March 2012
Available online 10 April 2012

Keywords:
Cysteine
Titanium oxide
Surface complexes
ATR-FTIR
Adsorption isotherm

ABSTRACT

Surface complexes of cysteine amino acid onto TiO_2 films at pH 2.0, 5.0 and 8.0 were characterized by IR spectroscopy. The FTIR spectra at pH 2.0 show the coexistence of surface complexes with protonated and deprotonated carboxylate groups. This points to the occurrence of electrostatic interactions between the zwitterionic form of cysteine and the positively charged TiO_2 film. A Langmuir isotherm analysis was performed to obtain the binding constants values, which are consistent with electrostatically adsorbed surface species. At pH 8.0, the TiO_2 film is charged negatively, and the amino acid molecules approach the TiO_2 surface through the amino protonated groups. This new arrangement generates a larger surface concentration at saturation coverage.

1. Introduction

Cysteine is an amino acid commonly found in many proteins and enzymes, playing an essential role in many biochemical and pharmacological redox reactions. Several of these reactions have been studied 'in vivo' [1–6], where it is more difficult to obtain the reaction parameters, and 'in vitro' [7,8] in a homogeneous solution [9,10].

In aqueous solutions, the functional groups of the amino acids are available to undergo possible chemical reactions. However, if some of them are linked to an inorganic holder in an adequate way, the resulting framework may be used as a protein model. Its characterization would allow for a useful understanding of the different reactions that take place in "in vivo" conditions.

Furthermore, titanium implants are often used in dentistry and medicine [11]. Titanium is recovered by an inert thin layer of TiO_2 and, in the case of implants, this surface is in contact with different physiological fluids.

Adsorption of biomolecules on implant surfaces is an important phenomenon regarding the biocompatibility of prostheses and osseointegration process [12].

Adsorption of carboxylic acids [13–16], amino acids [17–21] and polyamino acids [22] on metal oxides has been reported in a large number of publications. Most previous works suggest that amino acids bind to metallic surface atoms through different functional groups. Jang and Condrate [17] investigated the adsorption of lysine to cation-substituted montmorillonites using infrared spectroscopy. They concluded that bidentate coordination occurred through the amino and carboxylic acid groups. Okazaki et al. [18] proposed that the adsorption of lysine to TiO_2 occurred *via* coordination of the amino group. However, in an infrared spectroscopy study of the adsorption of lysine on TiO_2 from an aqueous solution, Roddick-Lanzillotta et al. [19] conclude that at pH ≈ 5–7 there is no experimental evidence of lysine acting as ligand to surface Ti(IV) ions. They suggest that lysine accumulation on TiO_2 arises from electrostatic interactions between cationic amino acid and an anionic film. Martra et al. [20] indicated that the amino acid α-alanine is bound to TiO_2 surface in its zwitterionic form and that the adsorption strongly depends on the pH of the aqueous medium. In another paper Roddick-Lanzillotta et al. [22] analyze the influence of the electrostatic interactions of the polylysine with the adsorbent TiO_2, and reported that these interactions are very important to the adsorption process.

Concerning the adsorption of mercapto-carboxylic acids, in a recent paper Roncaroli et al. [23] studied the adsorption of thioglycolic acid onto a titanium dioxide sample at pH 4, using ATR-FTIR spectroscopy. The authors suggested that the carboxylate plays a more important role than the —SH group in the adsorption

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http://dx.doi.org/10.1016/j.colsurfa.2012.03.064

processes. Two adsorption isotherms are required in order to explain the spectral data, even though only one spectral component is found. The adsorption of thioglycolic acid on CdS thin films has been studied by Young et al. [24]. In this case, based on the absence of the band corresponding to S—H stretching in the infrared spectrum, the authors postulate that the adsorption occurs *via* the deprotonated thiol group.

The cysteine adsorption on metallic surfaces has been studied through ultrahigh vacuum (UHV) techniques. Most previous papers [25–29] are about the bonding to the metal in the form of a thiolate, and the structure of monolayers deposited onto clean and ordered metal (Au, Pt, Cu) or metal oxide surfaces. The carboxylate group may play an important role in the bonding of cysteine on the TiO_2 surface [30]. Ataman et al. [31] studied the adsorption of cysteine on a rutile TiO_2 surface in UHV conditions, and the results show that the molecules bind to the Ti atoms through the carboxylic groups, and the majority of all thiol groups do not participate in the adsorption process.

The surface specific analytical tools used in UHV experiments are not applicable in wet environments [21]. Therefore, there is not much information on the cysteine adsorption processes on a TiO_2 surface in wet environments. For example, Rajh et al. [32] report a study about the surface modification of small particles of TiO_2 colloids with an aqueous solution of 0.1 M cysteine at pH 4. The results obtained by infrared spectroscopy suggest that the carboxyl group is involved in the binding of cysteine on the TiO_2 surface. However, the stretching vibration of the SH group was not affected by adsorption.

For a better understanding of the interfacial biological molecule/titanium dioxide interactions, we have studied the adsorption of cysteine aqueous solutions on TiO_2 film at pH 2.0, 5.0 and 8.0. The characterization of the surface complexes was carried out by attenuated total reflection (ATR) spectroscopy. The ATR mode allows for the exploration of the interfacial region with minimum interference from the dissolved species. The binding constants for cysteine adsorption on TiO_2 at the mentioned pH values were obtained from conventional adsorption isotherm experiments. The nature of the adsorption was discussed, taking into account the pH spectral dependence, and the binding constants were calculated by applying the Langmuir isotherm model.

2. Materials and methods

Titanium dioxide, Degussa P-25, has a particle size of *ca.* 25 nm. Its BET specific surface area is 51.4 $m^2\,g^{-1}$ and was determined from N_2 adsorption at 77 K. The sample is mainly anatase and the content of rutile is less than *ca.* 20%. Suspensions were prepared with bidistilled water and were aged for 15 days.

The cysteine amino acid, as cysteine hydrochloride dihydrate (Fluka) ($C_3H_7NO_2S{\cdot}ClH{\cdot}2H_2O$), was at analytical grade and used without further purification.

FTIR spectra were recorded using a NICOLET 560 instrument equipped with a liquid N_2 cooled MCT-A detector. A horizontal ZnSe-ATR unit (area = 10 mm × 72 mm) was used; the incidence angle was 45° and total number of reflections was 11. This ATR element was SpectraTech.

Layers of TiO_2 particles were deposited by placing 200 μl of TiO_2 suspensions (6 g dm^{-3}) on the surface of the ATR crystal and evaporated to dryness at room temperature. The coated crystal was mounted in an ATR cell, and was allowed to equilibrate with a ligand-free solution until the FTIR signal became stable, and a blank single-beam spectrum was collected. Then, the oxygen-free solution of cysteine was added, and the IR-absorption spectra were recorded at 5–10 min intervals until signal amplitudes reached stable values. The working temperature was 25 ± 2 °C. Spectral

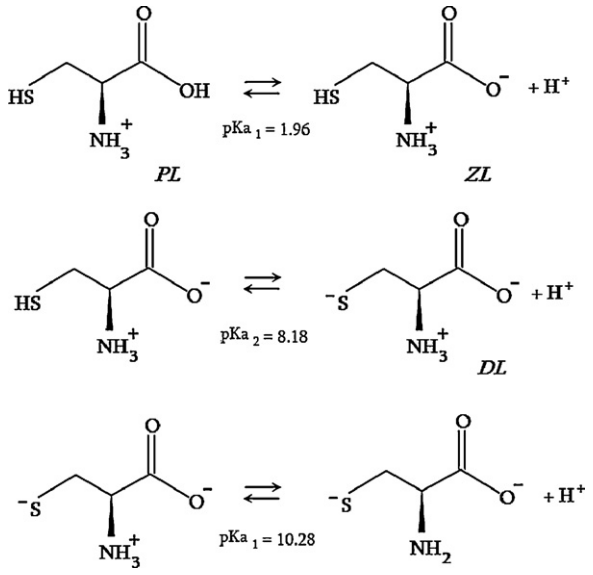

Fig. 1. Summary of pK_a of predominant cysteine species [34].

resolution was 2 cm^{-1}. Each of the final spectra is the average of 256 scans. Base line corrections were made in order to eliminate minor fluctuations due to instrumental instabilities. Under these conditions, since the cysteine concentration was always below *ca.* 1×10^{-3} mol dm^{-3}, no appreciable IR signal was detected from solution species. The FTIR-ATR spectra of free cysteine solutions of about 0.2 mol dm^{-3} were recorded as before.

The solutions at pH 2.0 were measured initially on a germanium crystal, but the absorbances were decreased relative to ZnSe. Therefore, spectra were recorded on a ZnSe crystal in the shortest time possible, in order to minimize the possible dissolution process of this crystal at pH 2.0. It should be noted that the spectra recorded with both materials were very similar, except for the much greater sensitivity in the case of that recorded on ZnSe.

For adsorption isotherms measurements, 25.0 cm^3 of TiO_2 suspension (20 g dm^{-3}) at adequate pH, was mixed with increasing concentrations of cysteine solutions of the same volume and pH. This was controlled by adding concentrated solutions of HCl or NaOH to the suspensions and to the cysteine solutions, as required.

The experiments were performed under N_2 atmosphere in a magnetically stirred vessel immersed in a thermostat at 15.0 ± 0.1 °C, in darkness conditions. The equilibration time was about 90 min. Under these conditions, the concentration of the free ligand became constant. Samples were taken and filtered through a 0.45 μm cellulose acetate membrane. Cysteine concentration in the filtrate was measured spectrophotometrically using the Ellman method [33], involving the reaction of 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB; $C_4H_8N_2O_8S_2$) with the thiol groups. 5.0 cm^3 of 0.1 mol dm^{-3} buffer phosphate at pH 8.0, EDTA 1×10^{-3} mol dm^{-3} and 0.100 cm^3 of DTNB (4 mg cm^{-3}) were added to the filtrated solution. The samples were measured after 15 min at a wavelength of 412 nm.

Cysteine blanks demonstrated that the amino acid concentration remains constant, showing that the oxidation is negligible in the experimental conditions.

3. Results and discussion

The overall charge of cysteine varies with the solution pH. Fig. 1 shows the predominant species present from pH 0 to 14 [34]: the fully protonated PL, the zwitterionic ZL, the thiol deprotonated DL and the amino deprotonated forms.