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Glycine and lysine adsorption and reactivity on the surface of amorphous silica

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Abstract: In order to better control the adsorption of proteins on mineral surfaces, it is useful to gain a thorough understanding of the behaviour of their constituting monomers, namely, amino acids. We have therefore investigated the adsorption of glycine, lysine, and other small biomolecules on well-characterised surfaces of silica. While similar systems have been studied before, these studies usually concentrated on evidencing the effect of surfaces on peptide bond formation in a phenomenological, mostly macroscopic approach. In contrast, we have considered the adsorption process from the view-point of the surface, using knowledge previously gained on the molecular identification of surface functional groups to better characterise their interaction with amino acids, both from the aqueous phase and from the vapour phase.

We combined macroscopic level information such as adsorbed amounts, pH dependence, or TGA, with molecular characterisation by vibrational spectroscopies and ^{13}C NMR of the adsorbed molecules. In parallel, we have carried out molecular modelling of candidate clusters containing the amino acid and the adsorption site by DFT. The structures of lowest energy were also those that best reproduced the observed spectroscopic properties.

Different adsorption mechanisms can be postulated, corresponding to different spectroscopic signatures of the amino acid/adsorption site complexes. On silica surfaces, this implies cooperative hydrogen bonding in a kind of molecular recognition. The existence of molecular recognition is also evidenced by experiments on the coadsorption of different amino acids, where strong selectivity effects may be observed. Such phenomena may have much practical significance in the fields of biofilms and prebiotic chemistry.

Key-words: oxides, amino acids, molecular recognition, adsorption mechanisms.

1. Introduction

Mineral surfaces have been suggested, already in the late '50s, to play a role in the activation of amino acids polymerisation that lead to the formation of peptides in many early prebiotic chemistry scenarii (Bernal, 1951). In particular, clays and other oxides were present in large amounts on the prebiotic earth crust after the formation of hydrosphere, and may have played an important role in the process of chemical evolution (Hazen, 2005 and references therein).

The earth crust consists primarily of silicate minerals, containing a number of metal cations (*e.g.*, aluminium, magnesium, calcium, iron, etc.) mainly structured in frameworks of (SiO_4) tetrahedra connected by siloxane bonds. In spite of relevant differences with natural silicates, the study of the adsorption and reactivity of amino acids on a pure silica system can be considered as a "model" system for studying the role that such surfaces may have played in the activation of peptide bond formation. In fact, the nature of the surface of amorphous silica is quite well understood nowadays, having been thoroughly studied in the past (*vide infra*, Part 3). Moreover, this kind of material may also present a high specific surface; a rather elevated number of surface adsorption sites can thus be present, allowing an increased sensitivity of the characterisation techniques for the detection of the adsorbed molecules and the study of their adsorption mechanism.

While the adsorption of such molecules has been studied before, these studies concentrated either on the adsorption on highly ordered metal surfaces in unrealistic conditions (ultrahigh vacuum, low coverages, etc.), or on evidencing the global effect of surfaces of mineral powders on peptide bond formation without investigating the adsorption mechanism at a molecular level (Bujdák *et al.*, 1994; 1995; 1996; Bujdák & Rode, 1997a,b). For metallic

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substrates, it has been widely recognised that the molecular structure of the surface is important in determining the adsorption mechanisms and thus the activation of these molecules and surface scientists have largely unravelled the subtleties of *e.g.*, amino acids adsorption on metals (Barlow & Raval, 2003 and references therein), while more complex biomolecules are beginning to be characterised at the same level (Barlow *et al.*, 2001). In contrast, for oxides such as silica, alumina, or titania, such molecular-level studies remain scarce (Giacomelli *et al.*, 1995; Roddick-Lanzilotta *et al.*, 1998, 2000; Soria *et al.*, 1999; Tzvetkov *et al.*, 2004; Fleming & Idriss, 2004; Reinholdt & Kirkpatrick, 2006), even though it would certainly be desirable to know not only if such non-patterned, non-designed surfaces can exhibit significant selectivities (may be even molecular recognition phenomena) for different biomolecules going from amino acids to proteins, but also whether the structure of the adsorption site may influence the reactivity of adsorbed biomolecules.

We have therefore undertaken a systematic study of the adsorption of simple biomolecules on several well-characterised oxide surfaces from the point of view of the surface, using knowledge previously gained on the molecular identification of surface functional groups (such as silanols, aluminols, silanolates, "nests" of silanols, ...) to better characterise their interaction with amino acids, both from the aqueous (Meng *et al.*, 2004) and from the vapour phase (Lomenech *et al.*, 2005). The situation in this domain is reminiscent of the studies of heterogeneous catalysts preparation, where it has been increasingly recognised in the last few years that molecular-scale characterisation of metal oxide supports surfaces can be useful in controlling technologically important properties of finished catalysts (Che, 2000). Recent results even seem to indicate that molecular recognition phenomena may be playing a role in this domain (Boujday *et al.*, 2003). In a second step, we try to take advantage of the knowledge of the adsorption mechanism to study the activation of the peptide bond formation at a molecular level.

At first, the textural and surface properties of the silica used for the adsorption were studied by N_2 physisorption and ^{29}Si solid-state NMR. Then the adsorption of different amino acids such as glycine, lysine, among others, and of mixtures of these amino acids on the well-characterised surface of amorphous silica was investigated. We combined macroscopic level information such as adsorbed amounts as a function of pH, co-adsorption experiments, or TGA, with molecular characterisation by vibrational spectroscopies (transmission, DRIFT, IRAS), UV-visible, and ^{13}C NMR of the adsorbed molecules. In parallel, molecular modelling of candidate clusters containing the amino acid and the adsorption site were carried out by DFT.

2. Experimental and computational approaches

2.1. Adsorption procedures

Two deposition procedures have been used: Chemical Vapor Deposition (CVD) (Lomenech *et al.*, 2005) and selective adsorption from water solutions (Meng *et al.*, 2004).

In the CVD-type procedure, an appropriate amount of amino acid (α-glycine or lysine hydrate, Aldrich, in the examples quoted here) is placed in the reservoir of a specially designed glass CVD reactor. About 1.5 g of silica (Aerosil 380, Degussa, 380 m^2/g), previously dehydrated at 100 °C overnight, are placed on the frit and the reactor is homogeneously heated at 160 °C for 15 h in argon flow (100 mL/min), then cooled down to room temperature. These conditions should insure silica dehydration without dehydroxylation.

In aqueous adsorption, 1.5 g of silica (Degussa Aerosil 380) were immersed in 50 mL of an aqueous solution of amino acid of known concentration (typically from 0.03 M to 0.5 M) and the suspension was stirred at room temperature for 2 h. The suspensions were either kept at natural pH, or their pH was adjusted to 6 or 7 using concentrated HCl or NaOH. After the adsorption, the solid was separated by centrifugation, washed once with 50 mL of distilled water and finally dried under vacuum at room temperature. In this way, excess amino acids in solution are mostly eliminated; we estimate that the amount of amino acid retained in the solution that wets the silica represents at most 10 % of the total amount finally present in the solid sample, and that only in the case of the most concentrated solution used (0.5 M in glycine). This procedure is inspired from the "selective adsorption" routinely used in supported catalysts preparation; its main inconvenient is that any reversible adsorption phenomenon will be perturbed by the washing step. The curves giving AA amounts in the final solid as a function of the solution concentration cannot be considered as adsorption isotherms in the thermodynamic sense: therefore, we will refer to these amounts as "deposited" amino acids.

2.2. Characterisation techniques

N_2 physisorption was performed on a Micromeritics ASAP 2010 instrument. The adsorption-desorption isotherms were registered after evacuation of the sample *in vacuo* at 200 °C. The surface area of silica was determined using the BET method.

FT-IR spectra were recorded in air at room temperature on a Vector 22 infrared spectrometer (Bruker). The spectra were measured in KBr pellets with a concentration of the sample in the pellet of several percents by weight. The absorption of a pellet of pure KBr was used as the background.

Powder X-ray diffractograms were recorded using the Cu K_α radiation (λ = 1.5418 Å) on a SIEMENS D500 apparatus fitted with a graphite monochromator and operating under a 30 kV tension. The powder samples were pressed in a borosilicate sample holder.

^1H, ^{29}Si and ^{13}C MAS NMR spectra were obtained on a Bruker Avance 400 spectrometer operating at ω_L = 400 MHz. Proton cross-polarization (^1H CP-MAS) was employed for the ^{13}C NMR spectra, with a contact time optimised at 7.2 ms. The samples were spun at the magic angle at a frequency of 5 kHz. The pulse length employed

for ^{29}Si and ^{13}C was 5 μs, whereas it was of 1 μs for single-pulse ^{1}H. The recycle delay was of 10 to 20 s for ^{13}C (CP-MAS). In the case of single-pulse ^{29}Si, the relaxation time is pretty long and thus a recycle delay of at least 100 s must be used.

Differential thermal gravimetry experiments were performed in air or dinitrogen flow (120 mL/min) and with a heating rate of 5 °C/min on a TG/DTA 220 thermal analyser (Seiko Instruments Inc.).

Quantitative identification of adsorbed amino acids was carried out by HPLC, after desorption using a 0.1 M $CaCl_2$ solution. The column was a XDBC8-ZORBAX from Agilent (4.6 × 150 mm) packed with octadecylsilica, the eluant was an aqueous solution of KH_2PO_4(10^{-2} mol L^{-1}) and sodium hexanesulfonate (5×10^{-3} mol L^{-1}), whose pH was adjusted to 2.5 with orthophosphoric acid.

2.3. Calculations details

DFT calculations were performed for the glycine/silica system with the B3LYP functional with a 6–31++G** basis set, using the Gaussian 98 package. Additional theoretical details may be found in two recently published papers (Lomenech *et al.*, 2005; Costa *et al.*, 2007).

Energies of adsorption, *i.e.*, energy of interaction between glycine and the silica surface, were evaluated for various configurations and experimental conditions. In order to obtain results that can be compared to experimental data, we have used and tested several different ways to calculate the energies of adsorption, detailed and named as follows:

1. *Electronic energy of adsorption from the gas phase at 0 K.* This energy corresponds to the adsorption of the amino acid in the gas phase on the hydroxylated/hydrated surface of silica at 0 K. In this case, the net energy of adsorption, $\Delta_{ads}E^{GAS}$, for a given configuration is defined as the energy of the final configuration (Zw, Silica, $m$$H_2O$ in kJ mol^{-1}), minus the sum of the energy of neutral glycine (G) and the energies of the optimised silanol clusters on which adsorption is taking place, either covered or not with water molecules (Silica, $m$$H_2O$):

$$\Delta_{ads}E^{GAS} = E_{ads}(0\,K) = E_{tot}(Zw - Silica, mH_2O) - E(G) - E(Silica, mH_2O). \quad (1)$$

It may also be interesting to evaluate if the amino acid can substitute water molecules adsorbed on silanolates; in order to have an indication on this process, we also calculated the $\Delta_{sub}E^{GAS}$, defined as:

$$\Delta_{sub}E^{GAS} = E_{tot}(Zw - Silica, mH_2O) - E(Zw, 2H_2O) - E(Silica, n\,H_2O) + E(H_2O)_{n+2-m}. \quad (2)$$

In this case the reference for glycine is the zwitterion micro-solvated by two water molecules. This energy corresponds to the electronic energy of the substitution of (n-2-m) water molecules around the silica surface sites by zwitterionic glycine. The (Silica, $n$$H_2O$) clusters are shown in Figs. 8b and 9c for the silanol and silanolate groups, respectively. The calculated energy of interaction of water with the silanol and silanolate are of –40 and –80 kJ/mol, respectively.

2. *Energy of adsorption from the liquid phase.* In the presence of an aqueous solution, the silica surface is initially covered with water molecules (for simplicity, the influence of the pH on the structure and charge of the first water layers on the surface is neglected) and glycine is solvated by more than two water molecules. It may be surmised that the adsorption of glycine implies the displacement of water molecules from its solvation sphere and from the first water layer on the hydroxylated silica surface. We have thus used a simple approximation to estimate whether the substitution of one or several water molecules by glycine is a favoured process or not. The free energies of solvation of the zwitterion and water are calculated with the PCM method for isolated molecules (Miertus *et al.*, 1981). The energies of adsorption of water on the silanols and silanolate were evaluated with discrete water molecules (see Figs. 8b and 9c). The free energy of adsorption from the liquid phase at room temperature is thus approximated by Eq. (3):

$$\Delta_{ads}G^{LIQ} = E_{tot}(Zw{-}Silica, mH_2O) - G(Zw)^{LIQ} - E(Silica, nH_2O) + (n - m)G(H_2O)^{LIQ}. \quad (3)$$

3. The surface of amorphous silica

The structure and the reactivity of the surface of amorphous silica have been thoroughly described for many decades now, and has been the object of a few monographs (Iler, 1979; Legrand, 1998; Devine *et al.*, 2000). Several models of the structure of amorphous silica, both from the point of view of the bulk (Devine *et al.*, 2000) and of the surface (Feuston & Garofalini, 1989; 1990; Garofalini, 1990; Civalleri *et al.*, 1999; Civalleri & Ugliengo, 2000) have been put forward. Among others, the works of Garofalini (1990) were used as the basis of many early studies, and one of his models is represented in Fig. 1.

Two kinds of surface groups of silica can be identified: siloxanes and silanols. The term siloxane can refer to either the Si-O-Si bridges or the rings formed by the same atoms (Fig. 1). These rings are generally formed by 3 to 8 edge-sharing (SiO_4) tetrahedra, even though highly constrained rings formed by only 2 Si tetrahedra (S2R, meaning Silicon-2-Ring) have been claimed to be present at high temperatures in highly dehydrated silica (Grabbe *et al.*, 1995). Rings larger than S2R are usually more flexible, being either planar or adopting other geometries, and their stability generally increases with the number of Si atoms. In fact, while S3R are quite reactive and are readily opened in the presence of water molecules at room temperature (Riegel *et al.*, 1997), larger rings are usually less reactive.

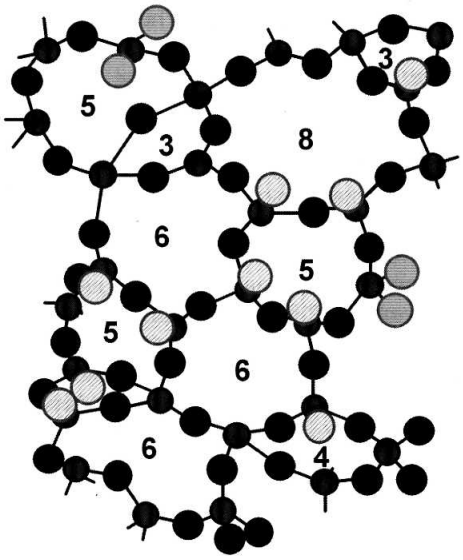

Fig. 1. The surface of hydroxylised amorphous silica, after a model of Garofalini (1990). Gray circles: silicon atoms, dark gray circles: bridging oxygens (siloxanes), oblique hatching: terminal silanols, horizontal hatching: geminal silanols.

Even though the relative amount of the different rings is specific to each amorphous silica, it can be safely assumed that S5R to S8R are mostly present when silica is exposed to ambient air or is suspended in an aqueous solution, as in the case of the samples studied in the present work. Rings larger than S4R are particularly interesting since they are very flexible and can adopt various possible conformations: they can be thus considered as good receptors, specifically relaxing to receive the substrate they are interacting with, and are sometimes compared to crown ethers (Lambert & Che, 1997). A S5R was therefore chosen for modelling a possible adsorption site of amino acids on silica by DFT calculations (*vide infra*, Part 6).

Surface silanols, *i.e.*, hydroxyl groups attached to four-coordinated silicon, were first suggested by Kisilev (1936). A few years later, Carman (1940) explained the formation of silanols as the result of the reaction of water with the surface of anhydrous silica. Then, in 1957, Belyakova *et al.* (1957) demonstrated that the adsorption of water and of other organic molecules bearing polar groups occurs via H-bonding to surface silanols, providing also a first estimate of their surface concentration.

Nowadays, several techniques such as infrared spectroscopy (Iler, 1979; Legrand, 1998) or ^{29}Si MAS NMR (Leonardelli *et al.*, 1992) are commonly employed to determine the concentration and the nature of exposed silanols. The latter method is particularly advantageous, since it allows one to directly observe the Si-O-H groups avoiding the interference of water molecules. In particular, it may distinguish among Si atoms bound to four siloxanes (Q^4, ($\equiv$Si-O)$_4$Si), or Si atoms bearing terminal (Q^3, ($\equiv$Si-O)$_3$SiOH) or geminal (Q^2, ($\equiv$Si-O)$_2$Si(OH)$_2$) silanols. These species give three well-separated peaks in the ^{29}Si NMR spectrum, and their deconvolution directly provides the relative amounts of the three species.

Associated silanols, *i.e.*, silanols interacting via H-bonds, can be distinguished by ^{1}H NMR or by vibrational spectroscopies in the absence of water (Iler, 1979; Legrand, 1998). In aqueous solution, according to the popular "1-site, 2-pK" model, silanols can be either protonated or deprotonated (giving silanolates, $\equiv$Si-O$^-$) depending on the pH. The PZC (Point of Zero Charge) of silica is commonly situated around pH 2, meaning that, except at very acidic pH, the surface of silica is negatively charged, a noticeable amount of the silanols being transformed into (hydrated) silanolates.

The silica used in the present study, Degussa Aerosil 380, is a commercial non-porous amorphous silica and has a BET specific surface of 380 m^2/g, as determined by N_2 physisorption. The ^{29}Si NMR spectrum (not shown) indicated the presence of three peaks at –89.5, –99 and –109 ppm referred to TMS. These peaks, which can be attributed to Q^2, Q^3 and Q^4 silicon atoms, have a relative intensity of 2.8, 18.2 and 79.0 % respectively. This gives an overall density of 5.1 silanols per nm^2, most silanols (4.5 per nm^2) being Q^3 terminal silanols.

Finally, the ^{1}H NMR spectrum (not shown) exhibits the presence of a dominant peak at 3.0 ppm, attributed to H-bonded associated silanols, and of a less intense peak at 1.4 ppm, typical of isolated silanols. This result shows that associated terminal silanols are the most common species at the surface of Aerosil.

4. A macroscopic view of the deposition of amino acids from aqueous solutions

4.1. Solutions of simple amino acids

The amount of amino acid deposited on silica is easily obtained from TG of the solid sample, by calculating the weight loss under air flow at temperatures between 110 and 600 °C and correcting for a blank consisting in a silica hydrated at the same pH as the adsorption solution, but in the absence of amino acid. It was found that samples calcined at 600 °C in these conditions did not contain any residual organic matter, so that one quantitatively measures the adsorbed amino acid.

One of the most important parameters that one must take into account to study the interaction of an amino acid with the surface in an aqueous environment is the pH of the solution in contact with silica. In fact, the simultaneous presence of (at least) one weak acidic and one weak basic function in the same molecule gives the molecule the possibility of existing in differently charged forms depending on the pH of the solution. As an example, the pH-dependent speciation of two amino acids, glycine and lysine, is shown in Fig. 2; glycine is the simplest α-amino acid, having no other substituent, whereas lysine has an additional ionisable amino-*n*-butyl substituent as a side chain. As a consequence, while glycine successively exists as three predominant forms as the pH increases (glycinium cation, glycine zwitterion, glycinate anion), lysine has an additional dicationic form which predominates at low pH, while the pre-

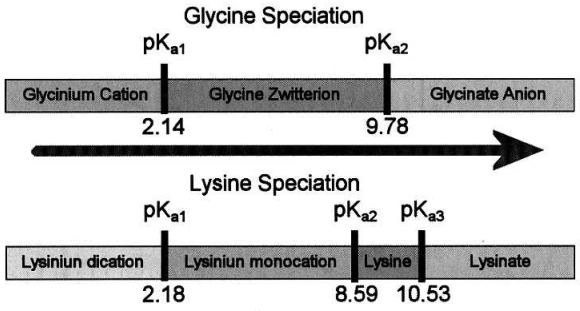

Fig. 2. Speciation of glycine and lysine with the pH. "Lysine" denotes the lysine zwitterion.

dominance domain of the remaining three forms is shifted to higher pHs.

Similarly, the pH determines the surface charge of silica (*cf.* Part 3), and thus the sign and magnitude of the electrostatic interaction between surface and adsorbate. In fact, if the amino acid-surface interaction was purely electrostatic, the adsorption should be possible only when the charges of the amino acid and the silica are of opposite sign, and changes in the pH of the adsorption solution would thus be the main factor controlling the amount of adsorbed molecules.

In contrast, if amino acids are subject to localised adsorption on specific sites, this electrostatic interaction would still be present, but would not be the only contribution, or may be not even the main contribution, to the adsorption energetics; in this case, one might observe a net adsorption even in conditions where the surface charge and the amino acid charge are of the same sign. Such an observation indeed constitutes a useful rule-of-thumb to identify a site adsorption mechanism.

From this point of view, it appears that the interaction of amino acids on silica is not, in general, a purely electrostatic affair: in other words, it is not a simple charge compensation, or ion exchange. For instance, glycine adsorption remains significant at pH 10, where speciation starts to favour the glycinate while the silica surface is strongly negative, and similar observations have been made for other amino acids.

Indications on the mechanism of adsorption and the affinity between a particular amino acid and a surface can also be obtained by studying the evolution of deposited amounts as a function of glycine concentration at a fixed pH. The corresponding curves for glycine and lysine on silica from aqueous solutions at their natural pH (pH 6 and 9 for glycine and lysine, respectively) are reported in Fig. 3. From these data, it is clearly visible that the two amino acids have different adsorption behaviours.

At low equilibrium concentrations, the deposition of separated adsorbed molecules of amino acid on the surface of silica can be inferred, whereas bulk deposition is supposed to start at higher concentrations. In the case of lysine, when the concentration exceeds 0.1 mol/L, the inception of additional deposition can be detected by looking at the deposition curve. In the case of glycine, this additional deposition is substantiated by XRD: when the equilibrium glycine

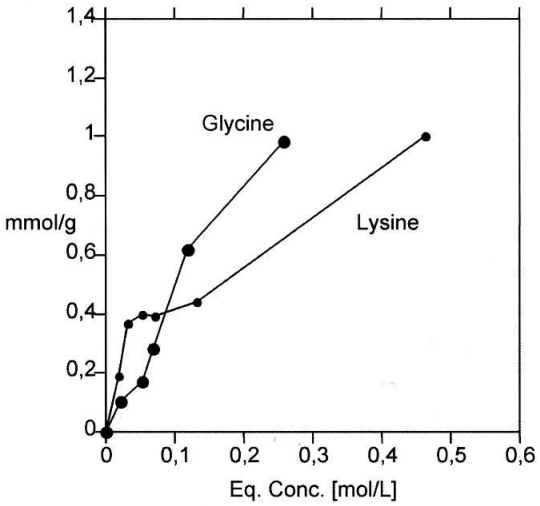

Fig. 3. Amounts of glycine and lysine on silica deposited from aqueous solutions at their natural pH (pH 6 and 9 for glycine and lysine, respectively).

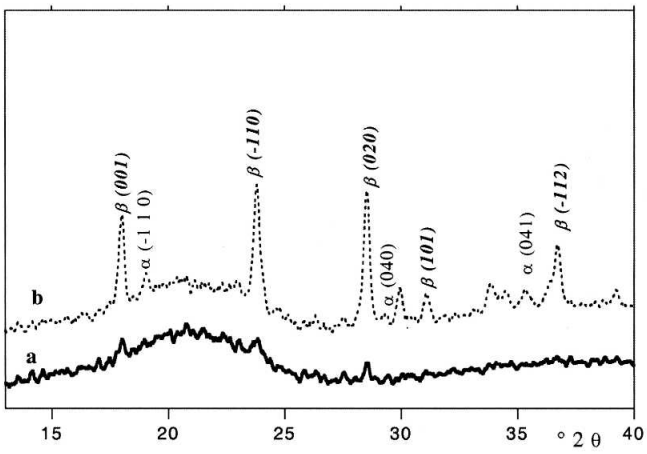

Fig. 4. Powder XRD pattern (λ = 1.54218 Å) of glycine deposited on silica at natural pH: (a) from a 0.08 M solution; (b) from a 0.5 M solution. Peak labels refer to the crystalline forms of bulk glycine.

concentration in solution is higher than about 0.03 M, precipitation of bulk glycine crystals is observed in addition to molecular adsorption, although the bulk solution is far below saturation levels, as shown in the pattern in Fig. 4. It is interesting to notice that, under these conditions, the β polymorph of glycine is observed, instead of the supposedly more stable α polymorph, which usually forms in aqueous solutions (Boldyreva *et al.*, 2003; Weissbuch *et al.*, 2005; the small amounts of the α form may be due to non-specific precipitation because of insufficient washing).

This phenomenon can be compared to what geochemists sometimes call "surface precipitation" or better "surface-induced precipitation", *i.e.*, the precipitation of a mineral exclusively on the surface adsorbent, under conditions where the bulk solution is undersaturated – this phenomenon is even observed in several procedures of preparation of supported heterogeneous catalysts (Tewari & Lee, 1975; Hermans & Geus, 1979; Farley *et al.*, 1985; Charlet

& Manceau, 1992). Even though with the techniques used so far, the precipitation of amino acids on silica is observed only after drying, which implies an intermediate increase in the concentration of amino acid at the solid/solution interface, a strong effect of the surface on the precipitated species is evident and seems to be specific to the interaction between an amino acid and the oxide surface. In fact, the formation of bulk crystalline amino acid on silica was observed, at comparable concentrations, only for selected amino acids, *e.g.*, for leucine and glycine, but not for glutamic acid or lysine. Formation of bulk glycine was previously reported by El Shafei *et al.* in glycine/boehmite (AlOOH) adsorption experiments (El Shafei & Philip, 1995), even though these authors gave a different and objectionable explanation of this observation. Interestingly, we did not observe any bulk glycine phase when contacting a solution of glycine with γ-Al_2O_3 at similar concentrations, indicating the complexity of the phenomenon.

In particular, as regards the formation of the β rather than the α polymorph of glycine, there are several conceivable ways in which the silica surface could cause the formation of specific condensation nuclei that would later grow to crystallites of the β form. In a previous work (Meng *et al.*, 2004), we underlined that electric field gradients in the diffuse layer close to the negatively charged silica surface are strong enough to cause specific orientations of the molecules, but other structuring effects such as H-bonding with surface groups could be operating as well.

To summarise, glycine adsorption on silica at least can best be rationalised by the simultaneous occurrence of two different mechanisms: site adsorption (*vide infra*) on a limited number of surface sites, and surface-induced precipitation.

4.2. Adsorption selectivity from mixtures of amino acids

In a lysine/glycine adsorption selectivity experiment, a definite preference for the adsorption of lysine from aqueous solutions was observed at pH 7. When solutions of equimolar mixtures of the two amino acids are contacted with silica, the lysine/glycine molar ratio in the adsorbed phase is always > 1, and strongly increases for more diluted solutions (Fig. 5).

This is not entirely unexpected if we consider the electrostatic contribution to the adsorption energy. As previously reported (*vide supra*, Part 4.1), the speciation of amino acids is pH-dependent as well as the surface charge. More precisely, in the presence of a solution at pH 7, the silica surface bears a significant negative charge, while glycine bears no net electric charge, and lysine is predominantly cationic. This situation should certainly favour lysine adsorption. However, once the negative charge of the silica (very approximately, 0.4 elementary charges per nm^2, or mmol g^{-1}) is saturated, this preference should disappear.

Surprisingly, the adsorption selectivities for different amino acids do not seem to have been investigated in detail so far. Yet, they may be relevant for protein adsorption as well; although most amino acids in the protein backbone

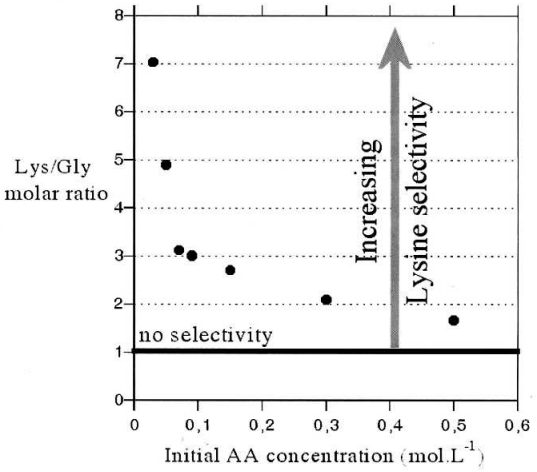

Fig. 5. Adsorption selectivity in the Glycine+Lysine/SiO_2 system (aqueous deposition), expressed as the Lysine/Glycine molar ratio in the adsorbed phase as a function of total amino acid concentration in the aqueous phase.

have lost their terminal groups in the formation of amide bonds, ionisable groups in side chains still play a fundamental role in directing protein reactivity; for instance, in the conditions we have tested, lysine-rich domains may be preferentially adsorbed on silica.

From a practical point of view, it is interesting to note that both pH and global concentration can serve as switches to control surface speciation and consequently adsorption selectivities for different biomolecules.

5. Molecular-level information on the adsorption mechanism of amino acids on silica

5.1. Vibrational spectroscopy

As can be seen in Fig. 6, the middle IR spectra of glycine adsorbed on silica can be markedly different according to the precise sample history. When adsorbed from the gas phase, all spectrum features can be assigned on the basis of neutral glycine molecules; in contrast, when adsorbed from water, the molecules are clearly present as zwitterions.

However, significant shifts are observed with respect to the known spectra of either neutral glycine or glycine zwitterion (Alper *et al.*, 1991; Stepanian *et al.*, 1998; Rosado *et al.*, 1998): the ν_{sCO} of the carboxylic acid moiety is shifted by –92 cm^{-1} in Gly/SiO_2 CVD, and the δ_{sNH3} of the ammonium is shifted by –36 cm^{-1} in Gly/SiO_2 adsorbed from aqueous solution.

This suggests that adsorbed molecules are strongly affected by the adsorption reaction. Unravelling the origin of these effects is not straightforward: DFT calculations (*vide infra*, Part 6) show that they cannot be correctly accounted for in a "localised" approximation; in other words, the molecule is globally modified by the adsorption, not only one of its functional groups.

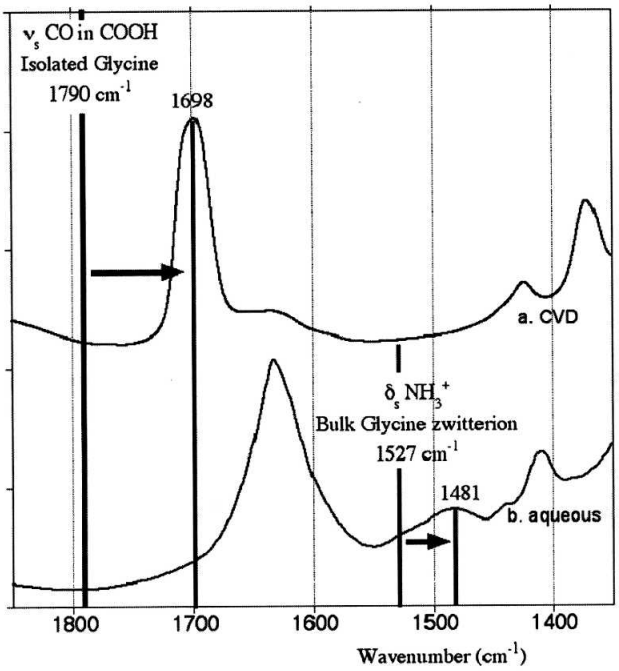

Fig. 6. Middle IR spectra (absorption mode) of (a) Gly/SiO_2 (CVD); (b) Gly/SiO_2 (aqueous deposition, pH 6, initial concentration 0.08 M). Diagnostic bands are compared with the positions of the corresponding vibrational modes in reference compounds (gas-phase monomolecular glycine and bulk α-glycine).

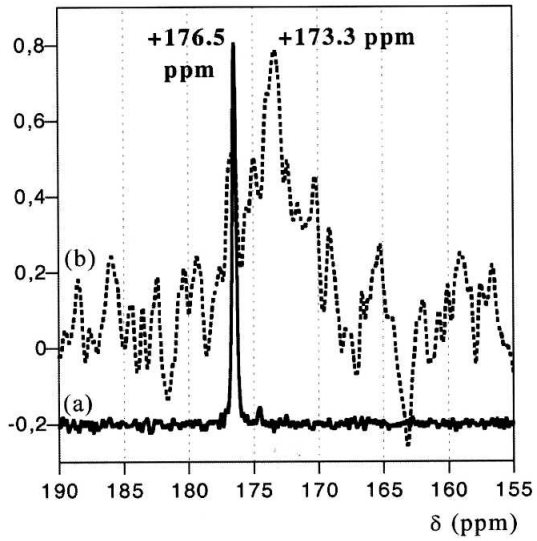

Fig. 7. Natural abundance ^{13}C CP-MAS NMR spectra of (a) bulk α-glycine; (b) Gly/SiO_2, aqueous deposition from 0.07 M solution at pH 6.

5.2. ^{13}C NMR

^{13}C NMR is not a technique of choice in studies of ideal surfaces, due to its low sensitivity that makes it unsuitable for the small quantities of molecules present in such systems. In contrast, in the case of adsorption on high-surface area powders, it can be carried out even in natural abundance, and provides a useful complement to vibrational spectroscopies, especially in the presence of water where the intense H_2O vibrations hide part of the vibrational information.

In samples where glycine was adsorbed from a low-concentration solution, a carboxylate signal can still be clearly observed, but it is shifted upfield by –3.2 ppm with respect to bulk α-glycine (Fig. 7; it was checked that this does not correspond to β-glycine either). Such shifts are typical of differences in the H-bonding of amino acids (Malkin *et al.*, 1995; Zheng *et al.*, 1997; Potrzebowski *et al.*, 1998). Here again, a more precise interpretation of the observed shifts is not straightforward. In particular, it has been demonstrated that the position of the carboxylate resonance can be significantly affected by H-bonding, not only of the carboxylate, but also of the $-NH_3^+$ moiety (Malkin *et al.*, 1995). Therefore, this observation is compatible with our previous model of glycine/surface interaction chiefly through the ammonium end (Meng *et al.*, 2004).

In spite of these difficulties, ^{13}C NMR has the potential to provide an independent validation of adsorption models. Thus, contrary to what usually occurs for adsorption on planar surfaces, vibrational spectroscopies do not constitute the only source of information on the molecular structure of the adsorbed molecules.

6. Molecular modelling

We have performed many simulations of glycine in interaction with various surface groups of silica (Lomenech *et al.*, 2005; Costa *et al.*, 2007), in the presence and in the absence of water molecules. Candidate structures for adsorbed glycine were evaluated along two criteria: energy minimisation and prediction of vibrational properties. The "best" structures from the energetic point of view were also those that reproduced spectroscopic features in the most satisfying way, as will be detailed hereafter.

As a first result, it was found that "grafted" molecules, *i.e.*, models involving the formation of a chemisorbed "surface ester" R-CO-O-Si, did not fit the criteria. This does not mean that surface grafting of amino acids on oxide surfaces is impossible in all cases, but that it does not happen in this system under the conditions employed in this study. This conclusion was independently confirmed by Ugliengo and co-workers (Rimola *et al.*, 2006) in a parallel study. Conversely, the adsorption mechanism seems to depend on specific patterns of hydrogen bonds between the amino acid and surface groups (including silanols and silanolates).

Secondly, the degree of hydration of the silica surface strongly influenced the structure of adsorbed glycine. In the absence of water (obtained by CVD, Lomenech *et al.*, 2005), the best possible structure corresponded to adsorption by a network of hydrogen bonds involving two neighbouring silanol groups of the silica surface, on the one hand, and the carboxylic acid and amine moieties of glycine, on the other hand (Fig. 8). The energy of interaction was calculated to be –68.7 kJ/mol (Lomenech

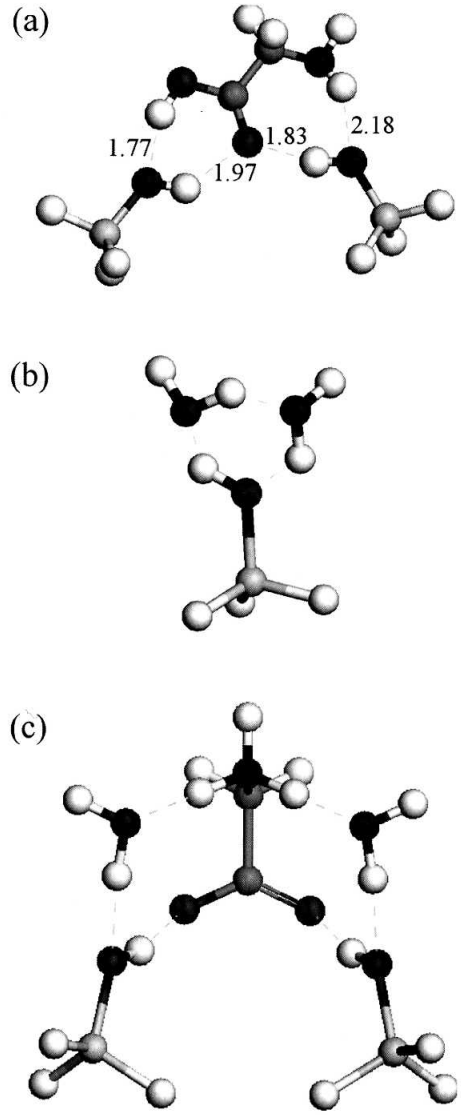

Fig. 8. Best DFT model of (a) Gly/SiO_2 in anhydrous conditions, such as may be obtained by CVD procedures. Narrow lines indicate the network of hydrogen bonds; the H-bond contact distances are indicated; (b) silanols with two water molecules; (c) zwitterionic glycine on silanols with two additional water molecules (see Costa *et al.*, 2007 for more details).

et al., 2005). The simulated IR spectrum for this adsorption model (ν_{CO} = 1701 cm^{-1}) agrees well with the experimental spectrum of glycine adsorbed on silica by CVD (ν_{CO} = 1699 cm^{-1}) (Lomenech *et al.*, 2005).

More recently, Rimola *et al.* (2006) simulated the adsorption of glycine on silica using periodic models, finding a similar adsorption pattern of hydrogen bonds. An even better agreement between the experimental CVD spectrum and the simulated data can be found in the latter case.

In contrast, the presence of water (Costa *et al.*, 2007) appears to favour a different pattern of hydrogen bonds. In fact, in the presence of an aqueous solution, the silica surface is initially covered with water molecules and glycine is solvated. It may thus be assumed that the adsorption of glycine occurs only with the displacement of water molecules from its solvation sphere and from the first water layer on the hydroxylated silica surface. The best conformation found for glycine solvated by two water molecules in interaction with two silanols is shown in Fig. 8c. In this case, the energy of adsorption ($\Delta_{ads}E^{GAS}$) is −146 kJ/mol (calculated using Eq. (1), see Sect. 2). Taking into account the displacement of the water molecules away from the silanols results in a ($\Delta_{sub}E^{GAS}$) of −12 kJ/mol (Eq. (2)). Indeed, the formation of four new H-bonds is partly compensated by the displacement of the water molecules. This suggests that silanols and water molecule act as ligands of similar strength for glycine, a trend developed in more details in Costa *et al.* (2007). Finally, taking into account the solvation energies of glycine and water leads to a free energy of adsorption from the liquid phase ($\Delta_{ads}G^{LIQ}$) = +49 kJ/mol. Under these assumptions, the adsorption of zwitterionic glycine from the liquid phase is therefore endergonic on one or several silanols.

The surface of silica at the water interface may also exhibit negatively charged silanolates. Silanolates are present at a rather low density on the surface of silica at neutral pH. In order to model this specific site, in a first step, we have studied the interaction of zwitterionic molecule of glycine interacting with one silanolate SiO^- (Fig. 9a). In this case, only the cationic end of the zwitterionic glycine binds directly to the negatively charged silanolate, producing a partial proton transfer from the NH_3^+ end to the silanolate (the N–H distance is 1.59 Å and the O–H distance is 1.04 Å). When the zwitterionic glycine interacts with a silanolate, the silanolate is virtually transformed into a silanol. In other words, the negative charge of the silanolate is largely transferred to the COO^- moiety, and a glycinate ion is stabilized. Complementary calculations on the interaction of neutral glycine with silanolate showed that the zwitterion (form I^- 0–NH) is 80 kJ mol^{-1} is more stable than the neutral state. Thus a single silanolate, which may establish a rather covalent bond with glycine, allows the stabilization of the zwitterionic state, whereas no less than four SiOH or water molecules are required for this stabilization (Costa *et al.*, 2007). Taking into account the solvation energies of glycine and water respectively, the free energy of the reaction:

$$Z_{solv} + (SiO^-, H_2O) \rightarrow (SiO, Z)^- + H_2O_{solv},$$

which corresponds to the substitution of one water molecule bond to the silanolate by a molecule of solvated glycine, is negative (–38 kJ/mol).

Now, the presence of a silanol in the vicinity of a silanolate is highly probable (*vide supra*, Part 3). Therefore, we have studied the possibility of the adsorption of the zwitterionic glycine on a site implying one silanolate and one silanol. To make this site reasonable, these functional groups were borne on a S5R cycle, which is known to be commonly present on the surface of amorphous silica. In a first step, the cycle was freely optimised; then, the bottom H atoms were kept fixed in order to mimic the constraints of the solid, and the cycle with the adsorbed glycine molecule was optimised. Again, one observes a spontaneous proton

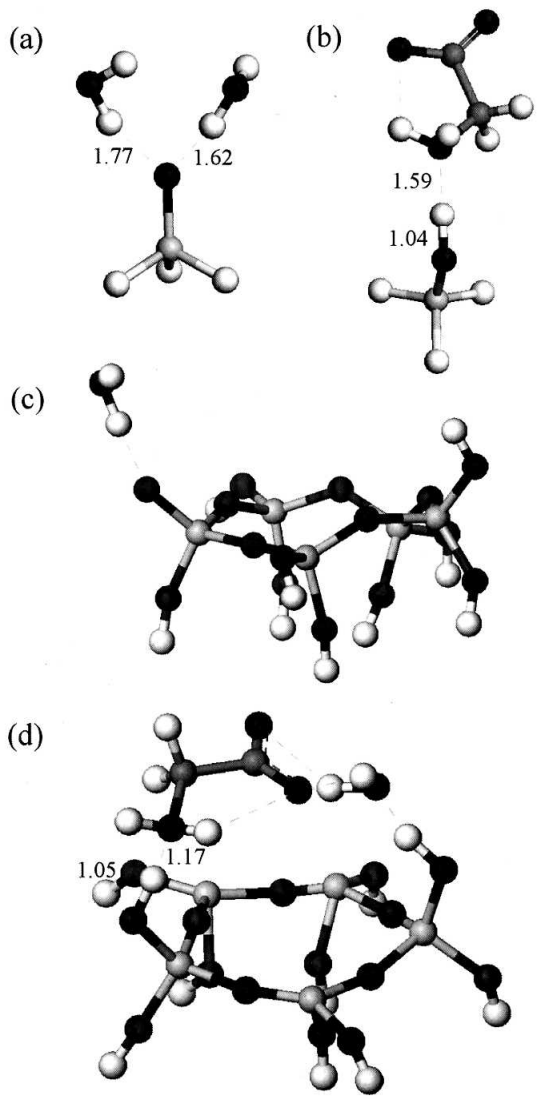

Fig. 9. Best DFT model obtained for (a) minimal H_3SiO^- cluster with two adsorbed water molecules; (b) glycine adsorbed on SiO^-; (c) silanolate in a 5R ring, with an additional water molecule; (d) glycine on a 5R ring bearing a silanolate group. Note the participation of one water molecule in the network of hydrogen.

transfer from the amine moiety to the silanolate. The carboxylate function interacts with the silanol in the same surface ring through a bridging H_2O molecule (Fig. 9d). The reaction:

$$Z_{solv} + (5SR, H_2O_{ads}) \rightarrow Z - S5R - H_2O$$

is exothermic by –74 kJ/mol, and appears until now as the most energetically favoured situation.

While these models can still be refined, these results suggest that amino acids adsorption is site-specific, at least at low molecular coverages, and that the molecular nature of the adsorption site depends on the adsorption conditions. In particular, in water, the best sites involve a negatively charged surface group (*vide supra*, Part 4.2), as well as the cooperation of one molecule of solvent: the adsorption is

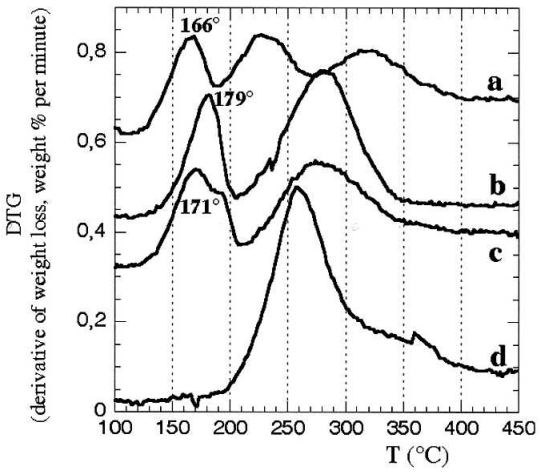

Fig. 10. Thermogravimetric traces (as DTG, derivative of weight loss) of: (a) Lys/SiO_2, (b) Ala/SiO_2; (c) Gly/SiO_2; and (d) DKP/SiO_2 (DKP = diketopiperazine or cyclic diglycine). Heating rate: 5 °C/min.

therefore a three-partner reaction, implying surface, adsorbate and solvent. This participation of solvent molecules is well known in other domains of supramolecular chemistry (Corbett *et al.*, 2005).

7. Heat-induced reactivity of adsorbed amino acids

It has been recognised by several authors that adsorption of amino acids on oxide surfaces can promote the formation of amide bonds without the need of any other additive or activating agent (Basyuk & Gromovoy, 1993; Bujdák *et al.*, 1995; 1996; Bujdák & Rode, 1997a,b – note that the term "catalysis" is improper here because the reaction is thermodynamically unfavourable in the absence of the oxides). The activation of peptide bond formation is obviously very important in prebiotic chemistry (Basyuk *et al.*, 1991; Bujdák *et al.*, 1994).

Indeed, we found that thermogravimetric analyses of glycine, lysine, alanine, or serine adsorbed on silica exhibit a thermal event around 150 to 170 °C, which was assigned to the loss of water upon formation of amide bonds. This thermal event is "clean" in that it is not accompanied by significant decomposition reactions; the promotion effect of the surface is evident as the temperature of amide bond formation is decreased by 80–90 °C as compared to the corresponding bulk solid amino acids. Figure 10 shows the thermogravimetric data for three silica-supported amino acids (a: lysine, b: alanine, c: glycine): all three show a clear weight loss before 200 °C that is attributable to amide bond formation. In contrast, the silica-supported cyclic glycine dimer DKP, which cannot form amide bonds anymore, shows no weight loss in this region.

These results were obtained for fast heating in the presence of a dry atmosphere. Other authors have studied different procedures of thermal activation, such as wetting-drying cycles at moderate temperatures (Bujdák & Rode,

1997b). If long enough equilibration times are applied, it seems that amide bonds may be formed at temperatures as low as 80 °C in some cases: clearly, continued studies of the effect reaction conditions (water activity, activation times) are in order.

An important question that we are presently trying to address is whether there exist significant selectivities as to the type of amino acids that may be bound together from an aqueous mixture. That would certainly increase the interest of this reaction for synthetic purposes. The existence of strong selectivities in the adsorption of amino acids suggests that selectivity might also be present in their further transformations.

Conclusions

Amino acids adsorption on mineral surfaces has been relatively little studied so far, perhaps because the apparent simplicity of the system leads one to believe that no interesting phenomena will be observed. However, this simplicity is deceptive, even for the most elementary amino acid, glycine. The use of well-known surface oxides, such as silica, can thus be of help for studying the role that such surfaces may have played in the activation of peptide bond formation. Under carefully controlled conditions, precise spectroscopic information could be obtained on glycine/silica systems, and compared with the results of molecular modelling based on a good knowledge of the mineral surface sites. The best models reveal a kind of interactional complementarity between the amino acid and specific adsorption sites, based in the studied case on a network of hydrogen bonds.

The idea that molecular recognition phenomena may be operating in these systems is corroborated empirically by the observation of significant adsorption selectivities from mixtures of different amino acids.

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