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Surface-Altered Protonation Studied by Photoelectron Spectroscopy and Reactive Dynamics Simulations

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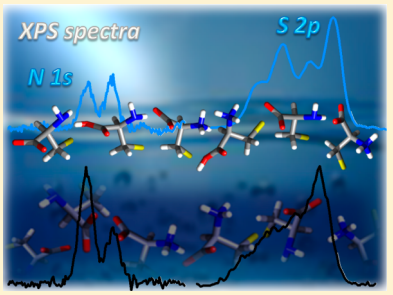
Supporting Information

ABSTRACT: The extent to which functional groups are protonated at aqueous interfaces as compared to bulk is deemed essential to several areas in chemistry and biology. The origin of such changes has been the source of intense debate. We use X-ray photoelectron spectroscopy and all-atom reactive molecular dynamics simulations as two independent methods to probe, at the molecular scale, both bulk and surface distributions of protonated species of cysteine in an aqueous solution. We show that the distribution of the cysteine species at the surface is quite different from that in the bulk. We argue that this finding, however, cannot be simply related to a change in the extent of proton sharing between the two conjugate acid/base pairs that may occur between these two regions. The present theoretical simulations identify species at the surface that are not present in the bulk.



In aqueous solutions, the acid–base chemistry at the surface is of paramount importance for several processes,[1,2] including efficient capture of environmentally meaningful gases,[3] catalytic effects due to different enzyme environments,[4] and charge transport across cell membranes,[5] to mention a few of many examples. The extent to which functional groups are protonated at aqueous interfaces are deemed essential to the examples given above. This has motivated much recent experimental and theoretical work devoted to identify the different chemical activity of protons at the surface as compared to that in the bulk.[1,2,6−9,] A number of experimental and theoretical studies have been interpreted in terms of a higher acidity of the neat water surface (pH around 5), though such an interpretation is in conflict with electrophoretic and titration measurements, which indicate a negative charge in the neat water surface.[10,11] The complexity of surface acid–base chemistry, connected to that of charging of the water surface,[12−14] may suggest that the most appropriate approach is to synergetically combine experimental techniques and computational chemistry.

Our investigation is focused on the protonation state of cysteine, which, in aqueous solution, is strongly affected by the acidity of the environment and may adopt various chemical forms. The hypothesis is checked by monitoring the distribution of cysteine species at different depths of the aqueous solution, sampled by using X-ray photons of different energies.[2,15] According to the Onsager–Samaras model,[16] all charged particles are depleted from a surface, but molecular dynamics simulations show a distinct behavior for larger and polarizable anions.[17] Intuitively, the observed changes at the surface of an aqueous environment could be ascribed to a change in the pH of the solution. In the paper by Ottosson and co-workers[15] on small carboxylic acids, it was argued that the observed changes cannot be connected with a surface propensity of hydronium atoms. However, the statement could not be proved also because only one protonated center was taken into account. In the present study, we clearly show that strong changes of the total amount of SH, S^-, NH_3^+, and NH_2 groups of the cysteine species in aqueous solution cannot be used to infer straightforwardly that the surface propensity of hydronium atoms is different from that of the bulk.

For the bulk of a cysteine aqueous solution, the link between pH and the distribution of chemical species is well-known. An exemplification of such a link, properly described as titration curves, is reported in Figure 1.[18] Around pH = 9.5, only four cysteine species are present: an oxygen zwitterion (ZO) with the groups COO^-, SH, and NH_3^+, anion–zwitterion (AZ) with the groups COO^-, S^-, and NH_3^+, an oxygen anion (AO) with the groups COO^-, SH, and NH_2, and a double anion (AA) with the groups COO^-, S^-, and NH_2. From the examination of

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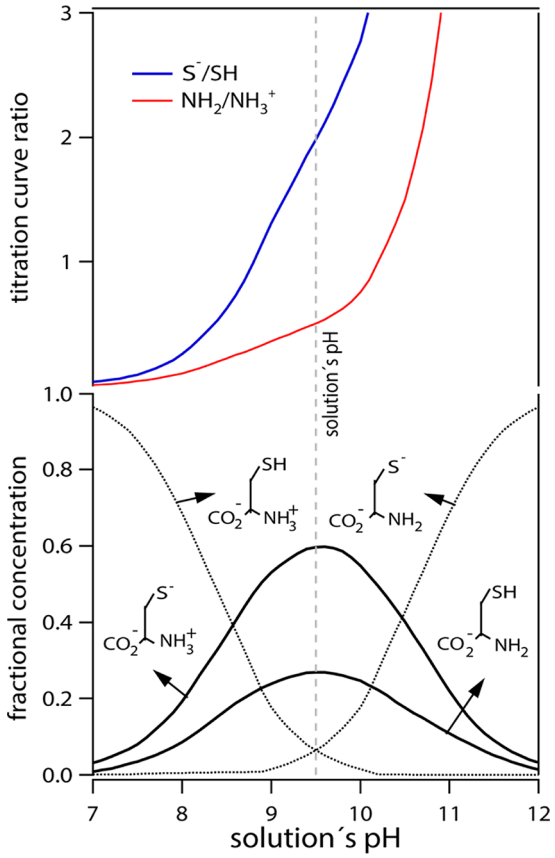

Figure 1. Tritation curves of cysteine in water.[18] Bottom: fractional concentrations of various cysteine species. Top: ratios between total concentrations of nonprotonated and protonated species.

Figure 1, it is evident that the pH of the solution is unambiguously inferred by knowing any of two concentration ratios, S^-/SH (blue line) and NH_2/NH_3^+ (red line) (in the upper part of Figure 1), obtained from the total amount of cysteines with the nonprotonated or protonated thiol and amino groups, respectively. In the following, we will consider an aqueous solution of cysteine where the pH has been fixed at 9.5 by addition of NaOH. According to the titration curves shown in Figure 1, the percentage of the four species present in solution at that pH is about AZ (56.5%), AO (27.5%), AA (8%), and ZO (8%).

All four species of cysteine, which are present in the bulk at pH = 9.5 (see Figure 1), have in common the COO^- group, and for all of them, an O 1s photoemission spectrum with a single broad band can be predicted. Instead, at both the S 2p and the N 1s edges, two peaks with a clear chemical shift, due to the protonation or deprotonation of the sulfur and amino groups, can be expected. The experimental S 2p and N 1s photoemission spectra of an aqueous solution of cysteine at pH = 9.5, displayed in Figure 2, confirm these expectations.

The S 2p spectrum (top) shows two bands (each one split into two peaks due to the spin−orbit coupling of the 2p hole) with a chemical shift of about 2.4 eV; the higher binding energy is naturally assigned to SH and the lower one to S^-. In the N 1s spectrum (bottom), there are also two bands with a chemical shift of about 2.6 eV, with the higher binding energy assigned to the NH_3^+ protonated species. This shift agrees well with that of 2.51 eV obtained for glycine.[19]

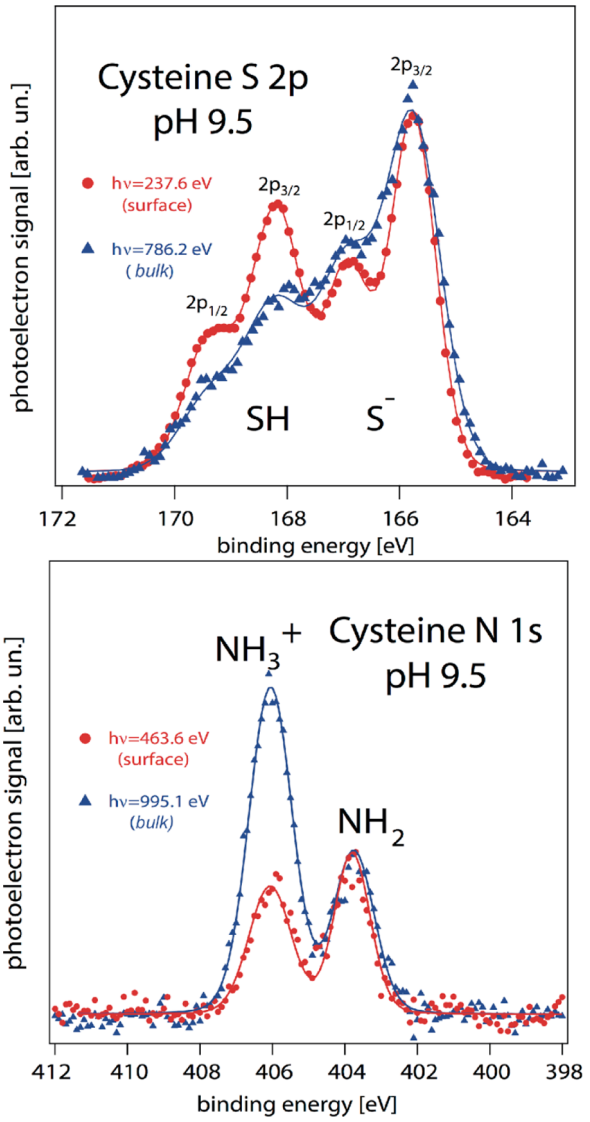

Figure 2. XPS spectra of an aqueous solution of 1 M cysteine at pH 9.5. In red (circles) [blue (triangles)] are spectra taken at lower [higher] photon energy compared to the corresponding atomic edge binding energy. Due to the electron scape depth, spectra in red (blue) are surface (bulk) representative. Protonated groups are indicated in the figure. For the sulfur edge, the spin−orbit split orbitals are also indicated. Ratios between S^- and SH (NH_2 and NH_3^+) areas are consistent with lower (higher) pH according to the upper part of Figure 1. See the text for further discussion.

As shown in Figure 2, the photoemission spectra have been collected at different photon energies. The dependence of the relative intensities of the two peaks (protonated versus nonprotonated species) from the photon energy is evidently very strong in both spectra. According to the localized, almost atomic character of a core orbital, the integrated photoemission cross section (PCS) should change very little between two core N 1s core lines separated by a chemical shift of 2.6 eV at a photon energy about 60 eV above threshold; an even smaller change is to be expected at 600 eV above threshold. Photoelectron anisotropy may present, in principle, a larger variation even for two core lines chemically shifted by a few eV. The same is valid for the S 2p lines. Experimental evidence, however, shows that this is not the case; see the Supporting Information for details. Conversely, the intensity ratio depends

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Table 1. Percentage Concentrations and Nonprotonated/Protonated Ratios for Different Cysteine Species in Aqueous Solution As Predicted by Tritation Measurements, Present Spectroscopical Measurements, and Present Reactive Dynamics Simulations, Bulk and Surface

	experiment			computation	
	titr_curve	high_ener.	low_ener.	bulk	surface
AO(SH,NH_2)	27.5%			20.2%	12.1%
AZ(S^-,NH_3^+)	56.5%			63.2%	19.3%
AA(S^-,NH_2)	8%			6.3%	27.0%
ZO(SH,NH_3^+)	8%			10.3%	6.4%
S^-	64.5%			71.8%	56.4%
SH	35.5%			28.2%	43.6%
S^-/SH	1.8	2.4	1.4	2.6	1.3
NH_3^+	64.5%			62.6%	48.4%
NH_2	35.5%			37.4%	51.6%
NH_2/NH_3^+	0.6	0.5	1.1	0.6	1.1

on the chemical composition of the solution, that is, on the ratio of the concentrations of protonated and nonprotonated species. Due to the fact that the XPS measurements are made on a solution exposed to the vacuum (and then with a surface), the variation of the intensity ratio could derive from a difference between the chemical composition of the "surface" and "bulk", which are differently probed by low-energy and high-energy X-rays. The effective mean-free path of the created photoelectrons, also described by the electron attenuation length (EAL), is relevant to the present discussion. The most recent study on the EAL shows that it does have a minimum of approximately 8 Å (40 Å) for photoelectrons with a kinetic energy of about 90 eV (600 eV).[20] A shorter minimum EAL (5 Å) was described in another study.[21] These values imply that by using our lower (higher) photon energy, our measurements are surface-sensitive (bulk-sensitive) and that the origin of the measured electrons averages from 5 to 10 Å (about 40 Å).

A simple picture, following the hypothesis that the surface behaves as "acidic" ("basic") in comparison to the bulk, would predict that the ratio (nonprotonated species)/(protonated species) is lower in the surface than in the bulk of the aqueous solution; see the blue and red lines at the top of Figure 1. This simple picture is in agreement (contrast) with the trend observed in the S 2p spectra in Figure 2 but contrasts (agrees) clearly with that observed in the N 1s spectra. The view that the surface is like a bulk with a different pH is too simplified.

Table 1 compares the number of cysteine molecules of different forms and the nonprotonated/protonated species ratios predicted by the present reactive molecular dynamics calculations for bulk and surface models to the intensity peak ratios obtained from a simple inspection of the XPS spectra. The values of the nonprotonated/protonated species ratios in the bulk obtained from the titration curve are also reported for comparison. It should be noticed that the percentage concentrations of the four cysteines species in the last column of Table 1 do not sum up to 100; this is due to the appearance, in the surface region of the simulated sample, of new cysteine species.

The trend in the variation of the ratios predicted by the computation is in agreement with the experimental observations for both the S 2p and N 1s spectra. In addition, an opposite variation of the intensity ratio for the S 2p and N 1s bands is now predicted.

While the XPS results can directly provide only concentration ratios, the theoretical modeling can also predict the concentration of each different species in solution (It is only true that XPS results can directly provide only concentration ratios if the angular anisotropy and PCS do not change between the two core lines separated by 2−3 eV. In the present case, the change in anisotropy is small, and changes in the PCS are expected to be even smaller. See the Supporting Information for more details.). In Figure 3, we present the percentage of different species of cysteine (Cys) versus the simulation time from the reactive dynamics of the "surface phase" model; the analysis refers to the surface slice of about 5 Å. A high "reactivity" is predicted starting from the chemical composition of the bulk (time 0) as obtained from the titration curves that contemplate only four species, namely, ZA (red), AO (green), AA (blue), and ZO (black). These are subject to proton exchange that is particularly intense during the first 10 ps and

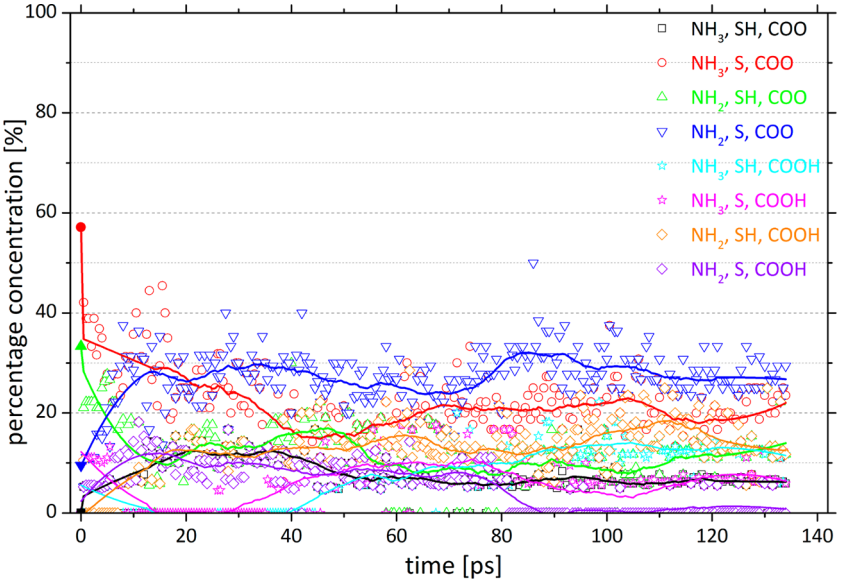

Figure 3. Percentage concentration of different Cys species in the surface slice during the reactive dynamics simulation.

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Table 2. Percentage Concentration of Cys Species from Tritation Curves (Exp.) and from Reactive Dynamics Simulations

	NH_3–SH–COO	NH_3–S–COO	NH_2–SH–COO	NH_2–S–COO	NH_3–SH–COOH	NH_3–S–COOH	NH_2–SH–COOH	NH_2–S–COOH
exp.	8.0	56.5	27.5	8.0	0.0	0.0	0.0	0.0
bulk	10.8	63.4	19.1	6.7	0.0	0.0	0.0	0.0
surface	6.4	19.3	12.1	27.0	12.7	7.1	14.2	1.1

that, at the end of the present simulation, tends to give a completely different chemical composition of the surface slice.

The percentages of the Cys species predicted at equilibrium in the different regions of the aqueous solution are compared, in the following Table 2, with the values obtained from the titration curves (Figure 1) at pH = 9.5.

The values obtained for the bulk slice are rather close to the experimental ones, while for the surface slice, the percentage of the four species (all having a COO^- group) that are the only components in the bulk sum up to about 64%. The remaining 36% is made up of "new" species all having the COOH group.

For the aqueous solution of Cys studied here, the changes in distribution of protonated species from the bulk to surface are well-reproduced by the theoretical simulations. Furthermore, the simulations predict the presence on the surface of species with COOH groups, which are not present in the bulk. Moreover, the four species of cysteine present in the bulk have concentrations at the surface region that cannot agree with any single value of pH according to the titration curves. In our view, this is because such titration curves derive from macroscopic (bulk) studies and are not adequate to describe the chemical equilibrium at the surface of the solution. A microscopic description of the surface, as offered by the present molecular dynamics calculations, predicts differences between bulk and surface, in good agreement with the experiment. Our experimental data do not allow for a direct measurement of the hydronium ion concentration, and our theoretical simulation should be extended for this purpose to a much larger model that is presently inconceivable. The present theoretical results do not prove the existence of a propensity of hydronium for the aqueous surface in neat water, as might be naively suggested by the appearance in the surface region of the Cys solution of protonated species (COOH group) that are missing in the bulk, but they do not rule it out either.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental methodology and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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