Guanidinium group is protonated in a strongly basic arginine solution

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Abstract: Knowledge of the acid dissociation constant of an amino acid has very important ramifications in the biochemistry of proteins and lipid bilayers in aqueous environments since charge and proton transfer depend on its value. The acid dissociation constant for the guanidinium group in arginine has historically been posited as 12.5, but there is substantial variation in published values over the years. Recent experiments suggest that the dissociation constant for arginine is much higher than 12.5, which explains why the arginine guanidinium group retains its positive charge under all physiological conditions. In this work, we use X-ray photoelectron spectroscopy to study unsupported, aqueous arginine nanoparticles. By varying the pH of the constituent solution, we provide evidence that the guanidinium group is protonated even in a very basic solution. By analyzing the energy shifts in the C and N X-ray photoelectron spectra, we establish a molecular level picture of how charge and proton transport in aqueous solutions of arginine occur.

Amino acids play important roles in living organisms, serving as the elementary building blocks of proteins and intermediates in metabolism. Arginine, an amino acid possessing a guanidinium and a glycine moiety, plays ubiquitous roles in protein interactions. For example, it is widely used for inhibiting protein aggregation,[1] protein refolding,[2] solubilization of proteins,[3] and protein formulation.[4] In these interactions, the guanidinium group of arginine is often involved because it has one of the highest acid dissociation constant (pK_a) values of all amino acids and is believed to be protonated and positively charged, even when buried in a highly hydrophobic environment.[5] This unusual ability of the guanidinium group has motivated scientists to revisit its pK_a value. The textbook pK_a value of guanidinium group of 12.5 has been used for many decades, but due to the difficulty in measuring multi-step acid-base equilibria under highly alkaline conditions, there has been substantial variation in published pK_a values over the years.[6] Recently it was found that the pK_a value of the guanidinium group is 13.8,[7] which has major ramifications on the stability of proteins in aqueous solutions,[8] the charge states of arginine in internal positions within a protein,[9] and charge carriers in voltage-sensitive ion channels.[10]

X-ray spectroscopic techniques are powerful tools in probing electronic structures of amino acids. To date, most X-ray studies of amino acids have been restricted to the solid state[11] or the gas phase.[12] However, these studies do not necessarily represent the biologically relevant electronic structures; biological chemical reactions occur in an aqueous environment. Amino acids exclusively exist in the neutral (molecular) form in the gas phase,[13] and prefer the zwitterionic form in the condensed solid-state phase.[11h, 14] However, depending on the pH of a solution, amino acids can exist in a wide variety of charge states in aqueous environment. They can exist dominantly as cations, zwitterions, and anions in acidic, neutral, and basic solutions, respectively. Several X-ray absorption,[15] X-ray emission,[16] and resonant inelastic X-ray scattering[17] studies have been conducted for glycine, proline, and lysine to investigate the change of their electronic and geometric structures by varying the pH of their solutions. Recently, the development of the micro-liquid jet technique has allowed X-ray photoelectron spectroscopy (XPS) to probe the electronic structure of highly volatile aqueous solutions.[18] To date, the XPS studies of lysine,[19] glycine,[20] and imidazole[21] (the side chain of histidine) in aqueous solutions have been performed. In these studies, the spectral energies of the C1s and N1s photoemission peaks shift as a function of pH due to changes in the protonation state of the amino acids. As a result, XPS can be used to detect the protonation and deprotonation forms of the arginine guanidinium group at different pH conditions.

In this work, we use a velocity map imaging spectrometer combined with an atomizer and aerodynamic lens (see experimental details in Supporting Information),[22] to measure C1s and N1s spectra of aqueous arginine nanoparticles generated from acidic, neutral, and basic solutions (pH values of 1, 7, and 13, respectively). By tracking the energy shifts of the C1s and N1s peaks at changing pH, we obtain a molecular-level electronic and geometric picture of arginine.

C1s photoelectron spectra of aqueous arginine nanoparticles, generated from solutions at pH of 1, 7, and 13 and measured with a photon energy of 310 eV are presented in Fig. 1(a, b, and c) with black dots. The spectra are expressed in terms of binding energy (BE). A “building block” approach is applied to the XPS analysis of arginine. Because one end of the arginine molecule holds a guanidine moiety and the other end is a glycine moiety, the building blocks are based on these units. We measured the XPS of glycine and guanidine hydrochloride nanoparticles generated from aqueous solutions with the same concentration (0.1 mol/L) and pH conditions as the arginine solutions. The BEs of the functional groups in glycine and guanidine hydrochloride are not exactly the same as the corresponding groups in arginine; however, these measurements enable the analysis of the C1s and N1s spectra of arginine.

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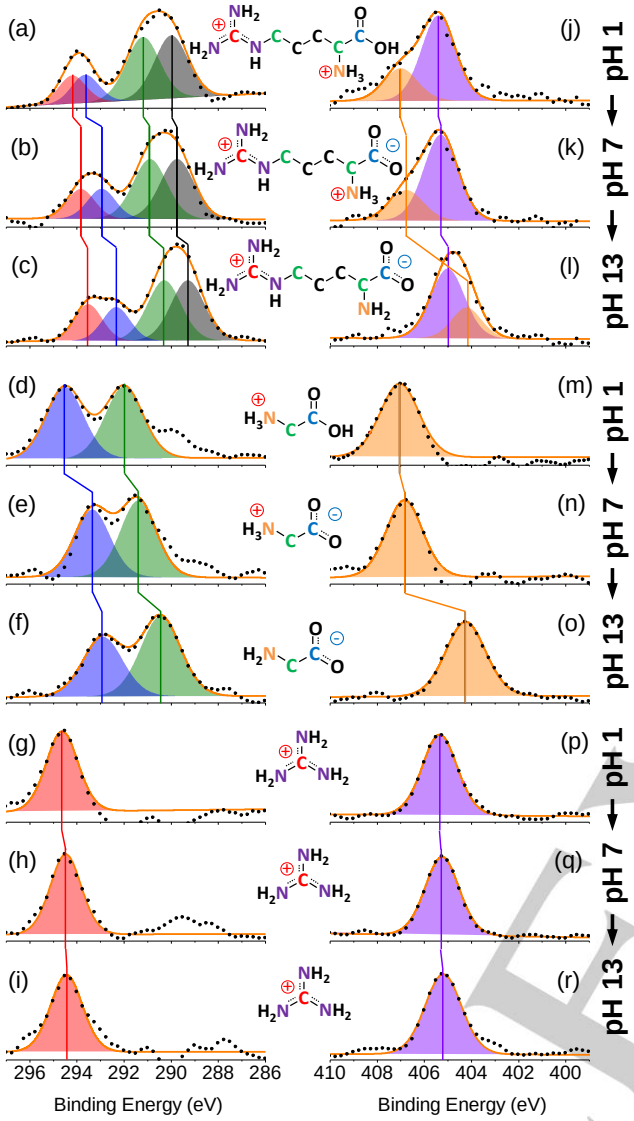

Figure 1. C1s (a-i) and N1s spectra (j-r) and corresponding molecular structures of arginine, glycine, and guanidine hydrochloride aqueous solutions at different pH conditions. The C atom in guanidinium group is labeled with red color, C atom in carboxylic group with blue color, C-N with green color and C-C with black color. The N atom in guanidinium group is labeled with purple color and N atom in amino group with yellow color. The experimental spectra are plotted with black dotted lines and the resulting fit to the entire spectrum is plotted with solid orange lines. The peak fits to the experimental spectra are overlaid with the same colors as the corresponding C atoms in the molecular formulas.

For glycine at pH 1, both carboxyl and amino groups are protonated (according to Fig. S1 in Supporting Information). The carboxyl C and the methylene C bonded to the protonated amino group in glycine give rise to the signals at 294.5 and 292.0 eV (Fig. 1d and Table 1), respectively. When the pH is increased to 7, the carboxyl group becomes deprotonated and a significant shift of 1.1 eV to lower BE is observed (Fig. 1e). Protonation of the amino group remains unchanged, resulting in a small shift of 0.6 eV. Increasing the pH to 13, the amino group becomes deprotonated, resulting in a negative shift of the C-N BE by 1.0 eV (Fig. 1f), while the carboxylate group has a smaller shift of 0.5 eV. The energy shifts of glycine engendered by changing pH in the current measurement are very close to those measured in Ref. 20 with the liquid jet method. The slight difference can be attributed to some uncertainties in the pH value measurement, and to temperature effects because the aqueous nanoparticles are thought to be super-cooled after evaporation.[23] To confirm the effectiveness of the nanoparticle method in probing the electronic structures of solvated species, we also measured partial electron-yield near edge x-ray absorption fine structure (NEXAFS) spectra (experimental details are given in Supporting Information) of aqueous glycine nanoparticles generated from acidic and basic solutions (Fig. S2) and compare them with those measured in Ref. 15b using the liquid jet method. At the C1s edge, the positions of the 1s → $\pi^*_{C=O}$ transition are reproduced very well: a red shift of 0.15 eV is observed in both our nanoparticle and the previous liquid jet[15b] measurements when the pH changes from 1 to 12. At the N1s edge, a large red shift in the main edge and an appearance of two sharp pre-edge resonances at 401.3 and 402.5 eV induced by the deprotonation of the amine group are also observed in both aqueous nanoparticle and liquid jet measurements. The good agreement between the XPS and NEXAFS spectra of glycine reported here and those from liquid jet suggests that the evaporative cooling of aqueous nanoparticles may not lead to a significant change of the structures of solute molecules in nanoparticles.

The previous study[24] suggested existence of species at the surface of a liquid jet different from those, observed in the bulk, because of the surface effect. In our study on glycine nanoparticles and in Ref. 20, the intensity of COOH (or COO-) peak is nearly the same as that of C-N peak under each pH condition, which is consistent with the stoichiometric ratio of corresponding C atoms in glycine molecule. As a result, the surface effect has nearly no influence on the protonation (or deprotonation) state of glycine at the surface of glycine nanoparticles in our method.

For guanidine hydrochloride solution at pH 1, the protonated guanidinium group gives rise to a signal at 294.6 eV (Fig. 1g). With a pK_a of 13.6, most of the guanidinium groups in guanidine hydrochloride solution are still protonated at both pH 7 and 13 (Fig. S3). As a result, its BE shifts to a lower value by only 0.1 eV (Figs. 1h and 1i), when pH is increased from 1 to 7 and from 7 to 13.

In arginine, there are four types of carbon in different chemical environments, labeled with different colors in Fig. 1: one C atom in a protonated guanidinium group (red color), one C atom in a carboxyl group (blue color), two C-C atoms (black color), and two C-N atoms (green color). According to the relative BEs of C atoms in glycine and guanidine hydrochloride, the small peak with higher BE in arginine C1s spectrum at pH 1 (Fig. 1a) is due to the overlap of signals from guanidinium group and carboxyl group, while the large peak with lower BE contains overlapped signals from C-N and C-C. Based on this hypothesis, the C1s spectrum of arginine at pH 1 is fit with four peaks (shown in Fig. 1 and overlaid with the same colors as the corresponding C atoms in molecular formulas). The peak positions obtained in fitting are listed in Table 1. When pH is increased to 7, the carboxyl group is deprotonated, while the

environments of guanidinium group, C-N, and C-C have nearly no change. The small peak in C1s spectrum becomes a little broader (Fig. 1b), which is due to the shift of the carboxylate group. After fitting, it is found that the BE of carboxylate group shifts by 0.7 eV, while those of guanidinium group, C-N, and C-C shift by 0.4, 0.3, and 0.2 eV, respectively. At pH 13, the amino group of arginine becomes deprotonated. If the pK_a of arginine is 12.5, then 75% of guanidinium group will be deprotonated (Fig. S4), but if the pK_a value is 13.8, then most of the guanidinium groups remains protonated (Fig. S5). It is obvious that the small peak becomes broader and splits into two peaks with BE of 293.5 and 292.3 eV (Fig. 1c). The BE of the carboxylate carbon at pH 7 is 292.9 eV. When the pH is increased to 13, it should shift to a lower BE because the carboxyl group remains deprotonated and the entire charge state of arginine decreases from +1 at pH 7 to 0 or -1 at pH 13, depending on whether the pK_a of guanidinium is 13.8 or 12.5, correspondingly. Hence, the peak at 292.3 eV is due to carboxylate group while the peak at 293.5 eV is due to the guanidinium group. Compared to that at pH 7, the BE of guanidinium group at pH 13 shifts to a lower value by only 0.3 eV. The small shift suggests that most of the guanidinium groups are still protonated at pH 13, which may indicate that the pK_a is larger than 13, consistent with the revised pK_a value of 13.8 in Ref. 7. Note that both pH and pK_a are temperature dependent. As far as we are aware, there has been no experimental study on the temperature of nanoparticles at the point of photoionization after evaporative cooling. According to the calculation in Ref. 23, nanoparticles are expected to exist in a super-cooled state at the photoionization point. When temperature decreases, the equilibrium concentration of hydrogen ion (H^+) in a basic solution decreases because the self-ionization of water and generation of H^+ is an endothermic process. As a result, the pH of aqueous nanoparticles will become larger. However, the concentration of hydroxide ion (OH^-) in the cooled, basic solution does not change significantly because most of the OH^- ions are from the solvation of NaOH. Thus, the change of pH should have nearly no effect on the protonation state of the guanidinium group at low temperature. For the pK_a of the guanidinium group, one study suggests that it increases from 11.74 to 12.01 when temperature decreases from 333 to 283 K.[25] However, there have been no studies on the pK_a of guanidinium group at lower temperature, and it is not possible to quantitatively determine the change of pK_a induced by the decrease in temperature. By comparing the XPS and NEXAFS spectra of glycine obtained in our nanoparticle experiment and those obtained with the liquid jet method, it appears that the temperature does not have a significant influence on the structure of amino acid molecules in aqueous nanoparticles. However, a quantitative determination of temperature effects is important and will be considered in future studies.

The N1s PE spectra of glycine, guanidine hydrochloride, and arginine aqueous solutions are shown in Fig. 1j-1r. The series of spectra are measured at exactly the same pH values as the C1s spectra with the photon energy of 425 eV. For glycine at pH 1 and 7, the amino group is protonated and gives rise to signals at BEs of 407.0 and 406.8 eV, respectively (Figs. 1m and 1n). The deprotonation of amino group at pH 13 leads to a large shift of 2.6 eV for its BE (Fig. 1o).

Table 1. Summary of all measured C1s and N1s binding energies (in eV) for guanidine hydrochloride, glycine, and arginine aqueous nanoparticles generated at different pH conditions. Gua, Gly, and Arg denote guanidine, glycine, and arginine, respectively. C_{gua} denotes C atom in guanidinium group, while N_{gua} and N_{amino} denote N atoms in guanidinium and glycine groups, respectively.

	Gua	Gly	
	C_{gua}	COOH	C-N
pH 1	294.6	294.5	292.0
pH 7	294.5	293.4	291.4
pH 13	294.4	292.9	290.4

	Arg			
	C_{gua}	COOH	C-N	C-C
pH 1	294.2	293.6	291.2	290.0
pH 7	293.8	292.9	290.9	289.8
pH 13	293.5	292.3	290.3	289.3

	Gua	Gly	Arg	
	N	N	N_{amino}	N_{gua}
pH 1	405.4	407.0	407.0	405.4
pH 7	405.3	406.8	406.7	405.3
pH 13	405.2	404.2	404.2	405.0

The guanidinium group in guanidine hydrochloride at pH 1 is protonated and gives rise a signal at a BE of 405.4 eV (Fig. 1p). It is still protonated at pH 7 and 13, so only small shifts (0.1 and 0.2 eV) are observed when pH is increased to 7 and 13 (Figs. 1q and 1r).

According to the BEs of glycine and guanidine hydrochloride at different pH conditions, the N1s spectra of arginine are fit using two peaks. The amino group and guanidinium group give rise to signals at 407.0 and 405.4 at pH 1, respectively (Fig. 1j). The spectrum at pH 7 (Fig. 1k) is very similar to that at pH 1, only the two peaks shift to lower BEs by 0.3 and 0.1 eV, respectively. At pH 13, the amino group is deprotonated and shifts to lower BE by 2.5 eV. The guanidinium group shifts by only 0.3 eV (Fig. 1l), suggesting that it is still protonated. This picture of the guanidinium being protonated even at very high pH is consistent with that observed in the C edge X-ray spectra.

In summary, X-ray photoelectron spectroscopy of aqueous arginine nanoparticles allows for determination of the protonation/deprotonation forms of amino acid at different pH conditions. We find that the guanidinium group in arginine is protonated in a strong basic environment, which may mean that the pK_a of the guanidinium group in arginine is larger than 13, consistent with the revised pK_a value of arginine in Ref. 7. This result explains why the guanidinium group can remain positively charged in a hydrophobic, confined environment, such as inside a protein or a lipid membrane. In addition, the temperature effect on the pK_a should be considered and will be the subject of future studies. The method of X-ray photoelectron spectroscopy of

aqueous nanoparticles promises to provide a window inside the chemistry of confined spaces, for instance, salt transport within a protein, or ion migration in synthetic systems such as nanotubes and vesicles.

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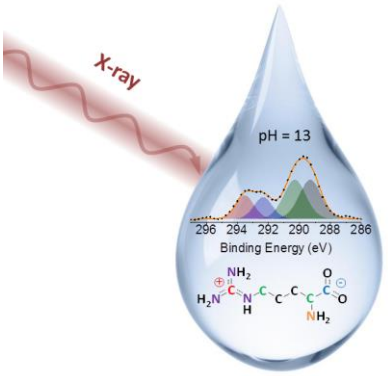
Entry for the Table of Contents

COMMUNICATION

Proton island in a sea of base:

X-ray photoelectron spectroscopy on aqueous arginine nanoparticles is used to probe the local electronic structure as a function of pH. The results reveal that the guanidinium group remains protonated even in an extremely basic environment, suggesting a revised pK_a value for arginine.



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