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pH-Induced Protonation of Lysine in Aqueous Solution Causes Chemical Shifts in X-ray Photoelectron Spectroscopy

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Abstract: We demonstrate the applicability of X-ray photoelectron spectroscopy to obtain charge- and site-specific electronic structural information of biomolecules in aqueous solution. Changing the pH of an aqueous solution of lysine from basic to acidic results in nitrogen 1s and carbon 1s chemical shifts to higher binding energies. These shifts are associated with the sequential protonation of the two amino groups, which affects both charge state and hydrogen bonding to the surrounding water molecules. The N1s chemical shift is 2.2 eV, and for carbon atoms directly neighboring a nitrogen the shift for C1s is ~0.4 eV. The experimental binding energies agree reasonably with our calculated energies of lysine$_{(aq)}$ for different pH values.

1. Introduction

Amino acids are the elementary building blocks of proteins. Particularly relevant in understanding biological activity is the effect of water on protein structure and how water molecules arrange around protein molecules under physiological conditions. This is highly influenced by the amino acid side chains at the surface of the protein. Importantly, the particular structure of the solvated amino acid is very sensitive to the pH of the solution, affecting biological activity; e.g., in one of the key steps to activate ribulose-1,5-bisphosphate carboxylase/oxygenase (responsible for carbon fixation in photosynthesis) a CO_2 molecule is added to the side-chain amino group of lysine 201. The carbamate formation is favored by alkaline pH and thus contributes to the increased activity at higher pH values.[5]

In the present work we use photoelectron (PE) spectroscopy, a powerful atomic site-sensitive technique,[6] to identify the electronic signature of individual nitrogen and carbon atoms of the amino acid lysine in a liquid water environment. We focus here on the changes of the PE spectra as a function of pH since biological activity is modulated by the pH of water, which decides whether a certain functional group is charged and hence drives structural modifications. In acidic aqueous solutions, amino acids exist as cations with their amino group being protonated while the carboxyl group is not protonated. At intermediate pH values amino acids form charge-neutral (dipolar) zwitterions, where the amino group is protonated. In basic solutions, amino acids exist as anions with an unprotonated, neutral amino group.

X-ray spectroscopic techniques, such as X-ray absorption (XA) and X-ray PE spectroscopy (XPS), are among the most versatile techniques for studying the local electronic structure of matter and have been extensively applied to the standard amino acids.[7−19] Yet, these investigations have long been restricted either to thin films or to the gas phase. Note that the aforementioned cationic and anionic forms are unique to the liquid phase. Amino acids in the gas phase exclusively exist in the neutral (molecular) form, and in the condensed solid-state

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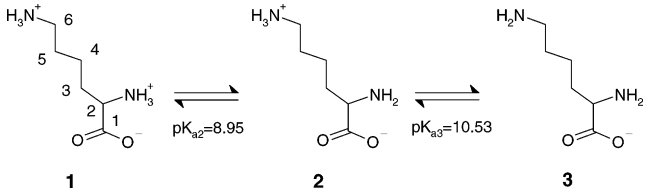

Figure 1. Lewis structure of the amino acid lysine at different pH values. Depending on the pH value two (**1**), one (**2**), or none (**3**) of the amino groups are protonated.

phase amino acids exist in the zwitterionic form. This makes liquid solvation predictions from the gas- and solid-phase spectroscopic data nearly impossible as condensation and charge-state contributions are hardly separable.

Two recent XA spectroscopic studies of aqueous amino acids measured spectral changes at the nitrogen and carbon K-edge of aqueous glycine and lysine;[20,21] these changes were suggested to reveal details on the ability of the ammonium moiety to form hydrogen bonds with surrounding water molecules. PE spectroscopic studies from aqueous solutions are in general scarce,[6] and to date, there have been no reports on amino acids in aqueous solutions. In the aqueous phase these studies have only recently become possible after overcoming problems with both the detection of electrons above the vaporizing water surface and the complexity of generating a stable free liquid water surface in high vacuum.[6] PE spectroscopy provides complementary information to XA spectroscopy, the former probing occupied rather than empty states. A major advantage of the former technique is its ability to access absolute electron-binding energies, even of the solvated species and their constituent atoms, which is demonstrated in the present work. Notably, the C1s energies can also be readily accessed, whereas the analysis of XA spectra at the carbon K-edge is often complicated due to adsorbed carbon on the optical elements.

Here we investigate aqueous lysine, which is one of the essential and proteinogenic amino acids. The Lewis structure of the molecule for different charge states is shown in Figure 1. The *n*-butylamine side chain is responsible for the basic reaction of lysine in aqueous solution. At pH 6 both amino groups are protonated (Figure 1, **1**). Near the isoelectric point, close to pH 9.5, only the amino group of the side chain is protonated, and the α-amino group is not (Figure 1, **2**). Above pH 10.5 both amino groups are deprotonated (Figure 1, **3**) (see also Figure 4). In the present study, we show that PE spectroscopy has the sensitivity to pick up these pH-induced local changes of both functional groups by spectral energy shifts (chemical shifts) of the N1s and even of the C1s photoemission peaks. The experimental N1s and C1s energy shifts will be compared with calculated energies, using ab initio methods.

In proteins the carboxyl and amino groups form peptide bonds, and the amino acid remains neutral unless the side chain is charged. These amide bonds will have a significant influence on atoms in the backbone and short side chains. Through-bond interactions are of short range (see Discussion), and for larger side chains as in lysine the effect of peptide bonds will be negligible. Furthermore, the charges are shielded by the solvent which reduces through-space interactions. Accordingly, free lysine versus lysine at the surface of a protein will probably show very similar results for the side-chain amino groups, whereas the properties of the backbone will be different. The investigation of the properties of the free amino acid will therefore be the first step for a better understanding of the effect of peptide bondings to individual properties of the amino acids.

We note that the XPS chemical shifts determined here directly reveal the different electron-binding energies of a given atom species, but located in different local environments. In contrast, nuclear magnetic resonance (NMR) chemical shifts that have been widely used to determine the degree of fractional protonation (including amino acids[22]) originate from molecular electron-cloud shielding effects on an external magnetic field. Compared to XPS chemical shifts, the shifts observed in NMR can be better resolved which allows the clear distinction of a much larger number of atoms of the same type. On the other hand, XPS gives a distinct access to all elements, e.g., sulfur or organometallic compounds. This could be exploited in future experiments by using labeling techniques. In addition, PE is a fast process ($\sim 10^{-17}$*s*) which can provide snapshots of fast-changing structures. Thus, XPS can provide information on issues where other methods, e.g., NMR, are either insensitive to the crucial elements or too slow for the process under investigation.

2. Methods

2.1. Experimental. Photoemission experiments on a 10-μm liquid jet were performed at the soft X-ray undulator U41 PGM beamline of the synchrotron radiation facility BESSY (Berlin, Germany). Photon energies in the present study were 380, 480, and 600 eV, which allows probing for carbon, nitrogen, and oxygen within the same probing depth of approximately 5−10 monolayers of water.[27] Although the measured signal is integrated over the whole probing depth, the contribution from lysine at the surface is negligible as lysine is not surface active. The liquid-jet velocity was 60 m/s; all data shown here were recorded at 4 °C jet temperature. For 3−5 mm downstream from the nozzle the jet flow is laminar with a smooth cylindrical surface. These conditions allow photoelectrons from the liquid phase to travel a sufficiently long distance without collisions with gas-phase water molecules. Furthermore, at this small diameter of the jet, irradiation-induced charging will not affect the PE spectra. Details of the experimental setup have been described elsewhere.[23,24] Briefly, photoelectrons pass through a 200-μm diameter orifice, which differentially pumps the jet main chamber (operating at 10^{-5} mbar from the electron detection chamber (operating at 10^{-9} mbar)) housing a hemispherical electron energy analyzer equipped with a multichannel detector. Electron detection was performed normal to both the synchrotron-light polarization vector and the propagation of the liquid jet with an electron spectrometer acceptance angle of 12°. The small focal size (23 × 12 μm) at the U41 PGM beamline allows for a good spatial overlap between the synchrotron light beam and the liquid jet. Energy resolution of the beamline was better than 0.2 eV at 600 eV photon energy. The resolution of the hemispherical energy analyzer was constant with kinetic energy (about 0.2 eV, at a pass energy of 20 eV). Typical count rates were 10^3 s^{-1} at a photon energy of 600 eV. Calibration of the electron kinetic energies is referenced to the O1s binding energy of liquid water, which can be measured simultaneously with the C1s and N1s spectra.

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S(+)-Lysine monohydrochloride (for synthesis) was purchased from Merck and was used without further purification. A 0.5 m solution of lysine monohydrochloride in highly demineralized water was used for all experiments. The pH value of the room-temperature solution was adjusted either with sulfuric acid or with sodium hydroxide; the pH was controlled with a pH meter (766, Knick) equipped with a single-rod measuring cell (SE 100, Knick), and an accuracy of ±0.1 pH units.

2.2. Theoretical Methods. To model the protonation and solvation effect on the N1s and C1s core-level BEs we have applied the recently improved solvation models of the Gaussian03 program package.[25] All calculations were carried out using density functional theory with the B3LYP functional and the aug-cc-pVDZ basis set provided by Gaussian03.

A standard approach to include solvent effects into high-level ab initio calculations are self-consistent reaction field (SCRF) methods. Here, the solvent is modeled as a continuum (called the reaction field), described by a uniform dielectric constant, and the solute is embedded into a cavity within the solvent. The different SCRF models differ in the definition of the cavity and the reaction field.

We have used Tomasi's polarized continuum model (PCM), where the cavity is constructed from a series of interlocking atomic spheres; the effect of polarization by the solvent continuum is calculated numerically which increases the accuracy of the calculations as compared to the simpler Onsager reaction field model, where the solute occupies a fixed spherical cavity within the solvent field.[26,27]

For each charge state the geometry was optimized separately, yielding extended structures (Structures and frequencies are available as Supporting Information.) with no through-space interaction between the side chain and the backbone. These optimized geometries were used for single-point calculations. Vibrational frequencies were calculated to identify saddle points. Molecular orbital energies were taken as BE because Gaussian03 cannot handle core−hole excited states which would give access to better methods for determining C1s and N1s binding energies. Program packages which can handle core−hole excitation do not implement a solvation model. We think that it is very important to include a solvation model for lysine. Without a solvation model the long chain of the molecule will form a ring-shaped structure. This causes a strong interaction of the positive and negative charge which is not present in solution. This is especially true for the zwitterion. The solvation model forces the molecule into a linear geometry and includes dipole interactions which is much closer to the conditions of the experiment. Only the energetical order of the binding energies is used for the interpretation of the experimental data, and this should be much more reliable for the linear structure.

3. Results

3.1. N1s Binding Energy. Figure 2 presents nitrogen 1s PE spectra of 0.5 m aqueous lysine solutions, at pH 13.0, 9.5, and 5.5, measured for 480 eV photon energy. The circles show the experimental data, the thinner solid lines are the individual peak fits, and the heavier solid lines are the resulting fits to the entire spectra (see below). Energies in the figure are given as electron-binding energies (BE), with the absolute energy scale being fixed relative to the O1s BE of liquid water (538.1 eV).[27] Intensities are normalized to the same maximum peak height.

All PE spectra were fitted with two Gaussians—one for each nitrogen. In the cases of pH 13 and pH 5.5, we assume the same cross sections, intensities, and peak width for both nitrogens, since both amino groups are either neutral or protonated. Accordingly, the fitted parameters were the two independent peak positions, and one peak width and height.

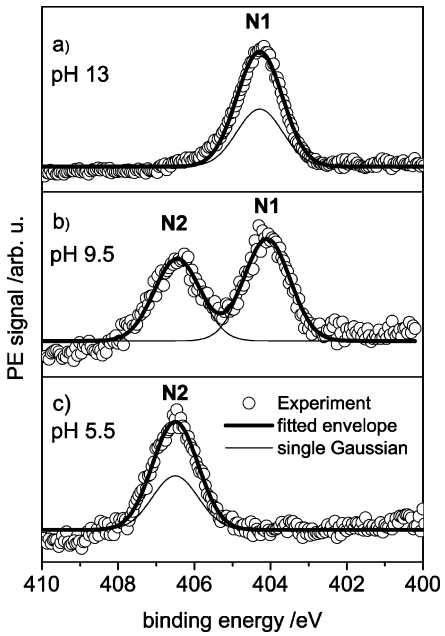

Figure 2. N1s PE spectra of aqueous lysine at different pH values. **N1**, **N2** are peak labels. The circles are the experimental data, and solid lines are fits. Intensities are normalized to the maximum peak height of each spectrum. (a) At pH 13, both amino groups are neutral and have the same BE of 404.3 eV. (b) Protonation of one amino group increases the N1s BE to 406.5 eV. As one amino group remains neutral, two peaks are observed. (c) At pH 5.5, both amino groups are protonated, which yields a single N1s peak with a BE of 406.5 eV.

Table 1. Fitted Experimental and Calculated N1s BEs[a] of Aqueous Lysine for Three pH Values

	pH 13		pH 9.5		pH 5.5	
assignment	fitted	calculated	fitted	calculated	fitted	calculated
N^2	404.3	389.1	404.1	389.2	406.5	392.8
N^6	404.3	389.4	406.5	393.4	406.5	393.5

[a] BEs are given in eV and assigned to the atom numbers in Figure 1.

At pH 13 (anion **3**), the PE spectrum exhibits a single peak, **N1**, at 404.3 eV BE, consistent with both amino groups being neutral. Apparently, under these conditions the N1s PE signals from the two nitrogen atoms are indistinguishable. At pH 9.5, i.e., for the case of one amino group being protonated and the other being neutral (zwitterion **2**), **N1** is shifted to a slightly lower BE (404.1 eV). In addition, a second peak, **N2**, appears at a higher BE, 406.5 eV. Lowering the pH to 5.5, at which point both amino groups are protonated (cation **1**), results in the single peak, **N2**, with the exact same peak position as found for pH 9.5, i.e., 406.5 eV BE.

For the **N1** peak structure in Figure 2a the best fit to the experimental PE spectrum was obtained if both Gaussians had exactly the same peak position of 404.3 eV. The same holds for the single peak **N2** in Figure 2c, except that the respective BE is higher, at 406.5 eV. For the PE spectrum at pH 9.5 (Figure 2b) the zwitterion **2** is dominant near the isoelectric point, but **1** and **3** are also significantly abundant (cf. Figure 4). Thus, the peak area was here fitted independently for both Gaussians, yielding BEs of 404.1 and 406.5 eV. The experimental full widths at half-maximum (fwhm) obtained from Figure 2 are 1.4 eV for pH 13 and pH 9.5, and 1.5 eV for pH 5.5. Calculated and fitted BEs are summarized in Table 1.

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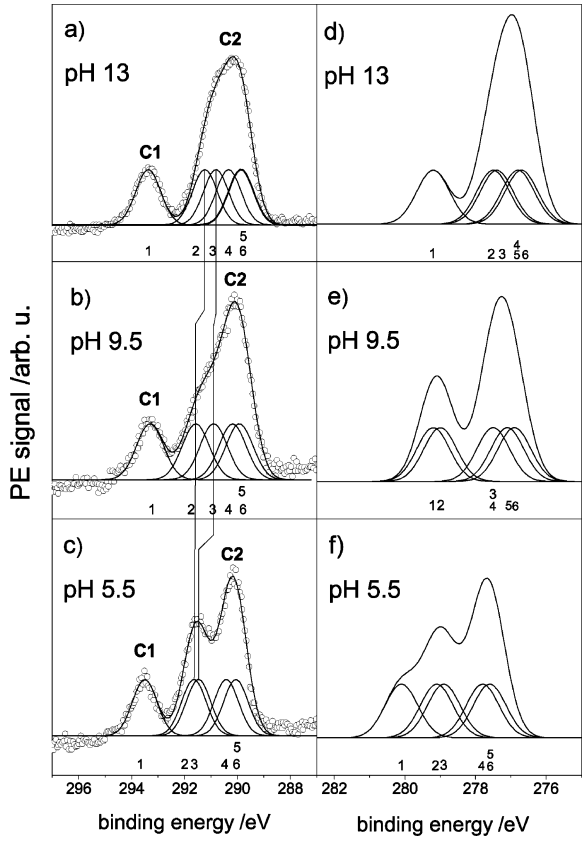

Figure 3. C1s PE spectra of aqueous lysine, measured (a−c) and calculated (d-f). Sequential protonation of the amino groups causes a shift in the BEs of the respective adjacent carbon atoms (indicated by vertical lines). Intensities of the experimental spectra are normalized at the peak height of **C1**, which is the C1s emission from the carboxylic carbon atom, C^1. Each experimental spectrum was fitted with six Gaussians (**G1**−**G6**) of identical widths and intensities. Small numbers are Gaussian peak labels, which should not be confused with the atom numbers in Figure 1. The simulated spectra were obtained by representing the emission from each carbon atom by a 1.1 eV wide Gaussian at the respective calculated BE.

3.2. C1s Binding Energy. The C1s PE spectra of aqueous lysine are shown in Figure 3. This series of spectra was measured at exactly the same pH values as the N1s spectra in Figure 2, using a photon energy of 380 V. The experimental spectrum (circles) at pH 13 exhibits a smaller peak (**C1**) at 293.4 eV, and a larger feature, **C2**, with a maximum at 290.4 eV BE. The broad shape of the latter immediately suggests that this feature contains contributions from the various carbon atoms, with different local environments, arising from slightly different BEs. For pH 9.5, a shoulder appears near 291.5 eV, while **C1** remains entirely unaffected. At pH 5.5 this shoulder has evolved into a distinct peak in the PE spectrum.

In order to analyze the measured C1s spectra, we have fitted each spectrum with six Gaussians (**G1**−**G6**) of identical width and intensity to represent the emission signal from each of the six carbon atoms. The procedure is facilitated by the existence of the single isolated peak **C1**. This peak, at 293.4 eV, can be associated with the carboxylic carbon (C^1), which is known to have the highest BE in amino acids.[12,15,19] Thus, one Gaussian was used to fit **C1**, and five Gaussians were used to fit **C2**. Photoionization cross-section variations are small and can be neglected. The results of the fit are given in Table 2. In all cases the fitted fwhm was 1.1 eV. For simulating the measured spectrum, the calculated BEs were represented by Gaussians with a fwhm of 1 eV, i.e., similar to the fitted Gaussians of the experimental data. The resulting envelope can be easily compared to the measured spectrum.

Table 2. Fitted Experimental and Calculated C1s BEs[a] of Aqueous Lysine for Three pH Values

peak no.	assignment	pH 13		pH 9.5		pH 5.5	
		fitted	calculated	fitted	calculated	fitted	calculated
1	C^1	293.4	279.2	293.4	279.2	293.4	280.1
2	C^6	291.3	277.5	291.7	279.0	291.6	279.1
3	C^2	290.8	277.4	291.0	277.5	291.3	278.9
4	C^5	290.4	276.8	290.3	277.5	290.4	277.8
5	C^3	289.9	276.8	290.0	276.9	289.9	277.6
6	C^4	289.9	276.7	290.0	277.1	289.9	278.6

[a] Energies are given in eV. The first column are the Gaussian numbers of Figure 3, and the second column are the atom numbers (Figure 1).

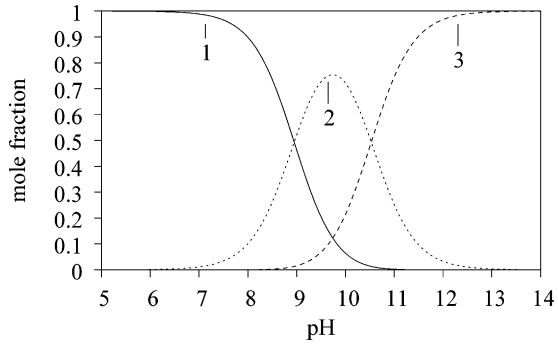

Figure 4. Mole fractions of the three different lysine charge states (cf. Figure 1) as a function of the pH value. The cationic **1** and anionic **3** charge state can be populated to nearly 100%. The zwitterion **2** has a maximum of 75% at the isoelectric point (pH 9.74) due to the similar pK_a values of the two amino groups.

4. Discussion

4.1. Nitrogen 1s. The observed two different N1s PE peaks with pH-dependent amplitude ratios in Figure 2 can be understood quantitatively by considering the fractional protonation of the different amino groups as a function of pH. According to the law of mass action the distribution of **1**−**3** depends on the pH value, as depicted in Figure 4. The mole fraction plots show that (with the known pK_a values 10.53 and 8.95[28] for N^6 and N^2) at pH 13 more than 99% of all lysine molecules exist as anions **3**, in agreement with the appearance of a single N1s core electron peak in Figure 2a. Both amino groups are neutral, and the two nitrogens appear to have the same BE of 404.3 eV, although they are not in exactly identical local environments.

From Figure 4, the zwitterion **2** near pH 9.5 is found to amount to about 70%; the remaining lysine molecules are in charge states **1** and **3**. At the isoelectric point (pH 9.74) both peaks (**N1**, **N2**) would have the same intensity. The experimental data (Figure 2b) show a marginally higher intensity for **N1** which corresponds to a pH of 9.9. We attribute the difference to some uncertainties in the measurement of the pH value, and to a slight temperature effect as the pH was measured at room temperature but the solution is cooled. The observed 2.2 eV higher binding energy (406.5 eV; see Figure 2b,c) of the protonated (side chain) amino groups is a result of the additional

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positive charge at the nitrogen site. The slightly lower N1s BE of the unprotonated amino group at pH 9.5 (404.1 eV) as compared to that at pH 13 (404.3 eV) is likely due to some rearrangement of the solvation shell. Alternatively, the lower BE might be associated with conformational changes, e.g. back-folding of the positively charged amino group toward the negatively charged carboxyl group. Through-bond effects would seem unlikely, given the large distance between the two amino groups (see also section 4.2). Finally, at pH 5.5 more than 99% of all lysine molecules adopt the two-site protonated form **1** which explains the existence of a single peak at the lower binding energy (406.5 eV).

Next, we compare the measured N1s energies with our calculated energies for the different charge states. In the experiment, the N1s BE for the two nitrogens are indistinguishable when both amino groups are either neutral (Figure 2a) or protonated (Figure 2c), in qualitative agreement with our calculations. The calculations for the solvated anion **3** yield 389.1 eV BE for N^2 and 389.4 eV BE for N^6 at pH 13; the respective experimental value is 404.3 eV. The computed N1s BE for the protonated amino groups are 392.8 and 393.5 eV, which compare with 406.5 eV measured here.

We notice that the calculated N1s chemical shift for the protonated vs unprotonated amino groups is 40% larger (4 eV), as compared to the experimentally determined energy shift (2.2 eV). We attribute this discrepancy to a considerable underestimation of the shielding of the positive charge by the solvent. We expect by the presently employed Tomasi-PCM model of the Gaussian 03 program package that the experimental liquid-phase energies, as reported here, will be important for calibrating and improving theoretical models that would include hydrogen-bonding and shielding effects explicitly. We point out that the N1s chemical shifts observed in the present liquid-phase study are in good qualitative agreement with corresponding thin film studies of amino acids.[9,16−19] However, a quantitative comparison is complicated by the fact that energies were given relative to the Fermi energy (an explicit value of which was not reported).

To date, no experimental N1s BE of gas-phase lysine has been reported. Gas-phase data exist for glycine, 405.58 eV,[29] and *n*-butylamine, 404.85 eV.[30] Since an aliphatic chain reduces the BE of an attached amino group, we would expect the N1s BE to shift to lower energies for lysine relative to glycine. Hence, in the comparison of the BEs of gas-phase amino acids and aqueous lysine we infer an approximate shift of 0.5 eV to lower binding energies, which can be attributed to the solvation free energy associated with the rearrangement of the water molecules that form the solvation shell. More insight into the microscopic details of hydrogen bonding between the ammonium moiety and the surrounding water molecules can be gained if we consider the large dipole moment of the water molecules, and also its amphoteric properties, all driving toward a strong interaction between water and the polar amino group. An important aspect is whether the amino group acts as a hydrogen-bond donor or acceptor. From recent XA spectroscopic investigations, it was suggested that the protonated amino group does indeed form hydrogen bonds with the solvent. For anionic structures, the intramolecular interaction between the α-amino and the carboxyl group might be strong enough to prevent hydrogen bonding with the solvent, where the amino group acts as a donor.[20,21] The lower N1s energy in solution, as observed here, would be consistent with an increased electron density at the nitrogen site in aqueous environment, i.e. the amine nitrogen in aqueous lysine probably does act as a hydrogen-bond donor. Thus, dipole−dipole interactions, or intra- or intermolecular hydrogen bonds with the amino group as donor may be responsible for the observed shift. Despite the different hydrogen bondings for the two amino groups, both amino groups appear to have the same N1s BEs in our experiment. Therfore, further investigations are necessary in order to arrive at a more detailed picture of solvation effects at high pH. This may comprise calculations which include the presence of OH^- anions, or experiments on molecules without carboxylic groups.

4.2. Carbon 1s. For all pH values studied in Figure 3, the fitting procedure with six equally shaped Gaussian peaks yields stable and highly unequivocal peak positions and peak amplitudes. Therefore, it is possible to assign the measured pH-induced energy shifts to protonation-induced structural changes at a specific carbon site. For further comparison the experimental (fitted) and calculated BEs are summarized in Table 2. Notice that two Gaussians, **G5** and **G6**, exactly overlap at the lowest binding energy.

To infer site-specific information we consider the most pronounced energy shifts, marked by the vertical lines in Figure 3, in more detail. **G2** is shifted by 0.4 eV to a higher BE when going from pH 13 to 9.5 in Figure 3b; this leads to the shoulder at 291.5 eV BE. Since protonation of the terminal amino group in the side chain is the main change at the former pH, **G2** can be associated with the nearby carbon atom, C^6. Due to the considerable distance to the positive charge at the nitrogen site, the carbon 1s chemical shift is much smaller as compared to the nitrogen 1s energy shift (see Figure 2). **G3** shows an initial smaller shift, 0.2 eV, and a 0.3 eV shift occurs between pH 9.5 and 5.5. In full analogy to the **G2** case, we assign the latter **G3** shift to the electron density change associated with the carbon atom C^2, as this is closest to the charged α-amino group. The former, smaller **G3** energy shift is attributed to the fact that close to the isoelectric point approximately 12% of the lysine molecules exist as cation **1** (Figure 4), which is not accounted for in our fit. Compared to the pH 13 case **G3** is shifted by 0.5 eV, which is similar to the shift observed for **G2**.

The assignment of **G4** is less obvious as no distinct energy shift is observed. Apparently, the through-bond distance, over which charge-state changes of the amino group can be sensed, is not larger than the size of a methylene group. As C^6 has the slightly higher BE than C^2 (both on contact with a nitrogen atom), we would also expect a higher BE for C^5 than for C^3 (both separated by a methylene group from the nitrogen). We hence attribute the lower BE of C^2 to the +I effect of the deprotonated carboxyl group[31] (which will affect C^3 more than C^5), and **G4** corresponds to C^5, in agreement with our calculations (Table 2).

The two carbons, having the same BE (peaks **G5** and **G6**), are assigned to carbon atoms C^3 and C^4, respectively. The

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agreement between relative experimental and relative calculated energy changes, both summarized in Table 2, is fairly good. Although absolute theoretical energies are underestimated by approximately 5%, the relative BEs which are used to assign spectral features to certain atoms are reliable. The assignment based on theory is the same as derived experimentally from the variation of the pH value (Table 2).

All chemical shifts induced by the charge are overestimated in the calculations. We attribute this to the neglect of hydrogen bonds which are not included in the current method. This agrees with the larger discrepancies for nitrogen energies. The amino groups are expected to form strong hydrogen bonds with water, compared to the much weaker interaction of the aliphatic chain with the solvent. The shielding of the charge on the amino groups is not being properly described and as a result the theoretical shift is much larger than the experimental one. Therefore, the through-bond interaction to the neighboring carbon atoms is also overestimated, and the chemical shifts are too high. The through-bond interaction decreases with the distance to the localized charge, and therefore, the nitrogen is more strongly affected than the carbon.

As expected, and in agreement with previous studies, the C^1 carbon atom of the carboxyl group has the highest BE, whereas the carbons inside the aliphatic side chain (C^3 and C^4) have the lowest BE and are not affected by the pH. The absolute BE for C^3 and C^4 is similar to the corresponding BE in *n*-hexane (290.09 eV),[32] i.e. they are not affected by the substituents. Furthermore, the calculations reveal the same order in BE obtained from the above qualitative discussion, and hence our calculations fully corroborate the assumption made in the spectral fit. Finally, the strong BE effect of carbon atoms adjacent to an amino group on charge states is in reasonably good agreement between experiment and theory. Yet, as we have already discussed for N1s, the C1s chemical shift is similarly overestimated in the solvation model used here.

5. Conclusion

Using the liquid microjet preparation technique for free liquid aqueous surfaces in vacuum in conjunction with PE spectroscopy we have demonstrated that individual nitrogen and carbon atoms of aqueous lysine can be identified by their respective core-level binding energies. The technique is shown to be sensitive to monitor charge densities modified by proton attachment. Moreover, PE measurements at different pH values reveal a 2.2 eV nitrogen 1s chemical shift toward higher BEs, associated with the protonation of any of the amino groups of aqueous lysine. The sensitivity of photoemission is found to be high enough to distinguish different carbon atoms of lysine in water. Here the C1s energies of carbon atoms next to the ammonium moiety are identified by a 0.5−0.3 eV chemical redshift upon deprotonation. All experimental energies are found to be in good qualitative agreement with our tentative, moderate efforts and density functional theory calculations using commercially available software. These results highlight the potential of liquid photoemission to unravel electronic and structural details of biological molecules in their natural aqueous environment.

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Supporting Information Available: Complete ref 25; extended structures (for each charge state where the geometry was optimized separately) and frequencies. This material is available free of charge via the Internet at http://pubs.acs.org.

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