# Investigation of the Amino Acids Glycine, Proline, and Methionine by Photoemission Spectroscopy

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The valence and core level photoelectron spectra of glycine, proline, and methionine in the gas phase have been investigated by VUV and soft X-ray radiation. The outer valence band photoemission spectra are similar to previously reported He I spectra, although relative peak intensities are different due to the different photon energy. We extended the spectral range to include the inner valence region. The carbon, nitrogen, and oxygen 1s as well as the sulfur 2p core level spectra of these amino acids have been measured and the states identified. Valence band spectra of proline have been recorded as a function of temperature, and they provide information about the relative populations of the lowest energy conformers.

## 1. Introduction

The physics and chemistry of small biological molecules, such as amino acids, are fundamental for many fields, such as their behavior in solution, in biotechnological applications such as sensors, and in astrochemistry and astrobiology. Theoretical methods are now very advanced, and it is possible to calculate the properties of complex systems, such as peptides and proteins, and predict their chemical and physical behavior, for example, their free energy, infrared spectra, or folding structure. Calculations of intrinsic molecular properties require experimental spectroscopic data to check their validity, and a frequent starting point is the free molecule, where solid- or liquid-state effects are not present. For this reason gas-phase photoemission studies of biomolecules are of current interest.

There have been many theoretical and experimental investigations of free amino acids. Several authors[1−4] have reported the He I and He II photoemission spectra of the valence bands of a series of amino acids in the gas phase, and more recently Powis[5] published the valence photoemission and circular dichroism spectra of L-alanine. The gas-phase core level photoemission spectra of glycine[6] and L-alanine[7] have been reported. The core electron energy loss spectra near threshold, equivalent to the near edge X-ray absorption spectrum, of glycine, alanine, phenylalanine, and glycyl−glycine have been measured by Cooper et al.[8] Other gas-phase X-ray absorption spectra at the carbon, nitrogen, and oxygen edges have been reported recently by Marinho et al.[9] for proline, by Morita et al.[10] for alanine, and by Plekan et al.[11] for glycine, methionine, and proline.

Not all biomolecules can be studied in the gas phase due to the effects of thermal decomposition. Spectroscopic studies of amino acids in the condensed state are more numerous as problems of thermal stability are avoided and sample densities are higher. X-ray absorption spectra of solid samples have been measured by Gordon et al.[12] and Zubavichus et al.,[13,14] and the carbon edge absorption spectra of amino acids were reported earlier by Kaznacheyev et al.[15] Photoemission studies include structural studies of glycine on Cu[16,17] and the work of Löfgren et al.,[18] who adsorbed glycine on Pt(111) as monolayers and multilayers and reported the core level energies. They interpreted the spectra as indicating that the molecule was in the zwitterionic state, as only a single O 1s core level was found. Bontcev et al.[19] reported the core photoelectron spectra of condensed proline. The photoelectron spectra of glycine on the surface of hydrophilic $Si(O_2)$ have been published by Wu et al.,[20] who concluded that the stable adsorbate layer of zwitterionic glycine molecules has a tendency for molecular orientation in a direction perpendicular to the substrate surface, with $COO^-$ groups away from the surface and the $NH_3^+$ groups toward the surface. Recently Nyberg et al.[21] and Jones et al.[22] adsorbed alanine and glycine, respectively, on Cu(110) and studied these molecules by a combination of near edge X-ray absorption fine structure (NEXAFS) spectroscopy, X-ray photoelectron spectroscopy (XPS), and density functional theory (DFT).

In the present work we studied the electronic structure of glycine, proline, and methionine. The schematic structure of these molecules is shown in Figure 1, and all three are known to exist in the neutral state in the gas phase, although they may exist as zwitterions in solution. Glycine is the simplest amino acid with only two carbon atoms. Proline is the only naturally occurring amino acid which contains a pyrrolidine ring. All carbon atoms are symmetrically inequivalent, but carbon atoms 3 and 4 are in very similar chemical environments, and carbon atoms 2 and 5 are also similar. The rigidity of the ring structure, compared with the greater rotational flexibility of other amino acids, is an important aspect when proline is incorporated in peptides and proteins. Methionine is one of the two amino acids which contain sulfur and contains two carbon atoms bonded to S (numbers 4 and 5), one carbon atom bonded only to carbon

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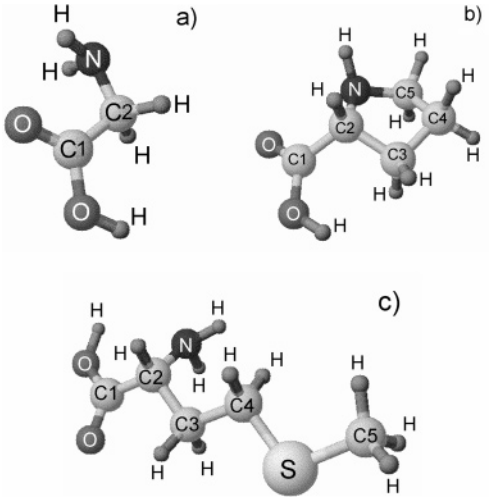


**Figure 1.** Schematic structures of (a) glycine, (b) proline, and (c) methionine.

and hydrogen (number 3), and one carbon atom (2) bonded to two carbon atoms and the amino group.

Amino acids are well known to exist as several conformers at experimental temperatures, and in this work only proline shows effects due to different conformers. These have been studied by a variety of experimental and theoretical methods,[23,24] and it is believed that the lowest energy conformers can be described by two structural motifs. The pyrrolidine ring is not flat and may be puckered up or down. The carboxylic acid group rotates easily, and the acid hydrogen may be oriented toward the amino nitrogen or away from it. These two structural elements can be combined to give the four lowest energy conformers.

## 2. Experimental Section

The photoemission spectra were taken at the Gas Phase Photoemission beamline, Elettra, Trieste,[25] using a 6-channel, 150 mm hemispherical electron energy analyzer. The electron analyzer was mounted in the plane defined by the (linearly polarized) electric vector of the light and the photon propagation direction at an angle of 54.7° with respect to the electric vector of the light. In this geometry the axis of the analyzer is set at the pseudo magic angle, and so measurements should be insensitive to the photoelectron asymmetry $\beta$ parameter.

The glycine, proline, and methionine samples were obtained from Sigma−Aldrich in the form of crystalline powder with a minimum purity of 99% and used without any further purification. The samples were evaporated from a noncommercial, noninductively wound furnace equipped with a chromel/alumel thermocouple. The materials used were aluminum or stainless steel for the crucible, stainless steel for the heated parts of the furnace and heat shield, and machinable glass ceramic for thermal and electrical insulation. Before the experiment, photoionization mass spectroscopy[26] was used to monitor evaporation and check sample purity. Typically, samples degassed water at low temperature, occasionally some other contaminants, and then the pure substance was found to evaporate. We monitored the mass spectra for several hours, and the ratio of the fragment ions was constant. In addition, the mass spectrum for photoionization at 21.2 eV photon energy was rather similar to electron impact ionization mass spectra.[27] For substances that decomposed (for example, if the temperature was too high), the mass spectrum usually varied with time and showed a very different fragmentation pattern from the electron impact spectra. The evaporation temperatures were 150 °C for glycine, 130−170 °C for proline, and 170 °C for methionine. The same temperatures and same furnace were used to produce samples for photoionization, where we additionally monitored the valence spectra for impurities. In valence photoemission, diatomic and triatomic impurities such as water or carbon dioxide are very easily detected as they give rise to sharp peaks, often with vibrational structure. We are therefore confident that our samples were pure and did not suffer from thermal degradation. Radiation damage is also unlikely as we did not use a gas cell, and so the residence time of the molecules in the beam is too short to allow photoproducts to be ionized.

The local vapor pressure was estimated by comparing the photoemission signal of the three compounds with the signal from nitrogen gas admitted to the chamber and measuring the nitrogen pressure with an ion gauge. The local pressures for glycine, proline, and methionine were $6 \times 10^{-6}$, $9 \times 10^{-6}$, and $5 \times 10^{-6}$ mbar, respectively. There may be a systematic error associated with this measurement as the nitrogen gas is distributed uniformly, whereas the effusive source is inhomogeneous. We conservatively estimate the possible error as a factor of 2. The densities are in any case low and in the molecular flow regime.

The evaporated material was collected on a shield, and after long evaporation times, charging and shifts in energy of the photoelectron peaks were observed. These effects were controlled by measuring the peak energies of calibrant gases simultaneously with the sample spectrum. After each sample, the shields and the chamber were cleaned. No other effects due to contamination of the instrumentation were observed.

The valence spectra of the studied biomolecules were recorded with an incident photon energy 99 eV or He I (21.21 eV) for proline. The S 2p, C, N, and O 1s core level spectra were measured at 256, 382, 495, and 628 eV photon energy, respectively, and referenced to published values of reference gases: S 2p of $SF_6$,[28,29] C 1s of $CO_2$,[30] N 1s of $N_2$, and O 1s of $CO_2$.[31] The spectra were measured at pass energies of 20 eV in fixed analyzer transmission mode. We did not calibrate the transmission of the analyzer, but it is expected to be constant over the narrow energy ranges of the core level spectra. Some variation in intensity may occur over the wider kinetic energy ranges of the valence spectra, about 30 eV, but we extract quantitative information only from the energies and not from the peak intensities. The spectra were measured with a total resolution (photons + analyzer) of 0.45, 0.25, 0.57, 0.59, and 0.53 eV at $h\nu = 99$, 256, 382, 495, and 628 eV, respectively. Spectra at the lowest energy of 99 eV were also taken at higher resolution (0.20 eV) but were not significantly better resolved, so we present here only the lower resolution data with better statistics. The values of resolution for 382 and 495 eV photon energies were calculated and then compared with measured spectra and found to be equal to the widths of the core level spectra of the calibrant gases, $CO_2$, $N_2$, and O 1s of $CO_2$. The C 1s and N 1s lines are narrow and in the most recent spectra[32,33] consist of slightly asymmetric Franck−Condon envelopes with a fwhm (full width at half maximim) of about 0.27 and 0.25 eV, respectively. The convolution of this line shape with our resolution contributes only about 50 meV to the measured width. The resolution for oxygen 1s measurements is calculated, and

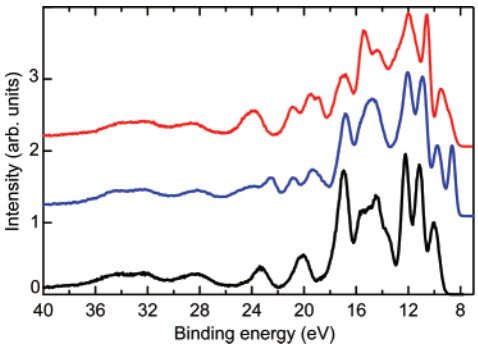


**Figure 2.** Valence band photoemission spectra of glycine (bottom curve, black), methionine (center curve, blue), and proline (top curve, red) at a photon energy of 99 eV.

the measured width for $CO_2$ is 0.9 eV. This is consistent with the reported width of the Franck−Condon envelope[34] at low resolution. Recent very high resolution measurements have resolved the vibrational components of the broad Franck−Condon envelope.[33]

More detailed information about the N 1s core level spectra of the amino acids studied here has been published elsewhere.[35]

## 3. Results

**3.1. Valence Photoemission Spectra.** The valence photoemission spectra of glycine, methionine, and proline, measured with a photon energy 99 eV, are shown in Figure 2. The energies of the peaks in the spectra are similar to those of previously reported He I spectra,[2−4] although the relative peak intensities are different due to the different photon energy. The experimental values of the valence band energies are given in Table 1. The energies of the bands of all three compounds are in very good agreement with the values of ref 3, and we follow their molecular orbital (MO) assignments for the first three orbitals. The first band in the valence photoemission spectra of glycine at a binding energy (BE) of 10 eV corresponds to excitation of the N lone-pair orbital ($n_N$). Within the COOH group, one nonbonding orbital ($n_O$) is localized on the oxygen atom of the hydroxyl group and another bonding orbital ($\pi_{OO}$) is localized on the oxygen atom of the carbonyl group. For bands at higher energy than the first three, we list the assignments of Falzon and Wang[36] for one conformer; the overall agreement is good. The highest binding energy peaks (18.7−34.5 eV) were not labeled by Cannington and Ham[3] or Falzon and Wang.[36] We assume that they are all substantially single-electron excitations (except for one very weak structure at 18.7 eV) and assign them by extrapolating the labels of Falzon and Wang. Since the 1a″ orbital lies at 17.6 eV, all orbitals at higher binding energy are labeled a′. The core levels of O, N, and C are labeled 1a′−5a′, while the states reported here are expected to be of predominantly N and C 2s character (see Table 1).

The first band of methionine at BE = 8.65 eV has mostly lone-pair sulfur character ($n_S$). The next orbitals correspond to excitation of the nitrogen lone-pair orbital ($n_N$), BE = 9.80 eV, and of an oxygen lone-pair orbital ($n_O$), BE = 10.90 eV. The data can be compared with the calculations of Dehareng and Dive,[37] which are known to be quite accurate for glycine, see above. They also correctly predicted the valence splitting of the HOMO of proline; see discussion below. They calculated the vertical ionization energies of three conformers of methionine, and their conformer CF1 had a first ionization energy of 8.09 eV. There is however no peak in the spectrum at this energy: the first peak is at 8.65 eV, consistent with their calculated energies of 8.60 and 8.58 eV for conformers CF2(1) and CF2(2). Thus, we conclude that CF1 is not significantly populated at the temperature of our experiment.

We assign the peak at 19.2 eV to S 3s emission, possibly overlapping other C 2s derived states. In dimethyl sulfide the bonding environment of the sulfur atom is similar, and Kishimoto et al.[38] found a peak at 19.02 eV assigned to S 3s and C 2s emission. Since methionine is a larger molecule than dimethyl sulfide, a small shift to higher binding energy is expected. The N 2s and O 2s peaks at 28.7−34.2 eV align well with the corresponding peaks of glycine, supporting this assignment.

For proline, the first two peaks of the valence spectra at 9.0 and 9.5 eV correspond to ionization from the HOMOs of different stable conformers, and both have nitrogen lone pair character ($n_N$).[39] This assignment is also consistent with the calculations of Dehareng and Dive,[37] although they calculated only two conformers. Cannington and Ham[3] assigned both of these to nitrogen lone pairs without further comment on why a single lone pair gave rise to two peaks. The subsequent bands at BE = 10.6, 11.5, and 12.0 eV correspond to excitation of lone-pair orbitals $n_O$, $\pi_{CO}$, and $\sigma_{CC}$, respectively.[3,37,39] We report four new peaks at high binding energies as well as the three O 2s and N 2s states. Again, the latter are at energies close to those of glycine and methionine.

We also recorded the valence band spectra of proline at a photon energy of 21.2 eV (He I) as a function of temperature. In this case the oven temperature was varied from 405 to 440 K. The goal of these measurements was to observe how the relative intensity of the first two valence peaks, characteristic of the lowest energy conformers at 8.9 and 9.5 eV, changed with temperature. The peaks were fitted in various ways by approximating the first three peaks by Gaussian functions, and then the intensity was evaluated as the area of the peaks. The temperature dependence of the ratio of intensity of the lone-pair orbitals ($n_N$) of different conformers of proline is shown in Figure 3 for a particular set of fit parameters. The analysis of this data is discussed below.

**3.2. Core Level Photoemission.** The carbon, nitrogen, and oxygen 1s as well as the sulfur 2p core level spectra of the three amino acids are shown in Figure 4, and their core level binding energies and widths are presented in Table 2. The peaks were fitted by simple Gaussian functions, and the relative intensities were obtained from the areas of the fitted peaks. The tabulated widths are the fitted Gaussian widths without deconvolution of the resolution.

The core level spectra of glycine have been measured and assigned by Slaughter and Banna[40] using Al Kα radiation, and the present binding energies are in good agreement. Due to superior resolution in our experiment, the peaks are better resolved in the present study. Two main peaks are obtained in each of the C 1s and O 1s regions. The peak at 295.2 eV is assigned to the carbon atom in the carboxyl group and the second peak at 292.3 eV to the carbon atom bonded to the amino group. In the oxygen core level spectrum the lower binding energy peak at 538.4 eV corresponds to the keto oxygen and the peak at 540.2 eV to the hydroxyl group.

The carbon core levels of methionine follow similar assignments: the first peak is due to the carboxyl carbon (294.7 eV) and the second to carbon bonded to the amino group (292.0 eV). The third peak is assigned to the carbon atom bonded to two other carbon atoms (291.5 eV), and the last to the two atoms bonded to the sulfur atom (290.9 eV). The two peaks in the

**TABLE 1: Valence Band Energies for Glycine, Methionine, and Proline[a]**

| glycine | | | | methionine | | | proline | | |
|---|---|---|---|---|---|---|---|---|---|
| binding energy (eV), present work, ±0.1 eV | binding energy (eV)[3] | binding energy (eV),[37] conformers CF1, CF2 | binding energy (eV)[36 a] | binding energy (eV), present work | binding energy (eV)[3] | binding energy (eV),[37] conformers CF1, CF2(1,2) | binding energy (eV), present work | binding energy (eV)[3] | binding energy (eV),[37] conformers CF1, CF2 |
| 10.0 | 10.0, $n_N$ | 9.82 CF1<br>9.98 CF2 | 10.0, 16a′ | 8.65 | 8.65, $n_S$ | 8.09 CF1<br>8.60 CF2(1)<br>8.58 CF2(2) | 8.9 | 9.0, $n_N$ | 8.75 CF1 |
| 11.2 | 11.1, $n_O$ | 11.16 CF1<br>11.11 CF2 | 11.4, 15a′ | 9.8 | 9.8, $n_N$ | 9.63 CF1<br>9.72 CF2(1)<br>9.77 CF2(2) | 9.5 | 9.5, $n_N$ | 9.36 CF2 |
| 12.2 | 12.2, $\pi_{OO}$ | 11.93 CF1<br>11.29 CF2 | 12.4, 4a″ | 10.9 | 10.9, $n_O$ | 10.61 CF1<br>10.81 CF2(1)<br>10.89 CF2(2)<br>10.91 CF1<br>11.09 CF2(1)<br>11.08 CF2(2) | 10.6 | 10.6, $n_O$ | 10.76 CF1,<br>10.57 CF2<br>10.88 CF2 |
| 13.7 | 13.6 | 13.16 CF1<br>13.40 CF2 | 13.6, 3a″ | 12.0 | 12.1 | 11.80 CF1<br>11.29 CF2(1)<br>11.02 CF2(2)<br>11.72 CF1<br>12.05 CF2(1)<br>12.18 CF2(2) | 11.3 | 11.6,$\pi_{OO}$ | 11.70 CF1 |
| 14.4 | 14.4 | | 14.8, 14a′ | 12.6 | 12.6 | | 12.0 | 12.0, $\sigma_{CC}$ | 12.00 CF1,<br>12.30 CF2 |
| 15.0 | 15.0 | | 15.1, 13a′ | | 13.1 | | 12.8 | 13.0 | |
| 15.8 | 15.6 | | 15.8, 2a″ | 14.8 | 14.5 | | 14.3 | 14.2 | |
| 16.6 | 16.6 | | | 16.8 | 16.6 | | 15.4 | 15.5 | |
| 16.9 | 16.9 | | 17.2, 11a′ | 19.2, S 3s | | | 16.7 | | |
| 17.6 | 17.6 | | 17.7, 1a″, 12a′ | 20.9 | | | 17.3 | 17.3 | |
| 18.7, satellite | | | | 22.5 | | | 18.7 | | |
| 20.2, 10a′ | 20.2 | | | 23.85 | | | 19.4 | | |
| 23.3, 9a′ | 23.2 | | | 28.2, N 2s | | | 20.9 | | |
| 28.3, 8a′, N 2s | | | | 32.4, O 2s | | | 23.9 | | |
| 32.3, 7a′, O 2s | | | | 34.3, O 2s | | | 28.7, N 2s | | |
| 34.3, 6a′, O 2s | | | | | | | 32.3, O 2s | | |
| | | | | | | | 34.2, O 2s | | |

[a] The values from ref 3 are experimental; the values from ref 37 are theoretical and calculated for two or three conformers and labeled as in that work, CF1, etc. [b] Reference 36 reported theoretical values for several conformers of glycine; we give only the values for conformer 1, calculated by the OVGF/TZVP method, Table 3 in the cited work.

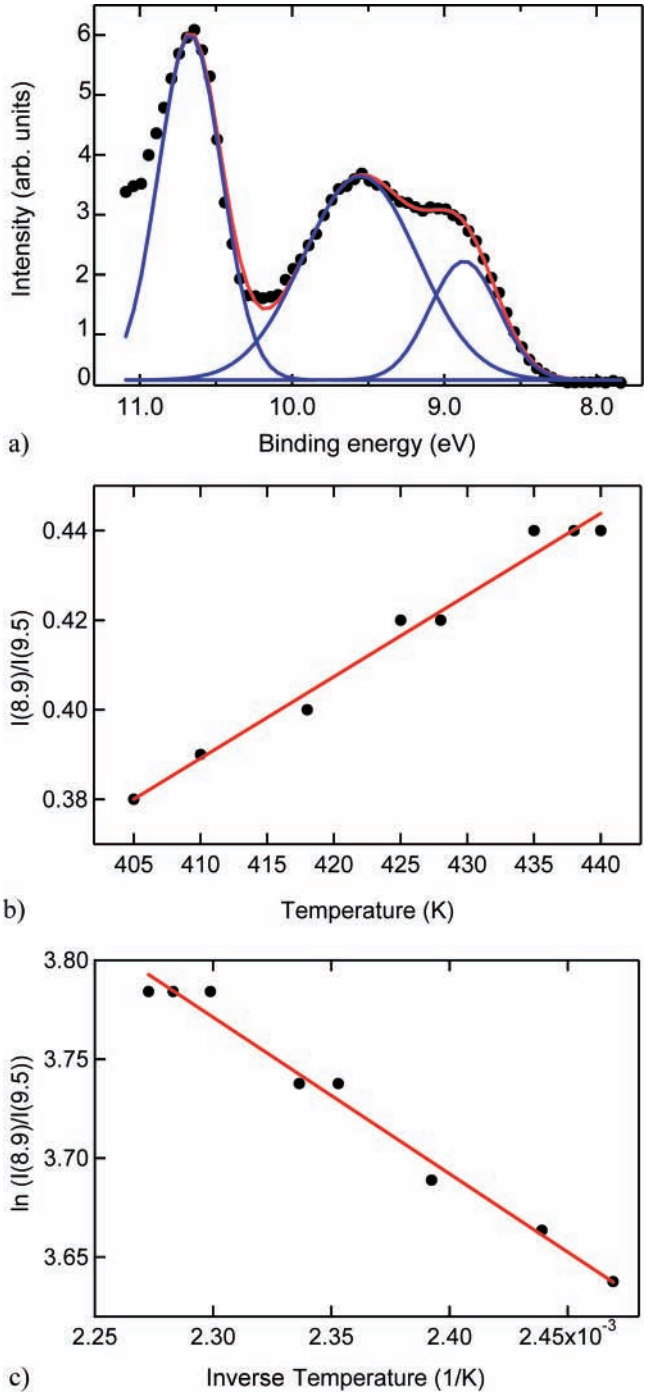


**Figure 3.** (a) He I valence band spectrum showing the three lowest binding energy peaks. Dots: data. Solid line: fitted Gaussian curves with energy, width, and intensity as free parameters. (b) Temperature dependence of the intensity of the peak at 8.9 eV, $I(8.9)$, divided by the intensity of the peak at 9.5 eV, $I(9.5)$, of proline. (c) Natural logarithm of the intensity ratio versus inverse temperature.

oxygen 1s core level spectrum of methionine have very similar binding energies to glycine and proline, and the assignments are the same.

The carboxyl carbon atom of proline has slightly lower binding energy than glycine. This difference is attributed to final state relaxation effects, which screen the core hole more effectively in larger molecules. For example, this core level shifts between acetic acid and propanoic acid by 0.39 eV[41] with addition of a $CH_2$ group. The measured widths of the C 1s peaks appear to be composed of similar contributions from the experimental resolution and the natural line width. They vary from 0.71 to 0.88 eV, compared with the estimated resolution of 0.57 eV.

As noted above, besides the carboxylic carbon in proline, there are four other carbon atoms, consisting of two pairs of chemically similar atoms. The spectrum in fact shows three peaks with intensity ratios of 1:2:2. The peak at 291.8 eV is assigned to the two carbon atoms bonded to N by analogy with the spectra of glycine and methionine but is shifted to lower binding energy. The binding energy is similar to that of alanine.[7] The peak at 290.9 eV is assigned to the two carbon atoms bonded to other carbon atoms in the ring structure. The oxygen core levels are located at 539.4 and 537.7 eV, and the spectrum shows a small difference: the O 1s peak at 539.4 eV is much broader than that of the other two amino acids, which may be a consequence of hydrogen bonding.

The N 1s spectra show single peaks for glycine at 405.4 eV and methionine at 405.1 eV which are assigned to the nitrogen atom bonded to carbon atoms. This shift is slightly larger than that of the N 2s level, underlining the different behavior of core and inner valence orbitals. Since the proline molecule contains only one nitrogen atom, a single peak might be expected as in the case of glycine and methionine, but in fact there are two. We assigned the two nitrogen core level peaks to two pairs of different conformers of the molecule,[35] labeled according to Ebrahimi et al.,[42] and we analyze this data further below. The N 2s level showed a shift of about 0.4 eV to higher binding energies with respect to the other two amino acids. The present result suggests that the 2s peak is composed of more than one peak which are not resolved because the peak is broad. The peak shapes of both glycine and methionine are clearly asymmetric and dominated by the intrinsic line shape rather than the resolution. However, the present data do not allow us to decide whether this shape is determined by a Franck−Condon envelope or the existence of conformers.

The intensities of the peaks in the carbon 1s spectra agree with the values predicted from the stoichiometry within 4%. This is expected as the experimental conditions should ensure that photoemission is quantitative: they were measured at the pseudo magic angle, about 100 eV above threshold, and therefore well away from resonances which may change the cross-section. For the oxygen 1s peaks the areas determined by fitting are however not equal in intensity but differ by 14−20%, and the OH peak is always weaker. This is probably due to the difficulty of fitting these closely spaced peaks and the simplification of using a Gaussian function to simulate the Franck−Condon envelope.

The binding energy of S $2p_{3/2}$ in methionine, 169.0 eV, is equal within the precision of the measurement to the value found in dimethyl sulfide, 169.05 ± 0.04,[43] as expected because the chemical environment of the sulfur atom is very similar. The amino acid group is fairly distant from the sulfur atom and does not appear to induce any additional screening of the core hole in the final state. The line shape is asymmetric and significantly broader than that of the calibrating gas, $SF_6$, and therefore dominated by the natural line shape.

The binding energies of the oxygen atoms differ by 1.8 eV for all three amino acids, and the keto O 1s widths are 1.07, 1.16, and 1.20 eV for glycine, methionine, and proline, respectively. This small variation is not very significant. However, the hydroxyl O 1s widths are 1.44, 1.16, and 1.68 eV, respectively, and this is a very significant variation. For a single conformer of each amino acid we do not expect strong variations in O 1s line shape because the carboxylic acid

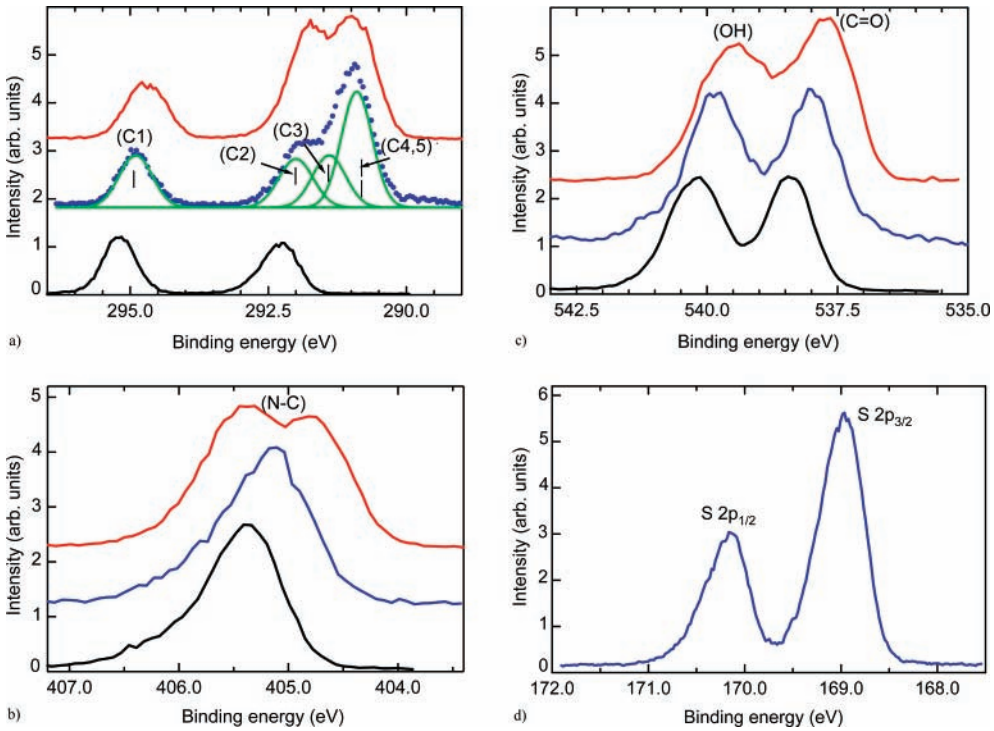


**Figure 4.** Core level spectra of glycine (lower curves, black), methionine (middle curves, blue), and proline (upper curves, red). (a) Carbon 1s. Fitted curves and their assignment (see Figure 1) are shown for methionine. (b) Nitrogen 1s. (c) Oxygen 1s. (d) Sulfur 2p of methionine.

**TABLE 2: C 1s, N 1s, and O 1s Binding Energies for Glycine, Methionine, and Proline**[a]

| molecule | core level | binding energies (eV), ±0.1 eV | fwhm of the Gaussian fit function (eV), ±0.01 eV | assignments | intensity (relative to C(1)*═O or C(1)═O*) |
|---|---|---|---|---|---|
| glycine | C 1s | 295.2 | 0.71 | C(1)*═O | 1 |
| | | 292.3 | 0.77 | N−C(2)*$H_2$ | 0.96 |
| | N 1s | 405.4 | 0.89 (asym.) | $H_2$N*−C(2) | |
| | O 1s | 540.2 | 1.44 | C(1)−O*H | 0.79 |
| | | 538.4 | 1.07 | C(1)═O* | 1 |
| methionine | C 1s | 294.7 | 0.70 | C(1)*═O | 1 |
| | | 292.0 | 0.86 | N−C(2)*$H_2$ | 0.96 |
| | | 291.5 | 0.86 | C(3)*$H_2$ | 1 |
| | | 290.9 | 0.77 | S−C(4,5)* | 2.08 |
| | N 1s | 405.1 | 0.9 (asym.) | $H_2$N*−C(2) | |
| | O 1s | 539.8 | 1.16 (asym.) | C(1)−O*H | 0.80 |
| | | 538.0 | 1.16 | C(1)═O* | 1 |
| | S $2p_{3/2}$ | 169.0 | 0.56 (asym.) | S*−C(4,5) | |
| | S $2p_{1/2}$ | 171.2 | 0.61 (asym.) | | |
| proline | C 1s | 294.7 | 0.85 | C(1)*═O | 1 |
| | | 291.8 | 0.88 | N−C(2,5)*$H_2$ | 1.94 |
| | | 290.9 | 0.82 | C(3,4)*$H_2$ | 2.06 |
| | N 1s | 405.5 | 0.92 (asym.) | $H_2$N*−C(2,5) | |
| | | 404.8 | 0.62 | | |
| | O 1s | 539.4 | 1.68 | C(1)−O*H | 0.80 |
| | | 537.7 | 1.20 | C(1)═O* | 1 |

[a] asym. in the width column indicates a markedly asymmetric peak.

chromophores are the same and differences in bonding occur at the third atom from the excited oxygen atom. The variation in the widths is assigned to the existence of multiple conformers in glycine, which are known to exist,[44] and support our interpretation of multiple conformers in proline.

## 4. Discussion

We model the valence band data by assuming first that a number $n_1$ of conformers contributes to the 8.9 eV peak and $n_2$ conformers contribute to the 9.5 eV peak. We assume that the conformers in each group are sufficiently close in energy to permit the assumption of an average free energy of $G_1 = H_1 - TS_1$ and $G_2 = H_2 - TS_2$, respectively. The enthalpies and entropies are assumed constant over the temperature range 405−440 K. The ratio of populations is given by the peak intensities, multiplied by the quotient of the two cross-sections, $r$.

Then the ratio of the intensities is given by

$$I(8.9)/I(9.5) = r\sum_{i=1}^{n_1}\exp(-G_1/kT)/\sum_{i=1}^{n_2}\exp(-G_2/kT)$$

$$\approx r^*\{n_1\cdot\exp(-G_1/kT)\}/\{n_2\cdot\exp(-G_2/kT)\}$$

The logarithm of the intensities is given by

$$\ln\{I(8.9)/I(9.5)\} = -G_1/kT + G_2/kT + \ln(r) + \ln(n_1/n_2)$$
$$= -H_1/kT + S_1/k + H_2/kT - S_2/k + \ln(r) + \ln(n_1/n_2)$$
$$= (-H_1 + H_2)/kT + (S_1 - S_2)/k + \ln(r) + \ln(n_1/n_2)$$

Thus, in the Arrhenius plot of Figure 3 the slope is equal to $1/k$ times the difference of the average enthalpies and the intercept is the difference of the average entropies plus a correction for the cross-section ratio and numbers of conformers. For the fit parameters used in Figure 3c the logarithm of the ratio of intensities plotted against inverse temperature has a slope of $-745 \pm 40$ K$^{-1}$, which yields an enthalpy difference of $6.2 \pm 0.3$ kJ/mol. This fit was performed with the peak heights, widths, and energies as free parameters. In another fit the peak heights and energies were left free, but the first two peaks were constrained to have the same widths: this gave a poorer quality fit and an enthalpy difference of 9.7 kJ/mol. This procedure gives an indication of the errors due to the assumptions in the model.

Turning now to the analysis of the nitrogen 1s core level data of proline, we performed the following steps. First, we used our calculated profiles[35] to model the peak shapes and we obtain a value of 1.12 for the ratio of the areas of peaks (1a + 1b) to (2a + 2b) at a temperature of 413 K. At the photon energy used (well above threshold), the cross-sections are equal, so the peak areas directly reflect populations. The value of 1.12 implies the average free energy difference, $G_1 - G_2$, is just 0.4 kJ/mol, so that the entropy difference $S_1 - S_2$ is about 14 J mol$^{-1}$ K$^{-1}$ for an enthalpy difference of 6.2 kJ/mol or 23 J mol$^{-1}$ K$^{-1}$ for 9.7 kJ/mol. The first value is a little larger than the maximum value calculated by Ebrahimi et al.,[42] which was 10 J mol$^{-1}$ K$^{-1}$ for conformer 1a, the ground state, but it is encouraging that the order of magnitude is correct. This whole analysis is of course rather approximate as it assumes average enthalpies and entropies for all conformers and that there are four conformers and as well being uncertainties associated with the peak areas. We also tried fitting with separate thermodynamic parameters, but the values obtained are constrained by the necessity to satisfy both the relative variation with temperature and the absolute populations, assuming only four conformers. If it is assumed that more than two conformers are present in the lower binding energy peak, the thermodynamic values are closer to the calculated values. Thus, as we suggested previously,[35] there may be more than two conformers contributing to this peak, and Ebrahimi et al.[42] discussed many more than just the four lowest energy conformers. We demonstrated that in principle thermodynamic information can be extracted and conclude that the differences between conformers for the free energy, enthalpy, and entropy are in qualitative agreement with the values calculated.

## 5. Conclusions

The core and valence level spectra of glycine, proline, and methionine have been measured and features assigned. New data has been reported for the inner valence region, while the outer valence region is in good agreement with existing spectra. The core level spectra of glycine are in agreement with reported data and better resolved, while new spectra have been obtained for methionine and proline. The carbon core level spectra were quantitatively fitted and assigned. By measuring the thermal dependence of the valence band spectra we were able to extract some approximate thermodynamic values for free energy and entropy, which are of the same order of magnitude as published calculations.

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