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Photoemission and the shape of amino acids

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Abstract

The nitrogen 1s core level photoionization spectra of the amino acids glycine, methionine and proline in the gas phase have been measured. The spectra of proline show two peaks separated by 0.5 eV, although the molecule contains only one nitrogen atom. Electronic structure calculations demonstrate that the peaks are due to two different types of conformer of proline, but the electronic structure differences giving rise to the core level shifts are complex. The relative intensities of the peaks at 413 K and 438 K set limits to the number of conformers and the values of their relative free energy.
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1. Introduction

Amino acids generally exist at laboratory temperatures as several conformers of comparable energy, and the determination of their properties is of fundamental importance in chemistry and biology. Methods for determining the conformation of free amino acids include electron diffraction [1] and low energy spectroscopies such as microwave and infrared techniques [2–4], but the high energy method of core level photoemission spectroscopy has not yet yielded significant structural information about free amino acids.

Microwave spectroscopy [5] and matrix isolation IR spectroscopy [6] together with theoretical analysis [7] have demonstrated the existence of two conformers of proline in both matrix isolated and jet cooled gas phase samples, with a calculated energy difference of 2.2 kJ/mol between the

two lowest energy conformers [8]. Ebrahimi et al. [9] and Stepanian et al. [6] predicted four conformers below 8 kJ/mol and the latter authors attributed the observation of only two to low energy barriers between conformers, which permitted interconversion. Dehareng and Dive [10] calculated energies of only two conformers, but they agree on their energy ordering. Tian and Yang [11] analysed theoretically the valence photoemission data [12], and concluded that two conformers of proline were present in thermally evaporated gas phase samples.

There have been many calculations of the energies of various free amino acids [e.g. 6–10], of their valence band electronic structure [10,13–16], and one calculation of the core level energies of glycine [17]. Several of these sought to relate their molecular structure to photoemission spectra. The valence photoionization energies of some conformers of proline [11,12] and phenylalanine [18] have been determined, but there has been no report of a dependence of core ionization energy on conformation. Powis et al. [19] searched very thoroughly for shifts of the C 1s lines of L-alanine and threonine, but the extra lines observed were assigned to peptide bonds for alanine (possibly due to catalytic reactions on the walls of the cell they

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used, or photolytic reaction) and thermal decomposition for threonine. In fact, although several core spectroscopic studies (including electron scattering) have been carried out [16,19,20], to our knowledge only one group has identified conformational effects in free organic molecules using core level techniques. Abu-samha et al. [21] recently reported that the methyl carbon 1s peak of ethanol was broadened due to the existence of *cis* and *trans* conformers in the gas phase. Since the photoelectron lines overlapped, rather subtle differences occurred in their vibrational envelopes. Thomas et al. [22] have also observed similar effects in other small molecules. This, and the lack of any other evidence, might suggest that conformational effects in core photoemission are generally very weak, but we show here that this is not necessarily true.

2. Experimental and theoretical methods

We investigated the core and valence level photoemission spectra of three amino acids, glycine, DL-methionine and L-proline. Since linearly polarized light was used, no chiral effects are expected and we drop the labels DL and L hereafter. The samples were evaporated from a home-built furnace with an effusive nozzle and were checked before the experiment using photoionization mass spectroscopy [23]. From this data we are sure that there are no effects due to thermal decomposition of the product, and since we use an effusive nozzle instead of a gas cell, we minimize the possibility of spurious effects like those reported in [19] (possible reactions on the surface of the cell, photolytic effects, concentration of reaction products in a cell). Evaporation temperatures were 430 K for glycine, 445 K for methionine, and 413 K or 438 K for proline. The photoemission spectra were taken at the Gas Phase Photoemission Beamline, Elettra, Trieste [24] using a 150 mm hemispherical electron energy analyser, equipped with six channel electron multipliers. The N 1s core spectra were taken at 495 eV photon energy and the binding energy calibrated to the N 1s binding energy of N_2 [25].

The N 1s photoemission spectra of each of the four conformers of proline shown in Fig. 1 were calculated theoretically by a ΔSCF procedure with separate geometry and electronic optimization of the ground state and of the core–hole state. It has been observed [6] that SCF calculations, as well as DFT/B3LYP calculations, cannot predict accurately the relative stability of proline conformers. This observation was confirmed by our present calculations that showed that in any case the effect of the SCF approximation on the ground state optimized geometries is very limited. The ΔSCF procedure was chosen because more accurate quantum mechanical methods, such as the Green's Function (GF) technique, are practically unfeasible for computing core level ionization potentials of such a large molecule as proline and that despite the neglect of correlation effects, the ΔSCF procedure takes into account correctly the relaxation effects that dominate in a core ionization process. Moreover, we assumed that the SCF
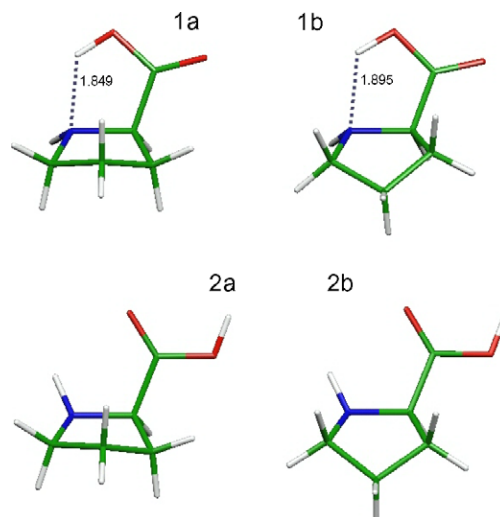

Fig. 1. The four lowest energy conformations of proline, labelled as in [9]. Green: carbon; blue: nitrogen; red: oxygen; white: hydrogen. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

approximation could provide sufficiently accurate energy potential surfaces for computing molecular vibrational states, in the harmonic approximation, of both the initial (ground) and the final (core–hole) states. Adopting the usual approximations for the dipole transition moment in a vibronic transition, the intensity for the ionization starting from the ground vibronic state and leaving the molecule in a specific vibrational state of the electronic core–hole state, was considered proportional to the Franck–Condon (FC) factor. A vibronic photoemission spectrum was then obtained as a bar diagram, where the set of FC factors was computed, by the MolFC code [26,27], taking into account the Duschinsky effect for transitions between vibrational states projected on different normal coordinate reference systems.

3. Results and discussion

The carbon and oxygen 1s core level spectra of the three amino acids were measured and will be presented elsewhere [28]. For glycine they are in good agreement with published data [29]. The N 1s spectra show single peaks for glycine and methionine Fig. 2, but in striking contrast, two peaks for proline, which is unexpected since the proline molecule contains only one nitrogen atom. We assign this additional structure to the existence of at least two conformers of the molecule at the temperature of the experiment. Our assignment was checked by DFT (density functional theory) calculations using the B3LYP density functional and the TZV basis set for the geometry optimization of the four conformers having the lowest electronic energy. In agreement with [6,9], we found that the four lowest energy conformers can be energetically distinguished as two pairs, Fig. 1. The lower energy pair is characterized by a N···H–O–C=O hydrogen bond, while the second one is characterized by
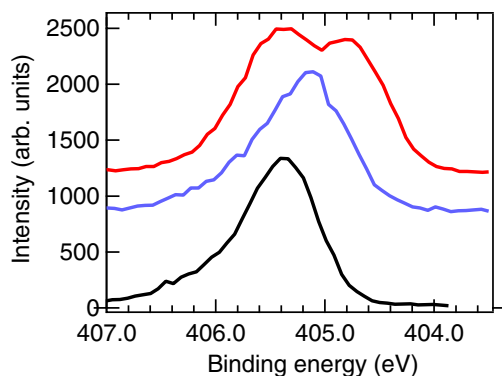
Fig. 2. N 1s spectra of glycine (lower curve), methionine (centre curve) and proline (top curve).

a weaker N–H···O=C–OH hydrogen bond. The two conformers in each pair differ by the ring puckering, as shown in Fig. 1. Our calculations Fig. 3 indicate that the conformers 1a (ground state) and 1b have very similar N 1s binding energies, while conformers 2a and 2b have binding energies 1.0 eV lower. Three of these conformers have very similar line shapes but conformer 1b is strikingly different.

The pairs of conformers have different hydrogen bonding and different N 1s binding energies, suggesting that a primary cause of the core level shift is the difference in chemical environment between nitrogen atoms in amino groups acting as donors or acceptors. Indeed calculations at the Koopman level of approximation predict that conformer 1 has a binding energy 0.94 eV higher than conformer 2 in the ground state. However a simple atomic population analysis shows no clear difference between conformers 1 and 2 attributable to changes in charge density due to hydrogen bonding. Thus, to understand the shift,

the whole electronic structure must be considered, and not just the local variations due to the presence of the hydrogen bond.

Curves(a) of Fig. 4 show N 1s spectra at two temperatures. The relative intensity of the lower binding energy peak has increased by about 8% for a temperature change of 25 K. Fig. 4 also shows simulated peak shapes which are the sums of calculated line shapes weighted by Boltzmann factors calculated using the (0 K) energies of Stepanian et al. [6] and broadened to more closely resemble the experimental spectra. Curve (b) shows the result assuming the presence of the four conformers discussed above. A two peak structure with a tail towards higher binding energy is produced by the simulation: this characteristic tail is consistent with the presence of conformer 1b. The energy separation of the two peaks is somewhat overestimated by theory, being 1.0 eV compared with an experimental value of 0.5 eV. The relative intensities of the two peaks are not reproduced: the low binding energy 2a + 2b peak has only about half of the intensity of the 1a + 1b peak, whereas experimentally the peaks are about equal. We expect the peak containing the high energy conformers to increase relative to the peak containing the ground state conformer with an increase in temperature. The calculations predict that the left hand peak contains the ground state, and this is indeed the peak which does not increase on heating, so we assign it to the ground state, conformer 1a, and the low energy conformer 1b.

Unlike some spectroscopic methods XPS is quantitative so that peak areas reflect populations directly. Stepanian et al. [6] performed high level calculations of the relative energies of four conformers but did not calculate the entropy, so their calculations cannot be used for a quantita-
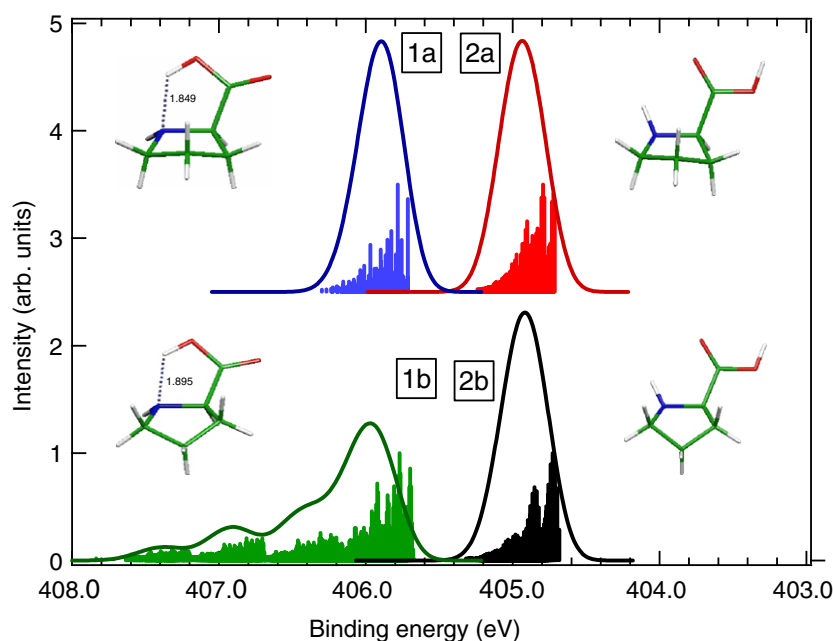

Fig. 3. Calculated N 1s spectra. The relevant conformer is shown beside each spectrum. Bars: calculated states; solid lines: overall spectra (convoluted with a Gaussian of width 0.25 eV).
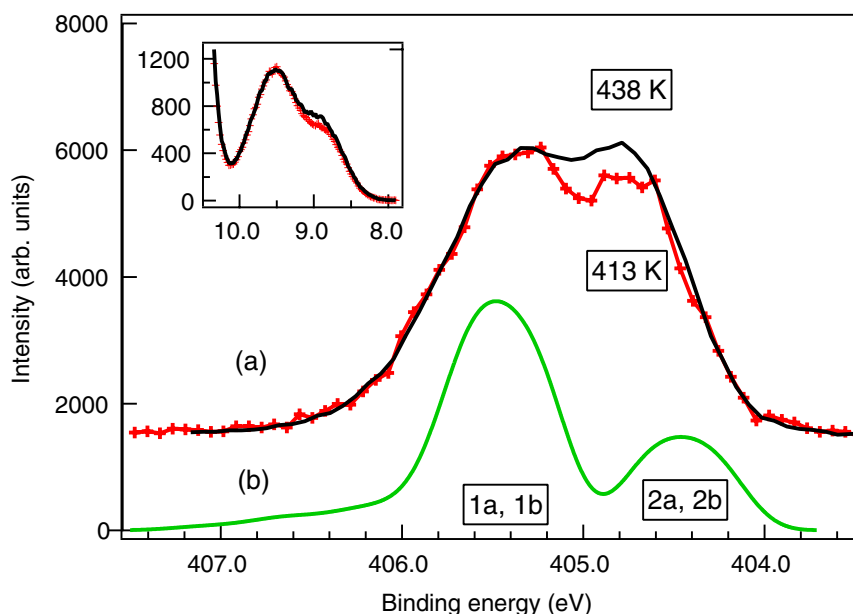
Fig. 4. (a) N 1s core level spectrum of proline at temperatures of 438 K (upper black curve) and 413 K (upper red curve). (b) Simulated spectra, 4 conformers. Inset: top of the valence band at 438 K and 413 K. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

tive comparison at the temperature of this experiment. Ebrahimi et al. [9] calculated the Gibbs free energy at 298.15 K [30], and we recalculated the free energy at the temperature of our experiment assuming constant entropy. We do not expect quantitative agreement between calculations and experiment because the MP2 MP2/6-311++G(d, p) level of calculations is known not to be sufficiently accurate. The values of Ebrahimi et al. give Boltzmann factors, which we used to model the data, namely nearly equal peak amplitudes, and an 8% increase in the second peak for a temperature difference of 25 K. We assume that the left peak consists only of the conformers 1a (the ground state) and 1b whose Gibbs free energy is calculated to be 0.99 kJ/mol [9,30] at 413 K relative to conformer 1a. Then the second peak would have the same peak height for two conformers and a relative average free energy of 1.2 kJ/mol at 413 K, or three conformers and an average free energy difference of 2.6 kJ/mol, and so forth. The values of entropy are restricted to certain values, but they fall in the range calculated by Ebrahimi et al. [9,30]. Thus we conclude that the right hand peak consists of two conformers with free energies quite close to the ground state and higher entropy, or more than two conformers, with fewer restrictions on the thermodynamic properties. Candidate conformers are not hard to find: Stepanian et al. [6] considered 15 conformer structures, and Ebrahimi et al. [9], 10 structures. We expect that those contributing to the second peak will be predominantly N–H–O=C hydrogen bonded, with a bonding geometry like conformers 2a and 2b.

Our result is a qualitative experimental test of calculated total energies at the present level of accuracy. This is more stringent than spectroscopies that use jet cooled or matrix isolated samples, which provide information about the existence of conformers, but not about their populations, as they are not performed at thermal equilibrium. While the conclusion is not quantitative, we hope that it will stimulate more high level calculations which can be compared with this data.

The valence band also shows effects of conformers, Fig. 4 inset. The valence spectrum of proline [12] contains bands at 9.0 eV and 9.5 eV binding energy, assigned to nitrogen lone pairs of conformers 2a + 2b and 1a + 1b, respectively [11]. Deharang and Dive [10] also predicted that the vertical ionization potentials of the two lowest energy conformers were separated by 0.6 eV. At a temperature of 438 K, the relative intensity of the lower peak increases by 8% with respect to $T = 413$ K, the same value as for the core levels, confirming that it is not due to the ground state HOMO. We assign the peak to the HOMO of the higher energy conformers, 2a and 2b (more extensive data will be published elsewhere [28]). We have also carried out SCF calculations for the ground state of the four lowest energy conformers confirming, at the low level approximation provided by Koopman's theorem (KT), the more accurate calculations cited above.

As noted above, microwave and infrared spectroscopy based on supersonic jet cooling, and matrix isolation techniques at low temperature have provided much of our knowledge of amino acid conformer structure. Photoemission, as applied here, has the advantages that the temperature of the sample is known and the method is quantitative, so that thermodynamic information is also accessible. Our results show that at least four low energy conformers have free energies in a range of 1.2 kJ/mol at 413 K, or that more than four conformers exist with free energies that are slightly larger.

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