Valence anions of *N*-acetylproline in the gas phase: Computational and anion photoelectron spectroscopic studies

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We report the photoelectron spectrum of anionic *N*-acetylproline, (*N*-AcPro)$^-$, measured with 3.49 eV photons. This spectrum, which consists of a band centered at an electron binding energy of 1.4 eV and a higher energy spectral tail, confirms that *N*-acetylproline forms a valence anion in the gas phase. The neutrals and anions of *N*-AcPro were also studied computationally at the B3LYP/6-31++G(*d*,*p*) level. Based on the calculations, we conclude that the photoelectron spectrum is due to anions which originated from proton transfer induced by electron attachment to the π^* orbital localized at the acetyl group of *N*-AcPro. We also characterized the energetics of reaction paths leading to pyrrolidine ring opening in the anionic *N*-AcPro. These data suggest that electron induced decomposition of peptides/proteins comprising proline strongly depends on the presence of proton donors in the close vicinity to the proline residue. © *2011 American Institute of Physics*. [doi:10.1063/1.3625957]

I. INTRODUCTION

Ionizing radiation or beams of high-energy particles, damage proteins through the production of radicals and low energy electrons, the latter being the most abundant.[1,2] For this reason the interactions between solvated electrons and small molecules mimicking peptides have received extensive study.[3–5]

Upon capturing an excess electron, otherwise stable peptides backbones may lose their structural stability.[6–13] Here, it is worth mentioning that proline seems to be quite an unusual amino acid as far as electron-induced N-C_α bond breaking is concerned. Indeed, in mass spectrometry experiments carried out for two dipeptides, Gly-Pro and Pro-Gly, no fragmentation was observed for the first case, whereas the characteristic dissociation products of the N-C_α bond were observed for the latter one.[9] This effect was ascribed to proline ring opening on N-C_α cleavage which still leaves the peptide connected in Pro-Gly but not in Gly-Pro.[9]

The conditions of mass spectrometry (MS) experiments are far from those existing in biological systems. Besides hydration effects that are usually present in the living cell and absent in the course of the MS analysis, peptides that enter the gas phase and then capture an extra electron are usually multiply protonated. Although in neutral water solution the *N*-terminal amino groups as well as the side chains of lysine, arginine, and histidine carry an extra proton, desorption of the corresponding cations into the gas phase via, e.g., electrospray results in activation of ions by collisions or IR heating leading to so called "mobile protons" and as a consequence in competitive transfer of those protons to the amidic functional groups, carbonyl oxygen, and amidic nitrogen atoms on the backbone.[14] As a matter of fact, the pulse radiolysis experiments on amino acids and simple peptides in an aqueous glass, carried out in the 1970's, demonstrated that immediate deamination follows the formation of anion radicals only if the *N*-terminal amino group is protonated.[5] Thus, presence of a proton donating group in the close vicinity to a center accepting an excess electron appears to govern the reactivity of nascent radicals which resulted from electron attachment to peptide based systems.

In this paper, we report photoelectron spectroscopic (PES) and quantum chemical modeling results pertaining to *N*-acetylproline (*N*-AcPro) anions, which results from electron attachment to the *neutral N*-AcPro. The molecular model of the current studies, *N*-AcPro (see Fig. 1), allowed us to describe the first steps of electron attachment to the *neutral* proline incorporated into the peptide backbone. In particular, *N*-AcPro, contains a cyclic structure which results in a unique N-C_α bond (none of other 20 naturally existing amino acids contains this type of the N-C_α bond). Moreover, *N*-AcPro mimics proline in a peptide context owing to the presence of the acetyl group at the N1 position (for atom numbering see Fig. 1). Furthermore, the free carboxyl group models the existence of proton donating species in the close vicinity to the N-C_α bond. Finally, the *N*-AcPro itself can be easily introduced into the gas phase, a property that allows gas phase studies to be carried out.

In our photoelectron experiments, as in mass spectrometry experiments, ions were created in the gas phase.

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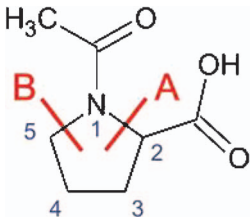

FIG. 1. Numbering of pyrrolidine's ring in *N*-acetylproline. The bond-cutting bars symbolize the considered breakages of the pyrrolidine ring in the *N*-AcPro anion: Path A – splitting of the N1-C2 bond, Path B – splitting of the N1-C5 bond.

However, in our work these ions (anions) were generated in a unique source, mass-selected, and the excess electron photodetached with a fixed frequency laser beam. The resultant photodetached electrons kinetic energy was measured which after accounting for the photon energy yields the electron binding energy (EBE) of the anion. Thus, mass spectrometry is a sub-component of the experimental method used in the present work. Moreover, the vertical detachment energy (VDE; a maximum EBE value in a given photoelectron feature) can be calculated using appropriate quantum chemical (QM) models.[15] Hence, by combining a PES experiment with QM calculations, one can obtain a clear picture of the structure of the resulting anion and thus elucidate the processes that may have produced that structure.

II. EXPERIMENTAL METHODS

Anion photoelectron spectroscopy is conducted by crossing a mass-selected, negative ion beam with a fixed-energy photon beam and analyzing the energies of the resultant photodetached electrons. This technique is governed by the well-known energy-conserving relationship, $h\nu = EBE + EKE$, where $h\nu$, EBE, and EKE are the photon energy, electron binding energy (transition energy), and the electron kinetic energy, respectively.

Negative ions of *N*-acetylproline were generated in an ion source before traveling through a linear, time-of-flight mass spectrometer which served to provide both mass analysis and mass selection (beside the parent $[N\text{-AcPro}]^-$, the deprotonated, $[N\text{-AcPro-H}]^-$, anions originating from the dissociative electron attachment process to the neutral *N*-AcPro (Refs. 16 and 17) were observed in our mass spectrum). The mass-selected anions of interest ($[N\text{-AcPro}]^-$) were then photodetached by light from a pulsed laser and their electron kinetic energies analyzed with a magnetically guided electron time-of-flight energy analyzer (a "magnetic bottle") with a resolution of 35 meV at EKE = 1 eV. Details of our apparatus have been described elsewhere.[18] The third (355 nm, 3.49 eV) harmonic of a Nd:YAG laser was used for the measurements reported in this paper. Photoelectron spectra were calibrated against the well known photoelectron spectrum of Cu^-.

N-acetylproline anions were generated using our pulsed, infrared desorption-pulsed visible photoemission anion source which we have described in details elsewhere.[19] In this source, fragile neutral molecules were brought into the gas phase by infrared desorption, while at almost the same time low energy electrons were generated by high intensity visible pulse (photoemission), and a pulsed jet of helium (99.994% purity) provided collisional cooling. Desorption of nonvolatile biomolecules was accomplished by directing a low power pulse of first harmonic (1064 nm, 1.16 eV) Nd:YAG laser light onto a translating graphite bar, thinly coated with *N*-acetyl-L-proline (98%, purchased from Sigma). Low energy electrons were emitted by coordinating a high power pulse of second harmonic (532 nm, 2.33 eV) Nd:YAG light onto a rotating yttrium oxide (work function of ~2 eV) disk. The helium jet was generated in a standard pulsed valve. This appears to be an especially gentle method of attaching electrons to molecules and forming their negative ions as evidenced by its production of several, otherwise unattainable, parent molecular anions. While there is no expectation of equilibrium conditions in the ion beam once fully formed, the possibility of a rapid equilibrium being established in the source region itself is a different issue. Infrared desorption is essentially a fast thermal vaporization, the photoemitted electrons are typically low energy electrons, and the burst of high pressure helium certainly provides many collisions which should approach an equilibrium condition. Indeed, it was demonstrated in the past that the most stable form of a given anion is measured with the photoelectron spectroscopy.[20] Further, our own experiments on various complexes between nucleobases and various proton donors as inorganic acids, amino acids, other nucleobases proved that the thermodynamically most stable anions are responsible for the main PES feature. [15]

III. COMPUTATIONS

As a matter of terminology, the names of anion radicals are preceded with a prefix "an-," as in an-endo-t, while their parent neutral structures are preceded by the prefix "neu-," as in neu-endo-t (see Fig. 2). Since *N*-AcPro may exist in several conformations, as demonstrated by Aliev *et al.* in their conformational NMR studies,[21,22] "-endo-" and -exo-" are used to differentiate between the C^γ-endo and C^γ-exo ring conformers, respectively, (see Fig. 2). Furthermore, the cis and trans rotamers of *N*-AcPro (related to the acetyl group rotation with respect to the *N*-AcPro ring) are indicated by a suffix "-c-" and "-t-," respectively (see Fig. 2). Finally, at the end of the names of cis conformers an additional "-c" or "-t" suffix is added reflecting the cis or trans position (with respect to the C2-H bond; for atom numbering see Fig. 1) of the carboxyl group (see Fig. 2). For instance, neu-endo-c-t stands for a neutral molecule in which the pyrolidine ring, acetyl and carboxyl group assume C^γ-endo, cis, and trans conformation, respectively. For energetic reasons, in the case of the trans conformation of the acetyl group, only structures with a hydrogen bond involving carboxyl's OH and acetyl's oxygen were considered. Therefore, the symbol related to the conformation of the carboxyl group was omitted in the names of these conformers (see Fig. 2).

We have applied the density functional theory method with the Becke's three-parameter hybrid functional (B3LYP) (Refs. 23, 24, and 25) and the 6-31++G(*d*,*p*) basis set.[26,27] The B3LYP method was found to be satisfactory for

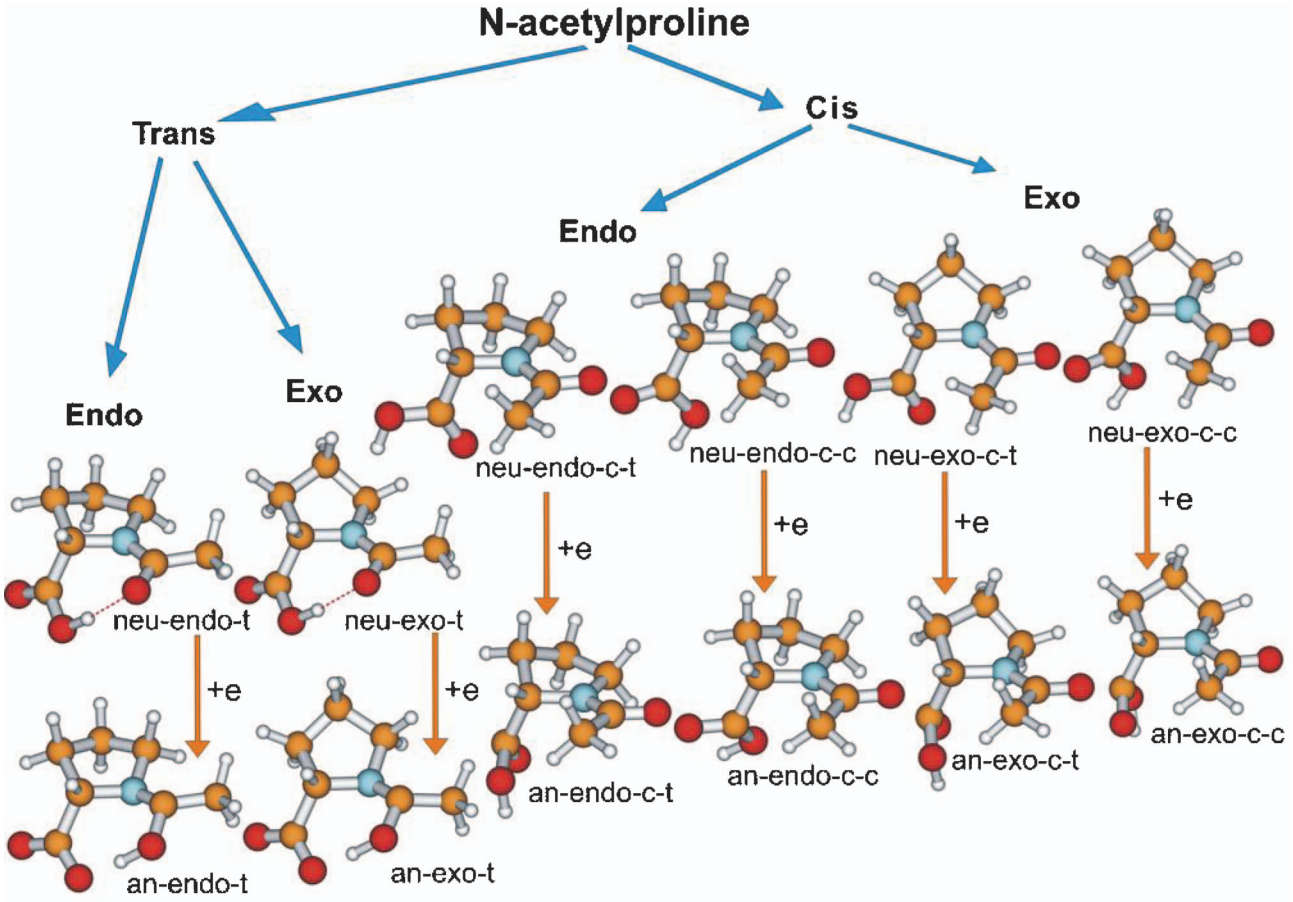

FIG. 2. Conformers of the neutral and anionic *N*-AcPro – from the most stable one on the left to the least stable one on the right.

predicting excess electron-binding energies for valence-type molecular anions[28] as well as for describing intramolecular hydrogen bonds.[29]

All geometries presented here were fully optimized without any geometrical constraints, and the analysis of harmonic frequencies proved that all of them are either structures at energetic minima (all force constants positive) or first-order saddle points (all but one force constant positive). The relative energies (ΔE), enthalpies (ΔH), and free energies (ΔG) of neutrals and anion radicals are defined with respect to the most stable neutral and anion radical, respectively. The relative enthalpies result from correcting the values of ΔE for zero-point vibration terms, thermal contributions to energy and pV terms, while the relative Gibbs free energies from correcting ΔH for entropy terms. These terms were calculated in the rigid rotor-harmonic oscillator approximation for $T = 298$ K and $p = 1$ atm.

To describe the stability of anion radicals we have compared two of their energetic characteristics. Electron vertical detachment energies (VDE), direct observables in our PES experiment, were evaluated as the difference between the energy of the neutral and anion radical at the geometry of the fully relaxed anion radical. Moreover, the difference in electronic free energies, enthalpies, and Gibbs free energies between the most stable neutral entity and its anion both in their correspondingly fully relaxed structures are denoted by AEA_E, AEA_H, and AEA_G., respectively.

All quantum chemical calculations have been carried out with the GAUSSIAN03 (Ref. 30) code on dual Intel Itanium 2 nodes at the Academic Computer Center in Gdańsk (TASK) and the pictures of molecules and orbitals were plotted with the GAUSSVIEW 5 (Ref. 31) and MOLDEN packages.[32]

IV. RESULTS AND DISCUSSION

A. *N*-acetylproline photoelectron spectrum

Figure 3 presents the anion photoelectron spectrum of $(N\text{-AcPro})^-$ measured with 3.49 eV photons. The shape (half width = 0.25 eV) and the position (maximum at EBE = 1.4 eV) of the spectrum suggest that under the conditions of our experiment only one type of anion is formed. Two types of anions are sometimes seen in photoelectron spectroscopic experiments: valence and dipole-bound anions.[20] Whereas the latter appear in photoelectron spectra as single narrow peaks with very low EBE values, the former typically exhibit broader features and have higher EBE maxima.[33] Thus, the spectrum depicted in Fig. 3 corresponds to the valence anion of *N*-AcPro, $(N\text{-AcPro})^-$. Due to collisional cooling in the source, one expects to make the most stable anions in these experiments, and thus the measured photoelectron spectra are those of relatively relaxed anion in most cases.[15] Thus, QM calculations meant to interpret these experiments typically focus on the thermodynamically most stable systems.

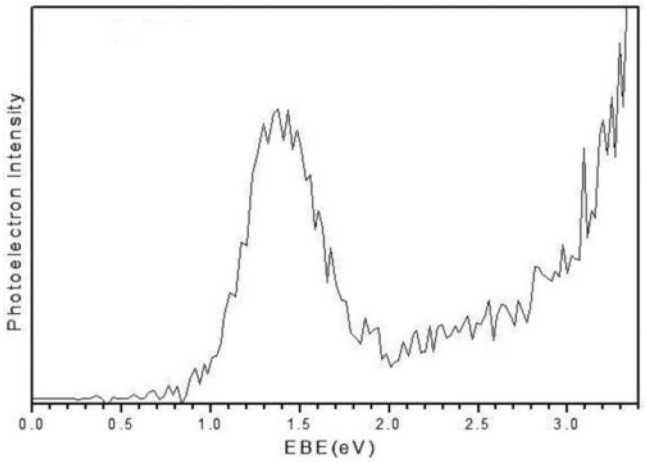

FIG. 3. Photoelectron spectrum of the (*N*-AcPro)$^-$ anion recorded with 3.49 eV photons (EBE = electron binding energy).

B. Neutral conformations

Employing the results of the above mentioned conformational NMR studies[21,22] we generated and optimized, at the B3LYP/6-1++G(*d*,*p*) level, six neutral structures of *N*-AcPro (see Fig. 2). These structures differ in the mutual orientation of the acetyl and carboxyl groups as well as with the conformation of the five-membered pyrrolidine ring. Although the combination of the two conformations of the ring (-endo and -exo) with the two conformations of the acetyl group (-*cis* and -*trans*) results in four possibilities, for the cis conformers we also considered two rotamers of the carboxyl group that led to the six mentioned above geometries (see Fig. 2).

The stability difference between the most stable acetyl-*cis* conformer (neu-endo-c-t) and the most stable acetyl-*trans* one (neu-endo-t), which is a global minimum, amounts to 4.2 and 2.7 kcal/mol in the electronic and free energy scale, respectively (see Table I). The calculated free energies imply that at 298 K ca 99% of *N*-AcPro is in the form of trans conformers. Thus, the equilibrated gas phase mixture of the neutrals is dominated by the geometries stabilized with a strong internal hydrogen bond between the carbonyl oxygen of the acetyl group and the hydroxyl group of the carboxyl function (see Fig. 2). Formation of this hydrogen bond is possible only in the acetyl-*trans* rotamers, which are therefore much more favorable. Moreover, it is worth mentioning that the C$^\gamma$-endo conformers are generally more stable than the C$^\gamma$-exo conformers (see Table I).

TABLE I. Energetic characteristics of the neutral conformers of *N*-AcPro calculated at the B3LYP/6-31++G** level. ΔE, ΔH, and ΔG stand for the relative electronic energy, enthalpy, and free energy, respectively, calculated with respect to the neu-endo-t structure (the lowest energy conformer); all values given in kcal/mol.

Structure	ΔE	ΔH	ΔG
Neu-endo-t	0.00	0.00	0.00
Neu-exo-t	0.98	1.07	1.12
Neu-endo-c-t	4.20	4.13	2.74
Neu-endo-c-c	4.54	4.52	3.12
Neu-exo-c-t	4.77	4.71	3.40
Neu-exo-c-c	4.98	4.93	3.56

C. Anion radicals

Attachment of an electron to the neutral form of *N*-AcPro leads to the formation of the valence anion radicals in which the excess electron is localized on a π^* orbital (see Fig. 4). This observation is consistent with the measured PES spectrum (cf. Fig. 3) whose shape and position corresponds to the valence anions.

To optimize the anionic structures at the B3LYP level we started from the six neutral geometries described in Sec. IV B and, consequently, ended up with the six anions presented in Fig. 2. In all acetyl-*cis* geometries the excess electron attaches to the π^* orbital localized on the carboxyl group of *N*-AcPro (see Fig. 4). As a consequence, this group loses its planarity (see Fig. 2), which diminishes the anti-bonding character of the singly occupied molecular orbital. On the other hand, the attachment of an electron to the acetyl-*trans* conformations triggers a proton transfer from the carboxyl to acetyl group. In this case, the excess electron occupies the π^* orbital localized on the acetyl group of *N*-AcPro and the formation of those anions is accompanied by the transfer of proton from the carboxyl group which additionally stabilizes the negative charge. Such barrier-free electron attachment induced proton transfer (PT) has been observed many times in the gas phase complexes between nucleobases and various proton donors.[15]

Table II presents the thermodynamic characteristics of the studied anions obtained at the B3LYP/6-31++G(*d*,*p*) level. The most stable anion, an-endo-t, is characterized by VDE of 1.53 eV that corresponds fairly well to the maximum on the PES spectrum (cf. Table II with Fig. 3). The second most stable structure, an-exo-t, is by 1.6 kcal/mol less stable, in the free energy scale (see Table II), than an-endo-t. It is calculated to have a VDE value of 1.49 eV (see Table II), and is also consistent with the experimental PES maximum, but it should not significantly contribute since it is less favored energetically. For the remaining anions which are of the acetyl-*cis* type, ΔG amounts to as much as 8.6–9.6 kcal/mol which implies that they should not appear in measurable amounts in the equilibrated gas phase mixture of the *N*-AcPro anions.

What is worth underlining, is that all of AEA values are negative, which, at the first glance, suggests that these anion radicals are not adiabatically stable (although they are vertically stable as indicated by their positive VDEs – see Table II). However, the most stable anion, an-endo-t, with $AEA_G = -0.41$ kcal/mol should be produced in amounts high enough to be observed in the PES experiment. The VDE of 1.53 eV calculated for this anion (see Table II) assures its kinetic stability so that once formed it easily survives over time needed for its transportation to the photodetachment region of spectrometer. A similar situation was observed for the CO_2^- anions which although characterized by negative adiabatic electron affinity were vertically stable.[34]

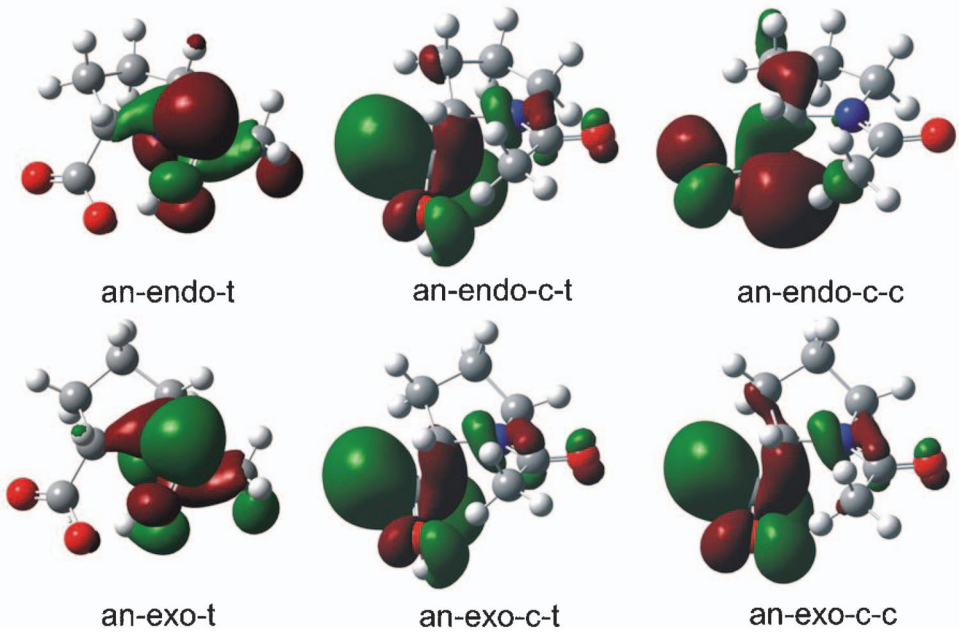

FIG. 4. Singly occupied molecular orbitals of *N*-AcPro anions plotted with a contour value of 0.03 bohr$^{-3/2}$.

D. Possible degradation pathways induced by an excess electron

It has been proposed that hidden proline rearrangements may play a role in the unusual behavior of multiply charged protein ions upon electron capture.[9] Indeed, a dissociation of the N1-C2 (path A) or N1-C5 (path B) pyrolidine ring bonds (see Fig. 1) within the respective radicals may follow electron attachment to proline-containing protein ions. We, therefore, modeled the above mentioned dissociation process in the anion radicals of (*N*-AcPro)$^-$. Thus, the energetics of two possible degradation paths was calculated for two chosen anions: an-endo-t, representing the acetyl-*trans* isomer and an-exo-c, being one of the acetyl-*cis* isomers. The stationary points for the considered reaction paths are depicted in Fig. 5, while their energetic characteristics are gathered in Table III and Fig. 5. Interestingly, the studied dissociation process is very sensitive towards the type of isomer involved in the reaction. For the acetyl-*cis* conformer, path A is a favored channel. Indeed, the thermodynamic driving force amounts to as much as −11.2 kcal/mol and the kinetic activation barrier is as low as 0.9 kcal/mol (see Table III). Simultaneously, the energetic characteristics for the path B indicate that this channel is closed. The situation is quite different for the acetyl-*trans* anion. Here, both paths, A and B, are associated with small negative free energies and comparable medium sized kinetic barrier (see Table III). These results remain in accordance with the experimental picture. Indeed, as was mentioned above, the gas phase equilibrium mixture of the considered anions mainly consists of an-endo-t. For this anion the activation barriers for both paths A and B (Table III) are sufficient to prevent appreciable dissociation during the time of the experiment (several dozen μs.) Thus, only a small fraction of the an-endo-t anions may decompose before electron detachment and most of them are registered as a PES signal with the maximum EBE of 1.55 eV (see Fig. 3). Finally, the VDEs of the dissociation products of the an-endo-t anions amount to 2.77 and 4.13 eV, respectively (see Table III). If these anions dissociated significantly one should observe the corresponding PES signal at ∼2.8 eV which is not the case, cf. Fig. 3.

The above described energetic characteristics for ring opening in the anionic (*N*-AcPro)$^-$ suggest that the fate of

TABLE II. Energetic characteristics of *N*-AcPro anion radical conformers along with their VDE and AEA values calculated at the B3LYP/6-31++G** level. ΔE, ΔH, and ΔG stand for the relative electronic energy, enthalpy, and free energy, respectively. The relative values are calculated with respect to the an-endo-t structure (the lowest energy conformer). ΔE, ΔH, ΔG, and AEAs in kcal/mol, VDE in eV.

Structure	ΔE	AEA_E	ΔH	AEA_H	ΔG	AEA_G	VDE
An-endo-t	0.00	− 3.75	0.00	−0.63	0.00	−0.41	1.53
An-exo-t	2.47	− 5.24	2.24	−1.81	1.62	−0.91	1.49
An-endo-c-t	9.69	− 9.25	10.13	−6.63	8.64	−6.31	0.55
An-endo-c-c	10.57	− 9.79	10.84	−6.95	9.17	−6.46	0.59
An-exo-c-t	11.19	− 10.17	11.69	−7.61	9.61	−6.62	0.62
An-exo-c-c	10.63	− 9.40	11.02	−6.72	9.63	−6.48	0.65

TABLE III. Thermodynamic (ΔE, ΔG) and kinetic (ΔE^*, ΔG^*) barriers for breaking pyrrolidine's ring in two chosen anionic conformers along with VDE of broken-ring products. Paths A and B indicate the cleavage of the N1-C2 and N1-C5 bond, respectively; VDE in eV, all other values in kcal/mol.

Substrate	Path	ΔE	ΔG	ΔE^*	ΔG^*	VDE
An-exo-c-c	A	− 8.52	− 11.18	2.22	0.90	2.93
	B	13.93	9.60	27.73	24.67	3.05
An-endo-t	A	1.58	− 0.73	17.59	14.37	2.77
	B	0.68	− 2.99	20.34	17.34	4.13

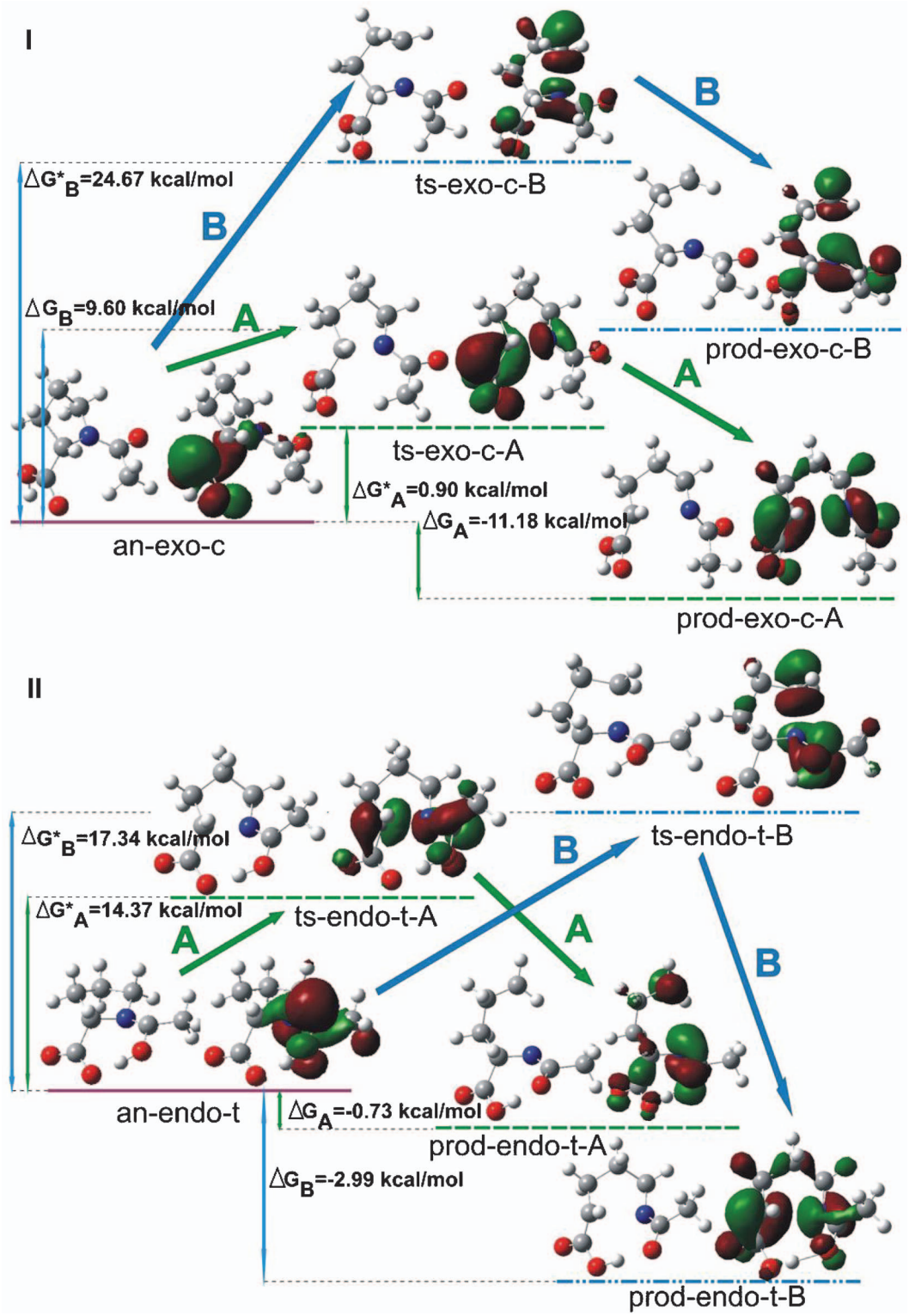

FIG. 5. Geometries and singly occupied molecular orbital (SOMO) distribution in stationary points (substrate, transition state, and product) on the reaction paths concerning the N-C bonds splitting (A – cleavage of the N1-C2 bond; B – cleavage of the N1-C5 bond) in the *N*-AcPro anions. Parts I and II refer to the cleavage of the pyrrolidine ring in an-exo-c and an-endo-t, respectively. ΔG and ΔG^{*} denote the free energy of reaction and the activation free energy, respectively.

proline incorporated in a peptide/protein immersed in a water solution under physiological *p*H should strongly depend on the peptide/protein sequence. Indeed, if a proton donor occurs in close proximity to the proline residue, then electron attachment should lead to proton transfer which disfavors ring opening reactions as was shown for the acetyl-*trans* anions of *N*-AcPro. Such a protein/peptide would probably dissociate into fragments due to electron attachment-induced cleavage of the N-C_α bond neighboring proline, and this would compete with pyrrolidine ring opening. If, however, an internal

proton transfer reaction triggered by electron attachment is not possible, proline would form the valence anions which would stabilize by ring opening as was demonstrated above for the acetyl-*cis* type anions of *N*-AcPro. As a result no fragmentation of protein/peptide would be observed at the proline residue.

V. CONCLUSIONS

N-acetylproline, a molecule which can be considered as a model of proline in a peptide/protein context, forms a valence anion, as shown by their anion photoelectron spectrum. The photoelectron spectrum of those anions exhibits a spectral band with a maximum measured at 1.4 eV. The fair agreement between this value and the VDE's of 1.53 and 1.49 eV calculated at the B3LYP/6-31++G(*d*,*p*) level for the an-endo-t and an-exo-t anions, respectively, indicates that the equilibrium gas phase mixture of anions is dominated by the acetyl-*trans* species. This conclusion is also supported by the thermodynamic arguments. Indeed, both the equilibrium mixture of the neutrals and anions is dominated by the acetyl-*trans* rotamers of *N*-AcPro. Thus, the anions which are observed in the photoelectron experiment are formed by electron attachment to the neut-endo-t conformer. The captured electron occupies the π^* orbital localized on the acetyl group and anion formation is facilitated by PT from the carboxyl group to the acetyl group, leading to the an-endo-t isomer.

The acetyl-*cis* anions, which do not undergo electron induced proton transfer and for which VDEs were calculated to be 0.5–0.6 eV, are by 9–10 kcal/mol less stable than the proton transferred structures and therefore do not contribute to the measured PES signal.

The $(N\text{-AcPro})^-$ anions may undergo thermal degradation. Taking into account literature suggestions the breakage of the C1-N1 and C1-N5 bonds has been considered. The medium kinetic barrier for these ring openings predicted for the an-endo-t anion indicate that its decomposition should not affect the photoelectron measurements.

The calculated kinetic characteristics suggest that the decomposition of proline incorporated in a peptide/protein should be sensitive towards its molecular surroundings under physiological conditions. Strong proton donors present in close vicinity to the proline residue are predicted to hinder pyrrolidine ring openings via electron induced proton transfer. On the other hand, electron attachment to the proline residue not interacting with a proton donor should promote rapid ring opening.

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[1] J. H. Miller, W. E. Wilson, and R. H. Ritchie, in *Computational Approaches in Molecular Radiation Biology*, edited by M. N. Varma and A. Chatterjee (Springer-Verlag, New York, 1994).
[2] B. R. Michael and P. O`Neill, Science **287**, 1603 (2000).
[3] M. Simic and E. J. Hayon, J. Phys. Chem. **77**, 996 (1973).
[4] D. Suryanarayana and M. D. Sevilla, J. Phys. Chem. **83**, 1323 (1979).
[5] M. D. Sevilla and V. L. Brooks, J. Phys. Chem. **77**, 2954 (1973).
[6] R. A. Zubarev, D. M. Horn, E. K. Fridriksson, N. L. Kelleher, N. A. Kruger, M. A. Lewis, B. K. Carpenter, and F. W. McLafferty, Anal. Chem. **72**, 563 (2000).
[7] E. A. Syrstad and F. Tureček, J. Am. Soc. Mass Spectrom. **16**, 208 (2005).
[8] J. J. Coon, B. Ueberheide, J. E. P. Syka, D. D. Dryhurst, J. Ausio, J. Shabanowitz, and D. F. Hunt, Proc. Natl. Acad. Sci. U.S.A. **102**, 9463 (2005).
[9] S. Hayakawa, M. Hashimoto, H. Matsubara, and F. Tureček, J. Am. Chem. Soc. **129**, 7936 (2007).
[10] N. D. Udeshi, J. Shabanowitz, D. F. Hunt, and K. L. Rose, FEBS J. **274**, 6269 (2007).
[11] F. Tureček, J. W. Jones, T. Towle, S. Panja, S. B. Nielsen, P. Hvelplund, and B. Paizs, J. Am. Chem. Soc. **130**, 14584 (2008).
[12] F. Tureček, J. Am. Chem. Soc. **125**, 5954 (2003).
[13] F. Tureček, X. Chen, and C. Hao, J. Am. Chem. Soc. **130**, 8818 (2008).
[14] R. N. Grewal, H. E. Aribi, A. G. Harrison, K. W. M. Siu, and A. C. Hopkinson, J. Phys. Chem. B **108**, 4899 (2004).
[15] J. Rak, K. Mazurkiewicz, M. Kobyłecka, P. Storoniak, M. Harańczyk, I. Dąbkowska, R. A. Bachorz, M. Gutowski, D. Radisic, S. T. Stokes, S. N. Eustis, D. Wang, X. Li, Y. J. Ko, and K. H. Bowen, in *Radiation Induced Molecular Phenomena in Nucleic Acids: A Comprehensive Theoretical and Experimental Analysis*, edited by M. K. Shukla and J. Leszczyński (Springer-Verlag, Amsterdam, 2008).
[16] H. Abdoul-Carime and E. Illenberger, Chem. Phys. Lett. **397**, 309 (2004).
[17] P. Sulzer, E. Alizadeh, A. Mauracher, T. D. Märk, and P. Scheier, Int. J. Mass. Spectrom. **277**, 274 (2008).
[18] M. Gerhards, O. C. Thomas, J. M. Nilles, W. J. Zheng, and K. H. Bowen, J. Chem. Phys. **116**, 10247 (2002).
[19] S. T. Stokes, X. Li, A. Grubisic, Y. J. Ko, and K. H. Bowen, J. Chem. Phys. **127**, 084321 (2007).
[20] D. Svozil, P. Jungwith, and Z. Havlas, Collect. Czech. Chem. Commun. **69**, 1395 (2004).
[21] A. E. Aliev and D. Courtier-Murias, J. Phys. Chem. B **111**, 14034 (2007).
[22] A. E. Aliev, S. Bhandal, and D. Courtier-Murias, J. Phys. Chem. A **113**, 10858 (2009).
[23] A. D. Becke, Phys. Rev. A **38**, 3098 (1988).
[24] A. D. Becke, J. Chem. Phys. **98**, 5648 (1993).
[25] C. Lee, W. Yang, and R. G. Parr, Phys. Rev. B **37**, 785 (1988).
[26] R. Ditchfield, W. J. Hehre, and J. A. Pople, J. Chem. Phys. **54**, 724 (1971).
[27] W. J. Hehre, R. Ditchfield, and J. A. Pople, J. Chem. Phys. **56**, 2257 (1972).
[28] J. C. Rienstra-Kiracofe, G. S. Tschumper, H. F. Schaefer, S. Nandi, and G. B. Ellison, Chem. Rev. **102**, 231 (2002).
[29] J. Rak, P. Skurski, J. Simons, and M. Gutowski, J. Am. Chem. Soc. **123**, 11695 (2001).
[30] M. J. Frisch, G. W. Trucks, H. B. Schlegel *et al.*, GAUSSIAN 03, Gaussian, Inc., Wallingford, CT, 2004.
[31] GAUSSVIEW *5*, Æ. Frisch, H. P. Hratchian, R. D. Dennington II, A. Todd, T. A. Keith, and J. Millam, Gaussian, Inc., Wallingford, CT, 2009.
[32] G. Schaftenaar and J. H. Noordik, J. Comput.-Aided Mol. Des. **14**, 123 (2000).
[33] A. Szyperska, J. Rak, J. Leszczyński, X. Li, Y. J. Ko, H. Wang, and K. H. Bowen, J. Am. Chem. Soc. **131**, 2663 (2009).
[34] D. Schröder, C. A. Schalley, J. N. Harvey, and H. Schwarz, Int. J. Mass Spectrom. **185/186/187**, 25 (1999).