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Detailed dissociative electron attachment studies on the amino acid proline

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ABSTRACT

We present a detailed study on the fragmentation of proline (Pro, $C_5H_9NO_2$) upon attachment of low energy electrons (<12 eV). Previous investigations on dissociative electron attachment to Pro are partly confirmed, extended and also in some points vitiated. A total of 11 fragment anions, with $[Pro\text{-}H]^-$ being the most abundant one, has been observed and the present results indicate that the formation of $[glycil\text{-}yl]^-$ from Pro cannot be induced by slow electrons. This work supplements existing work on amino acids and therefore improves the understanding of the interaction between electrons at sub-ionization energies and molecules of biological interest.

1. Introduction

Inspired by the pioneering work of Boudaïffa et al. [1], the interaction of free electrons with isolated molecules of biological relevance has attracted a lot of interest (e.g. DNA bases [2,3], amino acids [4,5], sugar [6], deoxyribose [7] and glycolaldehyde [8]). The bio-molecule proline is a non-essential amino acid, where the amine group is part of the five-membered ring (for chemical structure see Fig. 1). It belongs to the 20 DNA-encoded amino acids and plays an important role in the conformation of proteins and as an asymmetric catalyst in organic reactions. Existing knowledge of dissociative electron attachment (DEA) to simple amino acids (e.g. glycine [9,10], alanine [11] and valine [12]) is here extended to Pro, which shows a similar variety of fragment anions produced upon low energy electron interactions.

In 2004, Abdoul-Carime and Illenberger [13] already published a DEA study on proline, where they presented a total of six fragments arising from slow electron impact on Pro, namely $[Pro\text{-}H]^-$, $[glycil\text{-}yl]^-$, $(HCO_2)^-$, CN^-, OH^- and O^-. Due to the higher sensitivity in the present work, we were able to significantly increase the number of fragments observed and measured the anion efficiency curves for all of them with a very good signal to noise ratio. As the formation of $[glycil\text{-}yl]^-$, i.e. $(C_2H_4NO_2)^-$, from Pro would need a major chemical rearrangement in the molecule (e.g. $NH \rightarrow NH_2$), the observation of this anion in ref. [13] was an essential point. However, our detailed studies now indicate that this anion originated from a contamination of the sample used. Additionally, our high mass resolution allows us to separate isobaric fragments and therefore we can clarify the question whether ions observed with mass 26 m/z should be assigned as $C_2H_2^-$ or as CN^-.

2. Experimental

The present studies were performed utilizing two crossed electron-molecule beams instruments, which have been described in detail previously [14]. For high electron energy resolution measurements (70–100 meV; compared to ~150 meV in ref. [13]), a hemispherical electron monochromator apparatus (HEM) was used (Fig. 2b). The electrons emitted by a hairpin filament are monochromatized in a hemispherical electric sector and cross the neutral molecular beam perpendicularly. The resulting anions are then guided into a quadrupole mass filter without any further extraction fields and finally detected by a channel electron multiplier.

As this instrument is operated with a quite low electron current (10–30 nA), a double focusing two sector field spectrometer equipped with a standard Nier-type ion source (10 μA electron current (three orders of magnitude higher than for the HEM and for the instrument used in ref. [13]); ~1 eV energy resolution at electron energies below 4 eV) was used to measure the less abundant anions with a better signal to noise ratio (Fig. 2a), to confirm, respectively, prove the reproducibility of the data obtained with the monochromator instrument and to separate isobaric fragments. The latter is only possible due to the excellent mass resolution of up to $m/\Delta m = 120{,}000$ compared to about 300 for quadrupole mass filters as used in the HEM instrument and in ref. [13].

The L-proline was purchased from Sigma–Aldrich with a stated purity of 99.5% and heated up to 390–400 K in a home built copper oven to obtain a gas pressure in the collision region of about

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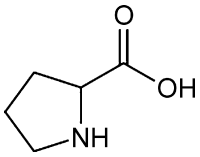

Fig. 1. Chemical structure of proline (Pro, $C_5H_9NO_2$).

5×10^{-5} Pa. This temperature range is well below the molecular decomposition temperature (490 K [15]) and has been proven not to thermally dissociate the molecule [13,16].

To calibrate the energy scale and determine the electron energy resolution, the anion yields of Cl^-/CCl_4 or $SF_6{}^-/SF_6$ which both show a narrow s-wave resonance at 0 eV were recorded. It has to be noted that whenever SF_6 had been introduced into the vacuum chamber, a sharp resonance at 0 eV appeared in the yield of the fragment $[Pro\text{-}H]^-$. The height of this resonance was in strong dependence to the amount of SF_6 that had been introduced and completely disappeared when there was no SF_6 present. We suggest that this was due to the so called "trojan horse effect" which was described in ref. [17] and therefore, we took special care that no SF_6 was in the chamber during the Pro measurements.

3. Results and discussion

An overview of all negative fragment anions of Pro is shown in Fig. 3. As in mass spectra of negative ions fragments only appear when they possess a resonance at the set electron energy, 11 spectra ranging from 0 to 10 eV have been taken in 1 eV steps and finally were summed up. The main purpose of this summary mass spectrum is to identify all existing fragments and to show which fragments are not observable. Only limited information about the relative abundances of the fragments can be obtained, as the heights of the peaks in the mass spectrum strongly depend on whether in these 1 eV steps a maximum of a resonance has been hit or not. Nevertheless, for a measure of the relative yield intensities of the fragments the count rates of the energy scans are stated in kilohertz (kHz; except for Fig. 6). As the experimental conditions were mainly the same for all measurements, these values are directly comparable. Relative attachment cross sections have been recorded (see Figs. 4–7) for all labelled masses in Fig. 3 and possible interpretations of the chemical compositions are given in Table 1.

In the work of Abdoul-Carime and Illenberger [13], a fragment at mass 74 m/z has been observed with an intensity of about 2% of the most abundant anion $[Pro\text{-}H]^-$ in the energy region between 0 and 3 eV. It has been interpreted as $[glycil\text{-}yl]^-$ that should arise from fragmentation of the five-membered ring. However, as one can clearly see in Fig. 3, this fragment is not observed in the present studies. Additionally, we carried out a detailed cross section measurement which confirmed that no fragment of Pro could be detected between 0 and 3 eV at mass 74 m/z. We suggest, as the experimental conditions were mainly the same (temperature, pressure, etc.), that in Abdoul-Carime and Illenberger's work either the sample was contaminated or there was still some glycine left in the machine from a previous measurement.

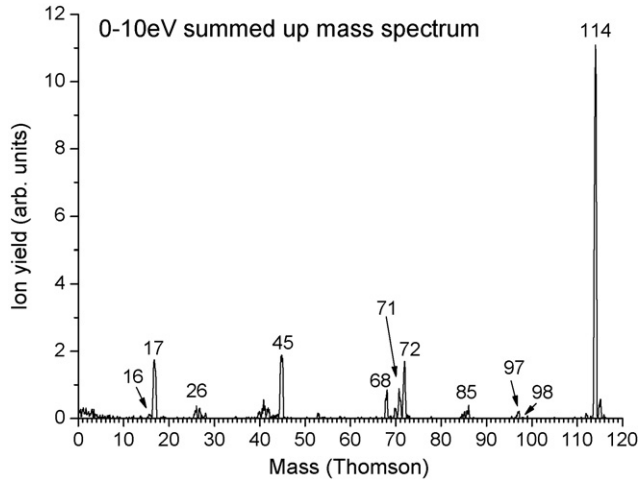

Fig. 3. Summed up anion mass spectrum of Pro recorded from 0 to 10 eV in 1 eV steps (see text).

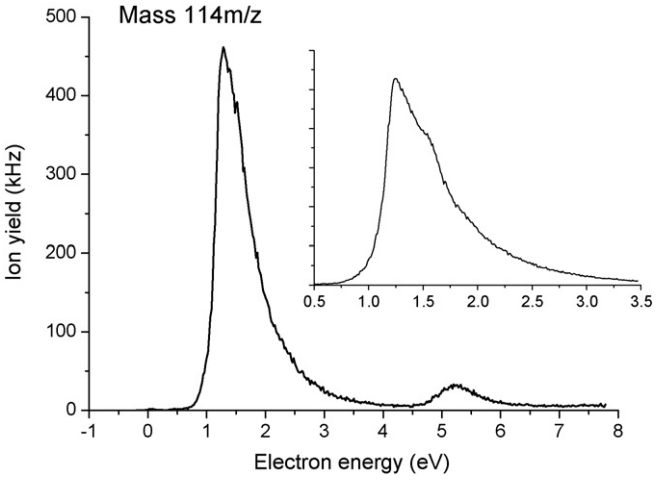

Fig. 4. Anion yield as a function of the incident electron energy for the closed shell anion $[Pro\text{-}H]^-$. The insert represents a long time measurement with high electron energy resolution in the region 0.5–3.5 eV to better identify the shoulder on the right slope of the main peak.

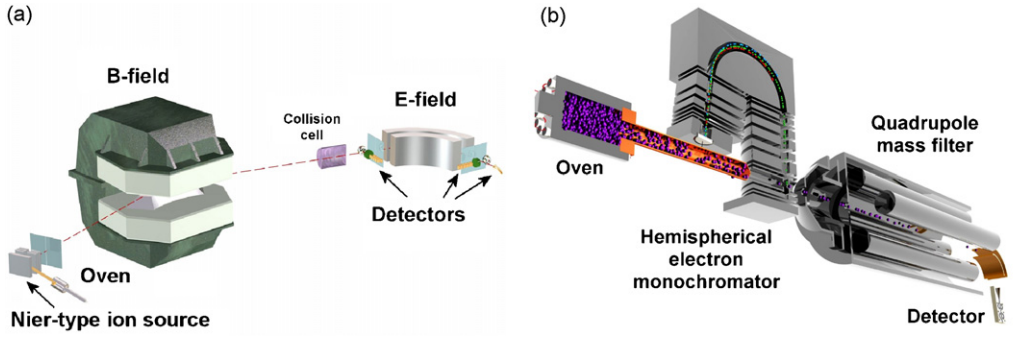

Fig. 2. Schematic view of the two mass spectrometer systems used for the present investigations, where (a) is the sector field and (b) the monochromator instrument.

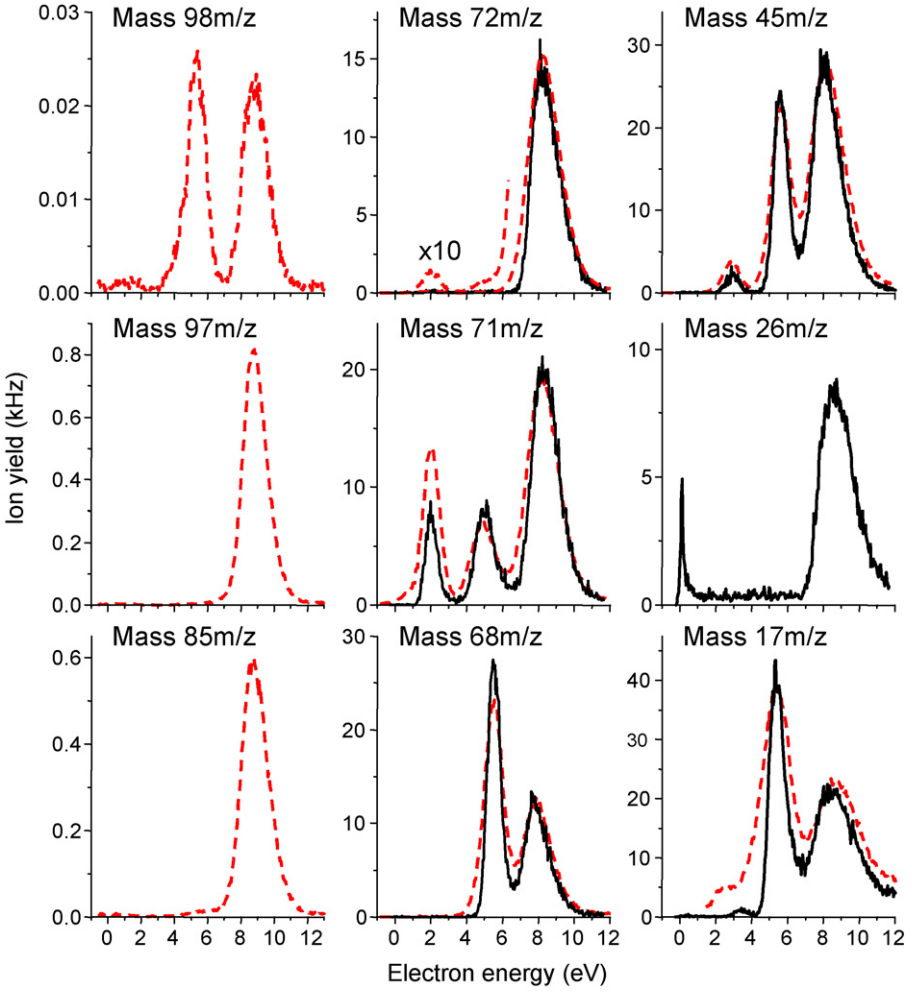

Fig. 5. Energy scans of additional fragments (98, 97, 85, 72, 71, 68, 45, 26 and 17 m/z). Possible chemical compositions of these fragments are given in Table 1. The dashed lines indicate ions that were recorded with the high sensitivity sector field instrument. Measurements from the HEM instrument are plotted as solid lines. *Note*: For the fragment at 72 m/z the low energy part covering the 2 and 5 eV resonance is multiplied by a factor of 10. For the 0 eV contribution at mass 26 m/z see text.

As for most molecules of biological interest (e.g. amino acids [4] and DNA bases [2,3]), also for proline the most abundant anion yield is observed for neutral H-loss. Fig. 4 shows the relative attachment cross section of $[Pro\text{-}H]^-$ recorded from 0 to 8 eV. To resolve the narrow structures in the low energy region an additional measurement with a smaller energy step width and an electron energy resolution of about 70 meV is also included in this figure. At least three resonances are clearly visible, the first one is at about 1.25 eV, the

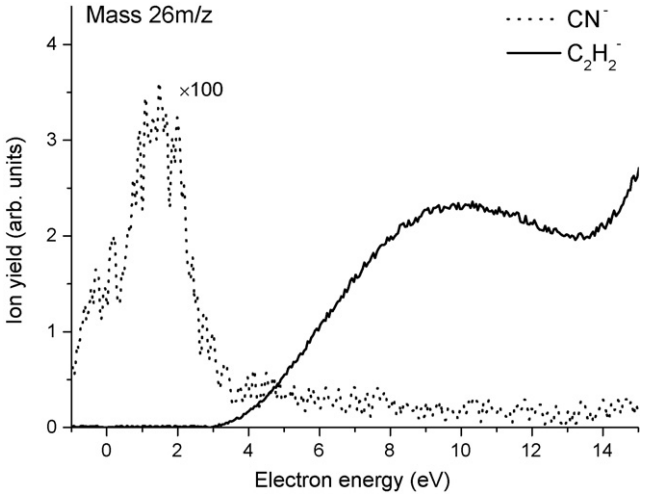

Fig. 6. High mass resolution measurements with the sector field instrument to separate the isobaric fragments at mass 26 m/z. The dotted line stands for CN^- and is multiplied by a factor of 100, whereas the solid line is $C_2H_2^-$.

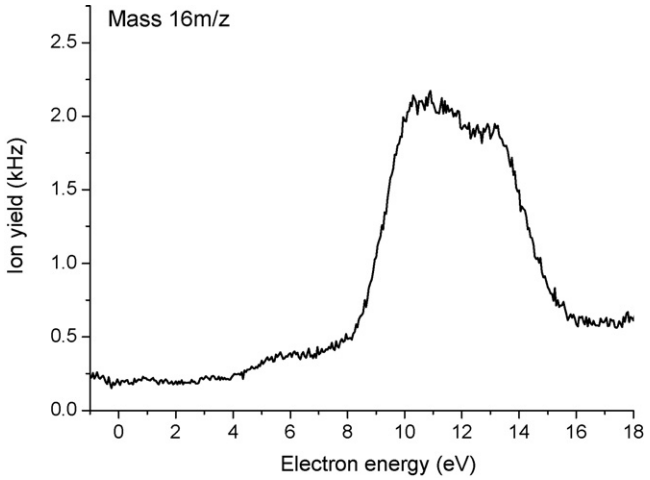

Fig. 7. Measurement of the relative attachment cross section of O^- (16 m/z) obtained with the sector field instrument. Possible contributions from residual gas at this mass have been taken into account and are subtracted.

Table 1
Positions of the resonances for all observed fragments

Mass (Thomson)	Composition	(1) Resonance (eV)	(2) Resonance (eV)	(3) Resonance (eV)
114	$[Pro\text{-}H]^-$	1.25/1.49	5.27	
98	$[Pro\text{-}OH]^-$		5.3	8.8
97	$[Pro\text{-}OH_2]^-$			8.8
85				8.8
72	$C_3H_4O_2^-$ and $^{13}C^{12}C_2H_3O_2^-$	2.0	5.0	8.4
71	$C_3H_3O_2^-$ or $[Pro\text{-}CO_2]^-$	2.0	5.0	8.4
68	$C_3O_2^-$ or $[Pro\text{-}H_3CO_2]^-$		5.5	7.9
45	HCO_2^-	3.0	5.6	8.1
26	$C_2H_2^-$			8.7
17	OH^-	3.4	5.4	8.5
16	O^-		6.2	10.2/13.0

The values have been obtained by Gaussian-fits (multiple Gaussian-fit for the first peak in $[Pro\text{-}H]^-$ and the second peak in O^-). Assuming that no complex rearrangements occur in the molecule, possible chemical compositions are given.

second one at 1.49 eV and the third one at 5.27 eV (values obtained by (multiple) Gaussian-fits).

For several organic acids including glycine [9], alanine [11] and valine [4,12] thresholds for H-loss from the different possible positions were determined by quantum chemical calculations. It is known that, e.g. acetic acid [18], which does possess a carboxylic but no amino group, shows only a resonance in the 1 eV region with a steep onset on the low energy side and vibrational structures on the high energy side for the dehydrogenated molecule. In comparison, DEA to the amino acid valine [4] resulting in $[valine\text{-}H]^-$ shows mainly two resonances, one at about 1 eV and another one at about 5 eV, where the low energy feature can be assigned as originating from the carboxylic group and the high energy resonance as originating from the amino group. Due to the similarity of the yield curves of dehydrogenated valine and proline and the fact that the 5 eV resonance is missing for acetic acid, we suggest the same assignment for Pro (i.e. ~1 eV resonance → O-site, 5.27 eV resonance → N-site). However, the final answer to the question, which H atom is removed at the 5.27 eV resonance, can only be given with experiments using labelled derivatives of proline.

In good agreement with Abdoul-Carime and Illenberger, no parent anion of Pro is observed at any electron energies in the present study. This fact was verified by quantitative measurements at the sector field instrument comparing the masses 114, 115 and 116 *m*/*z*. It turns out that the ion yields obtained at 115 and 116 *m*/*z* originate exclusively from dehydrogenated Pro molecules containing ^{13}C, ^{17}O, ^{18}O or ^{15}N (100% yield intensity for mass 114 *m*/*z*, 5.9% for 115 *m*/*z* and 0.5% for 116 *m*/*z*, which is in perfect agreement with the values calculated from the expected isotope ratios).

The advantage of additionally using a sector field instrument becomes obvious from Fig. 5, as these fragments could not be measured with acceptable signal to noise ratios with the HEM apparatus. Nevertheless, they can also be detected by the HEM instrument (see Fig. 3) which makes us sure that they do not originate from background contaminations in the sector field instrument. The energy scans at the masses 98, 97 and 85 *m*/*z* show a common resonance at about 8.8 eV and only mass 98 *m*/*z* possesses an additional contribution at about 5.3 eV.

Three quite intense fragments that have not been observed by Abdoul-Carime and Illenberger [13] are presented in Fig. 5. At the masses 72 and 71 *m*/*z*, a broad peak is visible in the high energy region at the same electron energy (8.4 eV), whereas mass 71 *m*/*z* shows two additional resonances (2.0 and 5.0 eV) at lower energies. The small contribution at ~2 eV for mass 72 *m*/*z*, that is only visible in the high sensitivity sector field measurement, is most probably the isotopic form of mass 71 *m*/*z* (with one ^{12}C replaced with ^{13}C). For mass 68 *m*/*z*, the first resonance peaks at 5.5 eV and the second one at 7.9 eV.

The formation of HCO_2^-, CN^- and OH^- upon dissociative electron attachment to Pro has been reported by Abdoul-Carime and Illenberger [13]. Comparing Fig. 6 with the data from the HEM for mass 45 *m*/*z* there is a constant shift in the position of all resonances of about 0.5 eV to higher energies (values obtained from ref. [13] are in brackets): 3.0 (2.6), 5.6 (5.2) and 8.1 (7.6) eV.

Measuring anions at mass 26 *m*/*z* is in general a difficult task as most common contaminations in the apparatus contain CN, respectively, C_2H_2. Therefore, one has to be very careful not to interpret background contributions as originating from the sample. In Fig. 5, for mass 26 *m*/*z* a peak at about 0 eV is visible that is very likely coming from the background, as its height was not corresponding to small changes in the temperature of the sample. So we suggest that the "real data" from Pro in Fig. 5 starts at around 1 eV. A similar problem probably occurred in Abdoul-Carimes measurements where a broad resonance at 1.6 eV is visible. This resonance coincides with the CN^- signal obtained from glycine [5] and therefore supports the suggestion, that during their measurements a contamination of glycine was present. The only resonance originating from DEA to Pro at mass 26 *m*/*z* is therefore at 8.7 eV and again about 0.5 eV higher than reported in ref. [12] (8.2 eV). This shift to higher energies is also observed for mass 17 *m*/*z* (OH^-; Fig. 5) where we obtained resonances at 3.4, 5.4 and 8.5 eV (in comparison to 3.0, 5.0 and 7.9 eV in ref. [13]; this surprisingly large shift may result from different extraction conditions of the two electron monochromator instruments).

With our sector field instrument we are able to separate isobaric fragments [5] and can therefore distinguish between CN^- (dotted line in Fig. 6) and $C_2H_2^-$ (solid line). If one compares now Fig. 6 with the data from the HEM (which represents a superposition of both isobaric fragments) in Fig. 5, it is immediately obvious that DEA to Pro exclusively leads to the formation of $C_2H_2^-$ in the region of mass 26 *m*/*z*. The very low yields for CN^- in Fig. 6 as well as the additional structures in the $C_2H_2^-$ peak originate from background contributions in the sector field instrument, as they are not visible in the HEM measurement.

This result fits nicely to studies we did on the amino acid glycine [5], where for this mass three resonances appeared, with the third one being in a similar energy region (~9–13 eV). In ref. [5], we showed that this resonance originates from $C_2H_2^-$, whereas the lower energy contributions (that are missing for Pro) could be assigned to CN^-.

Measurements on mass 16 *m*/*z* are again quite difficult to interpret, as O^- is formed from various background contaminations. The data in Fig. 7 exclusively represent the O^- formation out of Pro, whereas contributions from the background have been excluded by checking the dependence of the signal intensity on the temperature of the sample. Three resonances are visible for O^-, a very

small one at about 6.2 eV and two overlapping structures at 10.2 and 13.0 eV.

Table 1 gives an overview of the positions of all resonances and fragments observed. Anion formation in the energy regime below 4 eV is typically associated with shape resonances, which means that the excess electron is accommodated into a formerly unoccupied molecular orbital whereas the electronic configuration of the (neutral) molecule remains unaffected. Therefore, we suggest the low energy structures of the masses 114, 72, 71, 45 and 17 m/z are being produced via shape resonances.

Above ~5 eV fragments are usually formed via core-excited resonances (see ref. [19] for further details), which are found for all fragments presented in this work.

4. Conclusion

The present results show that low energy electrons effectively lead to fragmentation of the amino acid proline. Except for the most abundant anion $[Pro\text{-}H]^-$ all of the 10 other fragments are formed in the electron energy region between 8 and 10 eV. The masses 114, 98, 71, 68, 45, 17 and 16 m/z show additional resonances at an energy region between 5 and 6 eV, whereas dissociative electron attachment below 4 eV has only been observed for five fragments (114, 72, 71, 45 and 17 m/z).

The formation of a fragment at mass 74 m/z $[glycil\text{-}yl]^-$ has neither been observed at the HEM nor at the sector field instrument and we propose its origin in ref. [13] from a possible contamination of the sample.

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