Mass Spectrometric Investigation of Anions Formed upon Free Electron Attachment to Nucleobase Molecules and Clusters Embedded in Superfluid Helium Droplets

Stephan Denifl, Fabio Zappa, Ingo Mähr, Julien Lecointre,* Michael Probst, Tilmann D. Märk,† and Paul Scheier‡

Institut für Ionenphysik und Angewandte Physik and Center for Molecular Biosciences Innsbruck,
Leopold Franzens Universität Innsbruck, Technikerstraße 25, A-6020 Innsbruck, Austria

(Received 21 April 2006; published 26 July 2006)

Here we report the first mass spectrometric study of negative ions formed via free electron attachment (EA) to nucleobases (NBs) embedded in helium clusters. Pure and mixed clusters of adenine and thymine have been formed by pickup of isolated NB molecules by cold helium droplets. In contrast to EA of isolated molecules in the gas phase we observe a long-lived parent anion NB^- and, in addition, parent cluster ions NB_n^- up to size $n = 6$. Moreover, we show that a low energy electron penetrating into a doped helium droplet causes efficient damage of the embedded nucleobases via resonant, site selective, dissociative electron attachment.

DOI: 10.1103/PhysRevLett.97.043201 PACS numbers: 34.80.Ht, 82.33.Fg, 87.50.Gi

So far a large number of studies has been dedicated to free electron attachment (EA) to isolated nucleobases (NBs) in the gas phase (see a review in [1]). The main results were that no stable parent anions of NBs exist under single collision conditions. At electron energies below about 3 eV the NBs dissociate by H loss and above about 3 eV additionally the ring structures of the NBs are destroyed. These gas-phase experiments were motivated by studies of Sanche and co-workers [2,3], who had shown that electrons with subionization energies are able to induce single strand breaks (SSBs) and double strand breaks (DSBs) in plasmid DNA deposited on a surface. The efficiency curves for these strand breaks exhibit a resonant behavior with a maximum for DSBs at around 10 eV. In parallel, the electron induced desorption yield of light anions such as H^-, O^- and OH^- observed for various molecules had the same electron energy dependence, and this led to the suggestion that EA may be the initiating step in strand breaking [2]. One of the drawbacks with these surface experiments is that only the light fragments can be observed. In contrast, all product anions can be measured in gas-phase experiments. Nevertheless, in these experiments with isolated molecules it was not possible to study any environmental influence on the electron capture process (e.g., when the NB is surrounded by other NBs or water). Hence it is important to continue these first studies with isolated NBs by EA experiments with larger and more complex biomolecules such as nucleosides (e.g., see [4]) or possibly base pairs. The latter can be formed from the gas phase by pickup of single biomolecules into cold rare gas clusters where they can undergo weak electrostatic or van der Waals bonding. Helium droplets are known to pick up atoms or molecules efficiently and to be the perfect matrix for spectroscopic studies on atoms and molecules due to their extremely low temperature and the possibility for the creation of new molecular complexes [5]. Evaporative cooling (the binding energy of a helium atom in the cluster is only 0.6 meV) produces an isothermal low temperature environment in the droplet at $T = 0.37$ K, which is lower than possible temperatures for most solid matrices. This has been demonstrated via spectroscopy of pure and mixed doped clusters of atmospheric and biological relevance (see an extensive review about the spectroscopy and chemical reactions of molecules and complexes embedded in helium droplets in [5]).

In contrast, only a few investigations have been dedicated to negative ion formation in (doped) helium clusters (see a review in [6]). It was proposed that electron capture to pure helium clusters is only possible for large droplets ($N > 7.5 \times 10^4$) and that the EA produces bubbles in the interior of the cluster [6]. The smallest pure helium cluster anion observed so far by Toennies and co-workers [7] had a size of 9.3×10^4. Moreover, EA to SF_6, O_2, and H_2O embedded in He clusters was observed only indirectly by the decrease of the neutral cluster yield [8]. To our knowledge no direct mass spectrometric observation of anions formed upon EA to molecules embedded in helium droplets has been reported yet. Here we study in detail the processes initiated by low energy electrons in helium droplets doped with adenine and thymine molecules and pure and mixed clusters of these nucleobases.

The present measurements are performed with a He cluster source combined with a pickup cell containing the vapor of the biomolecules and a double focusing two sector field mass spectrometer (more details can be found elsewhere [9,10]). The helium cluster beam is formed by expansion of helium through a nozzle (orifice of 5 μm) into vacuum at a temperature of 10 K and pressures between 10 and 15 bar. The mean size of the helium droplets is estimated to be $N > 10^4$ under these operating conditions. The He droplets pass 1 cm downstream a skimmer with a diameter of 0.8 mm, and 2 cm downstream they enter a pickup cell. There the helium droplets are doped with the nucleobases being produced by vaporization in two resistively heated ovens filled with the corresponding powders. The ovens are directly connected to the pickup cell by copper capillaries to achieve a higher density of the biomolecules in the pickup cell. For the present measurements typical oven temperatures of about 420 K are used. The pickup cell is heated to 370 K to prevent condensation of the biomolecules on the wall of the pickup cell. The doped helium cluster beam enters the collision chamber of an ion source 20 cm further downstream from the cell, where electron attachment to the doped droplets takes place. The anions produced are accelerated by 3 kV and enter the mass spectrometer. The mass selected anions are finally detected by a channeltron-type secondary electron multiplier. The energy scale is determined by measuring the well known ion yield of SF_6^-/SF_6 under identical conditions. The ion yield of SF_6^- exhibits a sharp peak at 0 eV resulting from *s*-wave attachment to the neutral molecule [11]. The present experiments are performed with an electron energy resolution of about 1 eV and electron currents of about 5–15 μA. We estimate the accuracy of the reported peak positions to be ±0.25 eV. The composition of neutral He droplets doped with biomolecules is monitored by positive ion formation. Protonated nucleobase cluster ions, $(NB)_nH^+$, with n up to 10, are observed as the most abundant cations at sufficiently high concentrations of thymine. Besides proton transfer, no other fragmentation reactions are observed, indicating an efficient cooling of excited cluster cations. Therefore, we conclude that the protonated cluster distribution resembles closely the neutral cluster distribution. For the present conditions the dimer is the most abundant neutral cluster.

As mentioned above in free electron attachment under single collision conditions no long-lived valence NB^- can be observed, which is attributed to the short lifetime against autodetachment and dissociation [12]. This nonobservation of NB^- is in agreement with electron transmission spectroscopy [13], where a negative vertical electron affinity of NBs was observed, and theoretical calculations, which predicted an adiabatic electron affinity close to zero eV [14]. In contrast, dipole bound parent anions of isolated DNA bases were formed in charge exchange collisions between laser excited (Rydberg) Xe atoms and bare neutral NB molecules [15]. Furthermore, they were observed in anion photoelectron spectroscopy of mass selected NB anions generated in a microplasma [16]. It is also known that solvation of the NB (e.g., in water clusters or in rare gases) changes the characteristic of the excess electron binding (from dipole to valence bonding with a strong increase of the electron affinity) [15,17,18]. In our experiment we see a similar stabilization of the temporary negative ion state: the He/NB environment apparently allows one to dissipate the electron attachment energy, that is, the electron affinity plus the induced polarization energy. Hence we observe via EA to the doped He droplets the parent anion T^- (shown in Fig. 1) and also A^- (not shown).

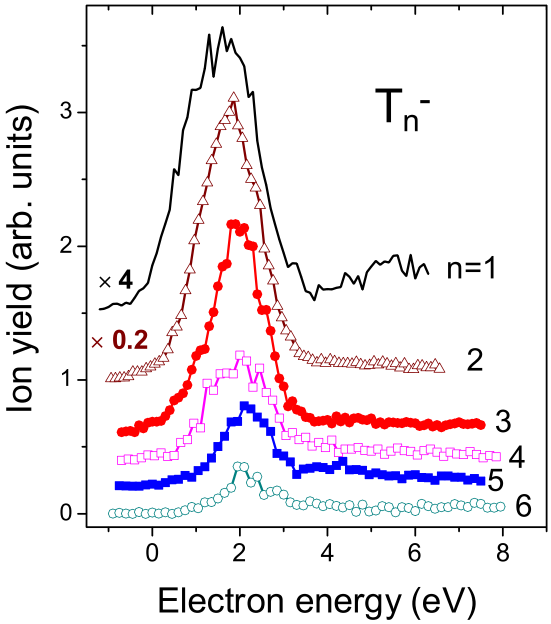

FIG. 1 (color online). Electron energy dependence of thymine cluster anions T_n^-, $n = 1$–6.

Both parent anions are formed via a resonance located at about 2 eV, which is rather surprising as an associative electron attachment process usually leads to a “zero eV peak” [4]. Hence it remains an open question to which degree the peak position may be influenced by an interaction of the incoming electron with helium. One way to elucidate this point is to measure the *s*-wave attachment cross section to CCl_4 embedded in helium droplets. In contrast to the gas phase, a stable parent anion CCl_4^- is observed in our experiments with doped helium droplets. The CCl_4^- anion efficiency curve shows two resonances at about 2 and 22 eV. It is interesting to note that negative ion formation of pure helium droplets also shows two resonances at the same electron energies [7]. Thus all these experiments indicate that zero eV peaks appear at 2 eV for electron attachment to pure and doped helium droplets.

The most abundant parent anions observed here, however, are the dimer anions like $(T_2)^-$ shown in Fig. 1. The formation of $(A_2)^-$, $(T_2)^-$, and $(AT)^-$ has been also reported in the studies of Rydberg electron transfer [19] and in anion photoelectron spectroscopy studies [17], the excess electrons showing for most of these anions valence bound character [17]. By embedding here the NB in cold helium droplets, larger clusters of NBs can be formed and we are able to observe cluster anions like for thymine of up to $n = 6$. The yields of all thymine cluster anions also show a single resonance at about 2 eV (see Fig. 1). We also measured the ion yield of $(AT)^-$ and $(A_2)^-$ (not shown in Fig. 1); both cluster anions are again formed in a single resonance close to 2 eV like $(T_2)^-$. AT base pair anions have been studied recently by means of anion photoelectron spectroscopy and theory [17]. The authors proposed from the measured and calculated vertical detachment energy that the captured electron is initially located at thymine and a subsequent barrier-free proton transfer from adenine to thymine results into a complex of a hydrogenated neutral thymine and deprotonated adenine. Hence they concluded that $(AT)^-$ cannot be characterized as T^- solvated by the adenine [17].

Besides parent anions we observe the dehydrogenated closed-shell anion $(\mathrm{NB\text{-}H})^-$ as another dominant product anion. The corresponding $(T\text{-H})^-$ and $(A\text{-H})^-$ ion yields are shown in Fig. 2. We tentatively propose that the attachment reaction leading to the first peak at about 2.7 eV is the same as for the isolated NB in the gas phase where $(\mathrm{NB\text{-}H})^-$ is formed in considerable amounts only below 3 eV with a threshold of 0.7 eV and a maximum at 1 eV [12]. The presence of the helium layer around the NB shifts these peaks again to higher energies (see above). For the resonances of $(\mathrm{NB\text{-}H})^-$ between 4 and 10 eV we suggest a different formation mechanism, i.e., a charge transfer reaction from a hydride anion, which is initially formed via dissociative electron attachment (DEA) to a NB in the cluster, to the complementary (NB-H) radical. This secondary process leads to attachment peak structures in the ion yield of $(\mathrm{NB\text{-}H})^-$ between 4 and 10 eV and it resembles that of the H^- resonances [20]. It is interesting to point out that in contrast to the gas-phase situation the structure between 4 and 10 eV is much larger than the low energy peak of $(\mathrm{NB\text{-}H})^-$. At this point we mention that DEA to NBs in the He droplet leads also to the formation of other fragment anions [e.g., for thymine $(T\text{-2H})^-$, $(T\text{-CNH}_3)^-$, and $(\mathrm{OCN})^-$] with an about 10 times higher cross section than in the gas phase [12]. We ascribe this to the presence of the solvation environment in the He droplet.

In addition to the structures between 4 and 10 eV we can observe peaks at about 22 and 28 eV in the ion yield of $(\mathrm{NB\text{-}H})^-$ shown in Fig. 2. These peaks most likely correspond to a reaction sequence with inelastic scattering events with helium atoms prior to the capture of the electrons at the NBs. This effect has been studied in EA to large pure helium droplets [7]. Since the lowest excitation energy of helium (2^3S state) is 19.82 eV [7], we conclude that the first scattering peak at about 22 eV corresponds to the first resonance of $(\mathrm{NB\text{-}H})^-$ at about 2.7 eV and the second much broader one to the ion yield between 4 and 10 eV.

Moreover, we find efficient hydrogen loss not only for the monomer but also for dimer anions. The corresponding ion yields of $(A_2\text{-H})^-$, $(AT\text{-H})^-$, and $(T_2\text{-H})^-$ shown in Fig. 3, however, differ substantially from those of the intact dimers (Fig. 1) and from $(\mathrm{NB\text{-}H})^-$ (Fig. 2). All dehydrogenated dimer anions show a common resonance at about 2.2 eV; however, the anion yield above 3 eV is quite different for the three anions shown in Fig. 3. It is not obvious whether the ion yield of $(AT\text{-H})^-$ can be interpreted as a superposition of the ion yield of $(T_2\text{-H})^-$ and $(A_2\text{-H})^-$; i.e., the electron capture occurs to a NB which is solvated by the other one. Taking into account the relative anion yields and peak strengths the present ion yield of $(AT\text{-H})^-$ would lead to the conclusion that electron attachment occurs preferentially via the thymine moiety, which is in line with the fact that the electron affinity of thymine is larger than that of adenine [17].

In order to further elucidate the hydrogen loss situation for these dimers, we also performed a pickup experiment using 1-methylthymine (m1T) instead of normal thymine. It is quite interesting to note in Fig. 4 that one of the three resonances in thymine (i.e., that at about 4 eV) is clearly absent in the DEA spectrum of m1T. In accordance with similar experiments and conclusions for gas-phase thymine

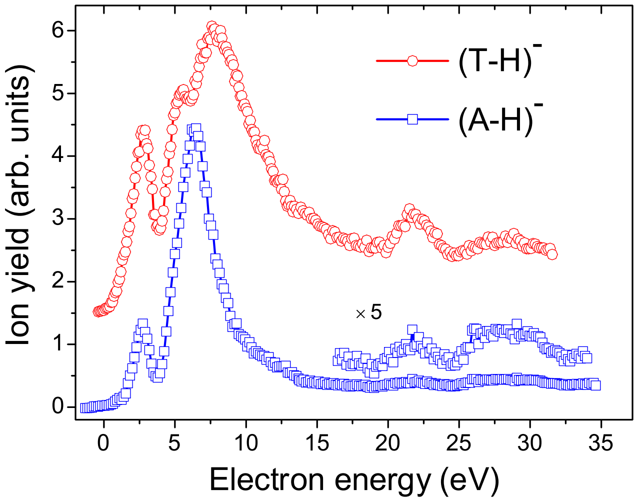

FIG. 2 (color online). Ion yield of $(T\text{-H})^-$ (circles) and $(A\text{-H})^-$ (squares) in the electron energy range from about zero to 34 eV.

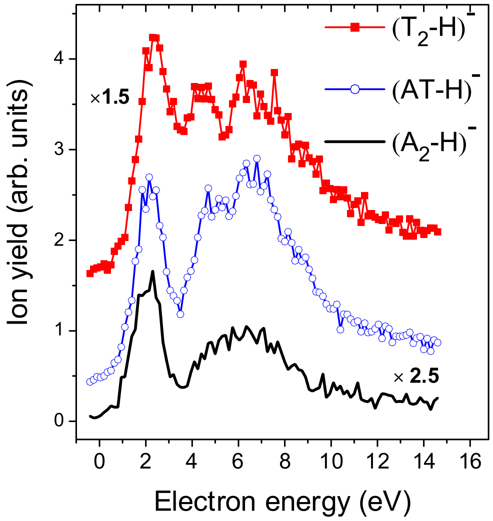

FIG. 3 (color online). Ion yield of $(T_2\text{-H})^-$ (squares), $(AT\text{-H})^-$ (circles), and $(A_2\text{-H})^-$ (line) in the electron energy range from about zero to 15 eV.

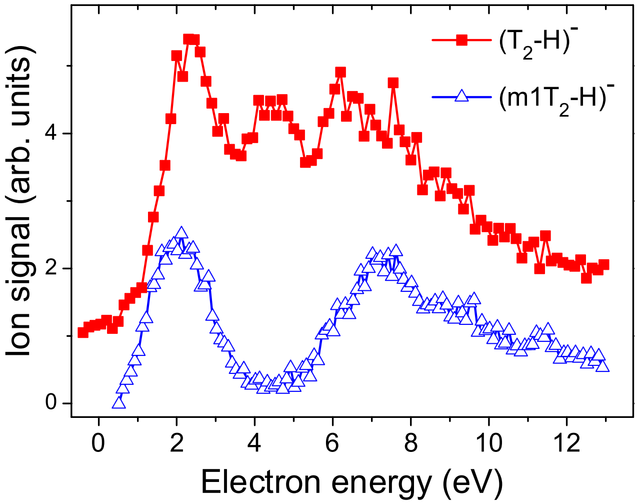

FIG. 4 (color online). Ion yield of (T_2-H)$^-$ (solid squares) and (m1T$_2$-H)$^-$ (open triangles) in the electron energy range from about zero to 13 eV.

[20] we conclude that this peak at 4 eV occurs via a site selective reaction involving the hydrogen at the N1H position (blocked in the m1T case via methylation, see [20]). We can exclude a charge transfer reaction as described above for (NB-H)$^-$ because the energetic threshold of H$^-$ formation from T is above the resonance position. The formation of H$^-$ becomes energetically possible only above 5 eV and thus the broad resonance at about 7.5 eV may be formed again via H$^-$ loss from the N3H and carbon sites in thymine (see Ref. [20]) with subsequent charge transfer back to the dimer radical. In analogy to gas phase T and m1T [21] we ascribe the resonance close to 2 eV to H loss exclusively from the N3H site.

In conclusion, in our study of negative ions formed by electron attachment to nucelobases embedded in cold helium droplets, we observe, on the one hand, stabilization of transient negative parent ions via solvation which are otherwise unstable isolated in the gas phase. On the other hand, we see an increase of the cross section of fragment anions above energies of electronic excitation. While other recent studies [3] have focused on the importance of low energy electrons (<3 eV) in inducing genotoxic damage by SSBs, we demonstrate here the importance of efficient dissociation of the nucleobases above electron energies of 3 eV, close to the energies where also SSBs and DSBs were observed [2]. This we conclude from an experiment which provides a reaction environment more complex than simple gas-phase studies, and closer to those in the condensed phase.

This work has been supported by the FWF, Wien, Austria, and the European Commission, Brussels. F. Z. gratefully acknowledges a grant from Brazilian agency CNPq.

---

[*] Permanent address: Université catholique de Louvain, 1348-Louvain-la-Neuve, Belgium.

[†] Also at Department of Plasma Physics, Comenius University, SK-84248 Bratislava, Slovak Republic.

[‡] Corresponding author.
Electronic address: Paul.Scheier@uibk.ac.at

[1] L. Sanche, Eur. Phys. J. D **35**, 367 (2005), and references therein.
[2] B. Boudaiffa, P. Cloutier, D. Hunting, M. A. Huels, and L. Sanche, Science **287**, 1658 (2000).
[3] F. Martin, P. D. Burrow, Z. Cai, P. Cloutier, D. Hunting, and L. Sanche, Phys. Rev. Lett. **93**, 068101 (2004).
[4] S. Ptasinska, S. Denifl, S. Gohlke, P. Scheier, E. Illenberger, and T. D. Märk, Angew. Chem., Int. Ed. **45**, 1893 (2006).
[5] J. P. Toennies and A. F. Vilesov, Angew. Chem., Int. Ed. **43**, 2622 (2004), and references therein.
[6] J. A. Northby, J. Chem. Phys. **115**, 10 065 (2001), and references therein.
[7] U. Henne and J. P. Toennies, J. Chem. Phys. **108**, 9327 (1998).
[8] M. Farnik and J. P. Toennies, J. Chem. Phys. **118**, 4176 (2003).
[9] K. Gluch, J. Fedor, S. Matt-Leubner, O. Echt, A. Stamatovic, M. Probst, P. Scheier, and T. D. Märk, J. Chem. Phys. **118**, 3090 (2003).
[10] S. Denifl, M. Stano, A. Stamatovic, P. Scheier, and T. D. Märk, J. Chem. Phys. **124**, 054320 (2006).
[11] L. G. Christophorou and J. K. Olthoff, Int. J. Mass Spectrom. **205**, 27 (2001).
[12] S. Denifl, S. Ptasinska, M. Probst, J. Hrusak, P. Scheier, and T. D. Märk, J. Phys. Chem. A **108**, 6562 (2004).
[13] K. Aflatooni, G. A. Gallup, and P. D. Burrow, J. Phys. Chem. A **102**, 6205 (1998).
[14] X. Li, M. D. Sevilla, and L. Sanche, J. Phys. Chem. B **108**, 19 013 (2004).
[15] V. Periquet, A. Moreau, S. Carles, J. P. Schermann, and C. Desfrancois, J. Electron Spectrosc. Relat. Phenom. **106**, 141 (2000).
[16] J. H. Hendricks, S. A. Lyapustina, H. L. de Clercq, and K. H. Bowen, J. Chem. Phys. **108**, 8 (1998).
[17] D. Radisic, K. H. Bowen, I. Dabkowska, P. Storoniak, J. Rak, and M. Gutowski, J. Am. Chem. Soc. **127**, 6443 (2005).
[18] J. Schied, R. Weinkauf, D. Neumark, and E. W. Schlag, Chem. Phys. **239**, 511 (1998).
[19] C. Desfrancois, H. Abdoul-Carime, C. P. Schulz, and J. P. Schermann, Science **269**, 1707 (1995).
[20] S. Ptasinska, S. Denifl, V. Grill, T. D. Märk, E. Illenberger, and P. Scheier, Phys. Rev. Lett. **95**, 093201 (2005).
[21] S. Ptasinska, S. Denifl, P. Scheier, E. Illenberger, and T. D. Märk, Angew. Chem., Int. Ed. **44**, 6941 (2005).