Collision-Induced Dissociation of Hydrated Adenosine Monophosphate Nucleotide Ions: Protection of the Ion in Water Nanoclusters

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Fragmentation of singly charged anions of adenosine 5'-monophosphate (AMP^-) induced by collisions with neutral atoms (Ne, Na) has been studied at a collision energy of 50 keV. The experiments were performed with isolated AMP^- as well as with AMP^- anions nanosolvated in a cluster with a given number m of water molecules. In the first case, the dominant fragmentation channels concern the loss of adenine, PO_3^- and $H_2PO_4^-$. In the latter, loss of water molecules becomes the dominating process, and the AMP^- ion is fully protected when m is larger than ~13. The observed fragment distributions are well described with the model of an evaporative ensemble.

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Radiation damage of biological systems has been studied for a long time in order to clarify the mechanisms and the phenomena occurring during the interaction of high-energy radiation with living cells [1,2]. Many experiments have been performed on a mesoscopic level, for example, irradiating cells or larger segments of DNA in solution and analyzing the damage with biological and chemical methods [3–5]. These studies clearly showed that the most severe consequences of irradiation are due to the damage of DNA, more precisely to the production of single and double strand breaks and, in particular, to the clustering of these lesions [6]. Furthermore, evidence has been reported [7] that base lesions are generated as well, which may be part of the clustered damage which consist of one or more modifications of the same or the two opposite strands within a helix turn. Another reactive species which induces modifications to cellular DNA has been shown to be the OH radical which is formed in the radiolysis of water molecules. Nevertheless, at present, the effect of very low irradiation doses is poorly understood.

More recently, studies of low-energy collisions involving isolated molecules of biological interest are performed either in the gas phase or with plasmid DNA deposited on a substrate to better understand the mechanisms of radiation damage on a molecular level [8,9]. In the case of electrons, Boudaiffa *et al.* [10] showed that already at very low electron energies, well below the ionization thresholds of the molecules involved, strand breaks occur due to the process of dissociative electron attachment. Along the primary ionization track also slow ions are formed as secondary particles. Their possible significance in the destruction schemes has been recognized only recently [11], when measurements have shown that recoil ion kinetic energies may be as large as 100 eV [12]. In the present work we use neutral Na and Ne atoms as collision partners, which are not necessarily relevant in the human body, but which allow the transfer of a certain amount of energy to the biomolecular ion and the study of energy-induced fragmentation of ''hot'' systems. However, the corresponding studies, based on gas phase targets and mass spectroscopic analysis, do have several drawbacks: (i) Owing to the difficulty to bring larger molecules intact into the gas phase, many of these studies are limited to smaller systems which still can be efficiently evaporated without fragmentation (e.g., nucleic bases). (ii) At present, many fragmentation studies are performed with isolated biomolecules in the gas phase. The question is raised, to which extent the obtained results can be applied to biomolecular systems in their natural environment, i.e., in an aqueous solution. (iii) Furthermore, in gas phase experiments irradiating dry DNA under normal atmospheric conditions, the presence of oxygen may strongly enhance the level of damaging and the biological consequences in radiation biology.

In order to clarify these questions, we have studied nanosolvated systems. Anions of nucleotides are produced with the aid of an electrospray ion source (ESI-source) [13] and the corresponding beams were used to study dissociation processes induced by collisions with neutral gas targets. Either inelastic collisions or electron capture processes (depending on the ionization energy of the target) may induce damage to the biomolecular system. It has been shown [14], that anions of adenosine 5'-monophosphate (AMP) can be prepared in ESI-sources with a certain number of water molecules attached, thus allowing us to

compare fragmentation patterns of the isolated and nanosolvated molecules. In the present work we consider this molecule as a model system, which contains the nucleic base adenine, the ribose sugar-part as well as a phosphate group, isolated as well as solvated.

Detailed information on the experimental setup is given elsewhere [15]. In brief, the ions were produced by electrospray of AMP dissolved in pure methanol (containing approximately 0.1% of water). The negative ions were accelerated to an energy of 50 keV and after a magnetic mass selection they passed through a cell containing the target gas. Primary as well as secondary ions and charged fragments were analyzed with respect to their mass-to-charge ratio with an electrostatic energy analyzer.

A typical fragmentation spectrum of isolated AMP anions is shown in Fig. 1. In addition to the primary anion, characterized by a mass-to-charge ratio of 346 a.u., many small-size fragments are observed, those formed by collisional energy transfer and those formed by electron transfer (for example the AMP^{2-} dianion less a hydrogen [16]). The dominant fragments are the negative phosphate groups (PO_3^- and $H_2PO_4^-$). The corresponding counterparts are not observed, as they most likely are formed as neutral fragments, which corresponds to the lowest energy fragmentation channel. Another important fragmentation process is due to the cleavage of the N-glycosidic bond between the adenine and the ribose part. This process leads to the formation of the adenine anion $[A]^-$ or the counterpart of the AMP molecule, the anion $[AMP\text{-}AH]^-$. These peaks are also dominant in collisions with a Ne target, where electron capture is unlikely to occur and, thus, the main underlying process is supposed to be collision-induced dissociation. The cleavage of the N-glycosidic bond between sugar and adenine, has been observed before as a prominent fragmentation channel, when thymidine or adenosine were irradiated with slow and fast ion beams [17,18]. The oxygen loss related to the formation of the $[AMP\text{-}O]^-$ fragment is attributed to dissociative electron capture as it is mainly present in the case of the sodium target.

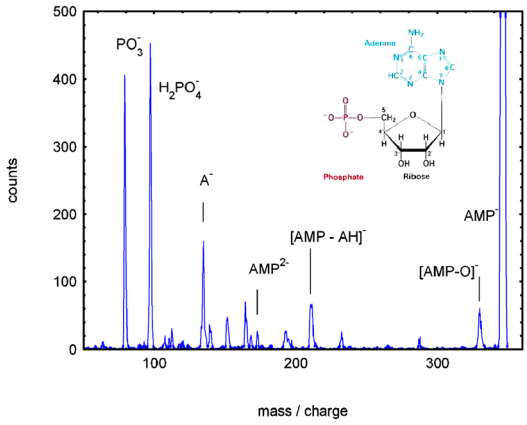

FIG. 1 (color online). Fragmentation mass spectrum obtained in 50 keV collisions of AMP^- anions with neutral sodium atoms.

In Fig. 2 we show the result of a semiempirical PM3 geometry optimization of the AMP^- system containing 20 water molecules, based on the GAUSSIAN98 program package [19]. There are several isomers of similar energy and the one shown is just one possible structure. The water molecules are attached close to the negatively charged phosphate group. The binding energy of a water molecule depends on its position. Within a water cluster the binding energy is of the order of ~0.43 eV [20], when more closely linked to the phosphate group this value is expected to increase to about 0.6 eV [21].

In Fig. 3 we compare fragmentation spectra where m water molecules ($m = 0 - 20$) were attached to the AMP^- anion. The spectra are normalized with respect to the total ion count rate (sum of primary and secondary ions), thus, allowing a direct comparison of the fragment intensities. When water molecules are added, a striking change of the spectrum is observed: the loss of the attached water molecules becomes the most important fragmentation channel. For a low value of m, it is rather likely that all water molecules are lost, whereas for larger systems the fragmentation probability decreases with increasing number of lost H_2O units. However, the intensity of the pure AMP^- fragment always shows a local maximum representing the end of the H_2O *evaporation chain*. The yield of AMP fragments does not vary strongly when increasing the water coverage; however, for $m > 10$ the overall intensity suddenly drops and only the loss of water molecules is observed. In this case, the AMP molecule seems to be fully protected by the surrounding water molecules. The energy, transferred during the collision to the solvated molecule, is used primarily to liberate the loosely bound water molecules, leading to a cooling of the residual system. Thus, the fragmentation probability for the AMP molecule itself is lowered.

In Fig. 4 the normalized intensities of the fragmentation spectra are shown for different m values as a function of the number n of water molecules attached to the AMP molecule in the final state. The intensity, given for $n = -1$, corresponds to the yield of fragmented AMP^- anions, including $H_3PO_4^-$, PO_3^-, A^-, and $[AMP\text{-}AH]^-$. For a

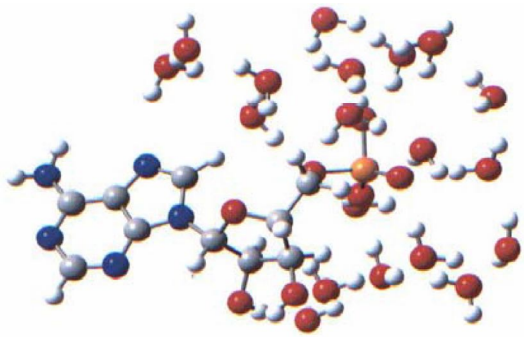

FIG. 2 (color online). One possible structure of the system $AMP^-(H_2O)_{20}$. Color code: gray (C atoms), blue (N atoms), red (O atoms), orange (P atom), small white spheres (H atoms).

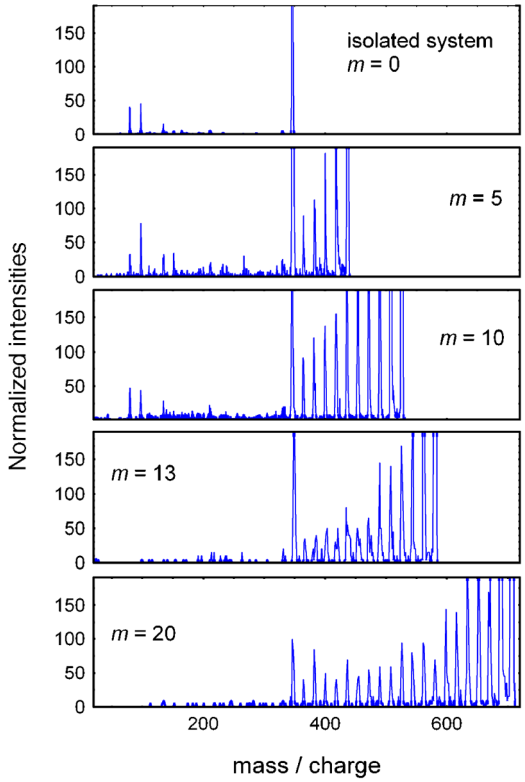

FIG. 3 (color online). Fragmentation spectra of $AMP^-(H_2O)_m$ ions for different m numbers of attached water molecules (obtained in collisions with sodium atoms at 50 keV). The spectra have been normalized to the total ion count rate.

given degree of solvation, the fragment intensity decreases with increasing loss of water molecules in a wide range nearly exponentially. However, for $n = 0$ and partially for $n = 5$, the intensity increases and shows a local maximum. The intensities of pure AMP-fragments are always smaller than those for $n = 0$.

The full curves, shown in Fig. 4, are the result of a model calculation, describing the loss of water molecules as an evaporation process, possibly followed by a fragmentation

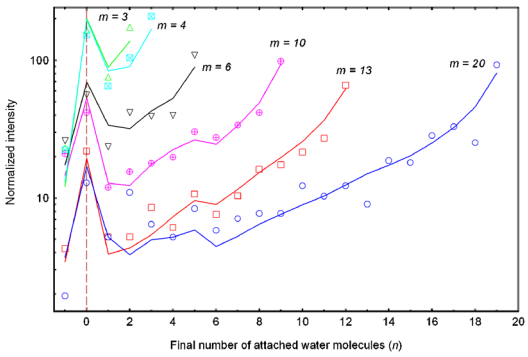

FIG. 4 (color online). Relative intensities of the fragmentation spectrum as a function of the number n of remaining water molecules. The intensity at $n = -1$ corresponds to AMP fragmentation (see text). The full lines are results from the evaporation model calculations.

of the biomolecule. This model is supported by the fact that no fragments with attached water molecules are observed. It is assumed, that during the collision a certain amount of energy is transferred to the projectile which is stored for a short time in the different degrees of freedom of the system. The loss of individual water molecules and the possible fragmentation of the biomolecule are characterized by different activation energies E_d. The sequential decay rates τ are given by

$$\tau = \nu \exp(-E_d/k_B T),$$

where ν is the prefactor characterizing the frequency for energy redistribution (assumed to be 10^{13} s^{-1}), k_B the Boltzmann factor and T the anion temperature. For $n > 5$ the activation energy for water loss is taken to be 0.43 eV, for $n \leq 5$ E_d is supposed to increase from 0.45 to 0.6 eV. The lowest activation energy, necessary to cause fragmentation of the AMP molecule, is of the order of 1.26 eV [22]. An increase in the activation energy causes an increase in the fragment intensity (*magic number* behavior) for $n = 0$ (and partially for $n = 5$). The internal energy (or temperature) after the collision is used as fit parameter to reproduce the fragment intensities.

Good agreement is obtained when the energy distribution is represented by two Gaussian functions. One is centered at lower energies (1.5 to 5 eV depending on m) representing the initial internal energy as well as the effect of glancing collisions. The second one occurring at somewhat higher energies, has a lower relative intensity and represents, according to our interpretation, contributions from penetrating collisions. The use of two Gaussians is an easy way to represent an energy distribution with a tail towards higher energies, resembling a so-called Airy function. A typical example is shown in the left part of Fig. 5.

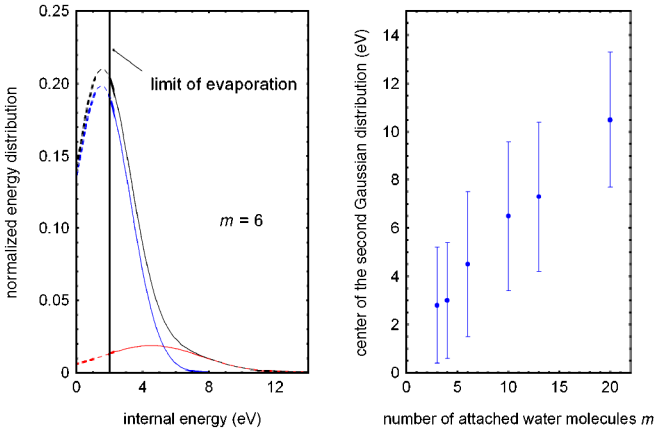

FIG. 5 (color online). Left: Internal energy distribution of $[AMP^-(H_2O)_6]^-$ anions, obtained from fitting the fragmentation spectrum in Fig. 4. The two Gaussian functions, used to approximate the energy distribution, are also given. The dashed part of the curves is not sensitive to the fitting procedure. Right: Center of the second Gaussian distribution as a function of m (see text). The vertical lines indicate the FWHM of the distribution.

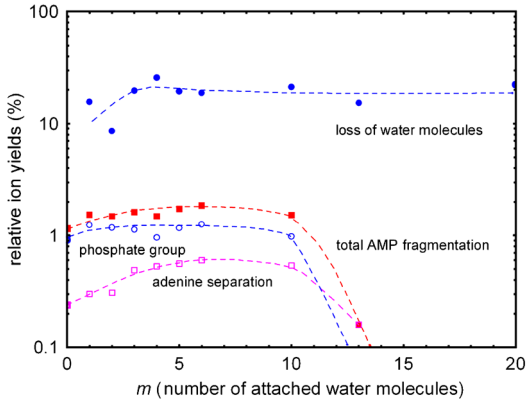

FIG. 6 (color online). Relative ion yields showing the importance of water loss channels and of biomolecular fragmentation channels. The dashed curves are to guide the eye only.

The fit is sensitive to the energy distribution only above a certain limit, as for lower values no evaporation occurs within the experimental time window. Therefore, the distribution shown in this region does not have any physical meaning.

Figure 5 (right part) shows the center energy of the second Gaussian distribution, which increases nearly linearly with increasing number of attached water molecules. The interpretation of such a linear dependence is difficult as the measurement averages over different impact parameters and as the initial internal energy, which increases itself with m, is contributing as well; however, not sufficiently to explain this linear increase.

The evolution of the energy distribution with m leads for a low water coverage to an increased fragmentation of the biomolecule (see Fig. 6). However, as soon as m becomes larger than 10, the increased energy transfer is well compensated by boiling off more water molecules and the biomolecule itself stays intact and is well protected. The nearly constant relative yield of the water loss channels (normalized to the total ion count rate) is surprising (see Fig. 6). However, as the amount of energy, dissipated by evaporation, increases with the number of evaporated water molecules and as this number increases on the average with m, the evaporative cooling increases with m as well. Furthermore, this behavior is well reproduced by the simulation. It seems that for a high degree of solvation the phosphate group is better protected than the adenine group. This can be explained by the structure of the system (see Fig. 2), which allows even for large m values a direct energy transfer to the adenine part of the molecule.

In conclusion, we have studied the collision-induced fragmentation of isolated and water-solvated AMP anions in collisions with Ne and Na atoms. We find that with increasing number of attached water molecules ($m = 3$–20) the internal energy of the system, after the collision, increases from about 3 to 10 eV due to penetrating collisions. This provokes first an increase of the fragmentation yield of AMP, but for $m > 13$ the biomolecule turns out to be well protected. The fragmentation distributions are well described by a statistical decay model leading to an evaporative cooling of vibrational *hot* AMP anions due to the loss of water molecules, thus protecting the biomolecule.

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