Collisions with biomolecules embedded in small water clusters

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Abstract. We have studied fragmentation of water embedded adenosine 5’-monophosphate (AMP) anions after collisions with neutral sodium atoms. At a collision energy of 50 keV, loss of water molecules from the collisionally excited cluster ions is the dominant process and fragmentation of the AMP itself is almost completely prohibited if the number of attached water molecules is larger than 13. However, regardless of the initial number of water molecules attached to the ion, capture of an electron, i.e. formation of a dianion, always leads to loss of a single hydrogen atom accompanied by evaporation of water molecules. This damaging effect becomes more important as the size of the water cluster increases, which is just the opposite to the protective behavior observed for collision induced dissociation (CID) without electron transfer. For both cases, the loss of water molecules within the experimental time frame is qualitatively well described by means of a common model of an evaporative ensemble. These simulations, however, indicate that characteristically different distributions of internal energy are involved in CID and electron capture induced dissociation.

1. Introduction
The effects of ionizing radiation on biological systems have been studied extensively during the last decades, and the most severe consequences are known to be mainly due to damage of cellular DNA containing the genetic information. However, the mechanisms and phenomena involved in processes leading to radiation damage of living cells are still not understood in detail. Biologically relevant damage may not only be induced by direct ionization of DNA, but, to a great extent, also by interaction with secondary particles, such as free electrons and radicals, created in large numbers along the ionization tracks of incident high energy quanta \cite{1,2}. Attachment of low energy electrons to nucleobases, for instance, is believed to play an important role for radiation damage of DNA and RNA \cite{3–7}. Often the problem is reduced to nucleobases or nucleotides, the basic building blocks of DNA and RNA, since a firm understanding of the processes at this molecular level is regarded as an important step towards a description of the even more complex processes taking place at the cellular level. In this work, we have studied Collision
Induced Dissociation (CID) and Electron Capture Induced Dissociation (ECID) of adenosine 5'-monophosphate (AMP), an RNA nucleotide containing the nucleobase adenine, a ribose sugar part and a phosphate group (cf. Fig. 1). Nucleotides are generally considered deprotonated in solution with the negative charge at the phosphate group. Here, we explicitly write AMP$^-$ in order to emphasize that it is a negative ion.

Gas phase experiments on single biomolecules have often been criticized as not really comparable to real biological systems due to the lack of the solvent environment which can be crucial for biochemical processes, the stability of the molecular conformation or the transfer of vibrational excitation. For DNA and RNA in aqueous solution, the primary hydration shell consists of about 20 water molecules per nucleotide [8] which are usually considered to be part of the biomolecular structure [9]. In order to find out if and how solvent molecules may play a role in CID and ECID processes we used beams of hydrated AMP$^-$, AMP$^-$·(H$_2$O)$_m$, with up to $m = 20$ water molecules attached to the nucleotide, and collided them with neutral sodium atoms. In gas phase, the attachment of a free electron would be hindered by a large Coulomb barrier of the AMP anion. Thus, the idea behind the experiment with sodium as collision target is to allow for a transfer of the Na valence electron and thereby mimic the situation in solution phase, where this Coulomb barrier is much lower due to polarization effects.

At center-of-mass collision energies of about 2–3 keV, a certain amount of energy is transferred to the cluster and both inelastic collisions and electron capture may induce damage to the biomolecular system. Information about the internal energies upon the two processes has been extracted from simulations of the water loss from the cluster, using a simple evaporation model, and a comparison with the experimental data. The model is the very same for CID and ECID but yields characteristically different excitation energy distributions for the two channels.

![Figure 1](image1.png)  
**Figure 1.** The nucleotide adenosine 5'-monophosphate (AMP).

![Figure 2](image2.png)  
**Figure 2.** Schematic layout of the experimental setup.

2. Experimental technique

The accelerator mass spectrometer setup used for the present experiments is illustrated in Fig. 2 and consists of an electrospray ion source [10] on a high voltage platform, an acceleration section, a double focusing bending magnet (1.5 m radius) for parent ion selection, a collision cell, and a 180° hemispherical electrostatic sector analyzer with a radius of 15 cm. Primary ions and charged fragments due to CID and ECID passing through the exit slit of the analyzer are counted by means of a channeltron detector. Mass-analyzed ion kinetic energy spectra are obtained by scanning the analyzer voltage. The flight times, $\tau_{flight}$, between the collision cell and the entrance of the energy analyzer are about 6–9 μs (depending on the number of water molecules, $m$, attached to the primary ion) and we thus probe the fragmentation within the...
corresponding time scale. The collision cell contains sodium vapor at low pressure such that the measurements are performed under single collision conditions.

3. Results

In Fig. 3, a typical mass-to-charge ratio spectrum for collisions of isolated AMP$^-^$ with Na is shown. The dominant fragments are PO$_3^-$, H$_2$PO$_4^-$, and the adenine anion [A-H]$^-$. The latter is due to the cleavage of the N-glycosidic bond between the adenine and the ribose part (cf. Fig. 1). The main underlying process here is assumed to be CID as these fragments are also dominant in collisions with neon, where electron capture is very unlikely to occur [11]. However, the loss of an oxygen atom, [AMP-O]$^-$, and formation of [AMP-H]$^{2-}$ dianions are channels attributed to fragmentation after capture of an electron [11, 12].

Fragment mass-to-charge ratio spectra for hydrated AMP$^-^$ are shown in Fig. 4. There, peaks of the AMP$^-^$·(H$_2$O)$_m$ primary ions with masses (346 +$m$·18) amu are followed by a series of monoanion peaks corresponding to loss of up to $m$ water molecules (the most intense peaks are off scale). At the end of the H$_2$O evaporation sequence, there is always a local maximum for the bare AMP$^-$. The intensities of AMP$^-^$ fragments decrease with increasing numbers of water molecules.

**Figure 3.** Mass-to-charge ratio spectrum of fragments from 50 keV AMP$^-^$+ Na collisions ($\tau_{\text{flight}} = 6$ $\mu$s). The dianion peak and the most prominent singly charged fragments are labeled ('A' denotes the adenine molecule). The intensity of the primary ion, with a mass-to-charge ratio of 346, is off scale by a factor of about 80.

**Figure 4.** Fragmentation spectra obtained from collisions of 50 keV AMP$^-^$·(H$_2$O)$_m$ with neutral Na atoms ($\tau_{\text{flight}} = 6.9$ $\mu$s ($m$=6), 7.4 $\mu$s ($m$=10), 7.8 $\mu$s ($m$=13), 8.6 $\mu$s ($m$=20)). The spectra have been normalized with respect to the total number of ion counts (sum of primary and secondary ions).
molecules attached to the AMP$^-$, and the peaks corresponding to the dominating fragments (PO$_3^-_2$, H$_2$PO$_4^-$, [A-H]$^-$) for the bare AMP$^-$ ion disappear if $m$ is larger than about 13. In these cases, the energies transferred to the cluster during the collision are primarily used to evaporate the loosely bound water molecules, which results in cooling of the system and protects the AMP$^-$ molecule itself from fragmentation.

A fragment spectrum from AMP$^-$·(H$_2$O)$_n$ + Na collisions with zoom-in on the dianion distribution is shown in Fig. 5. Dianion peaks due the process AMP$^-$·(H$_2$O)$_n$ + Na → [AMP- H]$^{2-}$·(H$_2$O)$_n$ + Na$^+$ + ···, i.e. to the loss of water molecules and a hydrogen atom upon electron capture, are clearly seen for $n = 0, 1$ and 2. Very little intensity, however, is observed for $n = 3$ and 4. The intensities close to and on the right hand side of the $n = 2$ and $n = 4$ peaks are due to singly charged fragments. Series of peaks corresponding to [AMP- H]$^{2-}$·(H$_2$O)$_n$ ($n \leq m$), are also observed for higher $m$, as more detailed scans of this mass-to-charge ratio range, like the ones in Fig. 6, reveal. No other doubly charged fragments could be identified, i.e. the dianion peaks in Figs. 5 and 6 are the only dianions that are stable on the experimental time scale of about 6–9 $\mu$s.

The loss of a single hydrogen atom from AMP$^{2-}$ has been identified as the completely dominant process for ECID by means of a precise calibration of the voltage scale of the energy analyzer [13, 14]. That is, dianion formation only occurs together with H-loss, independent of the number of water molecules attached before and after the collision. For all the experiments with different numbers $m$ of water molecules attached to the AMP$^-$, we always observe that at least a few of the water molecules are lost when the dianion is detected. This is a clear
contrast to the situation in collision induced dissociation where the cross sections increase with \( n \). This phenomenon may be explained by different energy transfers in the two processes, CID and ECID, as will be discussed below.

4. **Relative cross sections and the evaporation model**

In Fig. 7 we show relative cross sections as functions of the number \( n \) of water molecules remaining on the AMP\(^-\) and [AMP-H]\(^{2-}\) at the time \( \tau_{\text{flight}} \) after the collision for three different numbers \( m \) of initially attached water molecules. In the CID results (left panel), \( n = -1 \) represents the fragmentation of the AMP\(^-\) molecule itself. The full symbols are the experimental data and the open symbols, connected by dashed lines, are the results of model calculations. In the model, the loss of water molecules is described as an evaporation process, possibly followed by a fragmentation of the biomolecule. This approach is supported by the fact that no fragments with attached water molecules are observed.

4.1. **Dianion formation cross sections**

The ECID data in Fig. 7 have been normalized according to the absolute total cross sections (summed over all \( n \)), which were found to increase with increasing \( m \) [14], i. e. more attached

![Graphs showing relative cross sections and probability distributions.](image)

**Figure 7.** Left and middle: Relative cross sections as function of the number of remaining water molecules following CID, AMP\(^-\)·(H\(_2\)O)\(_m\) + Na → AMP\(^-\)·(H\(_2\)O)\(_n\) + ···, or ECID, AMP\(^-\)·(H\(_2\)O)\(_m\) + Na → [AMP-H]\(^{2-}\)·(H\(_2\)O)\(_n\) + Na\(^+\) + ···. In the left panel, \( n = -1 \) represents the fragmentation of the AMP\(^-\) molecule itself. Full symbols indicate experimental data points with error bars due to statistical and background subtraction uncertainties. Results from the model calculations are shown by open symbols, connected with dashed lines to guide the eye. Right: Probability distributions of the internal energy, \( f_m(E) \), used in the model calculations. Dashed lines indicate the two Gaussian components (without truncation). In the model, energies below the cut-offs (shaded areas) do not contribute to CID fragment intensities within the experimental time scales (cf. text).
water molecules lead to a higher probability for the potentially harmful H-loss process. This is in contrast to the behavior in the CID process where an increasing number of water molecules provides a better protection of the AMP\(^-\) against fragmentation. The increasing dianion cross section is probably due to the increase in geometrical size and polarizability, which favors larger dianion formation cross sections for larger numbers of attached water molecules. Further, dielectric screening of the adenine part from the negatively charged phosphate by the water nanodroplet makes the initially formed dianions more stable.

### 4.2. Model calculations

In the evaporation model, the sequential loss of individual water molecules from the parent AMP\(^-(\text{H}_2\text{O})_m\) and subsequently from the AMP\(^-(\text{H}_2\text{O})_n\) ions with \(0 < n < m\), or from the doubly charged systems [AMP-H\(^2+\)(\text{H}_2\text{O})_m] and [AMP-H\(^2+\)(\text{H}_2\text{O})_n], is characterized by their activation energies \(E_d\). The decay rates \(\Gamma_i\) are assumed to be given by Arrhenius type expressions,

\[
\Gamma_i = A \cdot \exp \left( -\frac{(E_d)_i}{k_BT_j}\right) = A \cdot \exp \left( -\frac{(E_d)_i}{E_j/(3N-6)} \right),
\]

with \(i\) indicating the evaporation step \((i = 1, \ldots, m+1)\), where \(i = 1\) represents the loss of one water molecule and \((m + 1)\) the fragmentation of the AMP\(^-\) molecule itself. Here, \(A\) is a prefactor characterizing the frequency for the dissociation mode (assumed to be \(10^{13} \text{ s}^{-1}\)), \(k_B\) is the Boltzmann constant and \(T_j\) is the ion temperature. The index \(j\) denotes the number of already lost solvent molecules before the \(i\)th step \((j = i - 1 = 0, \ldots, m)\). Following D. Liu and coworkers \([15]\), we take \(E_d\) values decreasing from 0.45 to 0.37 eV for the first four water molecules attached to AMP\(^-\). We then assume the binding energy to be constant at 0.37 eV for all additional water molecules. The activation energy necessary to cause fragmentation of the AMP\(^-\) molecule is about 1.26 eV \([16]\). The temperature is expressed in terms of the total number of atoms \((N)\) is the total number of atoms in the cluster) and which is used as a fit parameter. More precisely, different distributions of the internal energy prior to fragmentation, \(f(E) = f(E_{j=0})\), are assumed and after each evaporation step \(E\) is decreased by \((E_d)_i\). In order to model the measured fragment intensities \(c_j\) as functions of \(n\) \((which is related to \(j\) via \(n = m - j\))\), the following set of \(m + 1\) coupled differential equations, determined by the decay rates (Eq. 1), are solved:

\[
\frac{dc_0}{dt} = -\Gamma_1 c_0 \quad \text{and} \quad \frac{dc_j}{dt} = \Gamma_{i-1}c_{j-1} - \Gamma_i c_j \quad (j = i - 1 = 1, \ldots, m).
\]

Additionally, we define

\[
\frac{dc_{m+1}}{dt} = \Gamma_{m+1} c_m,
\]

where \(c_{m+1}\) refers to the summed intensities of AMP\(^-\) fragments (in CID only). The solutions are then evaluated at the time \(t = \tau_{\text{flight}}\). The modeled relative cross sections as functions of \(n\) depend mostly on the properties of the internal energy distributions (shapes, widths, and positions) but also on the rate constants (Eq. 1). For example, a typical uncertainty of 0.03 eV in \((E_d)_i\) gives a 0.3 eV uncertainty in the position of the assumed internal energy distribution.

Good agreement with all measured relative intensities is obtained if the internal energy distributions used for the simulations are described by the sum of two truncated Gaussian functions, with cut-off energies, relative intensities, widths, and positions of maxima as free parameters. Three examples of the resulting model distributions for CID, \(f_m^{\text{CID}}(E)\), and ECID, \(f_m^{\text{ECID}}(E)\), are plotted in the right hand panel in Fig. 7.
5. Discussion

There are considerable differences between the extracted internal energy distributions related to ECID compared to the ones from CID. In the latter case, the dominating parts at lower energies are believed to contain contributions from the initial internal energy and glancing collisions, while the much weaker high energy tails are related to closer collisions [11]. Ions with internal energies below the cut-off values do not contribute to fragment intensities within the experimental time scales as illustrated in Fig. 8, where the probabilities for the different cluster sizes after $\tau_{\text{flight}}$ are shown as function of internal energy for $m = 6$ as an example. Also a large fraction of the ions above the cut-off energy do not fragment. However, the detected primary anion intensity ($n = m$) is not included in the fit procedure and the model is thus not sensitive to the energy distribution below these limits.

The cut-offs in the energy distributions related to ECID, however, are at well defined and substantially higher values. They are needed to reproduce the very low measured intensities of peaks corresponding to the loss of no or few water molecules and, apparently, clusters with internal energies below $E_{\text{cut}}$ are not produced in the electron capture process. As a consequence, the distributions related to ECID are shifted to higher energies compared to the $f^\text{CID}_m(E)$. Further, the relative intensity of the two partial distributions is changed. For ECID, the broader partial Gaussian distribution contributes much more to the total distribution compared to the situation in CID, where it is only needed to create a longer high-energy tail. The reason for the shapes of $f^{\text{ECID}}_m(E)$ is not completely clear, but we find that it is not possible to reproduce the experimental distributions without the significant maxima. Possibly, these features are due to electron capture into electronically excited states and a rapid distribution of these excitation energies on the vibrational degrees of freedom. In other words, electron capture inevitably leads to a certain minimum internal excitation energy of the so formed dianions and therefore loss of a minimum number of the attached water molecules.

From the present study we do not gain any direct information on where in the dianions the H-atom is missing. However, in a previous ECID study [13] involving different isolated DNA and RNA nucleotides, including AMP$^-$, H-loss upon dianion formation was observed for all nucleotides and it was found that the cross section for this process is proportional to the number of hydrogen atoms bonded to nitrogen in the nucleobase. This indicates that the H-atom is probably removed from these sites. Quantum chemical calculations further show that electron capture to the phosphate group and H-loss from this site does not result in a stable dianion even if a water molecule is attached to it [14]. It is likely that this also holds for hydrated nucleotides, since calculations of the molecular structures indicate that the water molecules are preferentially localized close to the phosphate group. For AMP$^-$, this would mean that the H-
atom is lost selectively from the adenine NH$_2$-group, which is crucial for correct Watson-Crick base pairing, and H-loss from this site might therefore be particularly harmful.

6. Conclusion
To summarize, two different fragmentation processes – collision induced dissociation (CID) and electron capture induced dissociation (ECID) – have been studied in collisions between water embedded AMP$^-$ and neutral sodium atoms. We find that loss of water molecules is the dominating fragmentation channel and that the nucleotide anions are effectively protected from CID if sufficient numbers of water molecules are attached ($\approx$ 13 for the present collision energy). Very surprisingly, this protective behavior is not at all observed in the competing ECID reaction channel. A careful calibration of the mass-to-charge scale showed that ECID always leads to fragmentation in the form of single hydrogen loss. Even if the relative yield is low compared to CID this damage may be harmful as it most likely affects the nucleotide at a site which is crucial for correct Watson-Crick base pairing. In contrast to CID, the total dianion formation cross section increases with the number of initially attached water molecules which is explained by dielectric screening of the adenine part provided by the water cluster located close to the phosphate group and an increasing polarizability. Further, in the ECID processes there are large probabilities for losing at least some of the initially attached water molecules within the experimental time frame, a phenomenon not observed for CID but well described within a common simple water evaporation model. However, quite different internal energy distributions appear to favor the ECID and CID processes. This may be related to the capture into electronically excited states for ECID and the following, expected, rapid redistribution of this excitation energy among the internal degrees of freedom.

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