Photodestruction of Adenosine 5′-Monophosphate (AMP) Nucleotide Ions *in vacuo*: Statistical versus Nonstatistical Processes

S. Brøndsted Nielsen,[1,*] J. U. Andersen,[1] J. S. Forster,[2] P. Hvelplund,[1] B. Liu,[1] U. V. Pedersen,[1] and S. Tomita[1]

[1]*Department of Physics and Astronomy, University of Aarhus, Ny Munkegade, DK-8000 Aarhus C, Denmark*

[2]*Département de Physique, Université de Montréal, Montreal, Québec, Canada H3C3J7*

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The lifetimes of both deprotonated adenosine 5′-monophosphate (AMP) and protonated AMP, after 266-nm photon absorption and intramolecular vibrational redistribution, were determined to be 16 μs based on a newly developed heavy-ion storage ring technique. Protonation of the adenine nucleobase seriously affects the fragmentation mechanism: the major part of the photoexcited anions fragment in a statistical (ergodic) process, whereas nearly all the cations ($> 99\%$) fragment in a nonergodic process. In solution at natural *p*H the AMP anion is prevalent and photoproduct formation is therefore less important as the time for vibrational cooling is of the order of picoseconds.

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Mononucleotides are the building blocks of RNA and DNA and they consist of a nucleobase, a furanose sugar, and a phosphate group. The nucleobases strongly absorb ultraviolet (UV) radiation with a maximum at 260 nm. The $S_0 \rightarrow S_1$ transition is due to excitations to π^* orbitals [1]. The photophysics of nucleobases and nucleotides has been studied extensively, both in solution [2–6] and in the gas phase [7,8]. Gas phase investigations are important for a detailed understanding of the energy transfer processes in DNA since they reveal intrinsic molecular properties, and comparisons with the solution phase behavior may elucidate the role of the solvent.

After photoexcitation of the adenosine nucleoside (no phosphate group, abbreviated Ado) at 263 nm, internal conversion from the excited state (S_1) to the electronic ground state (S_0) has been shown to occur with a time constant of 290 fs [3]. The fluorescence quantum yield is very small, 10^{-4} or less [4], and intersystem crossing to a lower-lying triplet state is negligible [5]. Initially the vibrational excitation energy is deposited in the adenine base but intramolecular vibrational redistribution (IVR) is assumed to occur rapidly [3]. Vibrational cooling of Ado by solvent interactions then occurs in less than 2 ps [3]. It is unknown whether photoproduct formation competes with the energy dissipation and whether prompt processes are of significance. Prompt processes would be especially hazardous for a biological system if they were faster than solvent quenching of the excess energy. To address these issues the lifetime of the unsolvated molecule must be measured. We report lifetimes for statistical decay of deprotonated adenosine 5′-monophosphate (AMP) (AMP anion) and protonated AMP (AMP cation) *in vacuo* after 266-nm photon absorption, and demonstrate that the lifetime of the anion, the biologically relevant form, is far longer than the time required for vibrational relaxation in solution. Our technique only allows us to measure slow statistical processes on the microsecond time scale but indirect measurements indicate that, while a statistical decay process is dominant for the anions, statistical decay can account only for a minor fraction of the observed cation fragmentations.

The experimental setup is described elsewhere [9,10]. Briefly, an electrospray ion source is mounted on an accelerator, which is coupled to an electrostatic heavy-ion storage ring, ELISA. Molecular ions may be stored in the ring for several seconds and interactions with, e.g., laser light can be studied [11]. The AMP anions and cations [12] were formed by electrospraying AMP dissolved in either an ammoniated water/methanol (1:1) solution or an acetic acid water/methanol (1:1) solution. The ions were accumulated in a cylindrical ion trap for 0.1 s and equilibrated with a room temperature He buffer gas before being accelerated as an ion bunch to a kinetic energy of 22 keV. The structure of the cation is shown in Fig. 1. The anion is obtained by removing the proton on *N*3 and one of the protons of the phosphoric acid group [-OPO(OH)$_2$]. Ions of interest were selected by a magnet and injected into the storage ring. Collisions between ions and residual gas in the ring (mainly H_2) produce neutrals, which on the injection side were counted by a microchannel plate detector. The collisional lifetimes of both AMP anions and cations were measured to be 7 s at a pressure of 2×10^{-11} mbar.

The ions were irradiated by a nanosecond wide pulse of 266-nm photons (fourth harmonic of a Nd:YAG laser) in the straight section of the ring opposite to the injection side 70 ms after injection. For lifetime measurements, a storage time of 20 ms or more before irradiation is necessary to allow time for the decay of ions which have been excited in gas collisions during acceleration and injection. For both AMP anions and cations the irradiation resulted in a large production of neutral particles after bond dissociation (shown for the cation in Fig. 1). Again only neutrals formed in the injection side were counted by the detector.

The decay spectrum of the anion shown in Fig. 2 was fitted with an exponential with a time constant of 16 μs (broken line in the figure). However, this does not give a

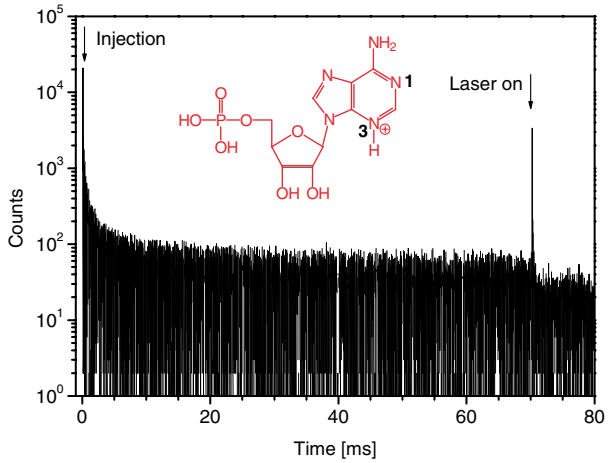

FIG. 1 (color online). Irradiation of AMP cations in ELISA. The initial count rate after injection is due to the decay of "hot" ions, but after ca. 20 ms the signal is dominated by collisional decay in the ring. Laser excitation after 70 ms of storage time causes a large increase in the count rate but within half a millisecond the signal is again due to collisional decay. The depletion of the ion beam is reflected in a lower rate of collisional decay after laser excitation.

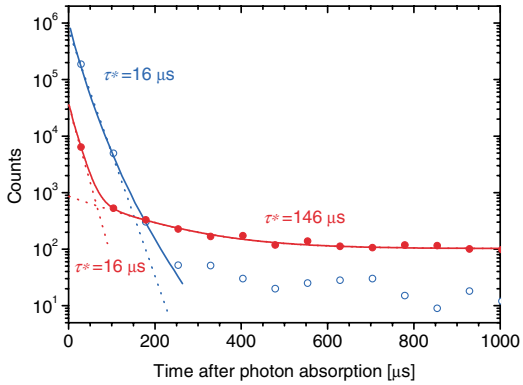

FIG. 2 (color online). Neutral count rate for AMP anions (open circles) and cations (filled circles) as a function of the time after 266-nm photon absorption. The circumference of the ring is 8.4 m and the corresponding revolution time of 75 μs is the spacing of the discrete points. Both ions have a component with a 16 μs lifetime. For the cation also a component with a larger lifetime (146 μs) is observed.

completely satisfactory description of the data since the ions do not all have the same internal energy and ions with higher internal energy will decay more quickly. To describe the data better we have represented the rate constant $k(E)$ by an Arrhenius expression [14],

$$k(E) = A\exp[-E_0/k_B(T - E_0/2C)]. \quad (1)$$

The activation energy E_0 was chosen according to the threshold experiments of Ho and Kebarle [15], which showed that dissociation into PO_3^- and adenosine is the process with the lowest activation energy ($E_0 = 1.26$ eV). The temperature T in Eq. (1) is related to the excitation energy E through the microcanonical caloric curve, and C is the temperature derivative of the function $E(T)$. To evaluate this function we have first calculated the average energy in thermal equilibrium in the harmonic approximation with vibrational frequencies obtained from a semiempirical PM3 calculation [16] and then used the relation $C \sim C_c - k_B$, where C_c is the canonical heat capacity and k_B is Boltzmann's constant. With the finite-heat-bath correction, $-E_0/2C$, the effective decay temperature is the average of the microcanonical temperatures in the initial and final states. As shown in [14] Eq. (1) then gives a good approximation to the energy dependence of the statistical decay rate as obtained from transition state theory or from detailed balance.

The energy distribution after absorption of a 4.66-eV photon was approximated by a Gaussian with a width equal to the rms fluctuation of the energy in the canonical distribution at room temperature T_0, $\sigma = (k_B C)^{1/2} T_0 = 0.17$ eV ($C = 0.0037$ eV/K). The average excitation energy at room temperature is 0.57 eV and after photon absorption it is 5.23 eV, and the average microcanonical temperature is $T_1 = 1085$ K while the heat capacity is $C_1 = 0.0073$ eV/K. The decay rate as a function of time was calculated with these parameters from the integral

$$I(t) = \int d\varepsilon \frac{1}{\sqrt{2\pi\sigma^2}} \exp(-\varepsilon^2/2\sigma^2) A \exp\left[-E_0/k_B\left(T_1 + \frac{\varepsilon - E_0/2}{C_1}\right)\right] \exp\left\{-tA\exp\left[-E_0/k_B\left(T_1 + \frac{\varepsilon - E_0/2}{C_1}\right)\right]\right\}. \quad (2)$$

The remaining parameter, A, was determined to fit the ratio of the first two data points in Fig. 2, $A = 1.4 \times 10^{11}$ s^{-1}, and the reciprocal of the rate constant in Eq. (1) for $T = T_1$ is $\tau^* = 16 \pm 2$ μs. The position of the P-O phosphoester stretch band is expected to lie in the 800–850 cm^{-1} region corresponding to a frequency factor of ca. 2.5×10^{13} s^{-1} [17]. The A parameter is expected to be lower than this value since the P-O bond breakage requires hydrogen atom transfer from P-OH to the oxygen linking the phosphate group to the sugar.

The decay spectrum for the cation is also shown in Fig. 2 (data from Fig. 1). The most favorable fragmentation channel is breaking of the glycosidic C-N bond between the protonated adenine base and the sugar unit followed by hydrogen atom transfer with the formation of protonated adenine and the corresponding neutral sugar fragment. This has been proposed to occur via a $E2$ elimination process [18] and is more favorable than for the anion since adenine is a better leaving group when protonated [18]. For the cation the spread in internal energy is not sufficient to account for the tail at long times and a fit with two exponentials is required. We have kept the time constant of the fast process fixed at

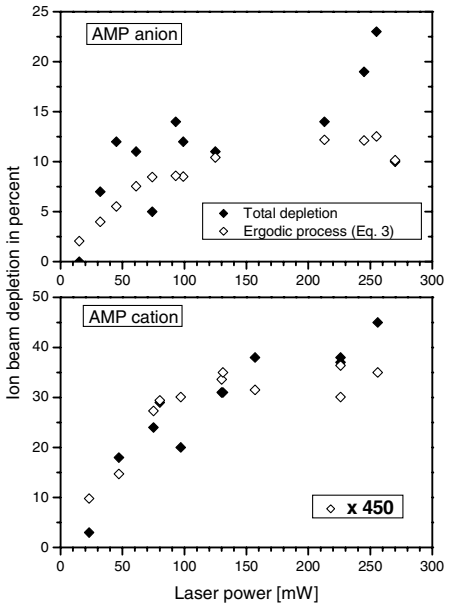

FIG. 3. Depletion of the ion beam, in percent, as a function of laser power. The experimental values are calculated from the ratio between the collisional decay rate before and after laser excitation (filled diamonds). The calculated values are obtained from Eq. (5) (open diamonds). For the AMP cation case the values have been multiplied by a factor of 450.

16 μs (*vide infra*), which results in a time constant of 146 μs for $(17 \pm 2)\%$ of the ions fragmenting via the slow process, based on the ratio of the preexponential factors multiplied by the ratio of the lifetimes.

After decay of ions which have been excited either in collision during injection or later by photon absorption, neutrals from collisions with the residual gas are observed. Their yield is proportional to the number of stored ions, and hence the beam depletion by the laser can be obtained from the ratio of the yields before and after laser excitation (Fig. 1). The depletion increases almost linearly with laser power (Fig. 3) but reaches a maximum due to excitation of all the ions in the region of overlap between the two beams (saturation). The depletion is observed to be larger for the cation than for the anion.

The large depletion of the cations allows us to determine the time constant of the fast decay process (cf. Fig. 2). Thus, the count rate of the first peak after laser excitation was measured for different time intervals between laser excitation and detection. The overlap between the ion bunch and the laser beam changed somewhat with laser delay but this could be corrected for through division of the count rate in the first peak by the beam depletion. The results are shown in Fig. 4 with a fit by a single exponential which gives a lifetime of 16 μs ($\pm$ 6 μs). This value was used in the fit to the data in Fig. 2. In a separate experiment we have cooled the ion trap in which the ions are stored before acceleration and injection into ELISA. The temperature of $-40\,^{\circ}$C corresponds to an internal energy which is lower by 0.2 eV than that at room temperature, where the lifetime for fragmentation after laser excitation is estimated to be shorter by about a factor of 1.6. We measured a lifetime of 21 μs at $-40\,^{\circ}$C, corresponding to a lifetime at room temperature of ca. 13 μs, which is in good agreement with the data shown in Fig. 4.

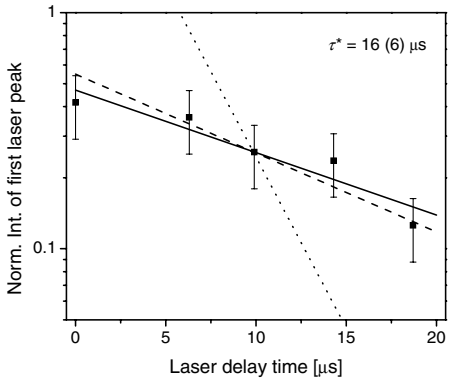

FIG. 4. Lifetime of AMP cations after 266-nm photon absorption. The intensity of the first laser-induced peak was measured as a function of laser delay time and normalized to the observed depletion of the stored ion beam. The solid line is for a 16-μs fit to the data. The dashed line shows results for a 13-μs decay and the dotted line for a 3-μs decay (see text for details).

After absorption of a 266-nm photon the electronic energy is converted into vibrational energy within a few picoseconds [19]. IVR followed by fragmentation accounts for the measured 16-μs lifetime of the anion and the cation. A fraction of $(17 \pm 2)\%$ of the observed decays of excited cations occurs with a longer lifetime of about 146 μs. This could be due to an intersystem crossing between S_1 and the lower-lying triplet state (T_1). These ions are trapped as the intersystem crossing to the electronic ground state is spin forbidden, which slows the decay by an order of magnitude.

Until now we have considered only statistical processes. However, an interesting question arises as to whether the ion beam depletion is completely accounted for by ions decaying via an ergodic process. The time constant for a nonergodic process, in which the C-N glycosidic bond between adenine and the sugar cleaves before the energy is statistically distributed among the vibrational modes of the whole molecule, is much smaller than the revolution time in the storage ring (75 μs), likely less than a picosecond (*vide infra*). Thus, a nonergodic process will cause a depletion of the ion beam but will not contribute to the neutrals count rate in our experiment.

In the following we estimate the ion beam depletion associated with the observed decay. The rate of neutrals formed in collisions is exponential,

$$n = -\frac{dN}{dt} = \frac{N_0}{\tau} e^{-t/\tau}, \tag{3}$$

where N and N_0 are the number of ions at time t and time zero, respectively, and τ is the collisional lifetime. Similarly, the rate of neutrals formed after laser excitation of

N_0^* ions with lifetime τ^* is given by

$$n^* = -\frac{dN^*}{dt} = \frac{N_0^*}{\tau^*} e^{-t/\tau^*}. \quad (4)$$

From the ratio of these two rates the proportion of depleted ions is

$$\text{depletion} = \frac{N_0^*}{N_0} = \frac{n^*}{n}\frac{\tau^*}{\tau} e^{t(1/\tau^* - 1/\tau)} \sim \frac{n^*}{n}\frac{\tau^*}{\tau} e^{t/\tau^*} \quad (\tau \gg \tau^*). \quad (5)$$

We have calculated the ratio n^*/n from the first point in the decay curve in Fig. 2, normalized to an average of the counts just before the excitation. With a lifetime of 16 μs for the excited ions and a collisional lifetime of 7 s, Eq. (5) results in the calculated values plotted in Fig. 3. A dramatic difference between the anion and the cation is evident. For the anion there is good agreement between the calculated and the observed depletion, indicating that an ergodic process is dominant. However, the large fluctuations in the experimental data in Fig. 3 (top panel) leave some room for fast, nonstatistical processes. The ratio of the ergodic depletion to the total depletion is on average 0.80 ± 0.20 based on 11 measurements assuming an uncertainty in τ^* of 2 μs and an uncertainty in t of 2 μs. The remaining fraction of 0.20 ± 0.20 would be the upper limit for prompt dissociation processes. For the decay of the cations, Eq. (5) underestimates the observed total depletion by roughly a factor of 450. The power dependence of the observed decay is consistent with single-photon absorption, and since the depletion ratio is essentially independent of the power it cannot be explained by multiphoton absorption. A lifetime of 3 ± 1 μs is required to account for the ion beam depletion but this value is inconsistent with the data in Fig. 4, being too short by a factor of 4 to 5. We therefore conclude that for cations the main fragmentation process is nonergodic, perhaps a prompt dissociation from an electronic excited state.

To summarize, we have provided data that support the IVR assumption for the dominant part of the AMP anions, whereas most of the AMP cations fragment before IVR, i.e., via a nonergodic process. As the pKa of protonated adenine is 4.1 the adenine base is neutral at natural pH. Therefore, the observation of ergodic fragmentation of the AMP anion (neutral adenine) implies that for a nucleotide in solution (pH 7) there is sufficient time for cooling by vibrational energy relaxation to water molecules to prevent its decomposition upon UV exposure. Further studies are required to understand the fast dissociation of the cation and how the electronic structure of the protonated adenine is perturbed from that of the neutral adenine. Most likely conical intersections explain the ultrafast conversion (< 1 ps) of electronic energy into the heat of nucleobases [3,8]. In the cation case, the excited-state potential energy surfaces may be located in such a way that conical intersections lead to prompt dissociation rather than to heat in the electronic ground state.

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*Corresponding author.
Email address: sbn@phys.au.dk

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