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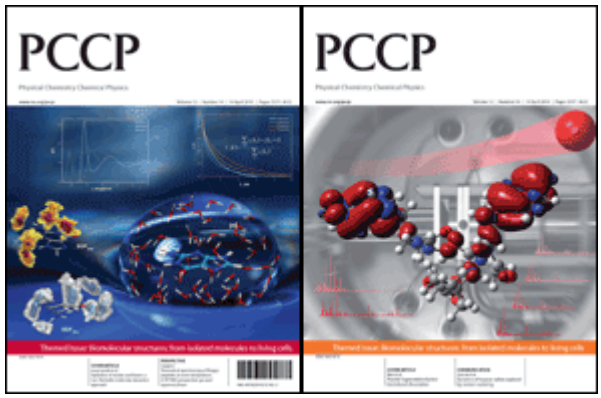
Biomolecular Structures: From Isolated Molecules to Living Cells

Guest Editors: Seong Keun Kim, Jean-Pierre Schermann and Taekjip Ha



Editorial

Biomolecular Structures: From Isolated Molecules to the Cell Crowded Medium
Seong Keun Kim, Jean-Pierre Schermann, Taekjip Ha, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/c004156b

Perspectives

Theoretical spectroscopy of floppy peptides at room temperature. A DFTMD perspective: gas and aqueous phase
Marie-Pierre Gaigeot, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924048a

Communications

Dynamics of heparan sulfate explored by neutron scattering
Marion Jasnin, Lambert van Eijck, Michael Marek Koza, Judith Peters, Cédric Laguri, Hugues Lortat-Jacob and Giuseppe Zaccai, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b923878f

Papers

Infrared multiple photon dissociation spectroscopy of cationized methionine: effects of alkali-metal cation size on gas-phase conformation
Damon R. Carl, Theresa E. Cooper, Jos Oomens, Jeff D. Steill and P. B. Armentrout, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b919039b

Structure of the gas-phase glycine tripeptide
Dimitrios Toroz and Tanja van Mourik, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b921897a

Photodetachment of tryptophan anion: an optical probe of remote electron
Isabelle Compagnon, Abdul-Rahman Allouche, Franck Bertorelle, Rodolphe Antoine and Philippe Dugourd, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b922514p

A natural missing link between activated and downhill protein folding scenarios
Feng Liu, Caroline Maynard, Gregory Scott, Artem Melnykov, Kathleen B. Hall and Martin Gruebele, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b925033f

Vibrational signatures of metal-chelated monosaccharide epimers: gas-phase infrared spectroscopy of Rb^+-tagged glucuronic and iduronic acid
Emilio B. Cagmat, Jan Szczepanski, Wright L. Pearson, David H. Powell, John R. Eyler and Nick C. Polfer, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924027f

Stepwise hydration and evaporation of adenosine monophosphate nucleotide anions: a multiscale theoretical study
F. Calvo and J. Douady, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b923972c

Reference MP2/CBS and CCSD(T) quantum-chemical calculations on stacked adenine dimers. Comparison with DFT-D, MP2.5, SCS(MI)-MP2, M06-2X, CBS(SCS-D) and force field descriptions
Claudio A. Morgado, Petr Jurečka, Daniel Svozil, Pavel Hobza and Jiří Šponer, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924461a

Photoelectron spectroscopy of homogeneous nucleic acid base dimer anions
Yeon Jae Ko, Haopeng Wang, Rui Cao, Dunja Radisic, Soren N. Eustis, Sarah T. Stokes, Svetlana Lyapustina, Shan Xi Tian and Kit H. Bowen, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924950h

Sugar–salt and sugar–salt–water complexes: structure and dynamics of glucose–KNO_3–$(H_2O)_n$
Madeleine Pincu, Brina Brauer, Robert Benny Gerber and Victoria Buch, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b925797g

Hydration of nucleic acid bases: a Car–Parrinello molecular dynamics approach
Alona Furmanchuk, Olexandr Isayev, Oleg V. Shishkin, Leonid Gorb and Jerzy Leszczynski, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b923930h

Conformations and vibrational spectra of a model tripeptide: change of secondary structure upon micro-solvation
Hui Zhu, Martine Blom, Isabel Compagnon, Anouk M. Rijs, Santanu Roy, Gert von Helden and Burkhard Schmidt, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b926413b

Peptide fragmentation by keV ion-induced dissociation
Sadia Bari, Ronnie Hoekstra and Thomas Schlathölter, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924145k

Structural, energetic and dynamical properties of sodiated oligoglycines: relevance of a polarizable force field
David Semrouni, Gilles Ohanessian and Carine Clavaguéra, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924317h

Studying the stoichiometries of membrane proteins by mass spectrometry: microbial rhodopsins and a potassium ion channel
Jan Hoffmann, Lubica Aslimovska, Christian Bamann, Clemens Glaubitz, Ernst Bamberg and Bernd Brutschy, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924630d

Sub-microsecond photodissociation pathways of gas phase adenosine 5′-monophosphate nucleotide ions
G. Aravind, R. Antoine, B. Klærke, J. Lemoine, A. Racaud, D. B. Rahbek, J. Rajput, P. Dugourd and L. H. Andersen, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b921038e

DFT-MD and vibrational anharmonicities of a phosphorylated amino acid. Success and failure
Alvaro Cimas and Marie-Pierre Gaigeot, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924025j

Infrared vibrational spectra as a structural probe of gaseous ions formed by caffeine and theophylline
Richard A. Marta, Ronghu Wu, Kris R. Eldridge, Jonathan K. Martens and Terry B. McMahon, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b921102k

Laser spectroscopic study on (dibenzo-24-crown-8-ether)–water and –methanol complexes in supersonic jets
Satoshi Kokubu, Ryoji Kusaka, Yoshiya Inokuchi, Takeharu Haino and Takayuki Ebata, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924822f

Macromolecular crowding induces polypeptide compaction and decreases folding cooperativity
Douglas Tsao and Nikolay V. Dokholyan, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924236h

Electronic coupling between cytosine bases in DNA single strands and *i*-motifs revealed from synchrotron radiation circular dichroism experiments
Anne I. S. Holm, Lisbeth M. Nielsen, Bern Kohler, Søren Vrønning Hoffmann and Steen Brøndsted Nielsen, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924076d

Photoionization of 2-pyridone and 2-hydroxypyridine
J. C. Poully, J. P. Schermann, N. Nieuwjaer, F. Lecomte, G. Grégoire, C. Desfrançois, G. A. Garcia, L. Nahon, D. Nandi, L. Poisson and M. Hochlaf, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b923630a

Insulin dimer dissociation and unfolding revealed by amide I two-dimensional infrared spectroscopy
Ziad Ganim, Kevin C. Jones and Andrei Tokmakoff, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b923515a

Six conformers of neutral aspartic acid identified in the gas phase
M. Eugenia Sanz, Juan C. López and José L. Alonso, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b926520a

Binding a heparin derived disaccharide to defensin inspired peptides: insights to antimicrobial inhibition from gas-phase measurements
Bryan J. McCullough, Jason M. Kalapothakis, Wutharath Chin, Karen Taylor, David J. Clarke, Hayden Eastwood, Dominic Campopiano, Derek MacMillan, Julia Dorin and Perdita E. Barran, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b923784d

Guanine–aspartic acid interactions probed with IR–UV resonance spectroscopy
Bridgit O. Crews, Ali Abo-Riziq, Kristýna Pluháčková, Patrina Thompson, Glake Hill, Pavel Hobza and Mattanjah S. de Vries, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b925340h

Investigations of the water clusters of the protected amino acid Ac-Phe-OMe by applying IR/UV double resonance spectroscopy: microsolvation of the backbone
Holger Fricke, Kirsten Schwing, Andreas Gerlach, Claus Unterberg and Markus Gerhards, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/c000424c

Probing the specific interactions and structures of gas-phase vancomycin antibiotics with cell-wall precursor through IRMPD spectroscopy
Jean Christophe Poully, Frédéric Lecomte, Nicolas Nieuwjaer, Bruno Manil, Jean Pierre Schermann, Charles Desfrançois, Florent Calvo and Gilles Grégoire, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b923787a

Two-dimensional network stability of nucleobases and amino acids on graphite under ambient conditions: adenine, L-serine and L-tyrosine
Ilko Bald, Sigrid Weigelt, Xiaojing Ma, Pengyang Xie, Ramesh Subramani, Mingdong Dong, Chen Wang, Wael Mamdouh, Jianguo Wang and Flemming Besenbacher, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924098e

Importance of loop dynamics in the neocarzinostatin chromophore binding and release mechanisms
Bing Wang and Kenneth M. Merz Jr., *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/b924951f

Structural diversity of dimers of the Alzheimer Amyloid-(25-35) peptide and polymorphism of the resulting fibrils
Joan-Emma Shea, Andrew I. Jewett, Guanghong Wei, *Phys. Chem. Chem. Phys.*, 2010
DOI: 10.1039/c000755m

Sub-microsecond photodissociation pathways of gas phase adenosine 5′-monophosphate nucleotide ions

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The sub-microsecond dissociation pathways for the protonated and deprotonated forms of adenosine 5′-monophosphate were probed in the gas phase using a linear time of flight spectrometer. The studies show two dissociation pathways for the AMP ions indicating dominant ergodic pathways in the photodissociation of these species. The photofragmentation was determined to be a single photon process for the AMP ions. Photodetachment of the AMP anion excited at 266 nm was not observed, leaving dissociation as the prominent pathway for relaxation of the excess energy in the biomolecule. The photofragments were analysed at the electrostatic ion storage ring (ELISA) and found to be similar to collision induced fragments in the case of anions but different in the case of cations.

I. Introduction

The adenosine monophosphate (AMP) nucleotide is found in RNA and consists of a phosphate group, sugar ribose, and the nucleobase adenine. It is an intermediate molecule formed during the process of energy production in the form of adenosine triphosphate (ATP) in our biological system. The cyclized form of AMP plays an important role in intracellular signal transduction in many different organisms. The structure of AMP is critically determined by H bonds at the phosphate group with the base as well as the ribose.[1,2] The significance of the nucleotides in fundamental biological processes motivates the study of its structural and functional properties. The structure and dynamics of nucleotides have been studied *via* collision induced dissociation (CID) using techniques such as Fourier transform ion cyclotron resonance mass spectrometry.[2] Dissociation studies on deprotonated dinucleotides are known to provide sequence information in nucleotides.[1] Yang *et al.*[3] have studied electron-capture and electron-detachment dissociation of oligoribonucleotides. There have also been theoretical and experimental studies on the photophysics related to the π–π* transition at the nucleobases in gas[4–8] and solution phase.[9–14] Guan *et al.*[15] reported the first observation of electron photodetachment from multiply charged nucleotides. Recently, Marcum *et al.*[16] studied UV photodissociation of deprotonated 2′-deoxyriboadenosine-5′-monophosphate in the gas phase and analysed the photofragments.

Nucleobases strongly absorb UV radiation due to the π–π* transition at the aromatic ring. Resonant absorption of about 5 eV in these biomolecules triggers several reaction pathways. Ultrafast dissociation occurring at a timescale faster than that for dissipation of excess energy to the environment would render these biomolecules vulnerable to UV radiation. Ultrafast internal conversion of the photon energy into vibrational excitation has been studied using femtosecond transient absorption spectroscopy.[9,10] Ionizing radiation could also damage these biomolecules with the generated energetic electrons and the effect of electron collisions has been studied previously at ELISA.[17] Photodestruction of DNA could cause hazards such as skin cancer[18,19] and it is therefore important to understand the intrinsic photoresponse of nucleotides, the fragmentation pathways, and the associated lifetimes. Gas-phase studies in general[20–27] enable us to understand the intrinsic properties of molecules devoid of environmental perturbations.

The study of dissociation lifetimes, along with the knowledge of the electronic energy levels and the intramolecular vibrational redistributions, is crucial for understanding the response of biomolecules to light. Photodissociation lifetime studies on the gas phase deprotonated and protonated AMP have been carried out in the millisecond regime at ELISA.[28] Henceforth the deprotonated and protonated forms will be mentioned as anion and cation, respectively. The study revealed that a large fraction of the AMP anions as well as the cations photodissociated too fast (within few tens of microseconds) to be detected at the applied "delayed" micro channel plate detector (MCP)[28] and only a depletion of the stored ion beam was registered. The depletion was found to be largest for cations and was thought possibly to be due to non-ergodic decay pathways. There have been reports on non-ergodic dissociation pathways in N–C bond cleavage in electron capture dissociation experiments, similar to the N–C bond found in the AMP.[29,30] However, there could be prominent fast dissociation pathways in the sub-microsecond regime that could not be perceived using ELISA. Excitation of large biomolecules could lead to many dissociation

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pathways.[31] CID of AMP ions reveal several dissociation pathways[1,2] and more than one mechanism are proposed to understand these pathways.[1]

In the present experiment we probed the submicrosecond regime photodissociation of AMP anions and cations using a linear time of flight spectrometer (TOF).[32,33] We observe more than one dissociation lifetimes for both the anion and cation in the submicrosecond regime. UV excitation of the nucleobase in the AMP anion was not observed to cause photodetachment, leaving dissociation as the dominant decay pathway since the flourescence quantum yield is known to be as low as 10^{-4}.[12]

II. Experimental

The AMP anions and cations in the gas phase were produced by electrospraying AMP dissolved in ammoniated water–methanol and acetic acid/methanol, respectively. The ions were trapped using a Paul trap with helium as buffer gas. The trapped ions were extracted at 20 Hz repetition rate and accelerated to 20 keV. A magnet was employed to mass select the AMP ions which were then guided to the interaction region with a 3° deviation in the path for elimination of neutrals formed *via* background gas collisions. The ions are collided at right angles with UV photons produced from the fourth harmonic of a Nd:YAG laser flashing at half the ion bunch extraction rate to enable background subtraction. Neutral fragments fly about 1.6 m before they hit a MCP detector. The ions are deflected away from the MCP using an electrostatic deflector and only the time of flight (TOF) of the neutral fragments created between the interaction point and the deflector is recorded.

To collect the sub-microsecond dissociation lifetimes, a uniform electric field is employed in the interaction region. Previously this electric field region, of about 26 cm length, had also been employed for photoelectrons.[33] A stack of electrodes, as shown in the inset of Fig. 1 were employed for creating a homogenous electric field inside the spectrometer. The ions entering the spectrometer are accelerated or decelerated depending on the charge and electric field. The interaction spot is about 5 cm downstream the entrance of the spectrometer and the neutral fragments formed at different times after the interaction have different velocities as the parent ion velocity varies across the spectrometer. The dissociation lifetime is hence tagged onto the TOF of the neutral fragments. All ions that fragment outside the spectrometer pile up to form a single TOF peak. For the AMP ions with 20 keV energy and about 3 kV spectrometer voltage (Vspec), dissociation times up to 2 μs are visible in this experiment.

The ionic fragment masses were analyzed at ELISA by performing UV photodissociation at 266 nm. The deflector voltages in the storage ring were switched to store the daughter ions and the mass analysis was done by scanning the ring voltages. In the current experiment the voltages were switched simultaneously with the appearance of the laser pulse. The method of daughter-mass detection at ELISA is described in detail elsewhere.[34] The fragment masses were identified using a modified linear-quadrupole-ion-trap-mass spectrometer (LTQ, Thermo Fisher Scientific, San Jose, CA) at Lyon.[35]

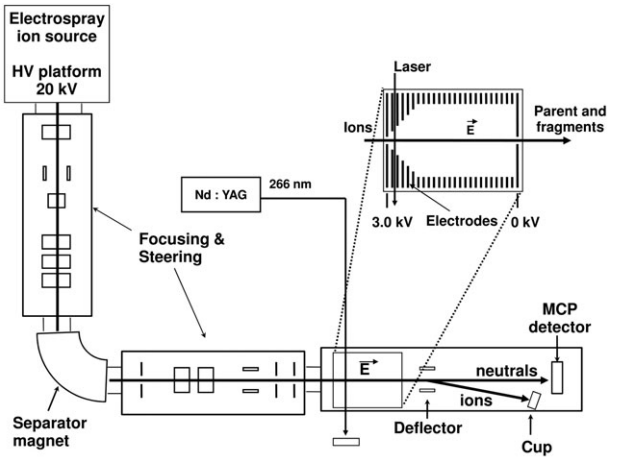

Fig. 1 Schematic representation of the experimental set-up with the ion-beam path and the laser set-up. The inset shows the spectrometer with a stack of electrodes that maintain a uniform electric field. The electric field region is about 26 cm while the distance from the interaction spot to the MCP detector is 1.6 m.

Ions produced using an electrospray source were trapped and photoexcited by 5 shots of 266 nm photons. The fragment ions were subsequently mass analyzed after about 100 ms of trap time.

III. Results and discussion

Fig. 2 shows the TOF of neutral fragments from the UV photodissociation of AMP anions recorded with the spectrometer voltage (Vspec) off. The width of the TOF stems from the kinetic energy release, the finite interaction volume, and the spread in parent ion energy. The kinetic-energy release (KER) is estimated to be 40 meV. Fig. 3 shows the TOF spectrum shifting to shorter times with Vspec raised to +3 kV. The TOF peak seen at about 15.15 μs corresponds to photofragments formed outside the spectrometer. The fragments formed inside the spectrometer yield the TOF peak around 14.4 μs with a decaying tail towards the peak at 15.15 μs. The TOF section corresponding to fragmentation inside the spectrometer has a fast and a slower component. Photodetachment could de-excite the system by ejecting an energetic electron. The simulation with zero kinetic energy release in Fig. 3 shows the absence of a photodetachment pathway, indicating randomization of the absorbed energy without electron detachment. The photon energy of 4.66 eV employed in the current experiment is lower than both the adiabatic and vertical detachment energies (VDE) for the dAMP anion[36] and lower than the estimated VDE of about 5.2 eV for the AMP anion.[17]

Fragmentation that occurs inside the spectrometer was found to be due to single-photon absorption as shown in Fig. 4.

Fig. 5 shows the TOF of photofragments from AMP cations measured with Vspec at +3 kV. Again, the peak with a tail corresponds to fragmentation inside the spectrometer. The spectrum shows a large fraction (94%) of the cations fragmenting within the spectrometer correlating with the large

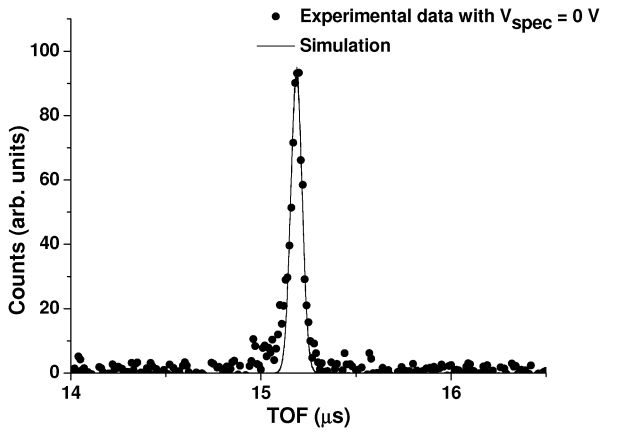

Fig. 2 The TOF of the neutrals from the photodissociation of AMP anions with the Vspec switched off. The solid line is the Monte-Carlo simulation for the time of flight (see text).

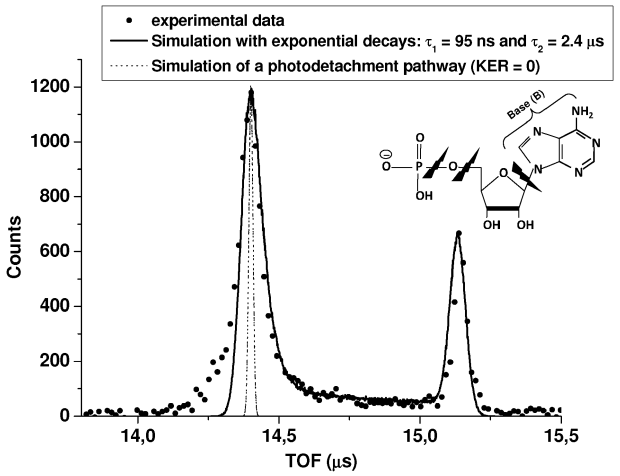

Fig. 3 The TOF of the neutrals from the photodissociation of AMP anions with Vspec raised to 3 kV. The solid and dotted lines are the Monte-Carlo simulations of the TOF for photodissociation and photodetachment pathways (KER = 0), respectively.

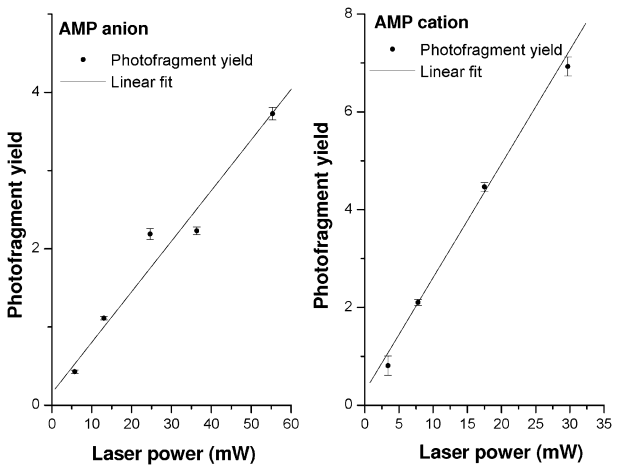

Fig. 4 Laser-power dependence for the yield of fragments from AMP anion and cations produced within the spectrometer.

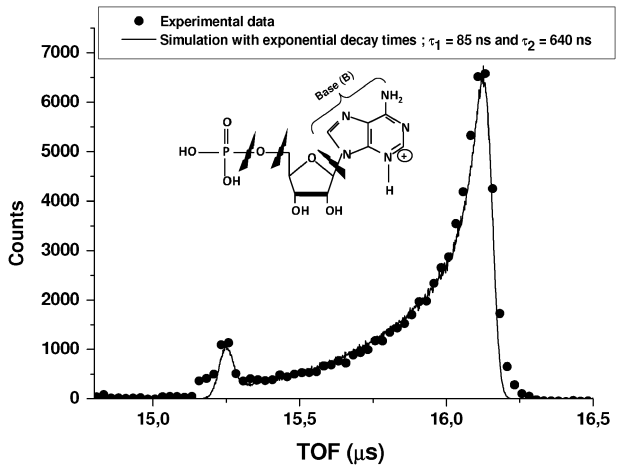

Fig. 5 TOF of the neutrals from the photodissociation of AMP cations with the Vspec raised to 3 kV. The solid line is the Monte-Carlo simulation of the TOF for the photodissociation pathways.

ion depletion for cations measured at ELISA.[28] These fragments were also found to be associated with a single-photon process as shown in Fig. 4. The TOF spectrum (Fig. 5) apparently shows components on a timescale of hundreds of nanoseconds.

Monte-Carlo simulations on the dissociation lifetimes

The dissociation lifetimes of the photofragmentation process were deduced through Monte-Carlo simulations. The simulation generates random interaction events within a finite interaction volume considering the kinetic energy released, which in turn was determined with Vspec off, the spread in parent ion energy, and the laser beam width. The neutral fragment ejection was assumed to be isotropic. For the anions, the simulated TOF yielded two exponential dissociation lifetimes: a fast fragmentation pathway with τ_1 = 95 ns and a slower component with a dissociation lifetime of τ_2 = 2.4 μs as shown in Fig. 3. About 54% of the ions fragmenting within the spectrometer have 95 ns lifetime, the rest having a decay time of 2.4 μs.

The simulation for the cation (Fig. 5) shows about 74% of ions, that fragment within the spectrometer, to have a dissociation lifetime of about 640 ns and the remaining of about 85 ns. Both components are too fast to be seen at the "delayed" MCP at ELISA. The large depletion of cations at ELISA was thought to be due to a non-ergodic process.[28] With the present technique, we show that the fast dissociation is indeed not prompt, but has a lifetime much shorter than what can be measured using ELISA. Clearly the processes are ergodic and fast enough (τ_2 = 0.64 μs) to go undetected at the delayed detector at ELISA.[28]

Fragment masses of the AMP anion

The UV photofragment ions from the AMP anion were mass analysed at ELISA. The measurement was performed by switching the deflector voltages along with the laser trigger and only fragments produced faster than about 15 μs would be stored in the ring. The daughter ions were collected after storing them for two revolutions. Fig. 6 shows the daughter

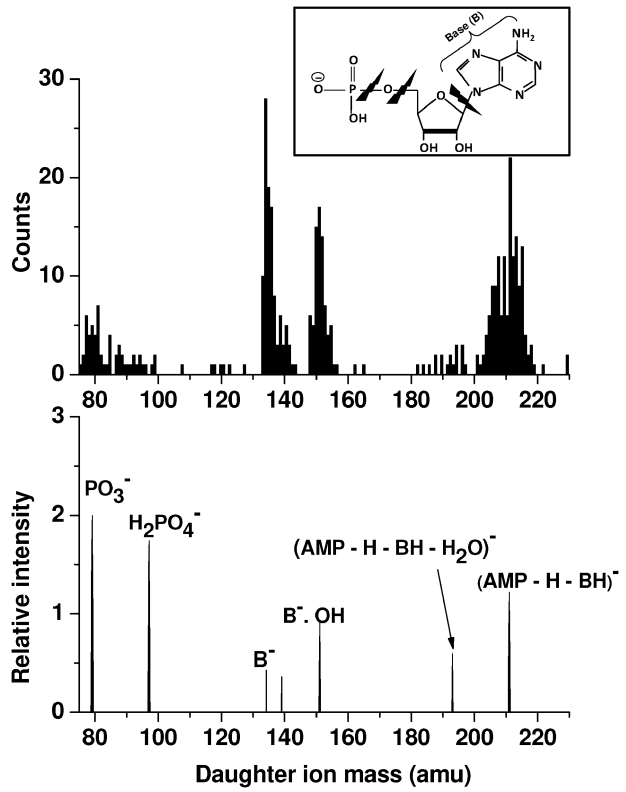

Fig. 6 The daughter-ion mass spectrum obtained from UV photodissociation of the AMP anion at 266 nm performed at ELISA is shown in the top panel. The assignment of the ionic fragments is provided from a high resolution mass spectrometer[35] (bottom panel).

mass spectra obtained at ELISA (top panel) along with the high resolution data (bottom panel) that we obtained using the mass spectrometer in P. Dugourd's laboratory in Lyon.[35] The present results on photofragments (see Fig. 6) are in agreement with the conclusion of Marcum *et al.*[16] that CID and photodissociation yield similar fragments. The relative yield of the anionic base (B^-) in the present measurement is found to be similar to that of $(AMP\text{-}H\text{-}BH)^-$ in contrast to the low relative yield of B^- in ref. 16. In addition, we observe an ionic fragment of mass 152 amu, which is likely to be hydrated B^-. The daughter mass measurement at ELISA has a discrimination against fragments much smaller than the parent ion, in this case, PO_3^- and $H_2PO_4^-$.

UV photoabsorption at the nucleobase leads to π–π^* excitation which could undergo ultrafast conversion to the ground electronic state *via* a conical intersection. The excess energy would then randomize, eventually leading to various fragmentation pathways. A prompt dissociation channel from the excited electronic state is also possible but with minor contribution since we can see no sign of it in the TOF. Prompt fragmentation pathways would be fatal as biomolecules would then fragment before the excess energy is exchanged with the surrounding environment.

Several mechanisms were proposed to explain collision induced dissociation of AMP anions.[1,37,38] The AMP anion, which is of biological relevance, is known to dissociate prominently at the phosphate–ribose bond yielding PO_3^- in CID.[1] The direct cleavage of the glycosidic C–N bond, following the photoexcitation of the base could be the fast (95 ns) dissociation component observed in the current experiment. The other fragments formed would require nuclear rearrangements after relaxation of the excited base and is hence likely to be the observed slower dissociation component (2.4 μs). Ho *et al.*[1] discuss an intermediate hydrogen-bond structure, formed after excitation, between the phosphate at the 3′-position and the base, with the negative charge on the base (B^-). This hydrogen-bonded structure either could undergo proton transfer to B^- expelling a neutral base (BH) or breakage of the hydrogen bond yielding a deprotonated base (B^-). B^- could be held with the remnant neutral to form an ion–dipole complex and the lifetime of dissociation to form B^- would depend on the shallow barrier holding the complex.[1] Cleavage of the strong hydrogen bond could be the rate determining step in this process. Such a process is also plausible through an intermediate with a H-bond between the base and the phosphate in 5′-position.

Fig. 7 shows the daughter mass spectrum obtained from UV photodissociation of the AMP cation performed at ELISA (top panel) along with the high resolution data (bottom panel) obtained using the mass spectrometer in P. Dugourd's laboratory at Lyon.[35] The dissociation fragments are found to be different from that obtained in CID experiments.[2] The cationic base formed by cleavage of the C–N bond, which is the

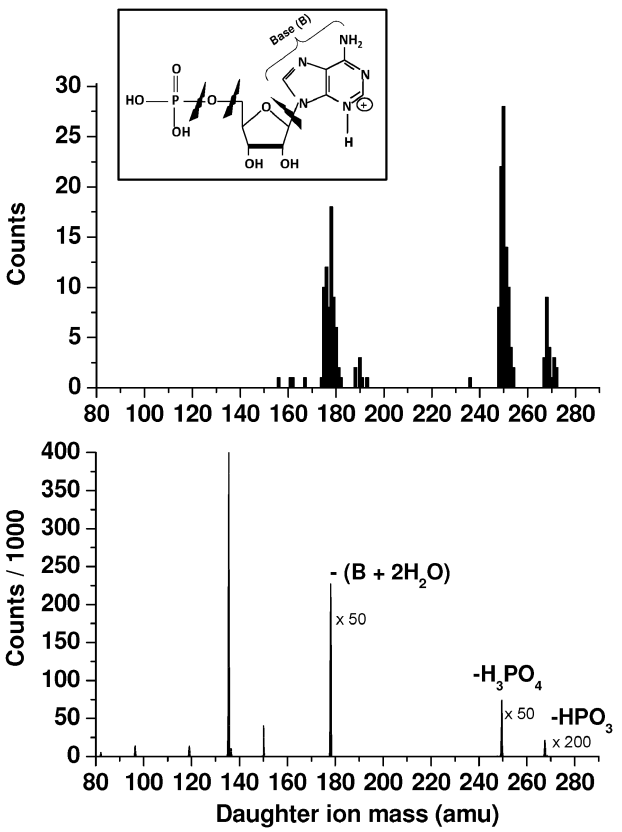

Fig. 7 The daughter-ion mass spectrum obtained from UV photodissociation of AMP cation at 266 nm performed at ELISA is shown in the top panel. The complementary neutral fragments are mentioned in the figure. The assignment of the ionic fragments is provided from a high resolution mass spectrometer[35] (bottom panel).

prominent fragment in CID, was not observed at ELISA. Our data from Lyon shows a prominent yield of the cationic base (136 amu) along with other masses at 82, 97, 119, and 150 amu, which are also unseen at ELISA. The difference stems from the fact that the data from ELISA is a mass spectrum integrated over a time window of about 10 μs after the excitation, while those obtained in Lyon are integrated over 100 ms. Apparently, the 136 amu fragment is associated with a rather slow dissociation and therefore not seen in the early, short time window using ELISA. The photofragment at 178 amu is likely to be the cation formed with loss of two water molecules along with cleavage of the C–N bond. Elimination of a water molecule from ribose is known from CID experiments.[2] It is not straightforward to relate the various observed fragmentation lifetimes with individual dissociation pathways.

IV. Conclusion

UV photodissociation of gas phase AMP cations and anions was performed at 266 nm. The dissociation lifetimes of the AMP ions were measured using a linear time of flight spectrometer. We see no evidence of an electron detachment pathway for AMP anions. Dissociation lifetimes were observed for both ions in the sub-microsecond regime corresponding to ergodic dissociation. The photofragmentation was determined to be due to a single photon process for both ions. The results indicate that the AMP ions can dissipate excess energy to the biological environment before they are damaged. The photofragmentation of the AMP anion was found to be similar to collision-induced fragmentation but this was not the case for the AMP cation. Several dissociation lifetimes, observed over a wide timescale, imply complex dissociation mechanisms, and relating the observed fragments to the measured lifetimes would require time-resolved mass spectrometry, which is presently being developed in our laboratory.

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