Scientific Report

Concerning the STSM action, COST P9:

Reference Code : COST-STSM-P9-00445 / 446

A) Purpose of the visit:

It was the aim of this short-term visit to perform common experiments at the ELISA facility with the objective to study
'Laser- and collision-induced fragmentation of isolated and solvated biomolecular systems'.

The participating scientists are coming from 3 different laboratories:

B. Liu, S. Broendstedt-Nielsen and P. Hvelplund - University of Aarhus, Denmark,
H. Zettergren and H. Cederquist - Stockholm University, Sweden,
B. Manil and B.A. Huber - CIRIL, Caen, France.

B) Description of the work and of the main results

Specific title of the studies:

Collision induced fragmentation of isolated and solvated anions of the Adenosine 5'-monophosphate (AMP, AMP*$(H_2O)_m)^-$ and of the dimer $(AMP)_2^-$

Recently, studies of low-energetic collisions with isolated molecules of biological interest are performed in the gas phase, in order to better understand the mechanisms of radiation damage on a molecular level[1]. Due 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 (like nucleic bases). Alternatively, larger systems can be produced in electrospray ion sources and beams of biomolecular ions can be used to study collision-induced processes when interacting with neutral gas targets. Either inelastic collisions or electron capture processes, in particular when targets with low ionisation potential are used (Na), may induce damage to the biomolecular system. However, the question is raised, to which extend these results are applicable to biomolecular systems in their natural environment, i.e. in aqueous solutions. This was the motivation for the present work, to study collision-induced fragmentation of isolated and micro-solvated nucleotides. Recent studies have shown[2], that anions of adenosine 5'-monophosphate can be prepared in ESI sources with a different number of water molecules attached. Therefore, in the present work we studied these biomolecules as a model system, which contains the nucleic bases adenine, the ribose-part as well as a phosphate group.

The ions are produced in an electrospray ion source using a solution of AMP and pure methanol. The negative ions are accelerated to an energy of 50 keV and after a magnetic mass selection they pass through a sodium cell, kept at a temperature of 220 °C. Primary as well as secondary ions and fragments are analysed with an electrostatic energy analyser allowing to

determine the mass/charge ratio of the charged particles. The preparation of solvated AMP-anions is still a delicate task and some of the important parameters are not well controlled at present. Although in the past up to twenty water molecules could be attached to the AMP-molecule, in the present experiment it was not possible to attach more than two H_2O molecules with beams of reasonable intensity. Variations of the spray solution and the spray conditions did not improve the situation. Therefore, the present study was limited to the fragmentation of (AMP, AMP*H_2O, AMP*2 H_2O)$^-$ and of the dimer $(AMP)_2^-$ in collisions with sodium neon targets. The corresponding primary beam signals have been of the order of 10^5, $3*10^3$, 10^3 and 10^3 particles per second. In the discussion below we include some results from earlier studies.

Fragmentation of $(AMP\cdot(H_2O)_m)^-$

A typical fragmentation spectrum of the pure $(AMP)^-$ anion after collisions with sodium atoms is shown in Fig 1. The dominant fragments correspond to the loss of the phosphate groups (PO_3^- and $H_2PO_4^-$) and to the breaking of the bond between the adenine and the ribose-part, leading to the formation of the negative fragment of adenine (A^-) and the counterpart of the AMP-molecule $(AMP-AH)^-$. Furthermore, oxygen loss from the AMP-molecule is observed. In addition, due to single electron capture, dianions of AMP are formed.

In Fig. 2 we compare the spectrum with those spectra obtained when several water molecules are attached. The intensity of the primary beams are normalised to the same value,

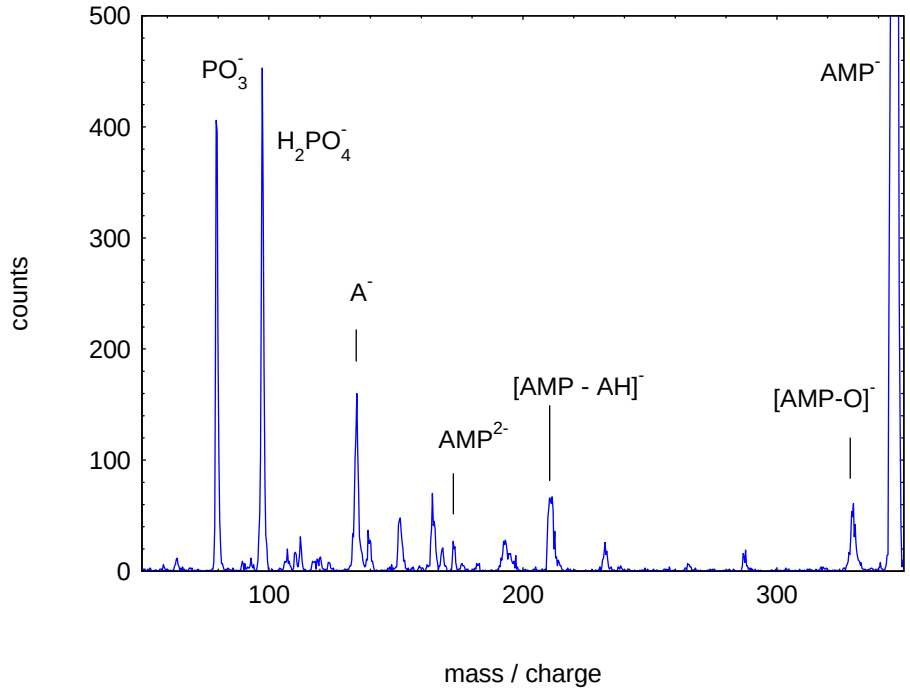

Figure1: Fragmentation spectrum obtained in collisions of $(AMP)^-$ anions with Na at 50 keV

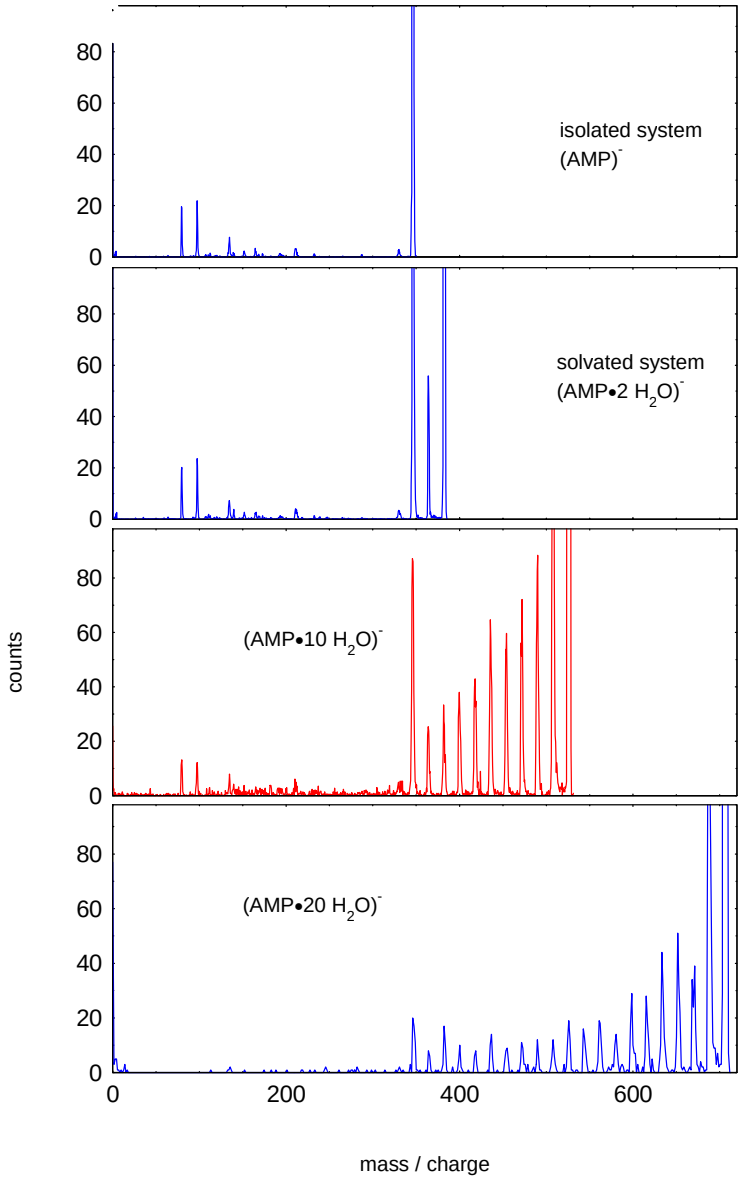

Figure 2: Comparison of fragmentation spectra for isolated and solvated AMP - anions

thus allowing a direct comparison of the fragment intensities. The striking change are the strong contributions from processes linked to the loss of the attached water molecule.

For a low number *m* of attached water molecules, it is most likely that all water molecules are lost, whereas for larger systems the fragmentation probability decreases with increasing number of lost H_2O-units Nevertheless, the intensity of the pure $(AMP)^-$ fragment still shows a local maximum as it represents the end of the 'H2O-evaporation chain'. The structure of the AMP-fragments of the AMP-molecules is nearly independent of the degree of solvation, in particular, no AMP-fragments with an attached water molecule are observed. This indicates that fragmentation of the AMP-molecule occurs only after all loosely bound water molecules have already been released. However, the intensity of the fragmentation channels changes with *m*.

In Fig. 3 we show the relative destruction cross sections as a function of the originally attached water molecules *m*. The total fragmentation cross section (upper part of Fig.3) increases strongly with *m* and saturates for larger systems (m>10). This increase can be explained by the increasing geometrical cross section, but also by the opening of new low-

energy channels linked to the loss of water molecules. For low *m*-values, the AMP-fragmentation does not vary strongly, the slight increase for *m*=1 is probably due to the increase of the total cross section and the weak energetic coupling between the water and the AMP molecule. However, for *m*>10, the AMP part seems to be well secured by the water surrounding and the intensity for AMP-fragmentation strongly decreases. Thus the damage to the AMP-molecule seems to be strongly reduced in a water environment.

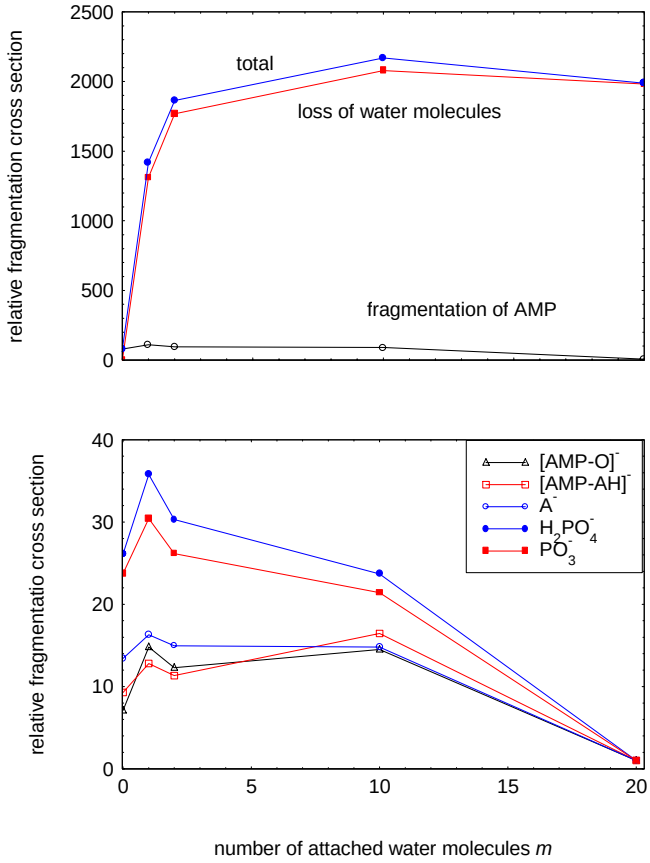

Figure 3: Relative fragmentation cross sections as a function of the number *m* of attached water molecules. Upper part: total destruction cross section, cross section for loss of water molecules, destruction cross section for the AMP-part. Lower part: Relative cross sections for individual AMP-fragmentation channels.

Fragmentation of the dimer $(AMP)_2^-$

Collision induced fragmentation of clusters and particularly of the dimer may yield information on the geometrical structure and the binding conditions in these complex systems. In Fig. 4 we compare the fragmentation spectrum obtained in the collision of the dimer $(AMP)_2^-$ with that of the monomer, already shown in Fig.1 and represented as inlet in Fig. 4 at its correct *m*/*q* position or shifted by 346 mass units. The most obvious differences are the following:

- First of all, the dominant fragment is the intact monomer $(AMP)^-$. Thus, it is most likely, that the dimer breaks apart into two monomer species.
- A more close analysis shows, that no dianions, in particular no $(AMP)^{2-}$, are formed in contrast to the monomer case. Thus, when capturing an electron and forming $(AMP)_2^{2-}$

dianions, thiese systems are unstable due to the Coulomb repulsion and contribute to the $(AMP)^-$ signal.

- Concerning the fragments with *m*/*q*-values smaller than 346 ($(AMP)^-$), we find a similar distribution as in the monomer case. Thus the anions like PO_3^-, $H_2PO_4^-$, A^-, $[AMP-AH]^-$ and $[AMP-O]^-$ are the dominant fragments. Also for larger fragments a similar distribution is obtained ($[AMP+PO_3]^-$, $[AMP+H_2PO_4]^-$, $[2\ AMP-AH]^-$, $[2\ AMP-O]^-$), however, the fragment $[AMP+A]^-$ is missing. Assuming that these fragments are formed by a simple bond breaking process, we may conclude from the observed fragmentation pattern, that the nucleic base adenine is not involved in the dimer bonding. In contrary, it should be placed at the opposite site, thus favouring a hydrogen bond between the negative phospate group and the neutral phosphoric acid group.

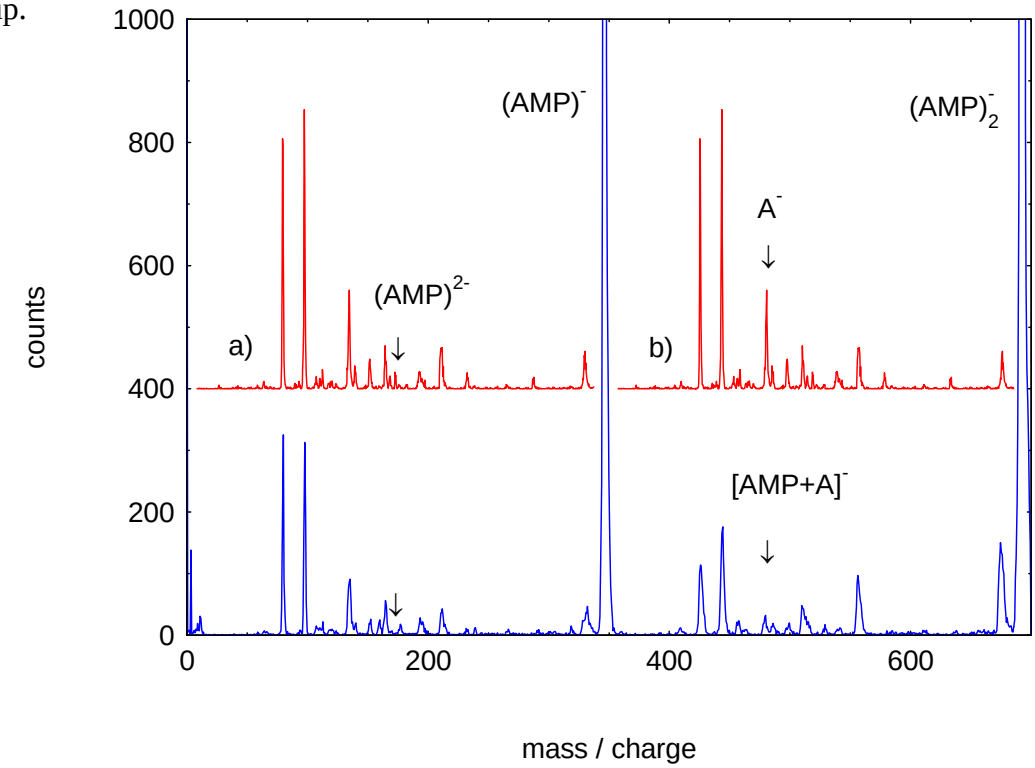

Figure 4: Fragmentation spectrum obtained in collisions of the (AMP) dimer anion with Na at 50 keV. The included inlet spectra (a) and (b) correspond to the fragment distribution of AMP^- monomers.

C) Conclusion and perspectives

The performed experiments have given first results on the radiation damage of larger biomolecular systems, such as adenosine 5'-monophosphate (AMP) induced by collision processes. The studies concerned the isolated AMP anion as well as solvated AMP and the anion of the AMP-dimer. It is shown that the fragmentation of the AMP^- anion is reduced with increasing number water molecules surrounding the biomolecular system, favouring the emission of the attached water molecules. The analysis of the fragmentation spectra of the $(AMP)^-$ dimer contains information about the geometrical structure of this dimer and proposes a possible non-covalent binding between the phosphate groups of the separated systems.

Further experimental studies have to be performed in the future in order to confirm these conclusions and to support these findings in more detail for a larger number of solvated systems.

D) Projected publications and articles

The obtained results are original and well suited to be published in a refereed journal, in particular when completed by additional measurements.

E) Confirmation by the host institution (host report)

As foreseen in the application, the participating scientists from Caen (B. Manil, B.A. Huber) and from Stockholm (H. Zettergren, H. Cederquist) arrived in Aarhus on Saturday, 23/10/04, and performed together with the local group experiments at the ELISA facility, ending on 29/10/04. Due to several technical problems at the storage ring ELISA, the main experiments have been performed at the mass separator installation. It was possible to study successfully the collision induced radiation damage of isolated and solvated systems. As model system we have used the anions of adenine 5'-monophosphate $(AMP)^-$. A first analysis of the data shows that more than ten water molecules have to be attached to the biomolecule in order to avoid its fragmentation. For twenty water molecules, practically no fragmentation signal was observed. Additional experiments have been performed with AMP-dimers. In this case the studies yielded information on the structure and binding conditions of the dimer system.

We plan to continue this type of studies in the framework of the established collaboration, also including experiments at the electrostatic storage ring ELISA.

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