Eur. Phys. J. D **43**, 117–120 (2007)
DOI: 10.1140/epjd/e2007-00093-9

THE EUROPEAN PHYSICAL JOURNAL D

Electron impact ionization of thymine clusters embedded in superfluid helium droplets

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Received 24 July 2006 / Received in final form 10 September 2006

Abstract. Embedding molecules in helium clusters has become a powerful technique for the preparation of cold targets for spectroscopy experiments, as well as for the assembly of complex, fragile molecular species. We have recently developed a helium cluster source and a pick-up cell to produce neutral beams of doped helium droplets, to be used as targets in studies on electron collisions with molecules of biological relevance. In the present work we present the results of a series of experiments on electron-impact ionization of helium clusters doped with thymine and 1-methylthymine, where several interesting phenomena were observed, i.e., (i) electron impact ionization of molecular clusters inside the helium droplets leads predominantly to protonated clusters; (ii) the appearance energies are close to the ionization threshold of the helium atom but ionization efficiency curves in addition extend down by several eV; (iii) ionized molecular clusters can undergo metastable decay via the loss of one neutral monomer.

PACS. 34.80.Gs Molecular excitation and ionization by electron impact – 36.40.Qv Stability and fragmentation of clusters – 82.33.Fg Reactions in clusters – 87.50.Gi Ionizing radiations

Obtaining a comprehensive description of the chemical and physical phenomena underlying radiation damage has become a goal of fundamental importance in recent years. This subject was brought to the attention of the electron-molecule collision community by the discovery that electrons can effectively damage DNA molecules [1–3] even at sub-excitation energies. This discovery is important because low-energy free electrons are among the most abundant secondary species produced by ionizing radiation. Furthermore, evidence was presented that an important process initiating the observed DNA strand break by electrons is the resonant formation of negative ions upon electron attachment [4–11].

In order to gain further insight into the role of secondary-electron interactions for radiation damage at a molecular level, it would be ideal to collect information about electron collisions to a variety of targets, ranging from the single DNA and RNA constituents to molecules with increasing complexity, including the sugar backbone components and possibly water. Doing so in the gas phase preserves all the advantages of the mass spectrometric techniques used so far, but also poses serious technical challenges. Namely, these compounds are non-volatile and it is necessary to heat them until sufficient vapor pressure is achieved. Under such circumstances, thermal excitation and decomposition has to be taken in account, which in turn complicates the interpretation of the results [12].

In recent years significant progress has been achieved in the mass spectrometric structural analysis of complex biomolecular anions and cations, in particular oligonucleotides, by the use of techniques such as MALDI and electrospray ionization [13]. However, little effort was extended to the study of the electron-impact ionization (as well as DEA) in such systems.

One successful approach to obtain complex isolated molecular targets is their assembly by independent evaporation of their constituents with subsequent condensation in a suitable carrier. Such a strategy was used for instance in spectroscopy experiments of paired nucleobase molecules, prepared by entrainment in a supersonic expansion where argon was used as carrier gas [14, 15]. Of special interest to us is the possibility of doping helium nanodroplets by crossing a supersonic helium beam with a vapor target of the desired molecular species

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("pick-up"). This technique has become a powerful tool for spectroscopy (see [16,17] for reviews on this subject); it was recently used in infrared spectroscopy experiments of mixed uracil-water complexes [18].

Concerning electron-impact ionization of doped helium clusters, considerable attention has been paid to the possibility of inducing "cold" chemical reactions [19], or obtaining "soft-ionization", i.e. decreasing fragmentation, either due to the transfer of excess energy of the molecular ion to the surrounding helium environment, or due to a "caging" effect [20–25].

Having considerable experience in electron-molecule collisions, particularly regarding negative-ion production, where characteristic resonances for different reaction channels are often observed [4–11] we recently explored the possibility that the observed resonances for DEA to biomolecules, in the gas phase, could be altered in the environment of a helium droplet, opening new possibilities for the investigation on the nature of such resonances.

We have therefore developed a new apparatus for the production of a beam of doped helium droplets to be used as target in our electron collision studies. The performance of the apparatus was investigated first by studying positive ion formation by electron impact. In the course of these investigations we have observed several interesting phenomena, which will be presented here. In particular, we will show results obtained with thymine and 1-methylthymine embedded in helium droplets. The two molecules showed essentially identical results, except where explicitly stated otherwise.

The main parts of our experimental setup have been described elsewhere [26,27]. Briefly, helium gas at 10 to 20 bar pressure is cooled down to 10 K by a closed cycle refrigerator, and expands into vacuum through a 5 μm nozzle. Under such conditions, the production of clusters of sizes ranging up to 10^4 can be expected [28]. The pressure in the expansion chamber is maintained below 10^{-2} Pa by a 1200 l/s turbo pump. The resulting supersonic beam is skimmed by a 0.8 mm conical skimmer, situated approximately 5 mm downstream, and then enters a pick-up cell. This cell is 10 cm long, 5 cm wide and has two 5 mm apertures for the helium beam passage. It is fed with nucleobase vapor produced by an oven, with temperatures adjustable between 373 and 410 K. The cell itself is kept at 373 K by resistive heaters in order to prevent condensation on the walls. The cluster beam is then skimmed again into the ionization chamber of a modified Varian MAT CH5-DF two- sector-field mass spectrometer. The pressure in the mass spectrometer is typically better than 8×10^{-6} Pa; in the ion-source it is approximately 10^{-4} Pa.

Figure 1 shows a mass spectrum obtained by electron impact ionization of helium clusters doped with 1-methylthymine (MT) molecules at an electron energy of 120 eV and 500 μA electron current. Evident in this mass spectrum is a decreasing baseline of helium clusters He_i^+, and 1-methyl-thymine clusters MT_n^+ up to size $n = 4$. Larger clusters up to $n = 9$ were observed but not included in the graph for the sake of clarity. The insert shows a higher-resolution mass spectrum in the MT dimer region, where it can be seen that the MT cluster peak is a composition of de-hydrogenated ($m = 279$), normal ($m = 280$) and protonated ($m = 281$) MT dimer ions, the protonated one being the dominant peak. This observation holds for all cluster sizes accessible with sufficient resolution and intensity, i.e. up to 4. In the case of the monomer, a comparison can be made with a standard gas phase mass spectrum, which shows a de-hydrogenated peak of negligible intensity. Such an increase in the de-hydrogenated molecular ion signal formed in helium droplets was already observed in the case of alcohols [24]. The protonated peak obviously originates from fragmentation of higher order molecular clusters.

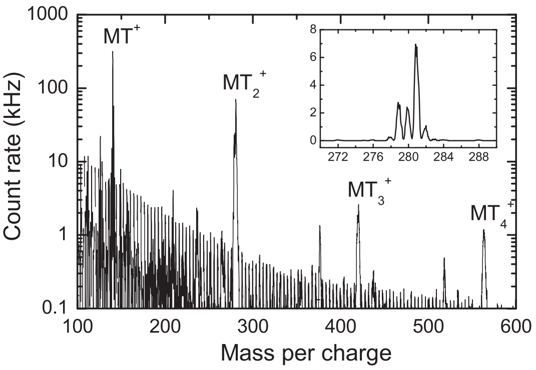

Fig. 1. Mass spectra of positive ions generated by electron impact of helium clusters doped with 1-methyl-thymine (MT).

Apart from the helium cluster background, the only other masses that appear clearly in the MT spectrum correspond to a group of peaks centered 44 amu below each of the molecular cluster ions. This is in contrast to normal thymine, where we did not observe any intermediate masses between the T_n cluster ions. It is interesting to note that in our gas phase measurement on the 1-methylthymine molecule there exists a group of fragments at a mass around 96 amu, but it is a minor contribution to the mass spectrum. The most intense peak in this case is, besides the parent ion, one at mass 43 amu, possibly the complementary counterpart of the ion at 96 amu. Further investigations with isotopically labeled molecules may elucidate this issue.

The relative intensities of the three peaks shown in the insert of Figure 1 are strongly dependent on the expansion conditions of the helium cluster source. In Figure 2 we plot their relative intensities with respect to the normal 1-methyl-thymine dimer ion, as a function of the nozzle temperature, for a fixed pressure of 15 bar. We interpret the strong drop of the protonated ion signal as a consequence of the pick-up probability for differently sized droplets: for higher temperatures the helium clusters are smaller involving a smaller pick-up probability. This effect is more important for the neutral trimer from which the protonated dimer ion originates, than for the neutral dimer. The approximately constant value for the de-hydrogenated dimer ion, on the other hand, seems to be an indication that it originates from the same neutral precursor as the normal dimer ion.

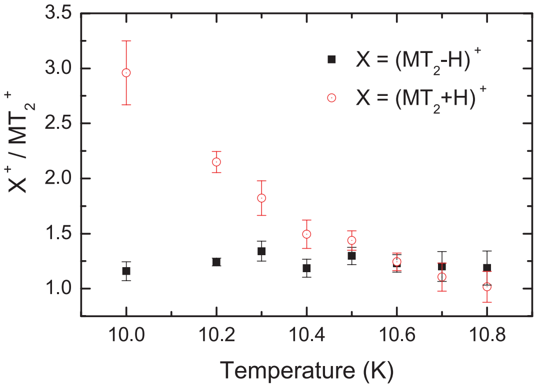

Fig. 2. Dependence of the ion yield on nozzle temperature for protonated and de-hydrogenated 1-methyl-thymine dimer ions normalized to the normal 1-methyl-thymine dimer ion.

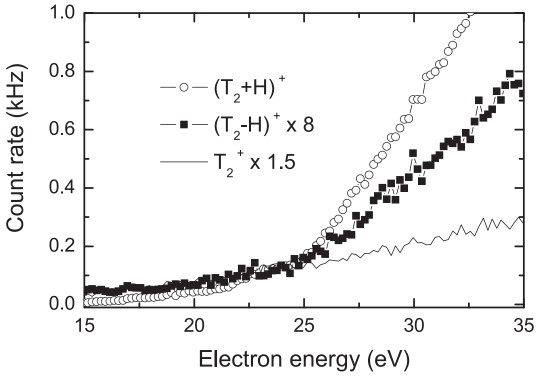

Fig. 3. Ion yield close to the ionization threshold for protonated, normal and de-hydrogenated thymine dimer ions. The intensities were normalized at 25 eV to emphasize the different slopes.

In order to characterize the ionization mechanism of the molecular clusters inside the nanodroplets, we have performed electron energy scans close to the ionization thresholds. Figure 3 presents the results for the de-hydrogenated thymine dimer, $(T_2–H)^+$, the normal thymine dimer, T_2^+, and the protonated thymine dimer, $(T_2 + H)^+$. The substantial increase of the ion yields above the ionization threshold of helium is striking. Analogous behavior was found for other dopants in helium droplets (see for instance [20,23]); it is attributed to a "charge hopping" mechanism that is effective when the dopant is located deep inside the droplet. On the other hand, if the dopant resides near the surface as in the case of alkaline metals, the ionization threshold appears at lower energies, close to the first excitation of the helium atom, and Penning ionization becomes a relevant process [21].

Further information can be inferred from the slope of the ion yields with respect to electron energy above the helium ionization threshold. The difference in the slopes for the protonated molecule in comparison to the normal molecule results from the differently sized neutral precursors for the two ions. However, in the case of the de-hydrogenated ion, the charge-hopping mechanism cannot account for the different electron energy dependence in comparison to the normal dimer. Our interpretation is that each product ion shows a different partitioning between the charge hopping and the Penning ionization mechanisms, depending on the excess energy available in each case. One may conclude from this analysis that the energy dissipation to the helium cluster during the ionization event of the dopant is not complete, leaving products with appreciable amount of internal excess energy.

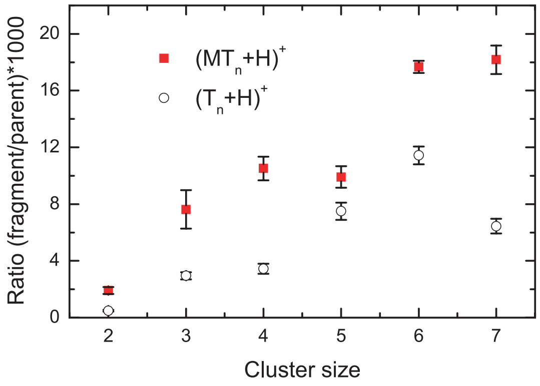

Fig. 4. Ion yield for monomer loss from thymine (T) and 1-methyl-thymine (MT) clusters, normalized to the respective parent cluster ion intensities ("metastable fraction").

The presence of excited, metastable states of the molecular ions can be investigated in our apparatus using the so-called MIKE (Mass-analyzed Ion Kinetic Energy) technique [27]. In short, the first sector of the spectrometer, with adjustable magnetic field, selects the desired molecular ion by momentum filtering. Scanning the electric field of the second sector of the mass spectrometer allows the identification of fragment ions resulting from dissociative metastable decay occurring between the magnetic and the electric sector. The width of the fragment peaks are generally associated with the kinetic energy release (KER) of the respective decays, but in our case some contribution of fragments differing by 1 amu is possible, as well as, to a minor extent, fragments coming from the adjacent masses (mass of molecular clusters ±1). Similar measurements were undertaken for molecular cluster sizes up to 7 for both thymine and 1-methyl-thymine. Where a clear distinction was possible, we chose always the protonated species as parent, since they are the most abundant. In all cases the only observable fragment ions correspond to the loss of one neutral monomer. For a quantitative comparison between the probabilities for these metastable decays we calculate the fraction between the total fragment ion yield and the parent ion intensity. The result is shown in Figure 4. The error bars account for the statistical fluctuation of the results, which is mainly caused by small instabilities in the nozzle temperature during each measurement.

It can be seen in Figure 4 that thymine clusters are always more stable than their methylated counterparts. This is an interesting result because the presence of a methyl group at the N1 position of the thymine molecule reduces the number of sites available for “hydrogen-bond-like” interactions between the molecules.

This work was partially supported by the FWF (Wien), by the European Commission (Brussels), EIPAM and COST-P9. F.Z. acknowledges support from the Brazilian agency CNPq and Universidade Estacio de Sá.

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