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Electron capture and loss by protonated peptides and proteins in collisions with C_{60} and Na

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Abstract. Electron capture and loss cross-sections have been measured for collisions between fast multiply charged peptide and protein ions $[M+nH]^{n+}$ and Na or C_{60}. The ions were produced in an electrospray ion source (ESI) and accelerated to an energy of $n \times 50$ keV. We find that the size of the cross-sections depend strongly on molecule size, ionization energy and projectile charge state n. For multiply charged ubiquitin projectiles, the cross-sections for electron capture are found to be several times larger with a Na target than with a C_{60} target. This observation is qualitatively explained by means of an over-the-barrier model for electron transfer using metal-sphere descriptions for the electronic responses of the collision partners.

PACS. 34.70.+e Charge transfer – 61.48.+c Fullerenes and fullerene-related materials – 87.15.-v Biomolecules: structure and physical properties

1 Introduction

Measurements of electron capture and loss is a classical discipline in ion atom collision studies and information about atomic structure and dynamics can be obtained from such studies. Recently there has been a growing interest in charge permutation reactions in collisions between cluster ions or large molecular ions with gases or with clusters. Electron capture and loss processes are reasonably well understood for atomic systems [1] but have not been fully investigated for collisions between more complex systems such as large molecule- or cluster-ions and atoms molecules and clusters in the gas phase.

Electron capture by multiply charged ions in collisions with C_{60} became an active field of investigation since the pioneering experiments of Walch *et al.* [2]. For further references see *e.g.* Cederquist *et al.* [3]. The over-the-barrier model [4] has been very successful in estimating capture cross-sections for such collisions and, recently, it has been generalized to be able to treat cases with polarizable projectiles and targets of finite geometrical dimensions [5]. An example here is the successful modeling of target charge state distributions in slow, non-fragmenting, C_{60}^{q+}–C_{60} collisions [5,6]. Electron capture in collisions between singly charged alkali clusters and Cs was studied as a function of cluster size and a strong dependence of the cross-section on the energy defect as well as on the collision energy was found [7]. Theoretical estimates for these cross-sections were performed within a microscopic framework called nonadiabatic quantum molecular dynamics [8], and the importance of the cluster temperature on charge transfer noted. A process dubbed electron capture induced decomposition (ECID) has been used successfully for studies of decomposition of organic dications resulting from electron capture in collisions with atoms or molecules [9].

A subgroup of electron capture and loss processes is the so-called charge inversion reactions. In these reactions the charge of the molecular ion is changed from positive to negative or *visa versa* in collisions with neutral gas atoms or molecules. Since the electron-transfer processes are nearly vertical, the charge-inverted ions are often highly vibrational excited according to the Frank-Condon factors and their fragmentation may provide structural information. These processes, which are important in molecular mass spectrometry have recently been reviewed by Danell and Glish [10]. Hayakawa [11] has described how alkali metal targets can be used in charge inversion reactions to form negative ions from positive precursor ions. Again these processes were studied in order to obtain information about dissociation of molecular projectile ions. Along the same lines, capture of free electrons by multiply charged biomolecules has recently attracted a great deal of interest since such processes lead to nonergodic fragmentation [12], and thus provides new information about the amino acid sequence in peptides and proteins.

The present study focuses on measurements of electron capture cross-sections in collisions between multiply charged peptides or proteins with Na or C_{60}. We find that the magnitude of the cross-sections depends strongly on the size of the collision partners, on ionization energies,

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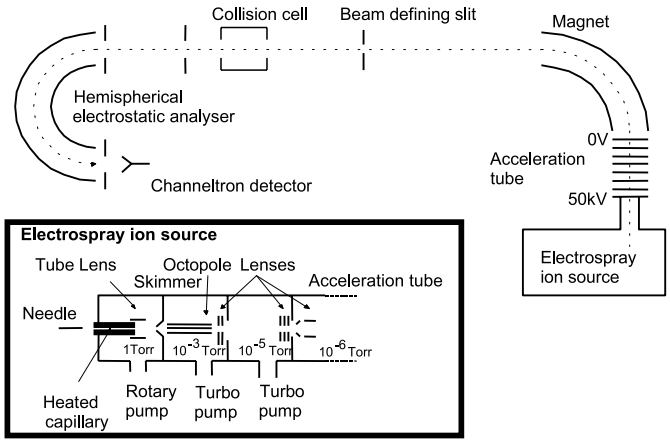

Fig. 1. Schematic diagram of the experimental apparatus; the insert shows the electrospray ion source.

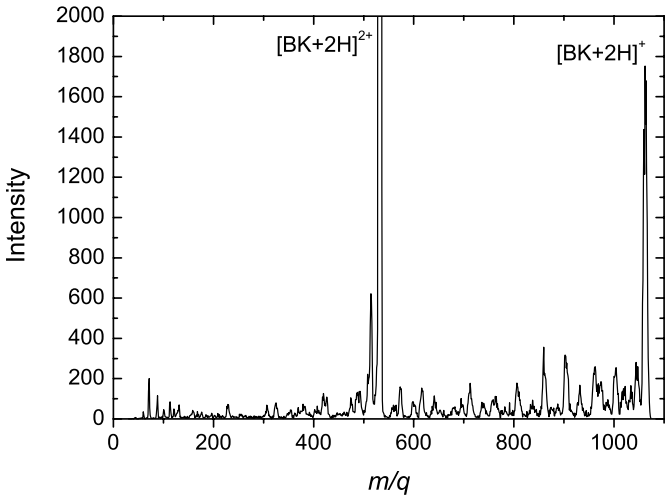

Fig. 2. Spectrum obtained for 100 keV collisions between $[BK+2H]^{2+}$ and Na.

on projectile charge state and on collision energy. We have applied the generalized over-the-barrier model [5] to qualitatively account for the measured dependencies of the geometrical extensions of the collision partners, the target ionization energy and the projectile charge states. We have, further, deduced approximate values for the critical distances within which ubiquitin projectiles fragment in collisions with Na and C_{60}.

2 Experiment

The experimental arrangement (Fig. 1) is described in detail in reference [13]. Briefly, protonated peptide or protein ions, formed by electrospray ionization (ESI), were accelerated to a kinetic energy of $n \times 50$ keV where n is the charge state of the ion. The precursor ions which are normally considered to be cold [14], were mass selected with a magnet and passed through a 3 cm long target cell. The target cell is a resistively heated 6 cm long stainless-steel tube where the 3 cm long central part is defined by 1- and 2-mm-diameter entrance and exit apertures, respectively. C_{60} powder or solid sodium was placed in the mid section of the tube between the two apertures. During the experiment C_{60} was heated to temperatures between 400 °C and 450 °C and sodium to 200 to 230 °C. The absolute target thickness was obtained from measured oven temperatures by use of vapor-*vs.*-temperature values for C_{60} [15,16] and Na [17]. The product ions exiting the cell were analyzed with an electrostatic hemispherical analyzer. Capture and loss cross-sections were deduced from the intensity of the product signal-*vs.*-pressure measurements, the so-called initial growth method. The experimental errors are mainly caused by beam instabilities, and are normally about ±10%.

3 Results

A spectrum showing electron capture in collisions between doubly protonated bradykinin $[BK+2H]^{2+}$ and Na is shown in Figure 2. The dominant product peak in the mass spectrum corresponds to $[BK+2H]^{+}$ but peaks corresponding to collision-induced dissociation CID are also observed. Our mass resolution is not sufficient for differentiating between the masses of $[BK+2H]^{+}$ and $[BK+H]^{+}$. We can therefore not directly exclude that the peak corresponds to proton loss instead of electron capture. Control experiments in a Ne target where this peak is absent however strongly suggests that the peak should be assigned to electron capture. The significance of electron capture with respect to the fragmentation pattern will be the subject of a coming article. Bradykinin is a peptide consisting of 9 amino acid residues with a mass of 1 062 amu. Spectra showing electron capture and loss in collisions between seven times protonated ubiquitin $[Ubi+7H]^{7+}$ and O_2, C_{60} and Na are shown in Figure 3. Ubiquitin is a protein consisting of 76 amino acid residues with a mass of 8.6 kamu. In all three spectra a background originating from CID is observed. On top of this, electron loss in O_2, loss and capture in C_{60} and electron capture in Na are observed. Electron loss by cations of amino acid peptides or proteins in collision with O_2 has been dealt with in previous articles [18,19] and there it was argued that electron transfer to O_2 resulting in O_2^- plays an important role. The electron loss cross-section for $[Ubi+7H]^{7+}$ colliding with O_2 was determined to be ∼100 Å^2 but only ∼6 Å^2 for collisions with C_{60}. Electron capture reactions of the type

$$[Ubi + 7H]^{7+} + T \rightarrow [Ubi + 7H]^{6+} + T^{+} \qquad (1)$$

are the dominating reaction channels for the targets (T) Na and C_{60}. Independent of theoretical approach the target ionization potential, the binding energy of the transferred electron and the relative particle velocity are important parameters. Since the target atoms (or molecules) are assumed to be in their electronic ground state before the collision, the ionization potentials of 5.138 eV [20] for Na and 7.6 eV [21] for C_{60} were assumed. The projectile ion has a quasi continuum of excited states and we

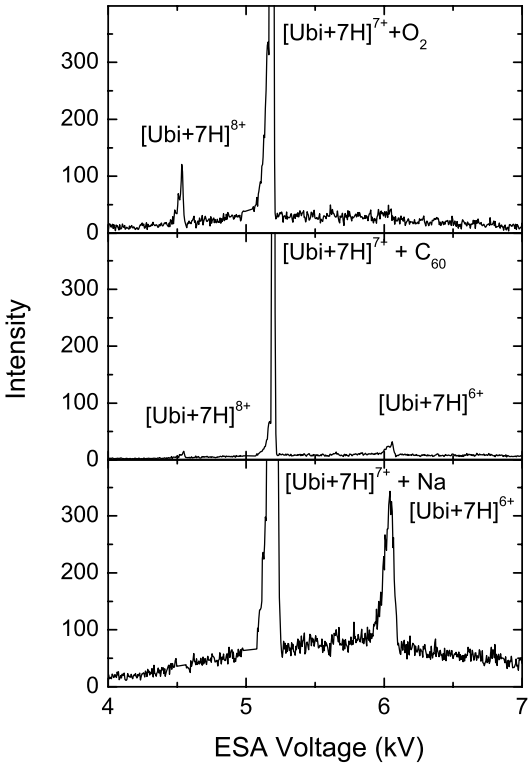

Fig. 3. Spectra obtained for 350 keV collisions between $[Ubi +7H]^{7+}$ and O_2, C_{60} and Na.

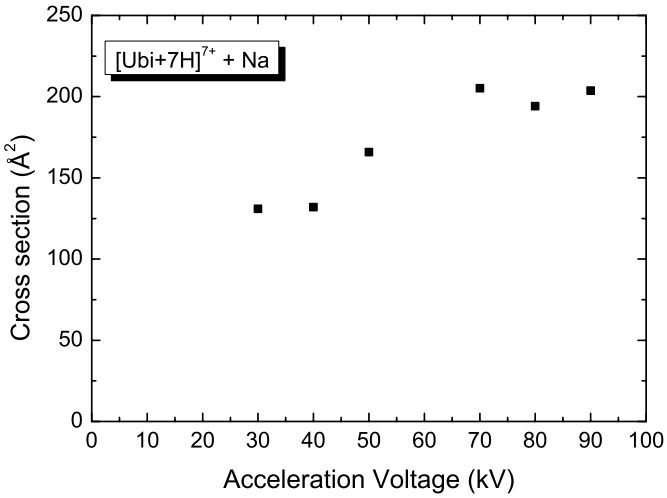

Fig. 4. Electron capture cross-section for collisions between $[Ubi+7H]^{7+}$ and Na as a function of acceleration voltage.

therefore expect the dominating electron capture process to be near-resonant which implies that the dependence on collision velocity is weak for low velocities. That was confirmed from the measured electron capture cross-section *versus* collision energy for $[Ubi +7H]^{7+}$ interacting with Na (Fig. 4). It should however be noted that since the electron transfer process in reality is non-resonant the capture cross-section will most likely approach zero at very low relative velocities. The two targets (Na and C_{60}) also differ with respect to geometrical size. The radius of the C_{60} cage structure is 3.5 Å while the Na atom in this comparison is a point-like particle.

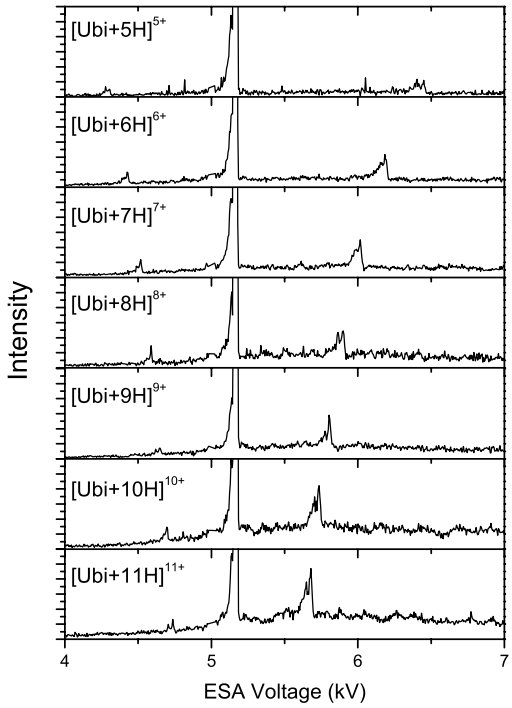

Fig. 5. Spectra obtained for collisions between protonated ubiquitin and C_{60} for charge states from 5 to 11.

The charge state dependence of the capture and loss cross-sections is illustrated in Figure 5, where m/q spectra are shown for ubiquitin with charge states from 5 to 11 interacting with C_{60}. The cross-section for electron loss is almost independent of charge state ($\sim$6 Å^2) while the electron capture cross-section increases with charge state.

In Figure 6 is shown the electron capture cross-section dependence on charge state for collisions between ubiquitin with Na or with C_{60}. The electron capture cross-section is around a factor of eight larger for interactions with Na compared to C_{60}, and increases in both cases almost linearly with the charge state of the projectile ion.

4 Model calculations and discussion

To obtain some insight in the electron capture mechanism we have applied the generalized over-the-barrier model for electron transfer between conducting spherical objects [5]. In this model it is assumed that electron transfer is possible when the over-the-barrier condition is fulfilled, *i.e.* when the potential seen by the electron moving from the target to the projectile equals the Stark shifted binding energy for the active electron at the target. The protonated, multiply charged ubiquitin ions are approximated by a conducting sphere with a radius of 16.0 Å. The estimate of the ubiquitin radius is based on its crystal structure which is determined by X-ray diffraction analysis. The multiply charged ubiquitin ions are assumed to possess enough internal energy to allow the excess protons

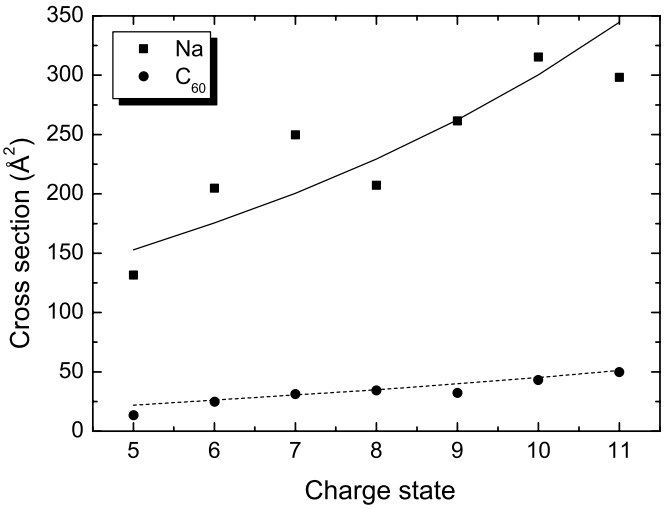

Fig. 6. Electron capture cross-sections for collisions between protonated ubiquitin and C_{60} (circles) or Na (squares) as a function of projectile charge state. Full curve and broken line: cross-sections calculated using the extended over-the-barrier model for electron capture.

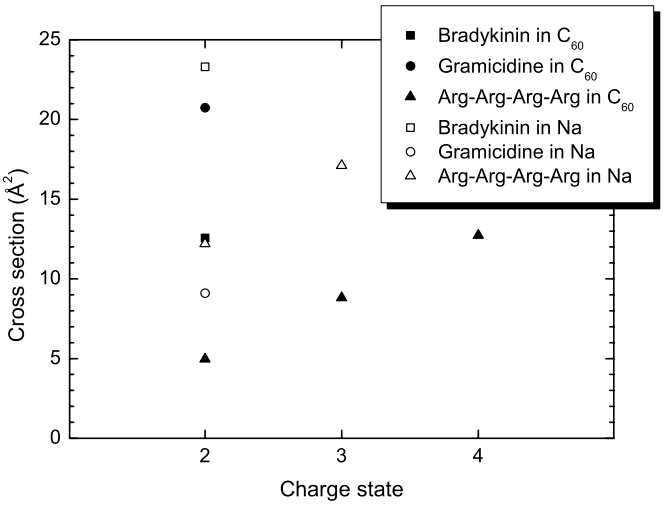

Fig. 7. Electron capture cross-sections for collisions between various protonated peptides and C_{60} or Na as a function of projectile charge state. The peptide masses are as follows: M(Arg−Arg−Arg−Arg) = 642 amu, M(gramicidin) = 1 214 amu, and M(bradykinin) = 1 060 amu.

Table 1. The critical distance R_c for the collisions between protonated ubiquitin, with charge states n between 5 and 11, and Na or C_{60}.

n	R_c(Na) (Å)	R_c(C_{60}) (Å)
5	21.66	21.95
6	21.84	21.99
7	22.04	22.02
8	22.27	22.05
9	22.52	22.09
10	22.81	22.12
11	23.13	22.17

to be mobile and to move fast between the basic groups. The ion is to a good approximation spheric and we therefore use a simple conducting sphere model to describe the ions. The radii of C_{60}^+ and Na^+ are 3.8 Å and 0 respectively. The cross-section for pure electron transfer from the target particle to the projectile ion, σ_c is defined as

$$\sigma_c = \pi \left(R_c^2 - R_f^2\right) \quad (2)$$

where R_c is the critical distance between the two particles at which the over-the-barrier condition is fulfilled *i.e.* the active electron can move from one particle to the other and R_f is the largest distance at which projectile fragmentation occurs. Calculated R_c values for collisions between protonated ubiquitin, with charge states n between 5 and 11, and Na or C_{60} are given in Table 1.

The maximum distance between projectile and target particles at which fragmentation takes place, R_f, was used as a fitting parameter and the curves shown in Figure 6 show the best fit for R_f values of 20.56 Å and 21.79 Å for Na and C_{60} respectively. These values are reasonable since for collisions between "spherical" ubiquitin and C_{60}, R_f corresponds to a surface-surface distance of about 2 Å. In such a collision several eV can be transferred to the ubiquitin ion leading to fragmentation [22]. In comparison for collisions with Na the fragmentation distance corresponds to a distance between the ubiquitin surface and the Na nucleus of around 5 Å. This value is somewhat larger than expected and could indicate deviation from a spherical shape of the ubiquitin ion. However the total fragmentation cross-section based on this R_f value $\sigma_f = \pi 20.56^2 = 1\,350$ Å^2 is in good agreement with cross-section values based on drift tube ion mobility spectrometry [23].

However the drift tube measurements also showed that the total fragmentation cross-section depended on the charge state of the ubiquitin ion and the use of the same R_f value for all charge states is questionable. On the other hand, the calculated R_c values also depend on the size of the ubiquitin ion and therefore size and charge variations cancel to first order. Again these model calculations are crude and only serve as a framework for a discussion of the important parameters which enter the description of electron transfer between such complex ions. We have not discussed to what extent a conducting sphere is a good model for ubiquitin and C_{60}, but assumed that its use is justified by its simplicity.

Charge transfer cross-sections for collisions between various protonated peptides and Na or C_{60} are shown in Figure 7 as a function of projectile charge. The conducting sphere model is meaningless here since the projectile conformation is known to be far from spherical and change from peptide to peptide. In collisions with C_{60} the capture cross-section increases almost linearly with projectile charge state and seems not to depend on the type of peptide. For Na as a target the capture cross-section varies

more than a factor of two for a given charge state and the structure or size of the individual peptide ions seems to play an important role in the electron capture process.

5 Conclusions

The study of collisionally induced electron transfer in collisions between protonated proteins or peptides and Na or C_{60} shows a dependence which is similar to that observed for atomic species. The cross-section for electron transfer between multiply charged ubiquitin and Na is as large as 200 Å^2 but the total fragmentation cross-section is about 1 300 Å^2 indicating that only glancing collisions leads to electron transfer without fragmentation. A precise estimate of the electron capture cross-section is difficult to obtain, but by describing the electron transfer process in the framework of the over-the-barrier model, we have obtained qualitative agreement with measured cross-section values for collisions between multiply charged ubiquitin with Na and C_{60}.

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