Neutral-particle emission in collisions of electrons with biomolecular ions in an electrostatic storage ring

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Abstract. Electron-biomolecular ion collisions were studied using an electrostatic storage ring with a merging electron beam device. Biomolecular ions produced by an electrospray ion source and accelerated to 20 keV/charge were injected into the ring after being mass-analyzed. The circulating ion beam was then merged with an electron beam. Neutral reaction products in collisions of electrons with ions were detected by a micro-channel plate outside of the ring. Electron-ion collisions were studied for multiply-deprotonated oligonucleotide and peptide anions as well as singly protonated oligonucleotide and peptide cations. For peptide cations, neutrals were resonantly emitted at an electron energy of around 6.5 eV, which was almost independent of the ion masses. This is deduced to come from electron-ion recombination, resulting in the cleavage of a peptide bond. For DNA oligonucleotide cations, resonant neutral particle emission was also observed. In electron and DNA anion collisions, neutrals started to increase from definite threshold energies, where the threshold energies increased in proportion to the ion charge. The same was found for peptide anions. The origin of this phenomenon is discussed.

1. Introduction
Since the late 1980s, many ion-storage rings with electron coolers have been constructed. Some of them have been used for atomic and molecular physics. The features of this type of research can be summarized as: 1) high luminosity, 2) high resolution, 3) variable relative energy, 4) low background and 5) quenching of vibrationally excited states for molecular ions. With these storage rings, many new phenomena in molecular physics were found, mainly for light ions [1]. It would be very interesting to expand this type of experiment to heavier macromolecules, like biomolecules. However, in the magnetic storage rings used so far, the strength of magnetic field has to be increased along with an increase of the ion mass, and it is not easy to store macromolecules. However, if we use an electrostatic field instead of a magnetic field, we can store heavy ions independently of their masses, although high resolution cannot be expected because electron cooling does not work for heavy molecules. This new type of electrostatic storage ring was first constructed at the University of Aarhus [2]. We also started the construction of an electrostatic storage ring [3,4] and an electron beam device [5,6] at the High Energy Accelerator Research Organization (KEK) after the shutdown of our magnetic...
storage ring, TARN II [7], in 1999. In this paper, we introduce electron-biomolecular ion collision experiments using the electrostatic storage ring at KEK.

2. Experimental setup

The experimental setup consists of an ion source, a mass-analyzer and a storage ring. Biomolecular ions are produced by an electrospray ion source (ESI) and accelerated to 20 keV/charge [4]. Ions are then injected into the electrostatic storage ring [3] with a circumference of 8.1 m after being mass-analyzed.

Figure 1 is a simplified experimental setup. The injected ions circulate in the ring with a revolution time on the order of 100 µs for biomolecules. The circulating beam collides with the residual gas molecules and emits charged particles as well as neutral particles. Among them, neutrals move straight without being bent by electrodes in the ring. Therefore, they can easily be extracted outside of the ring and detected by a micro-channel plate (MCP). The lifetimes for biomolecular ions are typically 10-20 seconds [4] under a vacuum of 3×10⁻¹¹ Torr.

In order to study electron and biomolecular ion collisions, we constructed an electron-beam device [6]. The structure of the device is the same as an electron cooler with an adiabatically expanded electron beam. Electrons were emitted from a thermo-cathode with a diameter of 3.5 mm in a solenoid field of 1 kG, and after acceleration the electron beam was expanded to a diameter of 20 mm in a magnetic field of 30 G. The electron beam was then bent by 90 degrees and guided to a merging section. The length of the merging region is 20 cm. The electron energy is variable from about 1 eV to 100 eV. Neutral products emitted in collisions with electrons as well as residual gas are detected by the MCP in the vacuum extension.

Although electron cooling is not the main purpose, the observation of electron cooling is interesting regarding the electron beam quality, and the tuning energies help to calibrate the electron energies. A proton beam with an energy of 20 keV was stored and merged with an electron beam. The natural beam lifetime without the electron beam was about 2 s. On the other hand, the lifetime increased by a factor of about 2 with a velocity-matched electron beam at an energy of 10.9 eV. This clearly indicates that the ion beam was cooled in the transverse direction [6].

In order to discriminate neutrals in ion-electron collisions from those in ion-residual gas collisions, the electron beam was chopped with a time width of 0.25 s. From these data, we can clearly identify the neutrals from electron-ion collisions. In order to deexcite vibrationally excited states ions were first stored for 0.5 s and then measurements were started. As the intensity of the ion beam decreased with time, the beam was dumped and refilled every 10.5 s. The electron current in this experiment was about 12 µA and the electron beam diameter was 20 mm, which was larger than the ion beam size.

The electron beam energy ($E_e$) at an acceleration voltage of $V_a$ is given by $E_e = V_a + V_0 - KI_e/\sqrt{E_e}$, where $I_e$ is the electron current, and $V_0$ and $K$ are constants at a fixed electron current. These constants were determined by measuring the rates of neutrals emitted from dissociative recombinations of H$_2^+$, D$_2^+$ and D$_3^+$ at 20 keV as a function of the electron energies, where the electron recombination rates have maxima at zero relative energies between the ions and the electrons, corresponding to electron...
energies of 5.45, 2.72 and 1.82 eV, respectively. The relative rate coefficient, $<\nu>$, was extracted from the neutral particle emission rate in electron-ion collisions while changing the electron energies in small steps.

3. Electron-positive ion collisions

3.1. Electron-peptide cation collisions

Atomic collisions with proteins or peptides are a very interesting subject. The masses of charged collision fragments have been extensively studied in the field of mass spectrometry. Recently, in electron-highly protonated peptide collisions, electron-capture-dissociation (ECD) via non-ergodic processes [8] was proposed, and confirmed with a Fourier-transform ion cyclotron resonance technique (FTICR). However, ECD or dissociative recombination (DR) phenomena for more fundamental singly protonated peptides has not yet been studied, mainly due to a lack of experimental means, including the detection of neutral particles. As is well known, peptides consist of amino-acid residues linked by peptide bonds. We studied electron-ion collisions for both singly protonated amino-acid and peptide ions.

Figure 2(a) shows the neutral particle production rate as a function of the relative energy for basic amino acids of Arg and His [9]. $E_r$ is given by $E_r = \sqrt{E_i - \frac{(m_e/m_i)E_i}}$, where $E_i$ is the ion energy, and $m_e$ and $m_i$ are the electron and ion masses, respectively. (b) Neutral-particle emission rate as a function of the relative energies in collisions of electrons with singly protonated angiotensin II [9] and bradykinin [10]. One-dimensional structures are given in the figure.

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Figure 2(a) shows the neutral particle production rate as a function of the relative energy for basic amino acids of Arg and His, which are the main sites for the protonation of peptide cations. As can be seen in the figure, a slight, but clear, increase of the rates at energies less than 2 eV can be recognized, which comes from DR at low energies. On the other hand, the rates at high energies increase only monotonically [9]. Figure 2(b) shows the results on the peptides angiotensin II and bradykinin, which consist of 8 and 9 amino-acid residues, respectively. As can be seen in the figure, the spectra are quite different from those of the amino-acids. Huge peaks appear at around 6.5 eV with a shoulder at around 9 eV. Each peak appears to consist of two gross components located at a relative energy of around 7 eV and 9 eV. These peak energies are almost the same for other peptides [9,10].

These remind us of our DR experimental results with a magnetic storage ring, TARNII [11]. In collisions of electrons with light molecular ions, large DR cross sections at high energies were discovered for HeH$^+$ [11], HD$^+$ [12], H$_2^+$ [13], and also for many heavier molecular ions, in addition to the DR at zero relative energy. The large cross sections at high energies were explained as being the Feshbach resonance of two-electron excited states that form Rydberg manifolds converging to the first and more highly excited states of molecular ions [11]. By analogy with these, it can be deduced that the resonances originate from electron capture resulting in dissociation. Assuming a two-body system,
the reaction is expressed as \( e^- + (AB)^+ \cdot (AB)^* \cdot A + B, A + B^+ + e^- \). If there is a highly positive charge-density region in a peptide bond at a protonated site, a free electron excites a bonding electron from the \( \pi \) state to the \( \pi^* \) state, and is captured in an autoionizing state, resulting in a two-electron excited state that can dissociate and re-ionize. In this case, if the incident electron is captured into the \( \pi^* \) state, neutrals are emitted at a relative energy of 4.7 eV [9].

On the other hand, the abundance of charged fragments vs electron energy in the collisions of electrons with multiply protonated peptides was measured with FTICR by Kjeldsen et al. [14]. Low and high-energy bumps were found at around 7 and 9.5 eV, which came from \( c-z \) and \( b-y \) cleavage of peptide bonds, respectively. Interestingly, the peak energies nearly agree with our results, although the intensity ratio is different.

3.2. Electron-oligonucleotide cation collisions

Electron-DNA collisions are also a very attractive subject related to radiation damage and radiation therapy. There have been many experiments on electron and neutral DNA collisions as well as collision-induced dissociation (CID) of multiply charged DNA anions in a gas. However, little has been reported on electron–DNA ion collisions, probably due to a lack of experimental means. We studied electron and DNA cation and anion (next section) collisions. Figure 3 shows the neutral particle production rate as a function of the relative energy in collisions of electrons with a singly protonated 3-mer oligonucleotide (sequence: d(AAA)). Interestingly a large peak was found at around 4.5 eV as well as an increase in the rate toward zero relative energies. It is deduced that this resonant neutral production comes from DR or ECD, including a break of the phosphate backbone. However, more work is required to explain the data.

4. Electron-negative ion collisions

4.1. Electron-oligonucleotide anion collisions

The neutral-particle emission process in the collisions of electrons with multiply deprotonated oligonucleotide anions was studied. Figure 4 shows the neutral-particle production rates as a function of the relative energy for DNAs with different charge states, lengths and sequences [15]. As can be seen in the figure, the rate of neutral particles emitted in collisions started to increase from definite threshold energies, which increased regularly with the ion charge in steps of about 10 eV. These threshold energies were almost independent of the length and sequence of the DNA, but depended strongly on the ion charge.

There are two possibilities for neutral-particle production: one is electron detachment, resulting in neutralization; the other is the cleavage of molecular bonds, including strand breaks, resulting in the emission of neutral fragments. In order to study the probabilities of these processes qualitatively, we measured the number of neutrals arriving simultaneously at the detector with a two-dimensional imaging technique using a charge-coupled device camera. The average number of neutrals arriving simultaneously at the detector clearly increased at electron energies above the threshold energies, compared with that at energies below the threshold [15]. This means that neutrals emitted at energies
higher than the threshold came mainly from breaks of the oligonucleotides, rather than electron detachment.

As a possible reaction mechanism, we assumed that electron collisions excite plasmons, which would lead to the breakage of DNAs. A plasmon is a quantum of collective oscillation of electrons in a metal, semi-conductor, dielectric, atom or molecule. One quantum of plasmon energy is given by

$$\hbar \omega_p = \frac{\pi e^2}{m} n_v$$

where $n_v$ is the electron density and $e$ and $m$ are the electron charge and mass, respectively. The $n_v$ is given by the number of valence electrons divided by the volume of the oligonucleotide. The calculated plasmon energies gradually increase with the length, and saturate, but it is on the order of 10 eV [15]. A comparison of the plasmon energies with the experimental threshold energies is shown in figure 4. Interestingly, the experimental threshold energies approximately agree with the corresponding plasmon energies multiplied by an integer number which is equal to the value of the corresponding DNA ion charge. In other words, high-energy electron hits seem to simultaneously excite a larger number of plasmon quanta, where this number increases with the DNA oligomer anion charge. However, the detailed mechanisms of this phenomenon are still unclear.

4.2. Electron-peptide anion collisions

The phenomenon of a regular threshold energy increase with the ion charge is not limited to DNA, but can also be observed for peptide anions. Figure 5 shows the results on a peptide of angiotensin II. The threshold energies also increase with the charge, with nearly the same intervals of about 10 eV. Thus, the phenomenon can be widely observed in peptide anions as well as DNA anions, and appears to almost be independent of the electron affinities.
Figure 5. Neutral-particle production rate as a function of the relative energies in the collisions of electrons with deprotonated angiotensin II anions.

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