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Coincidence studies of O_2^-, O^- and electron formation in electron-stripping of cationic biomolecules by molecular oxygen

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Abstract

In high-energy collisions between protonated biomolecules (amino acids, peptides and proteins) and molecular oxygen, electron-stripping of the cations occurs, i.e., they increase their charge state by one unit. The fate of the stripped electron depends on the cation under study. We have measured the negative recoils coincident with the electron-stripped ions to be O_2^-, O^-, and a free electron (which might be a result of autodetachment from an excited state of O_2^-). The ratio of the O_2^- recoils to electrons decreases in the following order: $[\mathrm{Lys}+9\mathrm{H}]^{\bullet 10+} > [\mathrm{Ubi}+7\mathrm{H}]^{\bullet 8+} > [\mathrm{GS}+2\mathrm{H}]^{\bullet 3+} > \mathrm{TyrH}^{\bullet 2+}$, $\mathrm{PheH}^{\bullet 2+}$, in accordance with decreasing interaction times (Lys: lysozyme, Ubi: ubiquitine, GS: gramicidin S, Tyr: tyrosine, Phe: phenylalanine). (Int J Mass Spectrom 214 (2002) 57–62) © 2002 Published by Elsevier Science B.V.

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1. Introduction

In neutralization–reionization experiments, molecular oxygen is often chosen as a target gas for reionization as several studies have shown that O_2 has a much higher ionisation cross-section than the gases: He, Ne, Ar, Xe, N_2, NO, NO_2, SF_6, and C_{60} [1,2]. Also, O_2 is very efficient in collisional electron-stripping of ions such as protonated amino acids [3] and proteins [4]. The reason for this special property of O_2 is still unknown. Even though a high electron affinity (EA) of the collision gas is probably favourable, gases such as NO_2, SF_6, and C_{60} have higher EAs (2.27, 1.07, 2.67 eV, respectively) than that of O_2 (0.44 eV) [5]. Additionally, the collision energy in the center-of-mass frame cannot account for the observations since the mass of O_2 is greater than that of N_2 but smaller than that of NO_2 (cf. $E_{\mathrm{COM}} = E_{\mathrm{LAB}}\,(m_{\mathrm{gas}}/(m_{\mathrm{gas}} + m_{\mathrm{ion}}))$). However, O_2 differs from the other gases in having two unpaired electrons (triplet ground state), which may be important in conserving the spin in an electron transfer reaction.

The purpose of this work was to elucidate the dynamics of the electron transfer process involving a cation and O_2 and more specifically, to address the question whether O_2^- is formed as a by-product together with the electron-stripped cation.

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The formation of O_2^- in neutral–neutral collisions has been observed [1] but its formation in coincidence with the final charge state of the projectile has not yet been probed. Hence, we have measured the negative recoils formed in coincidence with cations of one charge state higher than that of the precursor ions by mounting a time-of-flight (TOF) spectrometer at the gas cell perpendicular to the flight direction of the high-energy cation beam.

A non-adiabatic electron transfer process will generate O_2^- ions with four or more vibrational quanta because of the smaller bond length of the neutral molecule compared to that of the anion (Franck–Condon effect) [6]. Such anions are unstable toward autodetachment and have a predicted lifetime of 100 ps [7]. Therefore, since the flight time to the detector is of the order of microseconds, only O_2^- ions with less than four vibrational quanta formed in an adiabatic electron transfer process will survive. Electron transfer to O_2 may also produce O^- via a dissociative channel of electronic excited O_2^- [6].

The precursor cations chosen for this study were protonated phenylalanine (Phe) (*m* 166), protonated tyrosine (Tyr) (*m* 182), doubly protonated gramicidin S (GS) (*m* 1142), multiply protonated ubiquitin (Ubi) (*m* 8 kDa), and lysozyme (Lys) (*m* 14 kDa) as representatives for common amino acids, peptides, and proteins. Also, neutral Phe molecules were subjected to collisional electron transfer.

2. Experimental

A detailed description of the setup can be found in references [8–10]. Briefly, electrospraying water–methanol (1% acetic acid) solutions of Phe, Tyr, GS, Ubi, and Lys generated the protonated molecules in the gas phase: PheH^+, TyrH^+, $[\mathrm{GS} + 2\mathrm{H}]^{2+}$, $[\mathrm{Ubi} + n\mathrm{H}]^{n+}$ ($n = 5$–9), and $[\mathrm{Lys} + n\mathrm{H}]^{n+}$ ($n = 8$–10). Ions were accelerated through a 50 kV potential and those of interest selected with a magnet according to their mass to charge ratio. Collision experiments were performed by letting O_2 gas into the collision region. An electrostatic analyzer in line with the precursor ion beam selected cations of a given kinetic energy (proportional to the mass to charge ratio), which were counted by a channeltron. Negative recoils (e^-, O^-, O_2^-) formed in the collision region were extracted perpendicular to the flight direction of the high-energy cation beam by an electrostatic field of 100 V/cm and were detected by a channeltron after ~6 cm flight distance [11]. The total acceleration voltage for the negative recoils was chosen to be 1.2 kV, which is adequate to measure both electrons

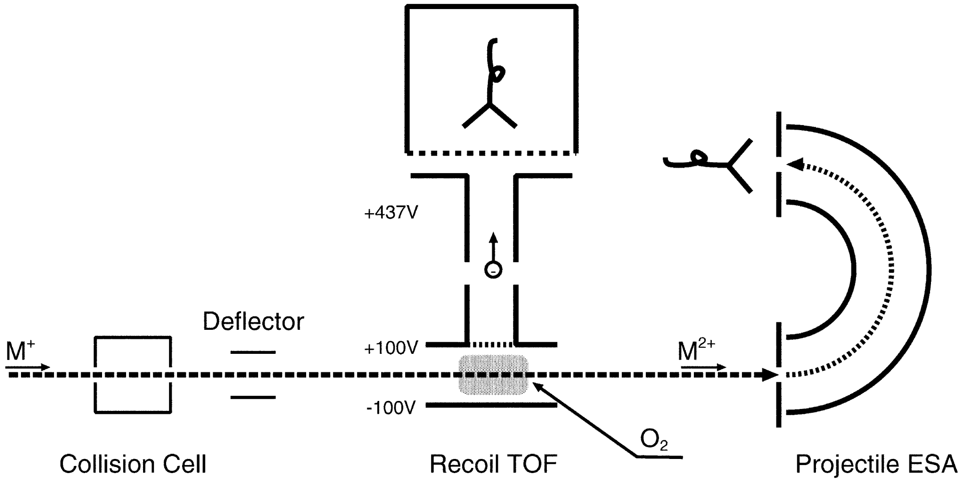

Fig. 1. The instrumental set-up used to measure coincidences between negative recoils and electron-stripped beam.

and negative ions. The voltage on the electrostatic analyzer was optimised to detect electron-stripped ions, and this signal was used as the start signal for the TOF spectrometer. Electrons reach the detector in nanoseconds while O^- and $O_2{}^-$ anions formed in coincidence with the stripped ions take 1.7 and 2.4 μs to reach the detector, respectively (cf. Fig. 1).

For the neutralization–reionization experiment of Phe, the protonated dimer of Phe was accelerated to a kinetic energy of 100 keV and collided with N_2 in a first collision cell resulting in a 50 keV beam of neutral Phe monomers. All products exiting the first cell experienced a deflection electric field to remove ions, and reionization of Phe with O_2 was carried out in a second collision cell and coincidences with $O_2{}^-$ as described above were measured.

3. Results and discussion

Typical daughter-ion mass spectra obtained for $TyrH^+$, $[GS+2H]^{2+}$, $[Ubi+7H]^{7+}$, and $[Lys+9H]^{9+}$ after interaction with O_2 are shown in Fig. 2. It is evident that electron loss is important in all cases. The results of measuring the negative particles formed coincidently with $TyrH^{\bullet 2+}$, $[GS + 2H]^{\bullet 3+}$, $[Ubi + 7H]^{\bullet 8+}$, and $[Lys + 9H]^{\bullet 10+}$ are presented in Fig. 3. Similar results were observed for the other charge states of Ubi and Lys. Interestingly, there are large differences among the TOF spectra: mainly electrons, which are produced either through direct collisional ionization or autodetachment from metastable $O_2{}^-$, are detected for $TyrH^{\bullet 2+}$ (and $PheH^{\bullet 2+}$, Fig. 5), electrons and $O_2{}^-$ for $[GS + 2H]^{\bullet 3+}$, whereas electrons and both $O_2{}^-$ and O^- are seen in coincidence with $[Ubi + 7H]^{\bullet 8+}$ and $[Lys + 9H]^{\bullet 10+}$. In addition, the coincidence spectrum obtained from a $[Ubi+7H]^{7+}$ fragment, revealed no O^- precluding its formation in a hard collision fragmentation process. The observation of the O^- indicates that a small fraction of the $O_2{}^-$ ions is formed in the repulsive $^2\Pi_u$ state possibly via a vertical Franck–Condon transition.

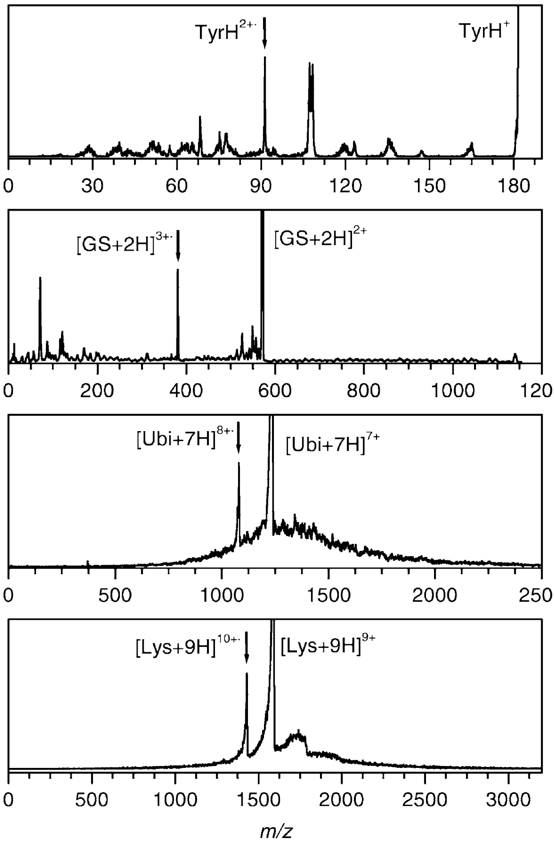

Fig. 2. Daughter-ion spectra (MIKE spectra) obtained when $TyrH^+$, $[GS + 2H]^{2+}$, $[Ubi + 7H]^{7+}$, and $[Lys + 9H]^{9+}$ collide with O_2. The primary beam and the electron-stripped components are noted. The arrow indicates the ion whose coincidence spectrum of recoil particle was studied.

The flight time in our instrumental setup for $O_2{}^-$ is 2.4 μs which means that the number of vibrational quanta, v, is less than four since the life-time of $O_2{}^-$ ($v \geq 4$) with respect to autodetachment of the excess electron is about 100 ps. If the electron-transfer occurs non-adiabatically from the cation to O_2 ($^3\Sigma_g{}^-$), the $O_2{}^-$ ($^2\Pi_g$ state) will initially be formed with $v \geq 4$ due to the much larger distance between the oxygens in the anion than in the neutral state [7]. Thus, the observed $O_2{}^-$ should be formed through an adiabatic process or by collisional cooling to a lower vibrational state ($v < 4$). For the case of $[Ubi+7H]^{\bullet 8+}$ cations, we measured the ratio between $O_2{}^-$ and electron counts as a function of the O_2 gas pressure but found no change in this number, which implies that the

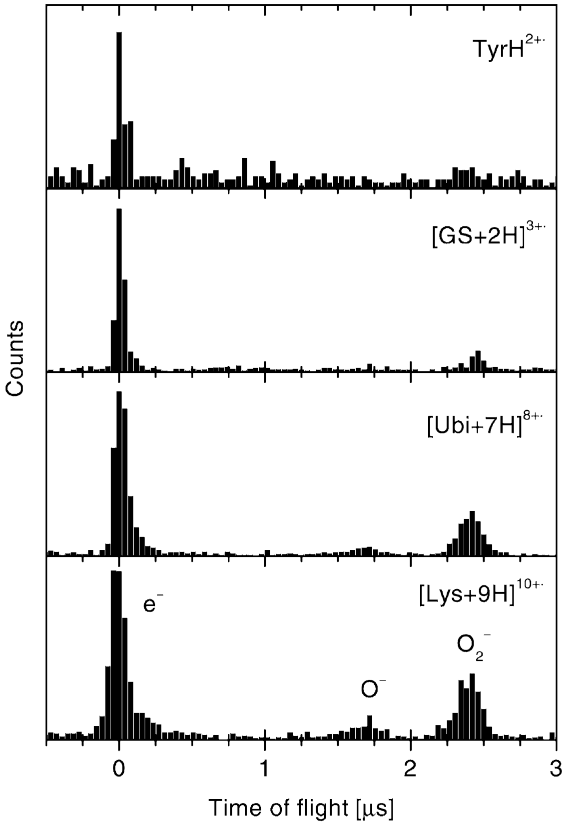

Fig. 3. Negative recoils observed in coincidence with the formation of $TyrH^{\bullet 2+}$, $[GS+2H]^{\bullet 3+}$, and $[Ubi+7H]^{\bullet 8+}$. The TOF is nearly 0 μs for electrons, 1.7 μs for O^-, and 2.4 μs for O_2^-.

O_2^- is formed with less than four vibrational quanta in interactions with the cation (adiabatic electron transfer). We emphasize that the absolute ratio between electron and O_2^- counts is unknown because of different detection efficiencies. However, by comparing the relative values it appears that a large cation more easily quenches the vibrational excitation of O_2^- than a small cation, resulting in negligible long-lived O_2^- for $PheH^+$ and $TyrH^+$ but a higher yield of O_2^- for $[Lys+nH]^{n+}$ and $[Ubi+nH]^{n+}$ than for $[GS+2H]^{2+}$. This trend can be explained by considering the collisional interaction time that is longest for $[Lys+9H]^{9+}$ ($\sim 2.6 \times 10^{-14}$ s for a collision interaction distance of 20 Å) and $[Ubi+7H]^{7+}$ ($\sim 1.9 \times 10^{-14}$ s) being of the order of a vibrational period of O_2^- (2.1×10^{-14} s), smaller for $[GS+2H]^{2+}$ ($\sim 6.7 \times 10^{-15}$ s) and smallest for $PheH^+$ and $TyrH^+$ ($\sim 2.0 \times 10^{-15}$ s). In other words, we attribute a longer interaction time to a more adiabatic interaction.

Danis et al. [1] also observed adiabatic O_2^- formation in the collision between O_2 and CH_3Cl or CH_3COCH_3. Further, an energy loss measurement by Durup et al. [12] revealed that O_2^- is formed adiabatically in high energy collisions between Ar and O_2. For comparison, the velocity of the neutral Ar projectile in their work was higher than that of $[GS+2H]^{2+}$ (O_2^- observed) but smaller than that of $[Tyr+H]^+$ (no or very little O_2^-) in accordance with the

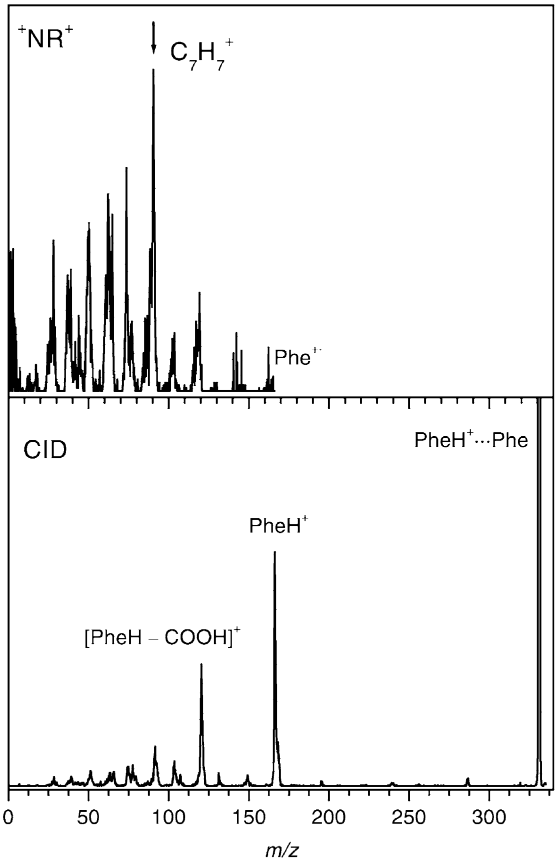

Fig. 4. Neutralization–reionization (top) and CID spectra (bottom) of the protonated dimer $PheH^+ \cdots Phe$. The arrow indicates the ion whose coincidence spectrum of recoil particle was studied.

collision interaction time scheme. Danis et al. [1] detected O^- in addition to O_2^- but in a higher proportion than we do. Although we did not find the O^- to O_2^- ratio to be strongly influenced by the charge state of $[\mathrm{Ubi} + n\mathrm{H}]^{n+}$, this ratio may depend much on the whole character of the projectile.

In neutralization–reionization experiments, O_2 is the preferred target gas. A neutral molecule polarizes the incoming O_2 to a much lesser extent than does an ion, and therefore, to probe whether this effect is important for adiabatic electron transfer, we have carried out a coincidence experiment for both protonated Phe and neutral Phe. The neutral Phe was made from collision-induced dissociations (CID) of the protonated dimer of Phe (Fig. 4). As can be seen clearly in the CID spectrum, the main fragmentation channel is formation of PheH^+ where neutral Phe is lost, which means that the main component of the neutral particle should be neutral Phe. To study the electron transfer collision between neutral Phe and O_2, all ions were deflected and reionization was carried out in a second collision cell (i.e., a neutralization–reionization experiment, $^+\mathrm{NR}^+$). However, the signal from $\mathrm{Phe}^{\bullet+}$ is too small for a coincidence experiment (Fig. 4), and instead coincidences between tropylium ($C_7H_7^+$) fragment ions and recoil particles were measured. The coincidence spectra of PheH^+ and Phe are similar (Fig. 5) and in both cases very few O_2^- reach the detector. The projectile charge state was thus found to have no influence on the coincidence spectra in this case.

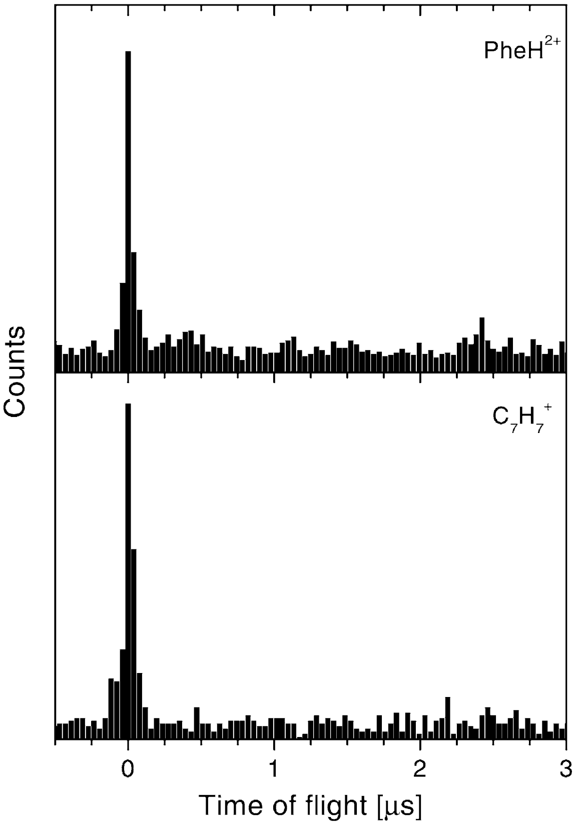

Fig. 5. TOF spectra for negative recoils observed in coincidence with PheH^{2+} from PheH^+ (top spectrum) and with the tropylium ion ($C_7H_7^+$) from neutral Phe (bottom spectrum).

4. Conclusions

We have demonstrated that long-lived O_2^- ions (lifetime $> 2\,\mu s$) are formed when cations pass through a gas of molecular oxygen within times of 10^{-14} s. However, some of the O_2^- anions possess high vibrational energy and autodetachment occurs rapidly (in about 100 ps) resulting also in the detection of electrons. The yield of O_2^- seems to increase with the size of the projectile and hence the collision interaction time. The charge state of the projectile does not seem to be an important parameter on account of the similar coincidence spectra obtained for Phe and PheH^+ and for Ubi and Lys in different charge states.

Acknowledgements

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