Electron loss and dissociation in high energy collisions between multiply charged oligonucleotide anions and noble gases

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Abstract

We have measured electron loss cross sections and fragment spectra for collisions between multiply charged oligonucleotide anions and noble gas atoms. The ions were produced in an electrospray source and accelerated through a potential difference of 50 kV before collisions with the target gas. The collision cross sections depend on the charge state of the projectile ions and on the target gas. The fragment spectra provide information about the sequences of oligonucleotides. The major sequence ions are w and $a - B$ ions. Radical ions as a result of electron loss lead to a ions and cleavage of the sugar ring. Double-electron loss is found to be the first step toward formation of b ions.
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1. Introduction

Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are chains of nucleotides. A nucleotide contains a nucleobase, adenine (A), guanine (G), cytosine (C) thymine (T), or uracil (U), a ribose or deoxyribose sugar and a phosphate group. The base is attached to the $1'$ carbon of the sugar and the phosphate group is attached to the $5'$ carbon. An oligonucleotide is formed when the $3'$ hydroxyl of the sugar of one nucleotide is joined to the $5'$ hydroxyl of an adjacent sugar by a phosphodiester bridge. By definition the sequence is written in the $5'$–$3'$ direction, using the different nucleobases as, e.g., $5'$-ACGT-$3'$. Electrospray [1] of nucleotides and oligonucleotides has been shown to be an effective method for forming anions of these molecules and a large number of collision-induced dissociation (CID) studies aimed at describing the fragmentation of these ions were performed earlier [2–5]. The preference for studies of oligonucleotide anions stems from the fact that the molecules are negatively charged in solution due to the phosphate groups but studies of fragmentation of oligonucleotide cations have also been reported [6]. McLuckey and Habibi-Goudarzi [2] pointed out that in low-energy CID two steps are involved in the fragmentation process: (1) loss of a charged or neutral nucleobase and (2) cleavage of the $3'$C–O bond belonging to the sugar ring that has lost its base. This fragmentation scheme results in formation of the sequence specific complementary w and $a - B$ (a ion minus base) ions that are labeled according to the nomenclature introduced by McLuckey and Habibi-Goudarzi [2] (see Scheme 1).

The present study focuses on measurements of electron loss cross sections in collisions between multiply charged oligonucleotide anions and noble gases. The kinetic energy of the projectile ions was 50 keV times charge state and the size was from 5- to 7-mers. We find that the electron loss cross sections depend almost linearly on the projectile charge, the probability for double-electron loss to be around 25% of that for single-electron loss and triple-electron loss to be almost non-existing. When it comes to projectile fragmentation, we also observed w and $a - B$ ions as in low-energy CID but other fragment ions that are most likely caused by fragmentation following electron loss are observed. We discuss the possible mechanisms leading to

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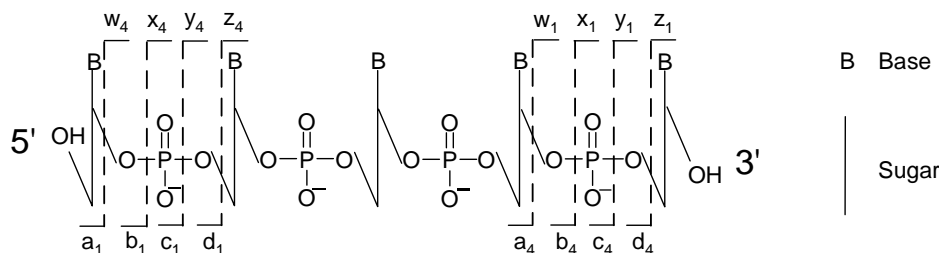
Scheme 1. Oligonucleotide fragment-ion nomenclature proposed by McLuckey and Habibi-Goudarzi [2].

formation of these ions and conclude that electron loss results in fragment ions that are not formed in low-energy CID.

2. Experimental

The experimental arrangement (Fig. 1) is described in detail in Ref. [7]. Oligonucleotide anions were formed by electrospray ionization (ESI). The oligonucleotides were obtained from DNA technique Ltd., Denmark and dissolved in a methanol solution (20 μM), which was sprayed with a flow rate of 2 μl/min. The ions formed by ESI were accelerated by an electrostatic potential of 50 kV. The precursor ions were mass selected with a magnet and passed through a 3-cm long gas cell with entrance and exit apertures of 1 and 3 mm in diameter. The He target gas pressure was monitored by a cold cathode gauge. For cross section measurements the pressure was varied between 0.05 and 0.2 mTorr which ensures single-collision condition. The product ions exiting the cell were analyzed through scanning the voltage of the electrostatic hemispherical analyzer and fragmentation spectra were obtained. This method is called mass-analyzed ion kinetic energy (MIKE) spectrometry. The electron loss cross sections were deduced from product signal intensity versus pressure measurements, the so-called initial growth method whereas the total destruction cross sections were found by recording attenuation of the primary beam as a function of target gas pressure. The experimental errors are mainly caused by beam instabilities and are about ±10%.
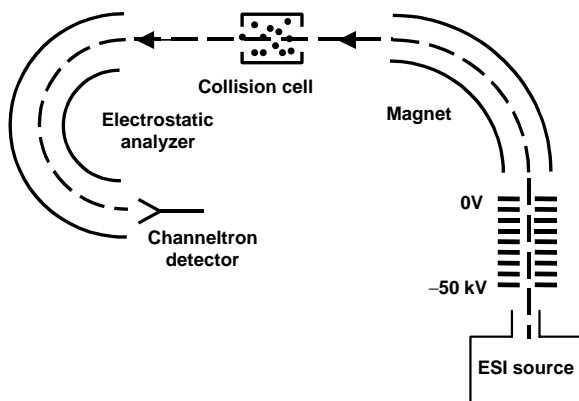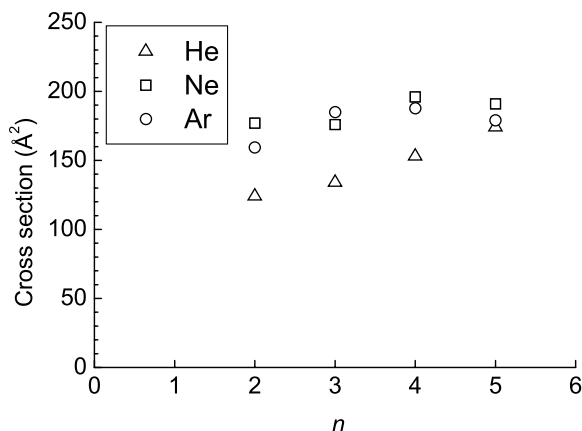

Fig. 2. Total destruction cross sections for $[d(A)_7\text{-}nH]^{n-}$ in He, Ne, or Ar gas as a function of n.

3. Results and discussion

3.1. Total destruction cross sections

The total destruction cross section was measured for $[d(A)_7\text{-}nH]^{n-}$ in He, Ne, and Ar by the beam attenuation technique for n from 2 to 5. The cross sections are shown in Fig. 2 and it should be noted that Ne and Ar give almost identical values whereas the cross sections in He are around 15% lower and that the cross sections increase slowly with projectile charge state. We have earlier observed similar behaviors for high energy collisions between lysozyme ions and noble gases [7] and will adapt the model then used to the present case. The $d(A)_7$ molecule (formula: $C_{70}H_{79}N_{35}O_{33}P_6$) is approximated by a sphere with a radius of 6.8 Å based on the total destruction cross section in collisions with Ar or Ne. The destruction cross section for collisions with He has to be modified since the molecule is somewhat transparent to He atoms. The cross section for transfer of more than around 5 eV (a typical binding energy) to an atom in the molecule in a collision with a He atom is $\sigma = 1.1$ Å^2. The average projected density of heavy atoms in the $d(A)_7$ molecule is around $t = 1$ atom/Å^2 and we estimate the transparency factor by its value for a random two-dimensional distribution, $T = e^{-t\sigma}$ which gives $T = 0.32$ for He and a negligible value for Ne and Ar. Multiplying the geometrical cross section by $(1 - T)$, we then arrive
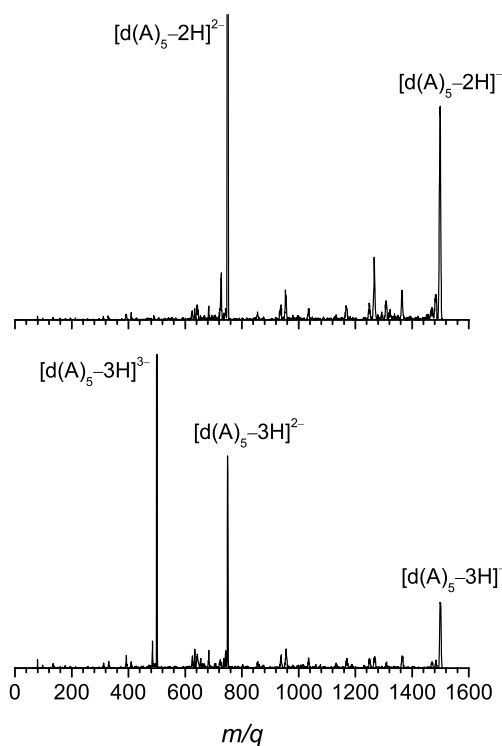

Fig. 1. Schematic drawing of the experimental apparatus.

Fig. 3. Spectra obtained for collisions between $[d(A)_5-2H]^{2-}$ ($m/q = 750.5$) and $[d(A)_5-3H]^{3-}$ ($m/q = 500$) and He. Note that the main "fragment" peaks correspond to ions formed after single- and double-electron loss.

at a cross section of $120\,\text{Å}^2$ for a He target as compared to around $200\,\text{Å}^2$ for Ne and Ar targets. We believe that this simple model in a qualitative way explains the difference in cross sections measured in light and heavy target gases, respectively. From Fig. 2, it is evident that the measured cross sections increase as a function of projectile charge state. A similar charge state dependence of cross sections measured by ion mobility was reported earlier by Hoaglund et al. [8] for charge states between 2 and 6 of 10-residue oligonucleotide anions comprised entirely of thymine bases. They ascribed the increase in cross section with charge state to an unfolding of the molecule as a result of Coulomb repulsion. We believe that the increase in total destruction cross section as a function of charge state which we observe can also be explained by unfolding due to Coulomb repulsion. But charge state dependent differences in the stabilities of the ions toward dissociation could also play a role.

3.2. Electron loss cross sections

Spectra obtained for collisions between doubly and triply deprotonated $d(A)_5$ ($[d(A)_5-2H]^{2-}$ and $[d(A)_5-3H]^{3-}$) and He are shown in Fig. 3. The dominant product peaks in the MIKE spectra correspond to single- and double-electron loss without fragmentation but peaks corresponding to CID are also present. Electron loss is normally not observed in low-energy collisions since a high energy is required in

order to satisfy the Massey criterion [9]. Single-electron loss cross sections of $[d(A)_7\text{-}n\text{H}]^{n-}$ are shown in Fig. 4 as a function of n for the collision gases He, Ne, and Ar. The cross sections increase linearly with n and the cross section is almost a factor of two larger in He as compared to the heavy gases Ne and Ar. We have not observed any signs of three electron loss. The ratio between double- and single-electron loss was found to be insensitive to both projectile charge state and target gas and amounts to around 0.25. Finally the single-electron loss cross sections were found to be identical for $[d(A)_7\text{-}3\text{H}]^{3-}$ and $[d(A)_5\text{-}3\text{H}]^{3-}$ ($18\,\text{Å}^2$), a finding which indicates that the size of the projectile is not influencing the cross section. The observations listed above allow us to propose a simple model for electron loss from nucleotide anions where the number of negatively charged phosphate groups is the decisive parameter. We assume that O^- ions attached to individual phosphor atoms are responsible for the final charge state of the nucleotide. It is thus tempting to model the nucleotide anion as an assembly of independent oxygen anions imbedded in a passive medium. By linear fitting, the single-electron loss cross section (σ_e) of an individual oxygen anion could be obtained from the slope of the line. In Table 1, a comparison is shown between our experimental data obtained as described above and previous experimental data obtained for O^- ions [10,11]. This comparison is rather crude since the data for the atomic ions are based on reasonable extrapolations of the original measurements. On the other hand the favorable comparison between the two sets of data indicates that our
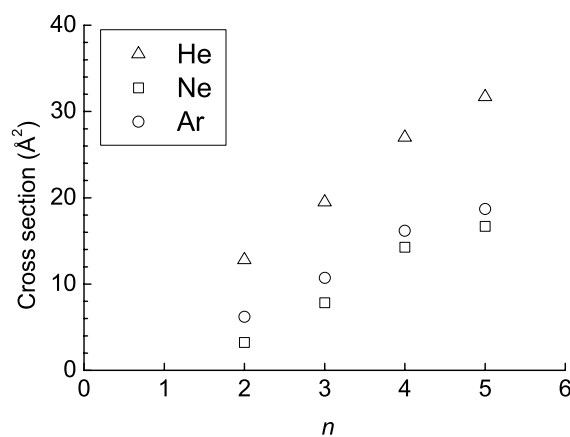
Table 1

Comparison of single-electron loss cross section of oxygen anion deduced from our experiments and the results from Matić and Čobić [10] and Penent et al. [11]

Target gas	σ_e (our exp.) (Å^2)	σ_e (O^- exp.) (Å^2)
He	6.5	4.5 [11]
Ne	3.2	2.0 [10]
Ar	3.8	3.5 [10]

Fig. 4. Electron loss cross sections for $[d(A)_7\text{-}n\text{H}]^{n-}$ in He, Ne, or Ar gas as a function of n.

simple model contains the essentials of the interaction between oligonucleotide anions and rare gas atoms in keV collisions.

The observation that all the cross sections for double-electron loss amount to 25% of the cross sections for single-electron loss indicates that the two electrons are lost from the same O^- ion in the molecule. A rough estimate shows that if two different O^- ions should be responsible for double-electron loss then the cross section should at most amount to a few percent of the cross section for single-electron loss and also depend on the charge state. Therefore, the double-electron loss process is most likely caused by autoionizing states in the phosphate group after one electron is lost from O^-. The biomolecules are transparent to He atoms as described in the section about total destruction cross sections. This transparency will also influence the electron loss cross section. A He atom may penetrate the molecule and cause electron loss without fragmentation whereas a larger atom like Ne or Ar can cause only pure electron loss in glancing collisions. This effect could explain why the measured electron loss cross section is around 40% larger for He than for Ne and Ar. We thus conclude that He is the ideal target gas if we aim at having large electron loss probabilities and small collision-induced fragmentation probabilities at the same time. Comparing Ar and He can tell us whether O^- is located on the surface or in the interior.

3.3. Electron loss-induced dissociation

CID spectra of two 5-mer oligonucleotides $[d(A)_5-2H]^{2-}$ and $[d(GCCCC)-2H]^{2-}$ are shown in Fig. 5. Peaks which were assigned to $a-B$ and w fragment ions are observed like they were in low-energy CID [2] experiments. Two kinds of fragment ions not seen in low-energy CID are observed in the present study, namely a ions and sugar ring cleavage ions dubbed Σ. In Scheme 2, the proposed cleavage site to form Σ is indicated where the charge resides on the 5′ fragment. When the charge is on the 3′ fragment, we denote the fragment ion by Δ. We cannot distinguish between Σ and Δ for cleavage of a sugar ring in $[d(A)_5-2H]^{2-}$ because these ions have similar mass. Therefore, we did an experiment on an asymmetric oligonucleotide $[d(GCCCC)-2H]^{2-}$ in which case no Δ ions were observed. Hence, we assume that Δ ions are not formed from $[d(A)_5-2H]^{2-}$ either.

A mechanism for production of a ions based on single-electron loss is proposed in Scheme 3. An ion radical is formed as a result of electron loss from the phosphate group in a collision with a He atom. A hydrogen atom from the sugar ring is then transferred to the oxygen radical and the 2′ carbon becomes the radical site. This then induces bond cleavage of the 3′C–O and a double bond is formed between the 2′C and the 3′C (elimination reaction) and therefore depending on the position of the charged phosphate group, a ions and w ions are produced. A mechanism which could lead to production of sugar ring cleavage ions
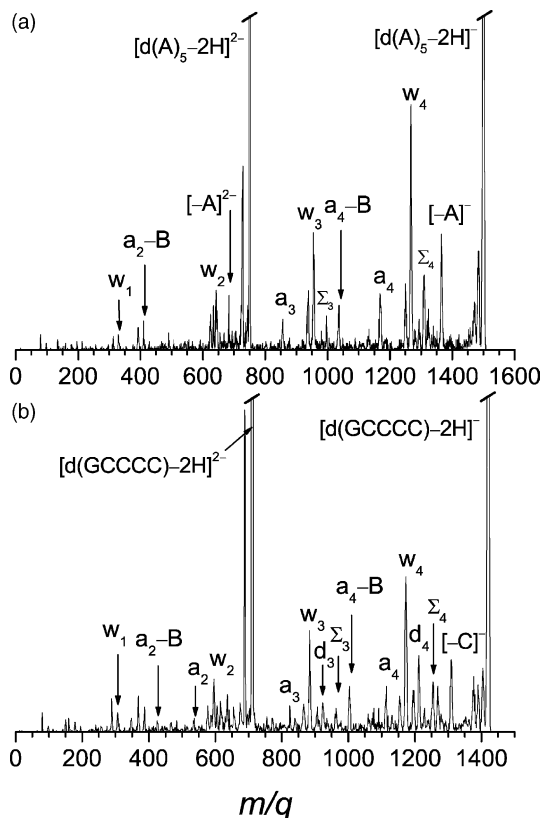

Fig. 5. CID spectra of two different 5-mer dianion oligonucleotides in collisions with He gas: (a) $[d(A)_5-2H]^{2-}$ ($m/q = 750.5$) and (b) $[d(GCCCC)-2H]^{2-}$ ($m/q = 710.5$).

is more complicated, but a possible mechanism is also based on initial single-electron loss from the oxygen atom. After the electron loss, again a hydrogen atom from the sugar ring is transferred to the oxygen radical and a 2′ carbon becomes the radical site. Instead of cleavage of the 3′C–O bond making a ions and w ions, a 1′C–O bond cleavage takes place. The O then becomes the radical site and a
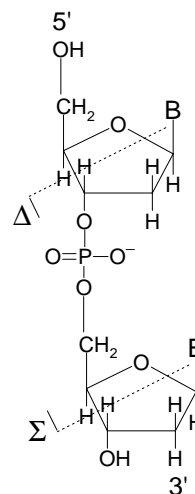
Scheme 2. The proposed sugar ring cleavage sites.
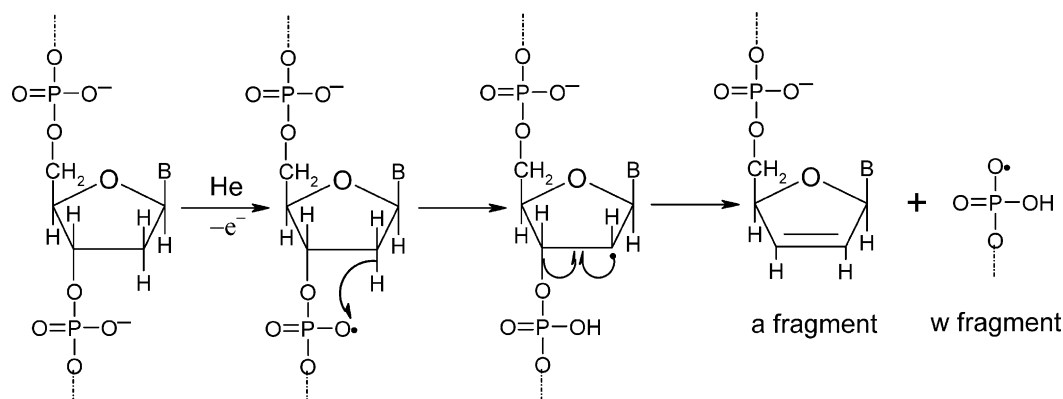
Scheme 3. The proposed mechanism for production of *a* ions.

double bond is formed between the $1'C$ and the $2'C$. The O radical then induces bond cleavage of the $3'C–4'C$ bond and the sugar ring is opened as a result of the cleavage of a C–C and a C–O bond.

The *a* ions and ring cleavage ions were not observed in low-energy CID, but in ion–ion electron transfer experiments by McLuckey and coworkers [12,13] oligonucleotide radical ions were formed that decay along the lines described above. However both in the present experiments and the experiments of McLuckey, Δ ions were not observed, which could be due to the instability of these ions to dissociation before detection.

It appears from Fig. 5 that *w* ions are the dominant fragment ions. This finding is also different from what is observed in low-energy CID where peaks assigned to *w* ions have the same intensity as other fragment-ion peaks assigned to $a − B$ or base loss ions [2]. Our spectra are also different from those of Weimann et al. [14] who performed CID studies at lab energies around 400 eV and found that the spectra were dominated by low m/q ions which makes sequencing difficult. But similar behavior was found in ion–ion reaction experiments [12]. The "excess" *w* ions would be produced by a single-electron loss process. The radical reactions lead to *a* ions, which in principle should produce the same amount of the complementary *w* ions, as indicated in Scheme 3. Dissociation of Δ ions can also make *w* ions. Our high-energy CID spectra which are dominated by *w* ions provide sequence information of oligonucleotides.

The CID spectrum of $[d(A)_5\text{-}4H]^{4-}$ is shown in Fig. 6a. In this spectrum, also *b* ions which were not seen in usual low-energy CID are observed. These fragment ions appeared in the CID spectrum of $[d(A)_5\text{-}3H]^{3-}$ but are nearly absent in that of $[d(A)_5\text{-}2H]^{2-}$. In Fig. 6b a plot of the cross section for *b* ion formation as a function of the charge state of the precursor ion. It is evident that *b* ions are not formed for ions with charge state 2− and that the cross section increases with charge going from 3− to 4−. This charge state dependence indicates that *b* ions are formed from ions that have lost two electrons but the reaction scheme is not understood at present.
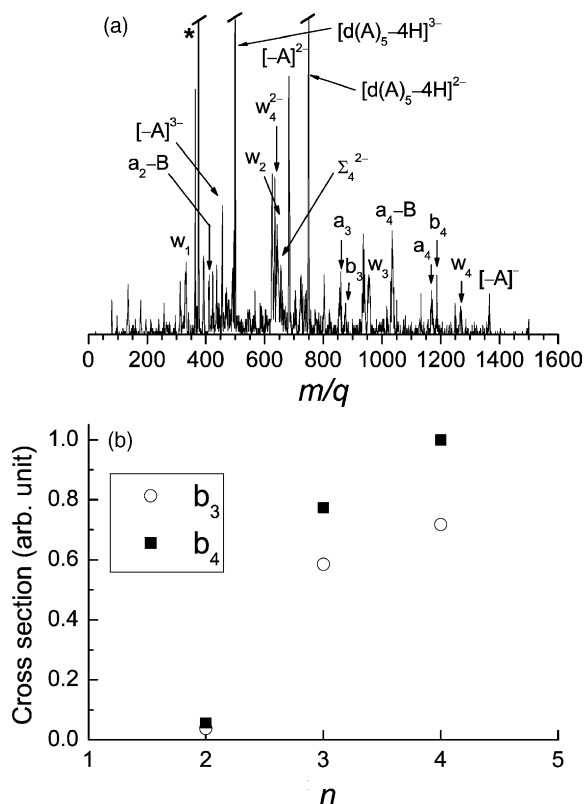

Fig. 6. (a) CID spectrum of $[d(A)_5\text{-}4H]^{4-}$ (*, $m/q = 375$) in collision with He gas. Note that *b* ions are observed in this spectrum. (b) Cross sections for *b* ions formation from $[d(A)_5\text{-}nH]^{n-}$ in He gas as a function of *n*.

4. Conclusions

Electron loss is the dominant reaction channel in high energy (100–250 keV) collisions between oligonucleotide anions and He. As a consequence of the importance of this reaction channel fragment peaks which are normally not observed in low-energy CID appear in our fragment spectra. A general observation is that *w* ions are much more prominent in the present experiments than in low-energy CID. We also observed *a*, sugar ring cleavage ions dubbed Σ, and *b* ions.

We have proposed a mechanism for the production of *a* and *Σ* ions based on loss of an electron from the precursor ion. Such ions were observed earlier in ion–ion reaction experiments [12,13]. We suggest that *b* ions are formed as a result of double-electron loss.

Acknowledgements

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