

Enhanced electron-anion interaction in electron-cation neutral plasma: sequencing of oligonucleotides by electron detachment dissociation

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ABSTRACT

We observed fast electron detachment dissociation (EDD) in an RF ion trap filled by nitrogen gas when we applied an intense electron beam with its energy higher than 25 eV. We proposed a "Plasma" EDD mechanism to explain why the EDD efficiency was dramatically enhanced. Plasma EDD sequenced oligonucleotides-including DNAs, RNAs, and modified oligonucleotide therapeutics-with the 100 % cleavage coverage.

INTRODUCTION

Applying electron capture dissociation (ECD), electron transfer dissociation (ETD) and other electron induced dissociation (EID) techniques to positively charged precursor ions can provide unique information about molecular structures that are challenging to obtain with collision-induced dissociation (CID). While electron detachment dissociation (EDD) and negative EID applied to negatively charged precursors is possible, it is difficult to perform because a precursor anion and an electron are repulsive by Coulombic force. The research presented here aimed to accelerate the reaction speed of negative electron-activated dissociation (negative EAD) to meet LC-MS/MS requirements. An intense electron beam does not help to increase the reaction speed because the electron beam and the precursor anions are spatially separated by Coulombic repulsion (Figure 1a).



Figure 1. (a) Anionic precursor distribution in an RF ion trap with a strong electron beam. (b) Anionic precursor can stay in the electron beam by introducing positive ions.

Plasma Electron Detachment Dissociation

The strong electron beam produces a repulsive Coulombic potential, and thus the precursor anions are pushed out from the electron beam (Figure 1a). To avoid this spatial separation, we introduced nitrogen cations (N_2^+) in the EAD device (Figure 1b). Now the precursor anions can stay inside the plasma, which is filled by the electrons, and the precursor anions interact with the electron beam frequently.

We produced the nitrogen ions using the electron beam for EDD in the EAD device in the ZenoTOF 7600 system (SCIEX) from nitrogen buffer gas filled in the EAD cell and Q2 cell (Figure 2 and Figure 3). Using the electron beam with kinetic energy (Ke) higher than the ionization energy of N₂ (15.58 eV), nitrogen is ionized. The electron beam is filled by N_2^+ instantly, so that the electron- N_2^+ neutral plasma is created. Surplus N_2^+ is quickly removed from the trap by the low-mass-cut-off of the RF ion trap. By this mechanism, the neutrality of the plasma is sustained.



Figure 2. DC potential along the electron beam path. The electron beam creates nitrogen ions inside the EAD device by an electron impact ionization from the nitrogen buffer gas.

MATERIALS AND METHODS

We used the ZenoTOF 7600 system without any hardware modification, and a research-grade EAD-TOF instrument (Figure 3). To allow higher electron beam energy (Ke) than 25 eV, the SCIEX OS software controlling the instrument acquisition was modified for research purpose. The EAD device is a branched RF ion trap and a strong electron beam is introduced along a branch called the electron beam path (ref. 1). 15-40 mer synthesized oligonucleotides were used as samples. Oligo $(dT)_{20}$ was obtained from Promega (Madison, Wisconsin). Other DNA and RNA samples were obtained from Integrated DNA technology (Coralville, Iowa) and GeneDesign, Inc. (Osaka, Japan). The typical concentration in infusion experiments shown in this presentation was 2 µg/mL in 50:50 (v/v) methanol/water with 10mM triethylamine (TEA) and 100 mM hexsafluoro-2-propanol (HFIP). The solution is ionized by electrospray ionization at a spray voltage of -4500 V.



EXPERIMENTS

(2) electron beam irradiation duration

Figure 5 shows the progress of plasma EDD. Deprotonated oligo (dT)₁₅ with the charge state of 7- was irradiated by an electron beam with Ke = 40 eV. The precursor was converted to CRSs consecutively, then fragments were produced. The electron detachment speed became faster when the charge state was decreasing (Figure 5e and 5f), which is evidence that the reaction is electron detachment-i.e., the Coulombic-repulsive interaction between the electron and the anions becomes weaker when the charge state of anions is decreasing [ref. 2]. The opposite occurs when ECD is applied to positively charged peptides and proteins, which shows reaction speed as the square of the precursor charge state.

Figure 3. EAD cell in the ZenoTOF 7600 system.



Figure 4. Consumption of the precursor and creation of CRSs. The sample was oligo $(dT)_{15}$. The ionization threshold of N_2 is 15.58 eV.

(1) electron beam energy dependence

Figure 4 shows the consumption of the precursor and the creation of charge reduced species (CRSs) when the electron beam energy was ramped from 0 eV to 50 eV. Strong electron detachment was observed above 15 eV, which is consistent with the ionization energy of nitrogen.

When the multiply deprotonated oligonucleotides were introduced into the plasma that was produced by an electron beam with 30 eV energy, a strong charge reduction—i.e., electron detachment—was observed by electron beam irradiation for 10 ms.



Figure 5. Dependence on duration of electron beam irradiation. The sample is Oligo (dT)15. (a)-(d) Plasma EDD spectra. (e) CRS production, where vertical scale is normalized. (f) A simulation result of electron detachment assuming that the electron-anion interaction weakens as the anion charge state decreases.



Figure 6. Sequencing of oligonucleotides by plasma EDD. (a) DNA (all PO), obtained by an infusion workflow without Zeno trap activation. (b) all PS DNA, obtained by an LC-MS/MS workflow with Zeno trap activation.

(3) Sequencing of DNAs

CONCLUSIONS

Fast dissociative electron-anion interaction in an electron-cation neutral plasma stored in a branched RF ion trap was observed. We demonstrated oligonucleotide sequencing using plasma EDD. The full information of this work is available in ref. 2 including the LC-MS/MS workflow.

Reference

Ref. 1: Anal. Chem. 2015, 87, 785-792; JASMS 2021, 32, 1964-1975 Ref. 2: Anal. Chem. 2022, 94, 15510-15517 Figures 1-5 are reused with permission from Anal. Chem. 2022, 94, 15510-15517, Copyright 2022, American Chemical Society.

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