Photodetachment and photodissociation mass spectrometry of DNA multiply charged ions

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We recently explored the effects of irradiating DNA polyanions stored in a quadrupole ion trap (QIT) mass spectrometer with an optical parametric oscillator (OPO) laser between 250 nm and 285 nm. We studied DNA 6-mer to 20-mer single strands, and 12-base pair double strands. In all cases, laser irradiation causes electron detachment from the multiply charged DNA anions (**Figure 1**). Electron photo-detachment efficiency directly depends on the number of guanines in the strand, and maximum efficiency is observed between 260 and 275 nm. Collisional activation of the radical anions results in extensive fragmentation, which can be used to sequence the DNA strands. It has therefore important potential applications in analytical chemistry [1].

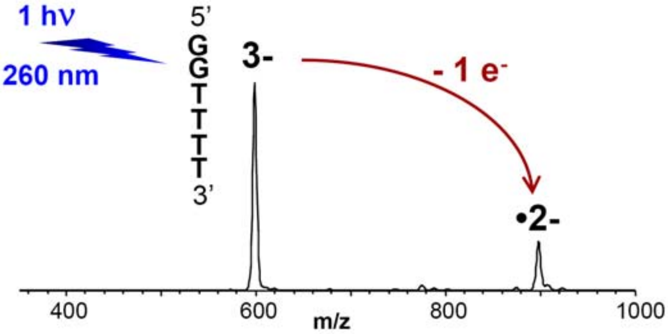

Figure 1: Example of electron photodetachment mass spectrum.

We will discuss the electron photodetachment mechanism (**Figure 2**). Electron photodetachment at 260 nm (4.77 eV) is a one-photon process. It is likely very fast, given that it is able to compete with internal conversion and/or radiative relaxation to the ground state. The DNA $[6\text{-mer}]^{3-}$ ions show a marked sequence-dependence of electron photodetachment yield [2]. Remarkably, the photodetachment yield ($dG_6 > dA_6 > dC_6 > dT_6$) is inversely correlated with the base ionization potentials ($G < A < C < T$). Sequences with guanine runs show increased photodetachment yield as the number of guanine increases, in line with the fact that positive holes are the most stable in guanine runs. This correlation between photodetachment yield and the stability of the base radical may be explained by tunneling of the electron through the repulsive Coulomb barrier. The calculations and the wavelength dependence suggest that the electron photodetachment is initiated at the bases and not at the phosphates. This also indicates that, although direct photodetachment could also occur, autodetachment from excited states, presumably corresponding to base excitation, is the dominant process at 260 nm.

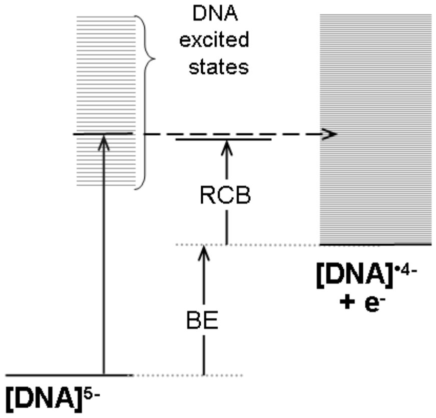

Figure 2: Photodetachment mechanism proposed for DNA excited around 260 nm. Slight changes in electron binding energy (BE) result in large changes in photodetachment efficiency because of the repulsive Coulomb barrier (RCB).

The wavelength-dependence of electron detachment yield was studied for dG_6^{3-}. Maximum electron photodetachment is observed in the wavelength range corresponding to base absorption (260-270 nm) [2]. This demonstrated the feasibility of gas-phase UV spectroscopy on large DNA anions. We recently have compared the action UV spectra of single-stranded and double-stranded DNA (**Figure 3**). Interestingly, we found that for a 12-mer duplex containing 100% of GC base pairs, action spectra are shifted in the duplex compared to the single strand. This shows that the bases' environment (possible the base stacking) plays a role in the absorption efficiency or in the photodetachment efficiency. This will be further explored in the near future.

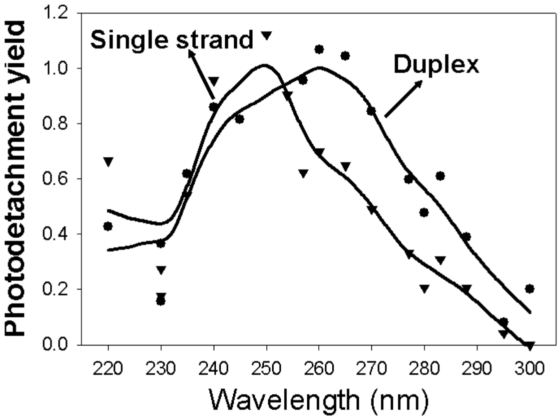

Figure 3: Action UV spectrum of single-stranded and double-stranded DNA ions: photodetachment yield as a function of the excitation wavelength for single-stranded $[dCGCGGGCCCGCG]^{3-}$ and the double-stranded $[(dCGCGGGCCCGCG)_2]^{5-}$ ions.

Finally, we also obtained preliminary results on laser irradiation of DNA coupled to other chromophores (covalently bound or noncovalently bound). Depending on the chromophore, three different behaviors are encountered [3]: (1) the photon energy can be redistributed in the molecule by internal conversion, (2) electron photodetachment was observed for a few chromophores, and (3) specific photodissociation was observed for a porphyrin chromophore (see poster presentation of F. Rosu). Interestingly, the chromophores resulting in electron detachment are all known fluorescent molecules, but the fluorophore needs to be noncovalently bound near the DNA bases for photodetachment to occur. Furthermore, photodetachment was found to be multiphotonic. The proposed mechanism (**Figure 4**) is therefore a two-step photon absorption by the ligand, followed by coupling to the DNA excited states, from which electron photodetachment can occur.

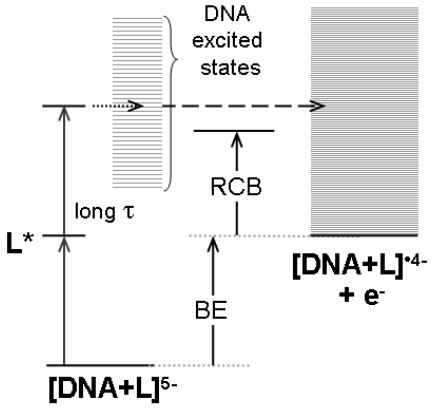

Figure 4: Photodetachment mechanism proposed for DNA complexes with noncovalently bound chromophore, excited at the chromophore absorption wavelength where DNA does not significantly absorb. (d) DNA with noncovalently bound fluorophore ligands L can absorb two photons and electron photodetachment occurs via coupling with the DNA excited states. (BE = electron binding energy; RCB = repulsive Coulomb barrier)

References:

[1] Gabelica, V.; Tabarin, T.; Antoine, R.; Rosu, F.; Compagnon, I.; Broyer, M.; De Pauw, E.; Dugourd, P. Electron Photodetachment Dissociation of DNA Polyanions in a Quadrupole Ion Trap Mass Spectrometer. *Anal. Chem.* **78**, 6564 (2006).

[2] Gabelica, V.; Rosu, F.; Tabarin, T.; Kinet, C.; Antoine, R.; Broyer, M.; De Pauw, E.; Dugourd, P. Base-Dependent Electron Photodetachment from Negatively Charged DNA Strands upon 260-nm Laser Irradiation. *J. Am. Chem. Soc.* **129,** 4706 (2007).

[3] Gabelica, V.; Rosu, F.; De Pauw, E.; Antoine, R.; Tabarin, T.; Broyer, M.; Dugourd, P. Electron Photodetachment Dissociation of DNA Anions with Covalently or Noncovalently Bound Chromophores. *J. Am. Soc. Mass Spectrom.* **18,** 1990 (2007).