Resonant neutral-particle emission in collisions of electrons with protonated and sodiated nucleotide monocations in a storage ring

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Abstract. Electron-ion collisions have been studied for protonated and sodiated single-strand dinucleotide monocations with various base compositions and sequences by using an electrostatic storage ring equipped with a merged-electron-beam device. The plots of neutral-particle production rates against collision energy show typical electron-capture-dissociation profiles, which are characterized by an increase in the production rate at energies close to zero and a bump at high energies. The height of the resonant bumps varies with the number of Na⁺ ions as well as the base composition and sequence; in most cases, the height increases with the number of Na⁺ ions. Molecular mechanics and semiempirical quantum-chemical calculations suggest that the rate is correlated with various base-base interactions.

1. Introduction
Collisions with low-energy electrons can cause severe damage to biological molecules because of the dissociation of molecular bonds. In particular, strand breaks of DNA are crucial and lead to the loss of genetic information. Thus, studies on electron-DNA collisions are very important for radiation damage as well as for gaining deeper insights into the fundamental processes governing these phenomena. Research on oligonucleotides and related molecules has extensively been performed experimentally for the collision-induced dissociation (CID) [1-6] and the electron capture dissociation (ECD) [7-9] processes by using mass-spectrometry techniques and also theoretically [10]. Studies on the mass-spectroscopic analysis of the ECD of doubly protonated nucleotides have conclusively shown that this process involves both sugar-phosphate backbone cleavage and base loss (glycoside bond cleavage). Besides, to clarify the microscopic mechanisms of these fundamental processes, electron collisions with neutral (or solid) DNA have been studied [11,12]. In particular, these studies have shown that the impact of low-energy electrons on DNA can cause single- and double-strand breaks via resonant electron attachment and formation of transient molecular radical-anions. Electron collisions with different types of ionic DNA have been investigated in our previous work [13-15]. In electron-DNA anion collisions, it has been observed that neutral product emissions start at definite threshold energies that regularly increase with the DNA anion charge in steps of about 10 eV. It was deduced that this
phenomenon could be attributed to collective electron excitation [13]. Furthermore, in electron-DNA cation collisions, the resonant electron attachments observed at a collision energy of 4.5 eV were found to be highly correlated with the well-known base stacking propensities of different DNA bases [14]. The fundamental mechanism underlying this phenomenon was considered to be Feshbach resonance in combination with the excitation of charge-transfer excitons [15], being promoted by the conformational transitions due to the incident electron capturing.

On the other hand, it is well known that significant changes in DNA structure occur also when DNA interacts with alkali metal cations, such as sodium or potassium which are ubiquitous in biological systems: however, in contrast to the many studies on protonated species, experimental and theoretical works on sodiated oligonucleotides are rather rare. DNA bases are known to aggregate in the presence of alkali metals [16]. The aggregation and conformational preferences of sodiated dinucleotides were studied in the gas phase by Baker et al. [17], who used a combination of experimental ion mobility data and molecular modeling findings. Unlike protons, which are always covalently bonded to appropriate functional groups, alkali cations can only electrostatically interact with π-electron systems or lone pairs of DNA bases. The origin of strong attraction in the Li⁺-π, Na⁺-π complexes has been theoretically attributed to the effects of polarization [18], denoted as induction forces [19].

Therefore, the physical-chemical consequences of electron-DNA cation collisions might be affected by the strength of the cation-π interactions resulting from conformational changes. Moreover, the exact location and physical-chemical consequences of the electron attachment to a DNA cation ought to depend on the DNA base composition and sequence, and also on the number of adducted alkali cations. In this study, we introduce our investigation on how the neutral particle production rate varies with the choice of bases and number of Na⁺ ions [20]. All the results are compared with those obtained for the corresponding protonated cations. Molecular-mechanical and quantum-mechanical computations help to suggest reaction mechanisms for the investigated dinucleotides.

2. Experimental setup and theoretical algorithms
Experiments were performed using an electrostatic storage ring and a merged-electron beam [21] at the High Energy Accelerator Research Organization (KEK). Figure 1 represents the experimental setup. Synthesized deoxyribodinucleoside monophosphates (hereafter referred to as dinucleotides) were dissolved in a water-methanol 50%-50% mixture with a biomolecule concentration of about 0.1 mM. A small amount of acetic acid was added to promote protonation. In order to adduct sodium, sodium iodide was added to each solution. The ions produced in the electrospray ion source were conveyed to an ion trap and accumulated there. After storage for 5.5 s, the ions were ejected as a bunch and accelerated to 20 keV. These ions were then mass-analyzed and injected into the storage ring. The circulating ion-beams were merged with the electron beam in one of the straight sections. The neutral particles produced in the merging section were detected using a microchannel plate outside the ring. As we study only singly charged positive ions, the addition of nNa⁺ ions coincides with the removal of (n-1) protons on the dinucleotides (M). In fact, [M+nNa-(n-1)H]⁺ ions were observed in the mass spectra.

Figure 1. Electrostatic storage ring equipped with an electrospray ion source, a mass spectrometer, an electron target and a neutral particle detector.
The injected ions were first stored for 0.5 s in order to de-excite the vibrationally excited ions. Then, the measurements were started. To estimate the background through collisions with residual gas, the electron beam was chopped at a time width of 0.25 s. The data can be expressed in terms of the rate coefficient, which is defined as $<v_e> = (N - N_B)v_i / N_e L$, where $N_i$ is the number of incoming ions, $N$ and $N_B$ are the numbers of neutral particles with and without the electron beam, respectively; $v_e$ is the relative velocity; $v_i$ is the ion velocity; $\rho_e$ is the electron density; and $L$ is the interaction length of 20 cm. The relative rate coefficients were estimated with the assumption that in the absence of the electron beam, the neutral-particle production rate is proportional to the number of stored ions.

Quantum-chemical calculations on the protonated and sodiated dinucleotides have been carried out at the PM3 level of approximation using the MOPAC-93 routine package [22]. For every protonated/sodiated nucleotide, its molecular geometry has been optimized in vacuo. All the possible prerequisites for the base-base coupling before the electron attachment have been qualitatively analyzed on the basis of ab initio quantum-chemical results obtained in our earlier work [14]. It should be noted that in the PM3 approximation, Na$^+$ ions are treated as classical point electrostatic charges.

3. Results and discussion

Figure 2 shows the production rates of neutral-particles vs. the relative energies for protonated and sodiated dinucleotide monocations with homo-nucleotide sequences. For the protonated dinucleotides, one of their both bases should covalently bind one proton to maintain the overall charge state of +1, except in the case of thymine (described later): the phosphate groups (which are the most acidic sites) are most probably neutralized by protonation. Meanwhile, for the sodiated dinucleotides, the number of Na$^+$ ions bound to a given dinucleotide correlates with the number of protons that can be removed from the bases. The most acidic hydrogens among the four bases are N1 in guanine and N3 in thymine: therefore, the dinucleotides containing G and T readily provide additional deprotonation sites, whereas there are no such favorable deprotonation sites for A and C.

Figure 2. Neutral-particle-production rates in collisions between electrons and dinucleotide cations of [M+nNa-(n-1)H]$^+$, where M represents (a) d(GG), (b) d(AA), (c) d(TT) and (d) d(CC). The protonated cation is shown as [M+H]$^+$ [20]. The rates for protonated dGMP and dAMP are also shown in (a) and (b), respectively.
Figure 2 shows strong resonant bumps with maxima at 4-5 eV for protonated d(GG) and d(AA). These bumps are enhanced by adding one or two Na$^+$ ions. For d(GG), the bump is further enhanced by adding three Na$^+$ ions. In contrast, for protonated d(CC), the strength of the bump is weak, and no bump at all can be observed for protonated d(TT). However, for sodiated d(TT), bumps appear, which are gradually enhanced with the addition of one and two Na$^+$ ions and are further amplified upon the addition of three or four Na$^+$.

Bump energies of 4-5 eV corresponds to the energy of the first singlet electronic excitation in unprotonated nucleic acid bases [23]. Thus, the bumps can result from Feshbach resonances: Incident electrons promote valence and/or core electron excitations in the target cation, by losing their kinetic energy and are thus captured. These highly excited resonance states should then have enough energy for reionization or even for molecular dissociation [14].

To identify the origin of the resonant bumps more clearly, we performed the same experiments on the mononucleotide cations of [dGMP+H]$^+$ and [dAMP+H]$^+$. As can be seen in figure 2 (a) and (b), no bumps were observed for these ions, nor were bumps observed for the sodiated mononucleotides of [dGMP+Na]$^+$ and [dAMP+Na]$^+$ (not shown in the figure). A comparison between the corresponding data for the dinucleotides in figure 2 (a) and (b) suggests that interaction between the bases causes the resonance.

As described in our previous paper [14], the presence of a thymine base in the protonated dinucleotide seems to hamper the resonance. This can be seen in hetero-nucleotides of d(AT) and

![Figure 3](image-url)  
**Figure 3.** Optimum *in vacuo* structure of the doubly protonated (a) d(AA) ([d(AA)+H]$^+$) and (b) d(CC) ([d(CC)+H]$^+$), according to PM3 geometry minimization. From here on, the protonation protons are shown as spheres of the largest radius [20].

![Figure 4](image-url)  
**Figure 4.** (a) Optimum *in vacuo* structure of the doubly protonated d(TT) ([d(TT)+H]$^+$); (b) Optimum *in vacuo* structure of the singly protonated and singly sodiated d(TT) ([d(TT)+Na]$^+$). Na$^+$ is shown as a blue sphere [20].
d(TA) as well as in the homo-nucleotide d(TT) shown in figure 2 (c). However, the situations change with the addition of Na$^+$ to d(AT) and d(TA): the bumps become clearly observable. Details are described in the reference [20]. Thus, the resonant attachment of incident electrons to these dinucleotides is critically dependent on whether the ions are protonated or sodiated and is also dependent on the base composition and sequence.

Our PM3 studies definitely show that, for all the protonated dinucleotides we study here, in the lowest-energy structures, the phosphate group is always protonated at one of its anionic oxygens. For example, figure 3 (a) depicts the lowest-energy structure of the [d(AA)+H]$^+$ monocation, resulting from our PM3 structure optimization. Here, the protonated phosphate establishes an H-bonding bridge to the 5'-end OH-group, whereas the protonated N1 site of the 3’-end Ade residue comes into contact with the amino group of the 5’-end Ade moiety. Thus, the over-all conformation of this monocation favors some contact between the both bases. Although such base-base contact in no way resembles the perfect stacking pattern found in DNA duplexes, it may nevertheless still be important in explaining the resonant bump observed for this monocation (cf. figure 2 (b)), as discussed in our previous report [14]. Some similar situation has been encountered in our PM3 studies on the doubly protonated d(CC) (cf. figure 3 (b)), although the degree of the Cyt-Cyt contact here is not that high as in the doubly protonated d(AA) (cf. figure 3 (a)). Consequently, in comparison with the corresponding d(AA) peak (cf. figure 2 (b)), the observed resonant bump (see figure 2 (d)) was also not pronounced to the same extent.

Remarkably, among all the dinucleotides studied, the d(TT) represents a special case, because its phosphate group should be doubly protonated, whereas none of the both Thy residues are expected to accept protons [1-3, 5, 6]. Our PM3 optimization studies are in full accordance with such a viewpoint. Figure 4 (a) presents the lowest-energy structure of the doubly protonated d(TT), where no contact is seen between the Thy residues in such a structure: this result corroborates our observation of the absence of a resonant bump for this species (cf. figure 2 (c)).

Bearing in mind the earlier data [17], in our PM3 computations, we have ensured that the proton is always attached to the phosphate group in the monosodiated monoprotonated dinucleotides. This pattern does in fact correspond to the lowest-energy structure in all the cases. For example, figure 4 (b) shows the lowest energy structure of the monosodiated monoprotonated d(TT), which is in good qualitative accordance with the earlier results obtained using different theoretical approaches [17]. It is clearly seen that in comparison with the structure of the doubly protonated d(TT) (cf. figure 4 (a)), the phosphate protonation enables the Na$^+$ to establish a weak coordination complex with both the Thy moieties, thus bringing them somewhat closer to each other. This finding can be readily used to explain some faint resonant bump still observable for the relevant species, unlike the case of doubly protonated d(TT) (cf. figure 2 (c)).

Finally, in order to study the effects of alkali metals other than sodium on nucleotides, we performed the same experiments also on lithiated and potassiated d(GG) monocations. In figure 5,
the results are compared with those obtained for protonated and sodiated d(GG). As can be seen in the figure, the rates are enhanced for sodiated and lithiated nucleotides, while the rate for the potassiated nucleotide remains almost the same as that for the protonated nucleotide. This tendency is similar to that of the ECD in alkali-cation-adducted peptides [24], and it reflects the difference in the nature of each alkali metal with regard to polarizability and electron configuration.

4. Conclusions
The experimental results and computational findings presented above show that the resonant attachment of incident electrons to single-stranded dinucleotides is critically dependent on whether these nucleotides are protonated or sodiated. Another important parameter is the number of Na⁺ in the sodiated adducts. It is deduced that the bump rate correlates with various base-base interactions.

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