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MS/MS Study of Deoxydinucleotides Bound with Alkali-metal Ions Using ESI-MS

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Abstract: Positive ion ESI-MS has been used to examine the fragmentation pathways of the complex ions of deoxydinucleotides with H^+, Na^+, K^+ by LCQ instrument. It had been found that the dissociation varied markedly due to the differences of the base sequence. The alkali-metal ion binding site and the characterization of dissociation were directed by the size of metal ion, the sequence of base and the steric hindrance.

Keywords: MS/MS, deoxydinucleotides, complex ions.

Electrospray ionization mass spectrometry (ESI-MS) has been shown to be an excellent means for the characterization of oligonucleotides[1,2]. Moreover, ESI-MS is a well suited technique to study solution formed ionic complexes, and it provides unambiguous identification of singly and multiply-charged ions in the deprotonated, protonated and metalated oligonucleotides[3,4]. In this work, positive-ion ESI-MS has been used to examine CID pathways of the complex ions of deoxydinucleotides, d(ApT), d(TpA), d(CpG) and d(GpC), with H^+, Na^+, K^+ by LCQ instrument. We report the characterization of the complex ions and the difference of the dissociation of $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions, using low-energy CID analysis with MS/MS experiments.

The experiments were performed on a Finnigan LCQ instrument with an ESI source (positive-ion mode). The samples were prepared with CH_3CN:H_2O (1:1+1%CH_3COOH) at a concentration of 10 pmol/μL. The mobile phase was CH_3CN:H_2O (1:1+1% CH_3COOH) at a flow rate of 5-10μL/min. The CID spectra were obtained in the presence of a collision gas of He with the relative collision energies varying from 12% to 40%.

The structures of the four deoxydinucleotides are shown in **Scheme 1.**

In addition to the $[M+H]^+$ ion (basic peak), the dinucleotides can produce complex ions, $[M+Na]^+$, $[M+K]^+$. In the same spectra, the formation of dimeric and trimeric ions, such as $[2M+H]^+$, $[2M+Na]^+$, $[2M+K]^+$, $[3M+H]^+$, $[3M+Na]^+$ and $[3M+K]^+$ was also observed for all four samples. Among all of the cluster ions the abundance of $[2M+H]^+$ in d(TpA) was the most intensive one. The observation of such a cluster ion suggests that the nucleoside oligomers have tendency to generate cluster through hydrogen bond.

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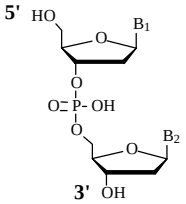
Scheme 1 The structures of deoxydinucleotides



	d(ApT)	d(TpA)	d(CpG)	d(GpC)
B_1	adenine (A)	thymine(T)	cytosine(C)	guanine(G)
B_2	thymine(T)	adenine(A)	guanine(G)	cytosine(C)

Figure 1 MS/MS spectra of $[M+H]^+$ and $[M+Na]^+$ ions of d(ApT) and d(TpA)

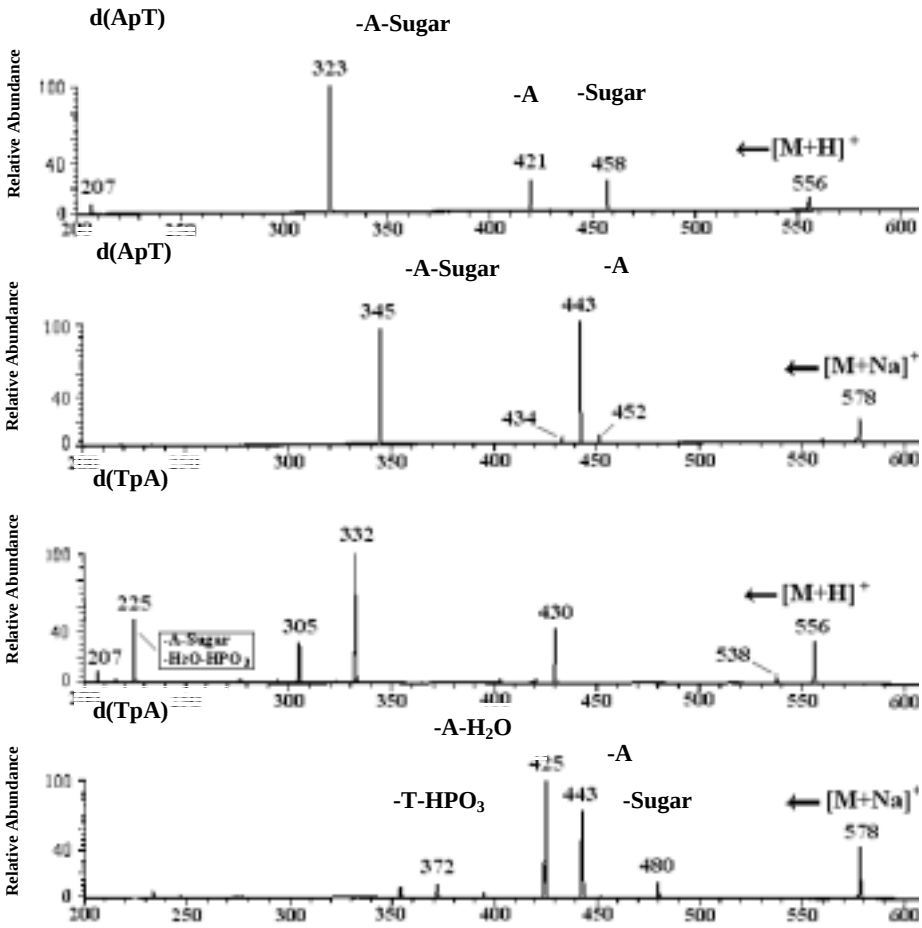
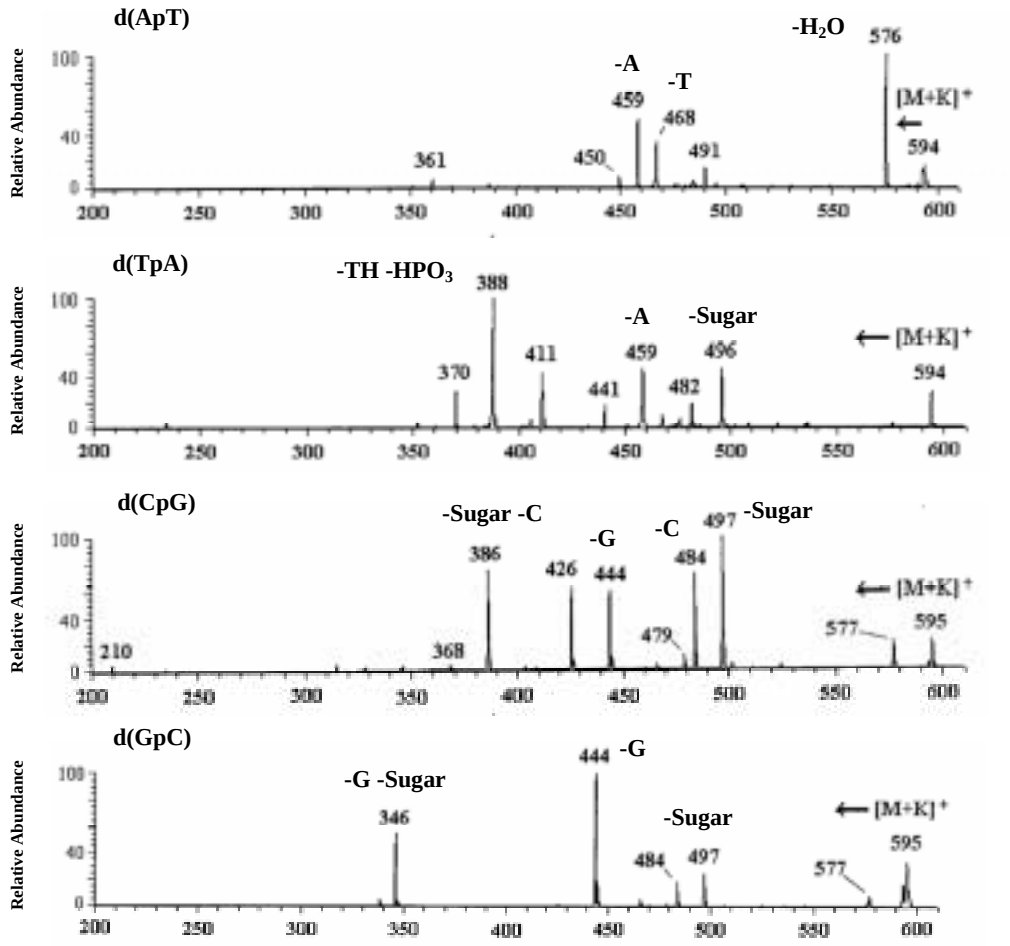


Figure 1 shows the MS/MS spectra of $[M+H]^+$ and $[M+Na]^+$ ions of d(ApT) and d(TpA), respectively. The major fragmentation pathways of first decomposition reaction observed in the $[M+H]^+$ systems are elimination of the terminal base, and the preferred loss of 5'-terminus base over 3'-terminus base. But this fragmentation is

directed by the site of different base. The major product ion was that resulting from loss of both 5'-terminus base and a sugar in the d(ApT) and d(TpA) systems. Furthermore, the followed fragmentation behaviors were quiet different.

In the $[M+Na]^+$ system, although the loss of the terminal base remains the major decomposition, the fragmentation behaviors have changed from that of $[M+H]^+$, and the loss of 5'-terminus base was not easy for all of the complexes. The major product ion of $[M+Na]^+$ precursor resulted in loss of the 5'-terminus adenine or loss of both the adenine and a sugar for the d(ApT) complex, while the major product ion resulted in loss of 3'-terminus adenine or both the adenine and water for the d(TpA) complex. On the other hand, there was a little difference between the fragmentation of $[M+Na]^+$ and that of $[M+H]^+$ precursors for the d (GpC), but the $[M+Na-GH-sugar]^+$ ion from the $[M+Na]^+$ is stronger. The major product ion resulted from loss of 3′-terminus guanine or from both the guanine and H_2O for the d (CpG).

Figure 2 MS/MS spectra of $[M+K]^+$ ion of d(ApT), d(TpA), d(CpG) and d(GpC)



Great changes have taken place in the fragmentation behavior of the $[M+K]^+$ complex ion, and the characterization of dissociation observed absolutely differs from each other in the four systems. The product ion spectra of $[M+K]^+$ precursor ion are shown in **Figure 2**. It can be observed that the dissociation was much more complex in the $[M+K]^+$ systems, and the loss of base or both the base and sugar was not the only major dissociation pathway. The major dissociation process involved loss of the water, sugar, base and metaphosphoric acid. The dissociation of the $[M+K]^+$ complex ions largely depended upon the sequence of bases and upon which led to different binding site for the K^+ ion in the deoxydinucleotides.

In the MS/MS experiment, the fragmentation of precursor ions, $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$, which came from the same dinucleotide seemed to be strongly dependent on the complex ion structures. In addition, the fragmentation pathway varied markedly due to the differences of the base sequence. Results from this study show that the alkali-metal ion binding site and the characterization of dissociation are directed by the size of metal ion, the sequence of base and the steric hindrance.

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