Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 3341–3344

TETRAHEDRON LETTERS

Low *Tm* DNA duplexes observed by cold-spray ionization mass spectrometry

Shigeru Sakamoto and Kentaro Yamaguchi*

Chemical Analysis Center, Chiba University, Yayoicho, Inage-ku, Chiba, 263-8522 Japan, and CREST, Japan Science and Technology Corporation (JST), Japan

Received 7 January 2003; revised 27 February 2003; accepted 28 February 2003

Abstract—Double-stranded oligodeoxynucleotides (6- to 14-mer) were observed by using CSI–MS. This method made it possible to observe very unstable species such as low *Tm* DNA duplexes which can not be detectable by conventional ESI–MS.

Electrospray ionization (ESI)[1] MS analysis has been generally used for the characterization of labile supramolecules in which non-covalent bonding interactions are important.[2] However, molecular ions were not detected in most cases because of their instability. Even if the molecular ions are observed by using this method, many fragment ions generated by thermal decomposition also appear in the spectrum. The heat giving off from the desolvation chamber was thought to be necessary for ionization in the gas phase in conventional ESI.[3] However, we recently developed a direct solution analysis method, cold-spray ionization (CSI)[4] MS, a variant of ESI–MS operating at low temperature, and we have applied this method to investigations of the solution structures of primary biomolecules,[5] labile organic species including Grignard reagents,[6] asymmetric catalysts,[7] and supramolecules.[8] This method allows reliable and precise characterization of labile non-covalent complexes, which are difficult to observe by conventional MS techniques including fast atom bombardment (FAB), matrix assisted laser desorption ionization (MALDI), as well as ESI.

In double-stranded DNA analysis, ion peaks of single-stranded oligonucleotide are always observed as a major component, together with the corresponding duplex, in gas phase by using ESI–MS. Further, non-covalent complexes of small (less than 10 base-pair) oligodeoxynucleotide-binding drugs are rather difficult to observe by the conventional method.[9,10] This is because of the low melting temperature (*Tm*) of the hetero-duplex. Here we report a facile and reliable analysis of double-stranded oligodeoxynucleotides that can detect precise structures in which non-covalent bonding interactions are important, by means of coldspray ionization mass spectrometry.

Negative CSI–MS measurements were performed with a two-sector (BE) mass spectrometer (JMS-700, JEOL) equipped with the CSI source.[4] Typical measurement conditions are as follows: acceleration voltage; −5.0 kV, needle voltage; −1.7 kV, orifice voltage; −100 to −60 V, ion source temperature; 15°C, spray temperature; 7°C, resolution (10% valley definition); 2000, sample flow rate; 8 μL/min, DNA concentration; 10 μM, buffer (NH_4OAc) concentration; 50 mM, solvent; H_2O:MeOH = 1:1.

First, hetero-duplexes (5′-dA_nG_n-3′)·(5′-dC_nT_n-3′) ((A_nG_n)·(C_nT_n)) ($n = 3 \sim 7$), annealed by heating to 90°C for 10 min and slow cooling to room temperature (2 h), were analyzed by CSI–MS. The estimated Tm_n ($n = 3 \sim 7$) values according to Wallace's theorem[11] were as follows. $Tm_3 = 18$, $Tm_4 = 24$, $Tm_5 = 30$, $Tm_6 = 36$ and $Tm_7 = 42$°C. The CSI–MS spectra of (A_3G_3)·(C_3T_3) and (A_4G_4)·(C_4T_4) at 7°C (spray temperature) are shown in Figures 1 and 2, respectively, as examples. Although ion peaks based on single strands (M_{ss}) were observed together with a major double-stranded (M_{ds}) nucleotide at m/z 1193 $[M_{ds}-3H]^{3-}$ (M_{ds}^{3-}) in the case of the 6-mer, a single major molecular ion peak of the duplex m/z 1203 $[M_{ds}-4H]^{4-}$ (M_{ds}^{4-}) was observed for the 8-mer. The corresponding duplex ion peaks of m/z 1512 M_{ds}^{4-}, m/z 1456 M_{ds}^{5-} and m/z 1704 M_{ds}^{5-} for the 10-,

Keywords: CSI; mass spectrometry; DNA; cluster; hydrogen bond.
* Corresponding author. Tel.: +81-43-290-3810; fax: +81-43-290-3813; e-mail: yamaguchi@cac.chiba-u.ac.jp

0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)00613-0

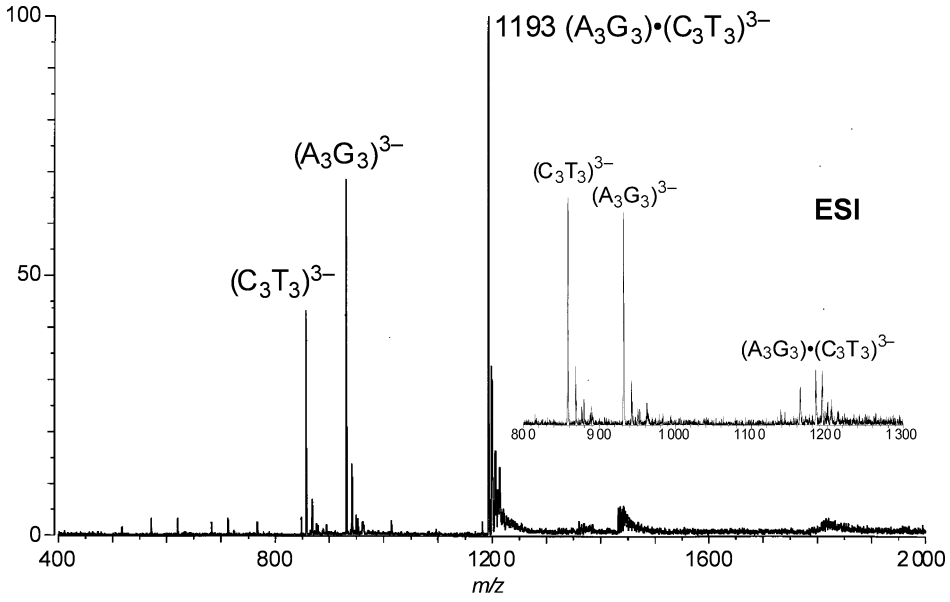

Figure 1. Negative CSI mass spectrum of duplex $(A_3G_3)\cdot(C_3T_3)$.

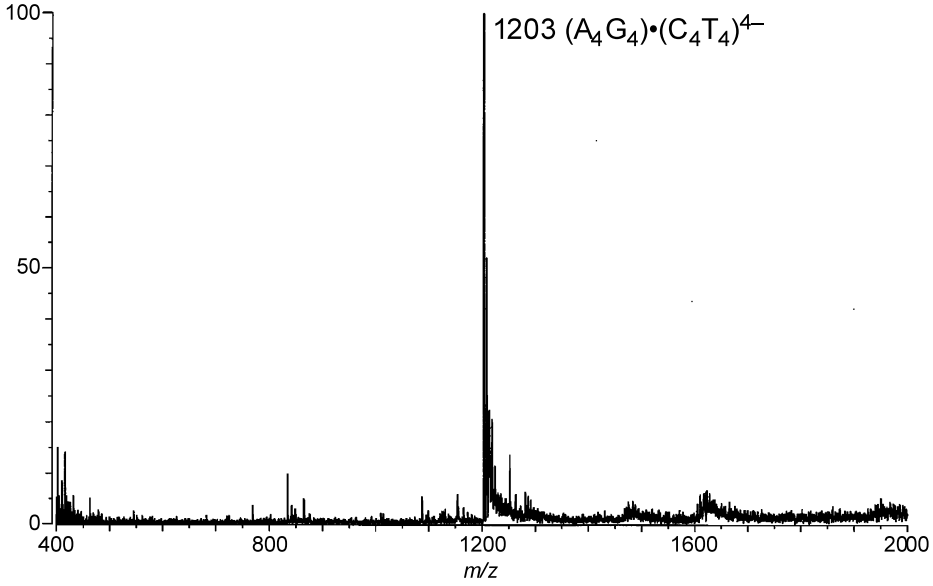

Figure 2. Negative CSI mass spectrum of duplex $(A_4G_4)\cdot(C_4T_4)$.

12-, and 14-mer, respectively, were also observed as the major species. The ion peaks of M_{ss} were the dominant species observed for these oligomers in conventional ESI–MS (not shown).

Furthermore, CSI–MS was applied to small oligodeoxynucleotides, 5′-dA_4C_4-3′ (A_4C_4), 5′-dG_4T_4-3′ (G_4T_4), 5′-dA_4G_4-3′ (A_4G_4) and 5′-dC_4T_4-3′ (C_4T_4). A clear ion peak m/z 1203 (M_{ds}^{4-}) due to the hetero duplex $(A_4C_4)'(G_4T_4)$ and $(A_4G_4)\cdot(C_4T_4)$ was observed in the mixtures of $(A_4C_4)(G_4T_4)$ and $(A_4G_4)(C_4T_4)$, respectively. Although the ion peaks of each M_{ss} were mainly observed in the mixtures of $(A_4C_4)(A_4G_4)$ and $(G_4T_4)(C_4T_4)$, the mismatched duplexes $(A_4C_4)\cdot(A_4G_4)$ m/z 1212 (M_{ds}^{4-}) and $(G_4T_4)\cdot(C_4T_4)$ m/z 1194 (M_{ds}^{4-}) were also observed in these mixtures. These are possibly based on C_4–G_4 Watson–Crick duplexes. The CSI–MS spectrum of the mixture $(G_4T_4)(C_4T_4)$ is shown in Figure 3. The ion due to the duplex was not observed in the $(A_4C_4)(C_4T_4)$ and $(G_4T_4)(A_4G_4)$ mixtures, presumably because of the lower affinity of A_4–T_4 duplex formation of the nucleotides having unfavorable mismatched sequences. The CSI–MS spectrum of the mixture $(A_4C_4)(C_4T_4)$ is shown in Figure 4. Finally, the mixture of all four oligodeoxynucleotides was analyzed. The CSI–MS spectrum of this mixture was quite simple. The ion peak m/z 1203 (M_{ds}^{4-}) based on the hetero duplexes $(A_4C_4)\cdot(G_4T_4)$ and $(A_4G_4)\cdot(C_4T_4)$, is the dominant species in the spectrum (Fig. 5). This confirms to the remarkable molecular recognition ability of DNA, which interacts highly specifically with the most favorable bases in solution.

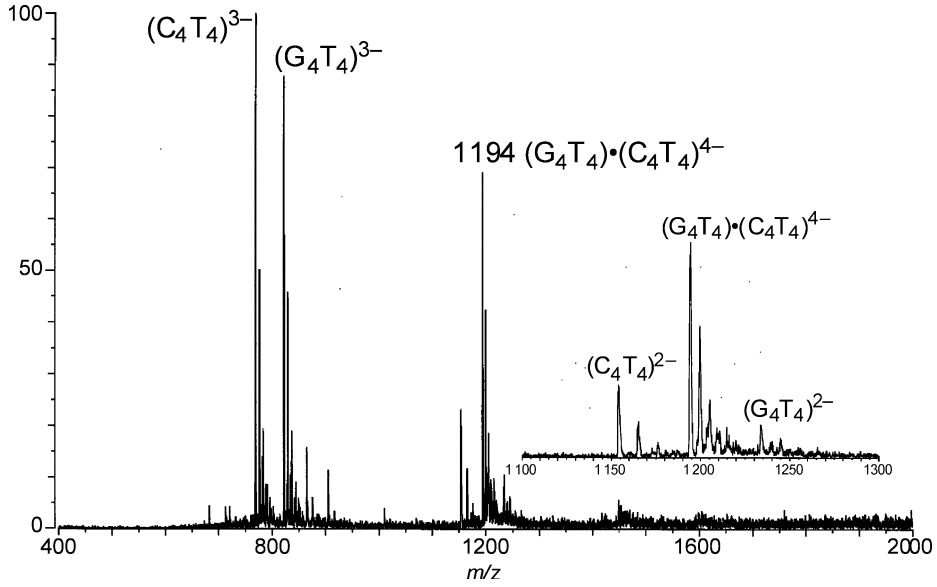

Figure 3. Negative CSI mass spectrum of the mismatched duplex $(G_4T_4)\cdot(C_4T_4)$.

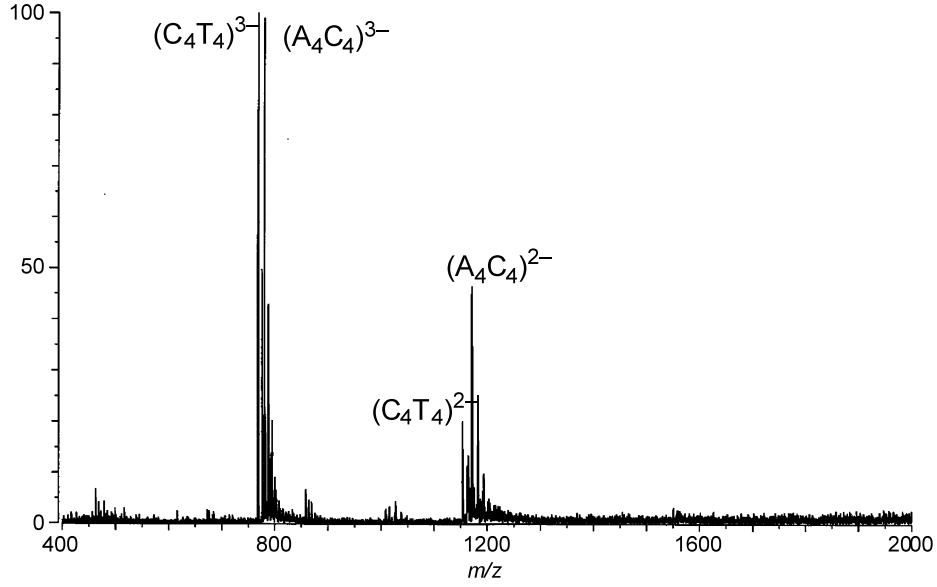

Figure 4. Negative CSI mass spectrum of a mixture of (A_4C_4) and (C_4T_4).

In summary, various complexes of oligodeoxynucleotides were observed by using CSI–MS. This method made it possible to observe very unstable species such as low *Tm* DNA duplexes which cannot be detectable by conventional ESI–MS. This method is remarkably effective to elucidate in detail the interactions of DNA complexes, being superior to other methods currently in use, such as UV melting methods. CSI–MS is also applicable to investigate labile solution structures of various primary biomolecules. The result shows the potential importance of this new MS technique for a wide variety of structural investigations in organic chemistry as well as biochemistry.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research (No. 13557198) from the Ministry of Education, Culture, Sports, Science and Technology. S.S. thanks JSPS Research Fellowship for Young Scientists.

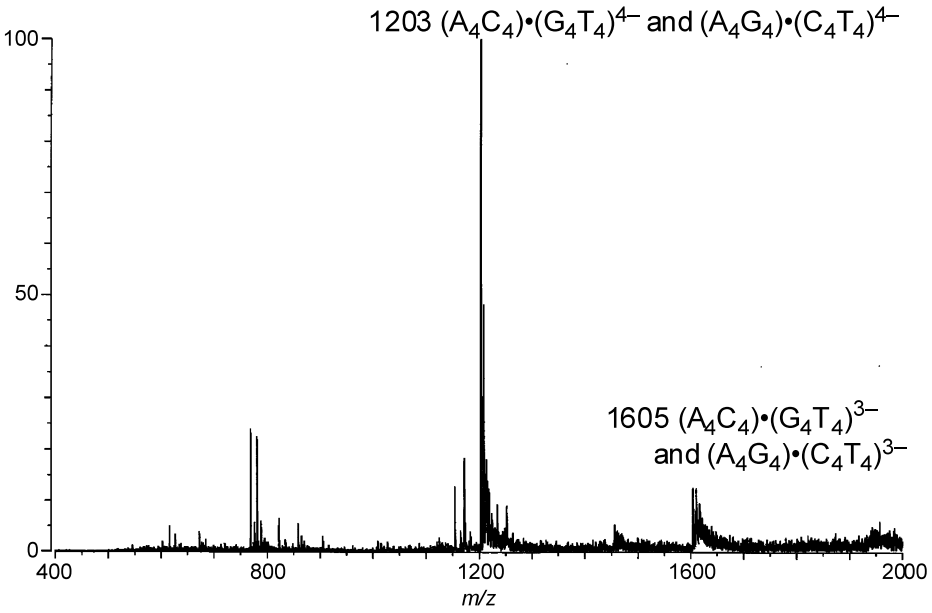

Figure 5. Negative CSI mass spectrum of a mixture of four 8-mers; (A_4C_4), (G_4T_4), (A_4G_4) and (C_4T_4).

References

1. Fenn, J. B.; Mann, M.; Meng, K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64.
2. Schalley, C. A. *Mass Spectrom. Rev.* **2001**, *20*, 253.
3. (a) Kebarle, P.; Tang, L. *Anal. Chem.* **1993**, *65*, 972A; (b) Bruins, A. P. *J. Chromatogr., A* **1998**, *794*, 345.
4. Sakamoto, S.; Fujita, M.; Kim, K.; Yamaguchi, K. *Tetrahedron* **2000**, *56*, 955.
5. Kunimura, M.; Sakamoto, S.; Yamaguchi, K. *Org. Lett.* **2002**, *4*, 347.
6. Sakamoto, S.; Imamoto, T.; Yamaguchi, K. *Org. Lett.* **2001**, *3*, 1793.
7. Suzuki, T.; Yamagiwa, N.; Matsuo, Y.; Sakamoto, S.; Yamaguchi, K.; Shibasaki, M.; Noyori, R. *Tetrahedron Lett.* **2001**, *42*, 4669.
8. (a) Yamanoi, Y.; Sakamoto, S.; Kusukawa, T.; Fujita, M.; Sakamoto, S.; Yamaguchi, K. *J. Am. Chem. Soc.* **2000**, *123*, 980; (b) Zhong, Z.; Ikeda, A.; Ayabe, M.; Shinkai, S.; Sakamoto, S.; Yamaguchi, K. *J. Org. Chem.* **2001**, *66*, 1002; (c) Sakamoto, S.; Yoshizawa, M.; Kusukawa, T.; Fujita, M.; Yamaguchi, K. *Org. Lett.* **2001**, *3*, 1601; (d) Dietrich-Buchecker, C.; Geum, N.; Hori, A.; Fujita, M.; Sakamoto, S.; Yamaguchi, K.; Sauvage, J.-P. *Chem. Commun.* **2001**, 1182; (e) Fujita, N.; Biradha, K.; Fujita, M.; Sakamoto, S.; Yamaguchi, K. *Angew. Chem., Int. Ed.* **2001**, *40*, 1718; (f) Kubota, Y.; Sakamoto, S.; Yamaguchi, K.; Fujita, M. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4854.
9. Reviews: (a) Beck, J. L.; Colgrave, M. L.; Ralph, S. F.; Sheil, M. M. *Mass Spectrom. Rev.* **2001**, *20*, 61; (b) Hofstadler, S. A.; Griffey, R. H. *Chem. Rev.* **2001**, *101*, 377.
10. (a) Kapur, A.; Beck, J. L.; Sheil, M. M. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 2489; (b) Gabelica, V.; Pauw, E. D.; Rosu, F. *J. Mass Spectrom.* **1999**, *34*, 1328; (c) Gabelica, V.; Rosu, F.; Houssier, C.; Pauw, E. D. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 464; (d) Wan, K. X.; Shibue, T.; Gross, M. L. *J. Am. Chem. Soc.* **2000**, *122*, 300.
11. Wallace, R. B.; Shaffer, J.; Murphy, R. F.; Bonner, J.; Hirose, T.; Itakura, K. *Nucleic Acid Res.* **1979**, *6*, 3543.