washing manipulations in the entire array. Significantly, the efficiency of the synthesis does not depend on the number of building blocks used along each axis. This feature provides a unique advantage of this method over the use of 2D arrays, representing a direct alternative to the split synthesis.[9] Although the operational simplicity of the split synthesis exceeds that of the present method, the ability to spatially resolve and positionally encode individual library members offered by the current approach compares favorably with the split synthesis, which requires additional encoding–decoding operations.[10,11] Extension of the work presented herein to the development of suitable systems for the synthesis of 1000–10000-membered small-molecule libraries is in progress and will be reported in due course.

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DNA Structure

Hyperstranded DNA Architectures Observed by Cold-Spray Ionization Mass Spectrometry**

*Shigeru Sakamoto and Kentaro Yamaguchi**

Formation of duplex, triplex, and quadruplex DNA structures has been a subject of extensive investigation because of their fundamental functional roles in living organisms. The structures of these multistranded DNAs have been examined[1–3] by electrospray ionization[4] mass spectrometry (ESI-MS). However, noncovalent complexes of multiply stranded DNA are difficult to observe by conventional methods because of their low melting temperature (T_m). The heat from the desolvation chamber is thought to be necessary for ionization in the gas phase in conventional ESI. However, we recently developed a direct solution analysis method, cold-spray ionization (CSI)[5] mass spectrometry, a variant of ESI-MS operating at low temperature, and we have applied this method to study various labile solution structures.[6] Here we report the characterization of triple- and quadruple-stranded oligodeoxynucleotides by means of CSI-MS.

First, triple-stranded oligodeoxynucleotides $T_n{\cdot}A_n{\cdot}T_n$ derived from 2:1 mixtures of 5′-dT_n-3′ (T_n) and 5′-dA_n-3′ (A_n) ($n = 8$, 10, 15, 20, and 25) were analyzed. Negative CSI-MS measurements were performed with a two-sector (BE) mass spectrometer (JMS-700, JEOL) equipped with the CSI source.[7] In the case of the 8-mer (Figure 1a), comparable

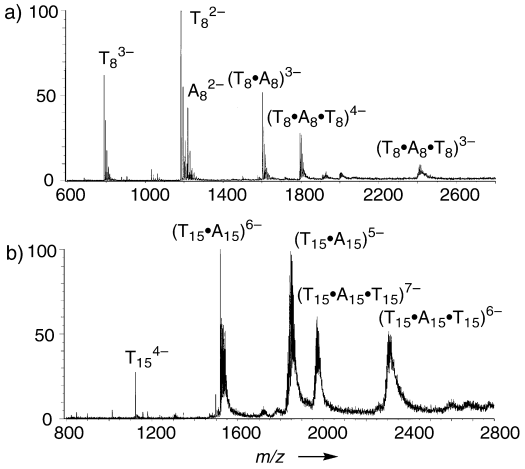

Figure 1. Negative CSI mass spectra of a) $T_8{\cdot}A_8{\cdot}T_8$ and b) $T_{15}{\cdot}A_{15}{\cdot}T_{15}$.

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ion peaks resulting from the duplex ($T_8{\cdot}A_8$) and a triplex ($T_8{\cdot}A_8{\cdot}T_8$) were observed, whereas the intensity of the ion peaks of the triplex became stronger when the number of base pairs increased. This might be explained by the higher stability of longer oligodeoxynucleotides with probably higher T_m values. The CSI mass spectrum of the 15-mer, exhibiting $(T_{15}{\cdot}A_{15}{\cdot}T_{15})^{x-}$ peaks ($x = 6,7$), is shown in Figure 1b. In the case of the 25-mer, ion peaks of the triplex were the major peaks, except for those of the single-stranded T_{25}^{5-}, and no ion peak of the duplex was observed (Figure 2a). The peaks of

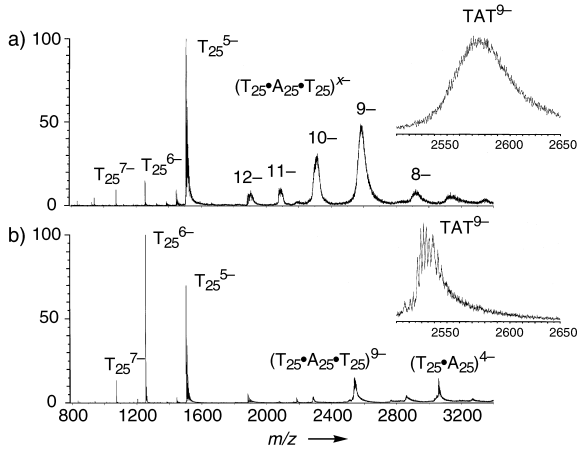


Figure 2. Negative a) CSI and b) ESI mass spectra of $T_{25}{\cdot}A_{25}{\cdot}T_{25}$.

the triplex ions $(T_{25}{\cdot}A_{25}{\cdot}T_{25})^{x-}$ ($x = 8$–12) are slightly broadened because of multiply attached NH_4^+ ions, which stabilize the triplex. From the broad $(T_{25}{\cdot}A_{25}{\cdot}T_{25})^{9-}$ peak follows that about 25 buffer molecules were attached to the triplex. The ions including buffer molecules can be interpreted as $[M-x\,H-y\,H+y\,(NH_4^+)]^{x-}$ (M: molecular weight of the multiply stranded DNA, x: charge state, y: number of buffer molecules attached). Only trace ion peaks resulting from the triplex and duplex, together with the major ion peaks of the single T_{25} strand, were observed by conventional ESI-MS in the case of the 25-mer (Figure 2b). The ESI-MS ion peaks were rather sharp compared to those of the CSI-MS, because NH_4^+ is dissociated by heat from the desolvation chamber in ESI-MS, and only two buffer molecules were left at the $(T_{25}{\cdot}A_{25}{\cdot}T_{25})^{9-}$ ion in the gas phase ($[M-9\,H-2\,H+2\,(NH_4^+)]^{9-}$). This destabilizes the triplex and promotes its dissociation. The triplex was thought to be formed by association of independently charged duplex and single-strand DNA in the presence of cationic buffer species. The complex $(T_{25}{\cdot}A_{25}{\cdot}T_{25})^{9-}$ might be formed from $(T_{25}{\cdot}A_{25})^{4-}$ and $(T_{25})^{5-}$, ions observed in the spectrum (Figure 2b). These experiments showed that CSI-MS is effective for characterizing unstable DNA complexes with low T_m values in solution.

Three main types of quadruplexes, composed of 1) one DNA strand folded intramolecularly,[8] 2) two DNA strands in various orientations,[9] and 3) four parallel DNA strands,[10] are known. We focused on the quadruplex type 3. Four DNA strands (8-mer) were prepared: 5′-d(AAAGGGAA)-3′ ($C_{80}H_{97}N_{40}O_{41}P_7$, $M_w = 2491.7$) (G3), 5′-d(AAGGGGAA)-3′ ($C_{80}H_{97}N_{40}O_{42}P_7$, $M_w = 2507.7$) (G4), 5′-d(AAGGGGGA)-3′ ($C_{80}H_{97}N_{40}O_{43}P_7$, $M_w = 2523.7$) (G5) and 5′-d(AGGGGGGA)-3′ ($C_{80}H_{97}N_{40}O_{44}P_7$, $M_w = 2539.7$) (G6). The negative CSI mass spectra of these oligomers at 7 °C are shown in Figure 3. In the case of G3 (37.5 % G), the ion peak of the

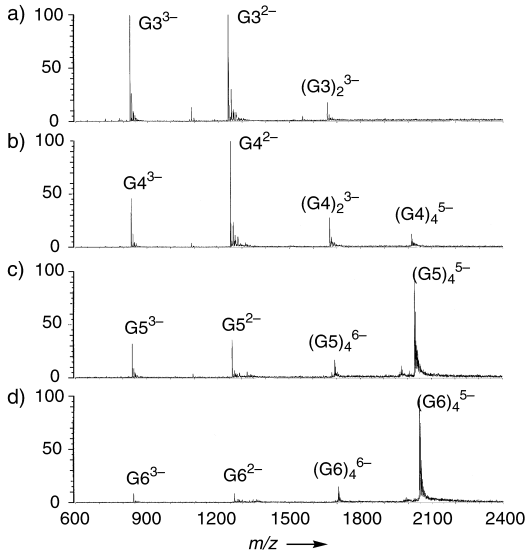
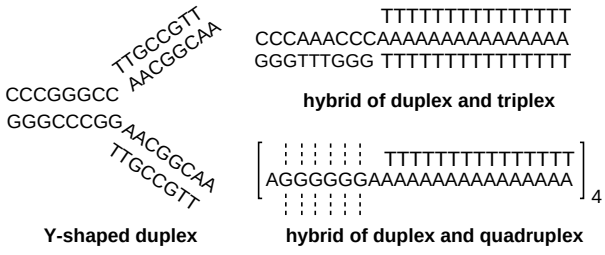


Figure 3. Negative CSI mass spectra of G3–G6.

quadruplex was not observed (Figure 3a). Trace ions of the quadruplex were observed for G4 (50 % G) together with major single- and minor double-stranded species (Figure 3b). Interestingly in the cases of G5 (62.5 % G; Figure 3c) and G6 (75 % G; Figure 3d), the ion peaks of the quadruplexes dramatically increased and became the major peaks. In addition, many NH_4^+ ions were attached to the quadruplexes, as observed in the case of the triplexes. We concluded that six bases G were necessary for stabilizing the telomeric G-quartet in 8-mer DNA.

Based on these results, several DNA molecules, which were expected to exhibit strong molecular recognition ability, were designed. Scheme 1 shows the schematic structures of the complexes identified in the CSI mass spectra of Figure 4. Figure 4a shows the forklike structure of a replicating DNA (Y-shaped duplex) consisting of a 1:1:2 mixture of 5′-d(CCCGGGCCAACGGCAA)-3′ ($C_{153}H_{193}N_{66}O_{90}P_{15}$, $M_w = 4861.2$) (**1**), 3′-d(GGGCCCGGAACGGCAA)-5′



Scheme 1. Schematic structures of the DNA complexes discussed in Figure 4.

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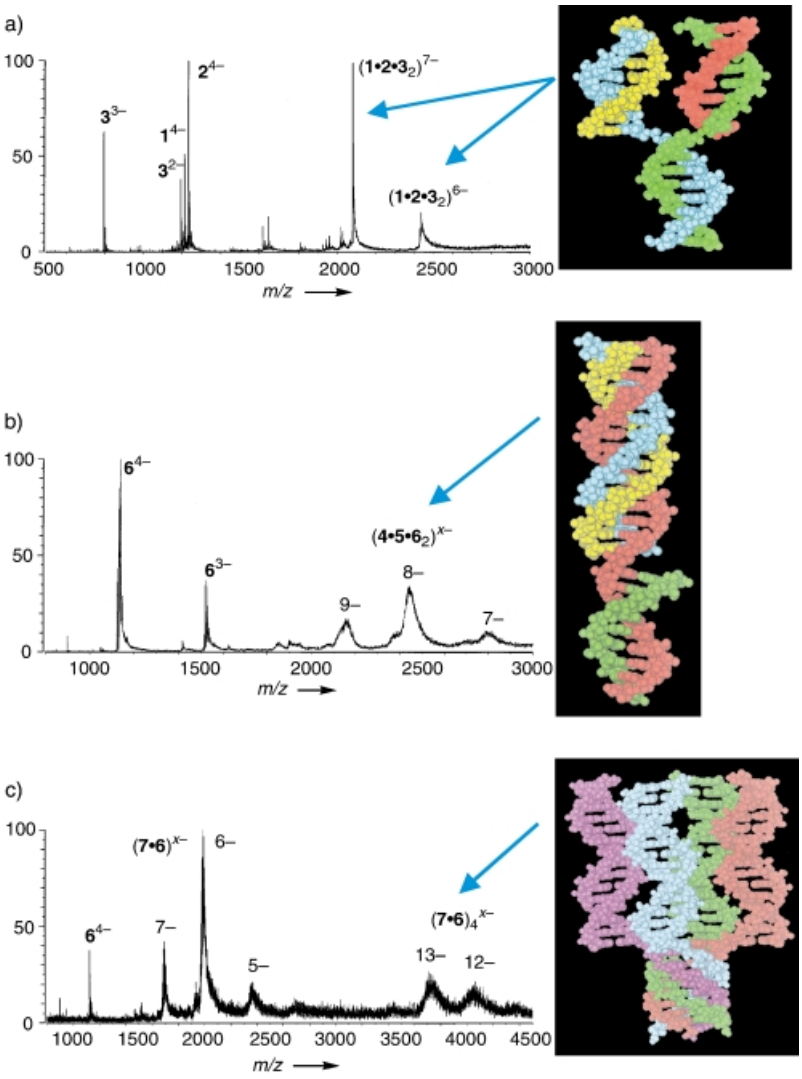

Figure 4. Negative CSI mass spectra of mixtures of a) **1, 2**, and **3**, b) **4, 5**, and **6** (1:1:2 ratios), and c) **7** and **6** (1:1 ratio) and the probable structures of the complexes.

($C_{155}H_{193}N_{70}O_{90}P_{15}$, $M_w = 4941.3$) (**2**), and 5′-d(TTGCCGTT)-3′ ($C_{78}H_{101}N_{24}O_{50}P_7$, $M_w = 2391.6$) (**3**). The expected negative ions (6− and 7−) of $\mathbf{1{\cdot}2{\cdot}3}_2$ were clearly observed in the spectrum (Figure 4a).The complex $\mathbf{1{\cdot}2{\cdot}3}_2$ consists of duplexes between **3** (8-mer) and the mismatched sites on **1** (16-mer) and **2** (16-mer), and between the sequences CCCGGGCC of **1** and GGGCCCGG of **2**.

When 5′-d(CCCAAACCCAAAAAAAAAAAAAAA)-3′ ($C_{234}H_{289}N_{108}O_{124}P_{23}$, $M_w = 7310.9$) (**4**), 5′-d(GGG-TTTGGG)-3′ ($C_{90}H_{112}N_{36}O_{55}P_8$, $M_w = 2825.9$) (**5**), and 5′-d(TTTTTTTTTTTTTTT)-3′ ($C_{150}H_{196}N_{30}O_{103}P_{14}$, $M_w = 4501.0$) (**6**) were mixed in 1:1:2 ratio, negative ions (7−, 8−, and 9−) of $\mathbf{4{\cdot}5{\cdot}6}_2$ were observed. Duplex formation between the sequences CCCAAACCC of **4** and GGGTTTGGG of **5,** followed by double coordination of **6** (T_{15}) to the sequence A_{15} of **4** led to a duplex–triplex hybrid (Figure 4b).

Finally, a 1:1 mixture of 5′-d(AGGGGGGAA-AAAAAAAAAAAAAA)-3′ ($C_{230}H_{277}N_{115}O_{119}P_{22}$, $M_w = 7236.9$)(**7**) and **6** was analyzed. The negative ions (12− and 13−) of $(\mathbf{7{\cdot}6})_4$, pointing to a duplex–quadruplex hybrid DNA structure, were clearly observed in the CSI mass spectrum of this DNA mixture (Figure 4c) together with the ions (7−, 8−, and 9−) of **7·6**. Various NH_4^+ attachments were also observed.

In summary, our results show that CSI-MS can be used to characterize triplex and quadruplex DNA as well as duplex DNA. This method is also applicable to unstable and complex species, hyperstranded DNAs, which are difficult to observe by other spectroscopic methods. The structure elucidation for a wide range of interesting biomolecules appears to be possible by using CSI-MS, including its negative mode.

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Typical measurement conditions and sample preparation procedures 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^{-1}, DNA complex 20 μM DNA in aqueous 100 mM NH_4OAc annealed by heating to 90 °C for 10 min and slow cooling to room temperature (2 h), then diluted with MeOH. DNA concentration 10 μM, buffer (NH_4OAc) concentration 50 mM, solvent H_2O/MeOH 1:1.

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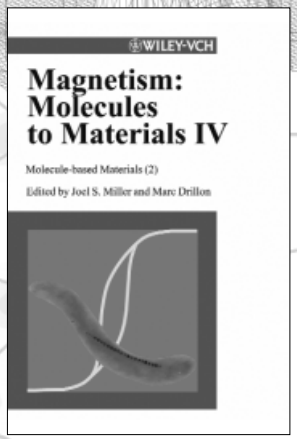

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