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Formation and destruction of the guanine quartet in solution observed by cold-spray ionization mass spectrometry†

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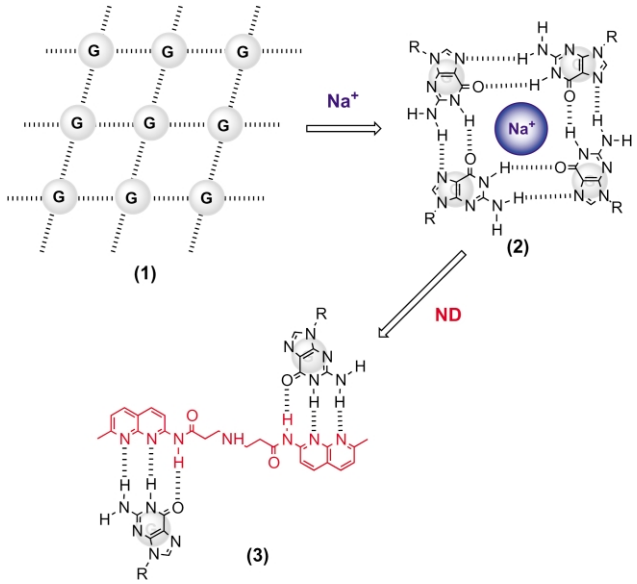
The dynamic solution behavior during formation of the 2′-deoxyguanosine tetrad (G-quartet) upon addition of alkali metal cations and destruction of the G-quartet upon addition of the guanine–guanine mismatch recognition molecule naphthyridine dimer was observed by cold-spray ionization mass spectrometry.

A direct solution analysis method, cold-spray ionization (CSI),[1] a variant of electrospray (ESI)[2] MS operating at low temperature (*ca.* −20 °C), allows facile and precise characterization of labile organic species, including supramolecules, in solution.[3] We have recently adopted this method for investigations of the solution structures of primary biomolecules such as nucleosides, amino acids, sugars and lipids.[4] Na^+ adducts were observed to form singly charged ions of large clusters (chain structures), presumably linked by non-covalent interactions, including hydrogen bonding and/or hydrophobic interactions. A large-scale-aggregated chain structure (**1**) was also detected by CSI–MS in the case of 2′-deoxyguanosine. This chain structure of 2′-deoxyguanosine was readily transformed by addition of an alkali metal cation to form a unit cluster, the guanine quartet (G-quartet) (**2**). It is suggested that the guanine-rich telomeric repeat in the end of human chromosomes is able to assemble into a four-stranded quadruplex structure, consisting of guanine tetrads stabilized by monovalent cations such as Na^+.[5] Interestingly, the G-quartet readily collapsed upon addition of the guanine–guanine mismatch recognition molecule naphthyridine dimer (ND), to form guanine adducts (**3**). In this report, the dynamic solution behavior during formation and destruction of the G-quartet was observed at the nucleoside level by using CSI–MS.

CSI–MS measurements were performed with a two-sector (BE) mass spectrometer (JMS-700, JEOL) equipped with the CSI source.[1] Typical measurement conditions are as follows: acceleration voltage; 2.5 kV, needle voltage; 0 kV,[6] needle current; 0 nA, orifice voltage; 60 to 100 V, ion source temperature; 5 °C, spray temperature; −20 °C, resolution (10% valley definition); 2000, sample flow rate; 17 μL min^{-1}, sample concentration; 1 mmol L^{-1}, additional alkali metal concentration; 0.25 mmol L^{-1}, solvent; H_2O:MeOH = 2:98.

The CSI mass spectra of the nucleosides (M) dG ($C_{10}H_{13}N_5O_4$, MW. 267.3), dC ($C_9H_{13}N_3O_4$, MW. 227.2), dA ($C_{10}H_{13}N_5O_3$, MW. 251.3) and dT ($C_{10}H_{14}N_2O_5$, MW. 242.2) clearly exhibited the ions of large-scale-aggregated chain structures, $[nM + Na]^+$ (n = dG: ~ 37, dC: ~ 43, dA: ~ 39 and dT: ~ 41) in solution.† The CSI mass spectrum of dG at −20 °C (spray temperature) is shown in Fig. 1. The chain structures gradually disappeared and characteristic ions were not detected when 0.25 eq. Na^+ was added to dT, dC and dA. However, in the case of dG, the chain structure totally disappeared upon addition of 0.25 eq. NaCl and a new ion peak of $[4dG + Na]^+$ (m/z 1091) became the dominant species, in addition to monomer and dimer, which points to the formation of the tetrameric cyclic cluster (**2**), the G-quartet,[7] as observed in the CSI–MS spectrum shown in Fig. 2.[8] The ion peaks of $[3dG + Na]^+$ (m/z 824) and multiply charged $[16dG + 3Na]^{3+}$ (m/z 1447) and $[12dG + 2Na]^{2+}$ (m/z 1625) were also observed as minor species in this spectrum.[9]



Scheme 1

Furthermore, the mismatch binding ligand naphthyridine dimer (ND), ($C_{24}H_{25}N_7O_2$, MW. 443.5), which has a strong binding ability to guanine[10] in DNA duplex was added to the G-quartet.[11] When 0.5 eq. ND was added, the G-quartet was

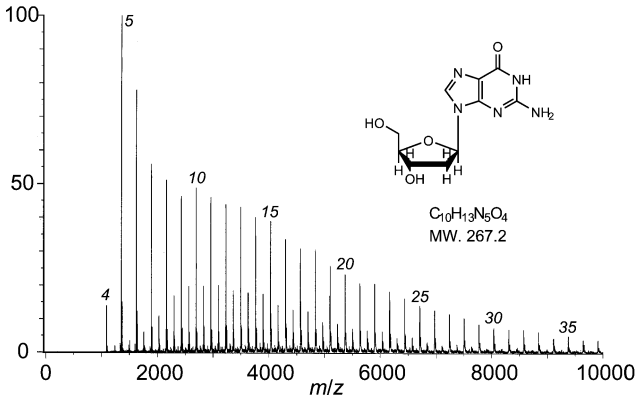


Fig. 1 CSI mass spectrum of 2′-deoxyguanosine (dG).

† Electronic supplementary information available: CSI and ESI mass spectra of dG, dC, dA and dT, and schematic diagram of the coldspray ion source. See http://www.rsc.org/suppdata/cc/b2/b212432g/

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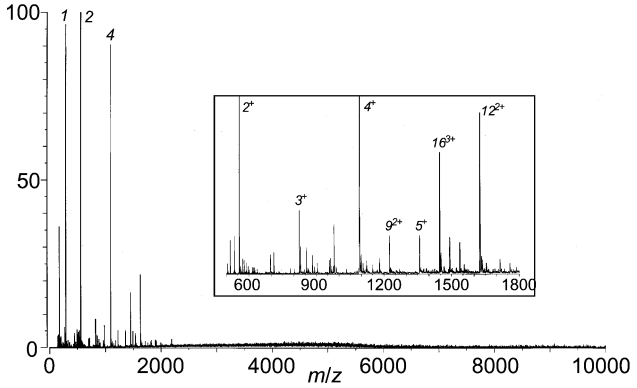

Fig. 2 CSI mass spectrum of dG (1 mmol L^{-1}) with NaCl (0.25 mmol L^{-1}).

destroyed to form the $[ND + 2dG + H]^+$ (*m*/*z* 978) ion (**3**) in solution (Fig. 3c). The CSI–MS spectra of the mixture were continuously recorded while the ratio of G-quartet : ND was increased from 1 : 0 to 1 : 1.25 , as shown in Fig. 3. The G-quartet was clearly apparent in 1 : 0.25 solution (Fig. 3b). The ion peak due to the G-quartet $[4dG + Na]^+$ (*m*/*z* 1091) disappeared when ND was added, and increasing ion peaks due to monomeric naphthyridine dimer $[2ND + H]^+$ (*m*/*z* 887) and $[2ND + Na]^+$ (*m*/*z* 909) were observed (Fig. 3d). It is suggested that the mismatch recognition molecule ND strongly binds to the guanine–guanine structure to collapse telomeric DNA.[5]

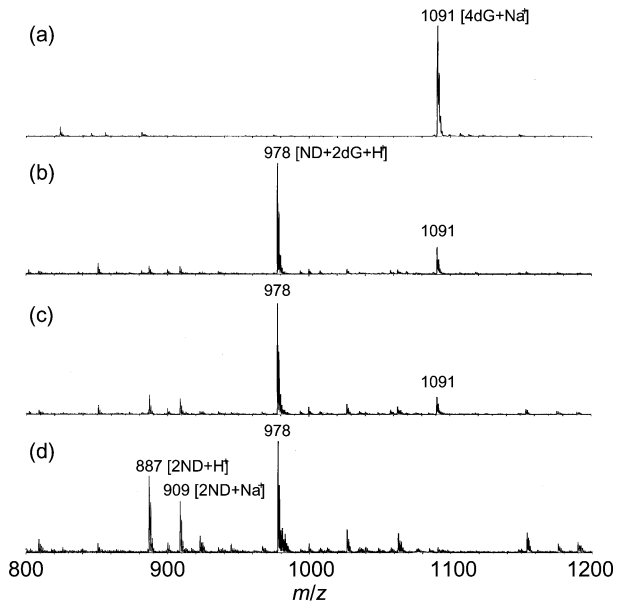

Fig. 3 CSI mass spectra of G-quartet solution (dG: 1mmol L^{-1}, NaCl: 0.25 mmol L^{-1}) plus 2-amino-1,8-naphthyridine dimer (ND): (a) 0 eq., (b) 0.25 eq., (c) 0.5 eq. and (d) 1.25 eq.

In summary, aggregation of nucleosides in solution, including induction and collapse of the G-quartet as well as large-scale-aggregated chain structure, could be observed by using CSI–MS. Although the chain structures gradually disappeared and no characteristic ion was observed in the case of dT, dA and dC upon addition of alkali metal cations, dG was transformed to the cyclic tetramer, the G-quartet, in solution. Destruction of the G-quartet upon addition of naphthyridine dimer was observed. This interesting dynamic solution behavior of DNA was directly observed at the nucleoside level for the first time.

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