Cold-spray Ionization Mass Spectrometric Observation of Biomolecules in Solution

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Investigations of the solution structures of primary biomolecules such as nucleosides, amino acids, sugars and lipids by using cold-spray ionization mass spectrometry (CSI–MS) are shown. Singly charged Na⁺ adducts of large clusters (chain structures) for these biomolecules, presumably linked by non-covalent interactions, including hydrogen bonding and/or hydrophobic interactions were observed. Detailed DNA duplex observation using this method is also presented.

Introduction

Recently we reported a new ionization method, cold-spray ionization (CSI) [1], a variant of electrospray (ESI) [2] MS operating at low temperature, which allows facile and precise characterization of labile organic species. The optimum spray temperature is estimated to be around –20°C. Generally, solvents show a higher dielectric constant at low temperature. This fact, as well as the solvation of the molecule, promotes electrolytic dissociation to form molecular ion in solution, because desolvation should not occur readily at such a low temperature. Therefore, it should be possible to detect extremely labile complexes without decomposition. The CSI apparatus has been refined and applied to characterize various kinds of organometallic compounds [3–4] and supramolecules [5–9]. Furthermore, CSI–MS revealed the dynamic assembling nature of coordination box type complexes [10].

This ionization method should also be applicable to biomolecules. In this report, we wish to describe solution structures including large-scale-aggregated chain structures of simple biomolecules such as nucleosides, amino acids, sugars and lipids observed by CSI–MS.

CSI–MS measurements were performed with a two-sector (BE) mass spectrometer (JMS-700, JEOL) equipped with the CSI source. Typical measurement conditions are as follows: acceleration voltage; 1.0 to 5.0 kV, needle voltage; 0 to 2.0 kV, needle current; 0 to 700 nA, orifice voltage; 60 to 100 V, ion source temp.; 5°C, spray temp.; –20°C, resolution (10% valley definition); 1000, sample flow rate; 17 μL/min, sample concentration; 1 mmol/L, solvent; H₂O: MeOH = 2:98.

Chain Structure

Fig. 1 shows the CSI mass spectrum of thymidine (M1) in aqueous MeOH solution. Asymptotically reducing ion peaks, which appear to represent large-scale hydrogen-bonding aggregates, or chain structure, were observed up to around m/z 10000. The major ion peaks were assigned as [nM1 + Na⁺]⁺ (n = 1 to 41). The minor ion peaks located in the valleys between the major ion peaks in the spectra were assigned as doubly charged ions [nM1 + 2Na⁺]²⁺. X-ray crystallographic analysis of the nucleoside has clearly revealed a chain structure in the crystal, linked by hydrogen bonds [11]. In the case of another...
nucleoside, 2′-deoxyguanosine, ions corresponding to quaternary aggregate structure, a G-quartet [12], were also observed together with chain structures.

Similar chain structures were also observed in amino acids. The CSI–MS spectrum of L-valine (M2) in solution is shown in Fig. 2. The major ion peaks were also assigned as [nM2 + Na]⁺ (n = 1 to 42) together with doubly charged ions with in the range of m/z 0 to 5000. Other amino acids such as leucine, isoleucine, lysine and serine also exhibited similar mass spectra suggestive of large-scale-aggregated chain structures. X-ray crystallographic analysis of these amino acids has indicated presence of hydrogen-bonding chain structures in the crystal [13].

Characteristic chain structures of monosaccharides were also clearly observed in solution. In the case of D-glucose (M3), ion peaks assigned to [nM3 + Na]⁺ (n = 1 to 133), together with doubly charged ions, were clearly observed in the range of m/z 0 to 24000. (Fig. 3). The crystal structure of D-glucose is known to exhibit strong intermolecular hydrogen bonding [14]. Chain structures with different abundances of aggregation states were observed with other isomers.

Furthermore, the membrane structure of the L-α-phosphatidylcholine, dilauroyl (C12:0) (M4) lipid bilayer [15] was directly observed, preserving the hydrophobic intermolecular interactions, in solution by means of CSI–MS (Fig. 4). Ion peaks assigned as [nM4 + Na]⁺ (n = 1 to 42), together with doubly charged ions, were clearly observed in the range of m/z 0 to 26000, the current limit of the mass spectrometer. Chain structure was also observed for steroids as well as other similar lipids and micelles.
Next subject is a facile and reliable analysis of double-stranded oligodeoxynucleotides that can detect precisely structures in which non-covalent bonding interactions are important, by means of cold-spray ionization mass spectrometry. 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 [16, 17]. This is because of the low melting temperature (Tm) of the hetero-duplex.

Negative 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; –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 (NH₄OAc) concentration; 50 mM, solvent; H₂O: MeOH = 1:1.

Hetero-duplexes (5’-dA₃G₃-3’) • (5’-dC₄T₄-3’) ([(A₃G₃) • (C₄T₄)]) (n = 3 to 7), annealed by heating to 90°C for 10 min and slow cooling to room temperature (2 hr), were analyzed by CSI–MS. The estimated Tmₙ (n = 3 to 7) values according to Wallace’s theorem [18] were as follows. Tm₃ = 18, Tm₄ = 24, Tm₅ = 30, Tm₆ = 36 and Tm₇ = 42°C. The CSI–MS spectra of (A₃G₃) • (C₄T₄) and (A₄G₄) • (C₄T₄) at 7°C (spray temperature) are shown in Figures 5 and 6, respectively, as examples.

Although ion peaks based on single strands (Mₙss) were observed together with a major double-stranded (Mₙds) nucleotide at m/z 1193 [Mₙds –3H]⁻ (Mₙds⁻) in the case of the 6-mer, a single major molecular ion peak of the duplex m/z 1203 [Mₙds –4H]⁻ (Mₙds⁻) was observed for the 8-mer. The corresponding duplex ion peaks of m/z 1512 Mₙds⁻, m/z 1456 Mₙds⁻ and m/z 1704 Mₙds⁻ for the 10-, 12-, and 14-mer, respectively, were also observed as the major species. The ion peaks of Mₙss were the dominant species observed for these oligomers in conventional ESI–MS (not shown).

Furthermore, CSI–MS was applied to small oligodeoxynucleotides, 5’-dA₄C₄-3’ (A₄C₄), 5’-dG₄T₄-3’ (G₄T₄), 5’-dA₄G₄-3’ (A₄G₄) and 5’-dC₄T₄-3’ (C₄T₄). A clear ion peak m/z 1203 (Mₙds⁻) due to the hetero duplex (A₄C₄)•(G₄T₄) and (A₄G₄)•(C₄T₄), was observed in the mixtures of (A₄C₄)(G₄T₄) and (A₄G₄)(C₄T₄), respectively. Although the ion peaks of each Mₙss were mainly observed in the mixtures of (A₄C₄)(A₄G₄) and (G₄T₄)(C₄T₄), the mismatched duplexes, (A₄C₄)•(A₄G₄) m/z 1212 (M₉ds⁻) and (G₄T₄)•(C₄T₄) m/z 1194 (M₉ds⁻) were also observed in these mixtures. These are possibly based on C₄-G₄ Watson-Crick duplexes. The CSI–MS spectrum of the mixture (G₄T₄)(C₄T₄) is shown in Figure 7.

The ion due to the duplex was not observed in the (A₄C₄)(C₄T₄) and (G₄T₄)(A₄G₄) mixtures, presumably because of the lower affinity of A₄-T₄ duplex formation of the oligonucleotides having unfavorable mismatched sequences.

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 (M₉ds⁻) based on the hetero duplexes (A₄C₄)•(G₄T₄) and (A₄G₄)•(C₄T₄), is the dominant species in the spectrum (Figure 8).

This confirms the remarkable molecular recognition ability of DNA, which interacts highly specifically with the most favorable bases in solution.

Thus, CSI–MS has revealed non-covalent clustering or aggregation of biomolecules in solution for the first time. In addition, 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 technique has many potential molecular-biological applications, including studies of dynamic interactions at very high resolution. 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.

References


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Fig. 7. Negative CSI mass spectrum of the mismatched duplex (G(T4))•(C(T4)).*