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Alkali Metal-Mediated Proline Aggregation in Solution Observed by Coldspray Ionization Mass Spectrometry

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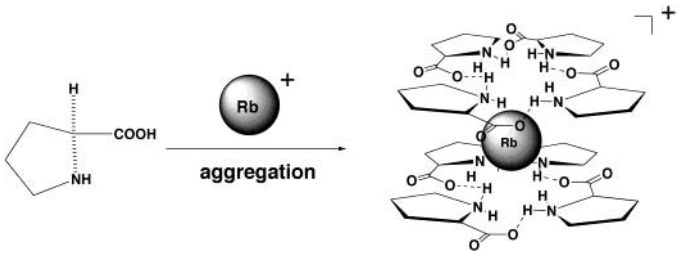
ABSTRACT



L-Proline aggregates to form cyclic clusters in the presence of alkali metal ions in solution, whereas a large-scale-aggregated chain structure was observed for several other amino acids. The major cyclic clusters are suggested to be constructed from trimeric and tetrameric subunits on the basis of direct solution analysis by coldspray ionization mass spectrometry.

Coldspray ionization (CSI),[1] a variant of electrospray (ESI)[2] MS operating at low temperature (ca. −20 °C), was recently developed by some of us.[1] This method allows facile and precise characterization of labile organic species in solution. The CSI apparatus has been used 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 for examination of the solution structures of biomolecules, since desolvation should not occur readily at such low temperature.

Self-directed polymerization of serine was observed by Cooks et al., by means of ESI-MS, and octameric clusters were detected.[11] We recently observed large-scale-aggregated chain structures of simple biomolecules such as amino acids. L-Serine exhibited chain structure in addition to a strong ion peak of the octamer in solution when examined by CSI-MS (Figure 1a). The major ion peaks were assigned as $[n\text{Ser} + \text{Na}]^+$ ($n = 4-38$) together with $[n\text{Ser} - \text{H} + 2\text{Na}]^+$ and multiply charged ions in the range of m/z 0−5000. A chain

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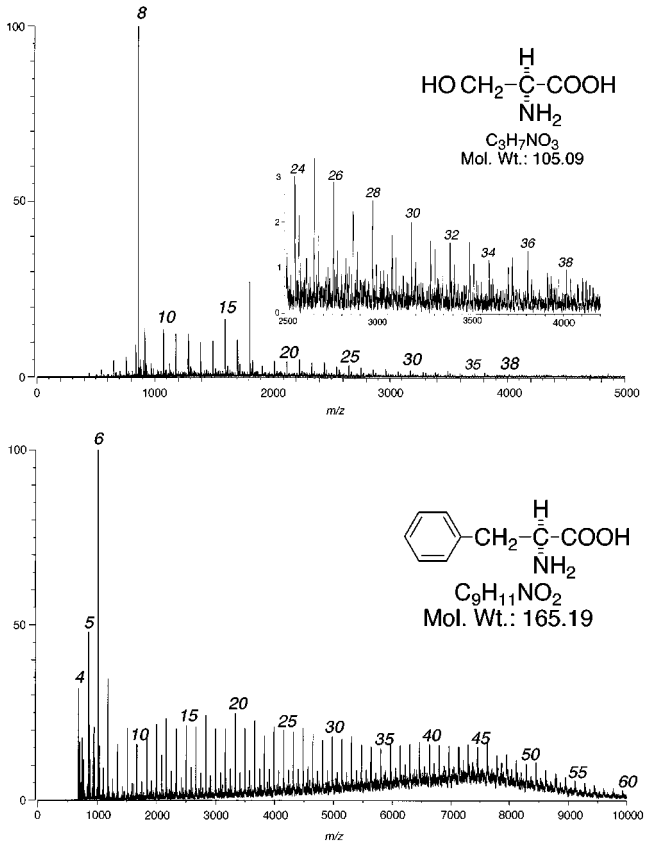

Figure 1. CSI mass spectra: (a, top) L-serine, italic numbers [*n*Ser + Na]$^+$; (b, bottom) L-phenylalanine, italic numbers [*n*Phe + Na]$^+$. Measurement conditions: acceleration voltage, (a) 5.0 kV, (b) 2.5 kV; needle voltage, (a) 0 kV, (b) 0 kV; orifice voltage, (a) 35 V, (b) 90 V.

structure in solution was also observed for other amino acids, such as glycine, L-valine, L-methionine, and L-phenylalanine, by means of CSI-MS (the spectrum for L-phenylalanine is shown in Figure 1b, as an example).[12]

X-ray crystallographic analysis of some of these amino acids has clearly revealed a chain structure in the crystal, linked by hydrogen bonds.[13−15] However, a chain structure was not observed in the case of L-proline in solution. We found that addition of various alkali metals ions induces periodic aggregates of L-proline in solution similar to that of serine observed in ESI-MS, as mentioned above. In this report, we describe alkali metal ion-mediated L-proline aggregation in solution observed by coldspray ionization mass spectrometry.

CSI-MS or CSI-MS/MS measurements were performed with a four-sector (BE/BE) tandem mass spectrometer (JMS-700T, JEOL) equipped with the CSI source. Typical measurement conditions are as follows: acceleration voltage, 2.5−5.0 kV; needle voltage, 0−2.6 kV; needle current, 0−700 nA; orifice voltage, 0−95 V; ion source temperature, 5 °C; spray temperature, −20 °C; resolution (10% valley definition), 1000−2000; sample flow rate, 8.3 μL/min; sample concentration, 1 mmol/L; additional alkali metal, 0.02 mmol/L; solvent, H_2O:MeOH = 2:98; collision gas, Xe.

The CSI mass spectrum of L-proline, to which NaCl was added, is shown in Figure 2. Three major cluster ions, [3Pro + Na]$^+$ (m/z = 368), [4Pro + Na]$^+$ (m/z = 483), and [6Pro + Na]$^+$ (m/z = 713), were observed. The degree of aggregation is estimated to be 3 or 4 in this case.

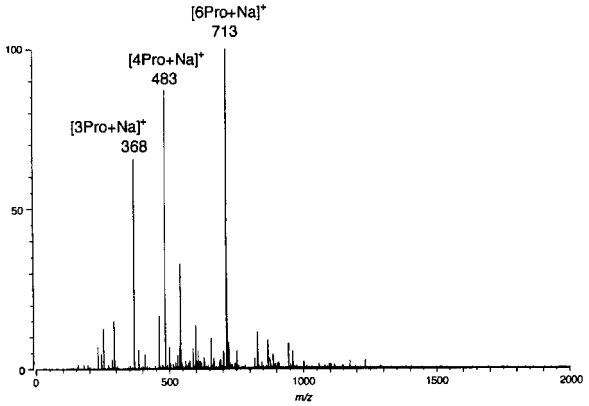

Figure 2. CSI mass spectrum of L-proline + NaCl. Measurement conditions: acceleration voltage, 5.0 kV; needle voltage, 2.5 kV; orifice voltage, 0 V.

The possible structures, trimeric (3Pro + Na), bitrimeric (3Pro + Na + 3Pro), and tetrameric (4Pro + Na) clusters, are shown in Figure 3. These structures are consistent[16] with the results obtained from semiempirical molecular orbital calculation[17] using the ZINDO/1-RHF[18] (restricted Hartree−Fock) procedure[19] as implemented in MOPAC.[20]

The effects of addition of KCl, RbCl, and CsCl to L-proline were also analyzed by the use of CSI-MS (the spectrum of the RbCl adduct is shown in Figure 4, for example).[21] The most intense ion peak was that of the octameric aggregate,

(12) The monomeric molecular ion, [M − H + 2Na]$^+$, was mainly observed by conventional ESI-MS for these amino acids. Ion peaks in the higher mass range, including the chain structure, are presumably not observed because of thermal decomposition caused by the heat of the desolvation chamber.

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(16) This result suggests an induced fit between the cyclic L-proline cluster (host) and metal cation (guest). If the guest fits in the middle of the host cavity (tetramer), the complex would exhibit a rather flat shape (Figure 3b) and no other aggregation should be observed. However, when the guest is too large to fit in the middle of the cavity, the guest should be located out of the host plane (Figure 3a). Then, the metal ion of this complex will be able to aggregate with another cyclic cluster (trimer) (Figure 3c). The structures appear to depend on the radius of the metal cation and the size of the cavity in the cyclic L-proline cluster.

(17) All calculations were carried out using the HyperChem ver. 5.01 program system with a Gateway Select personal computer.

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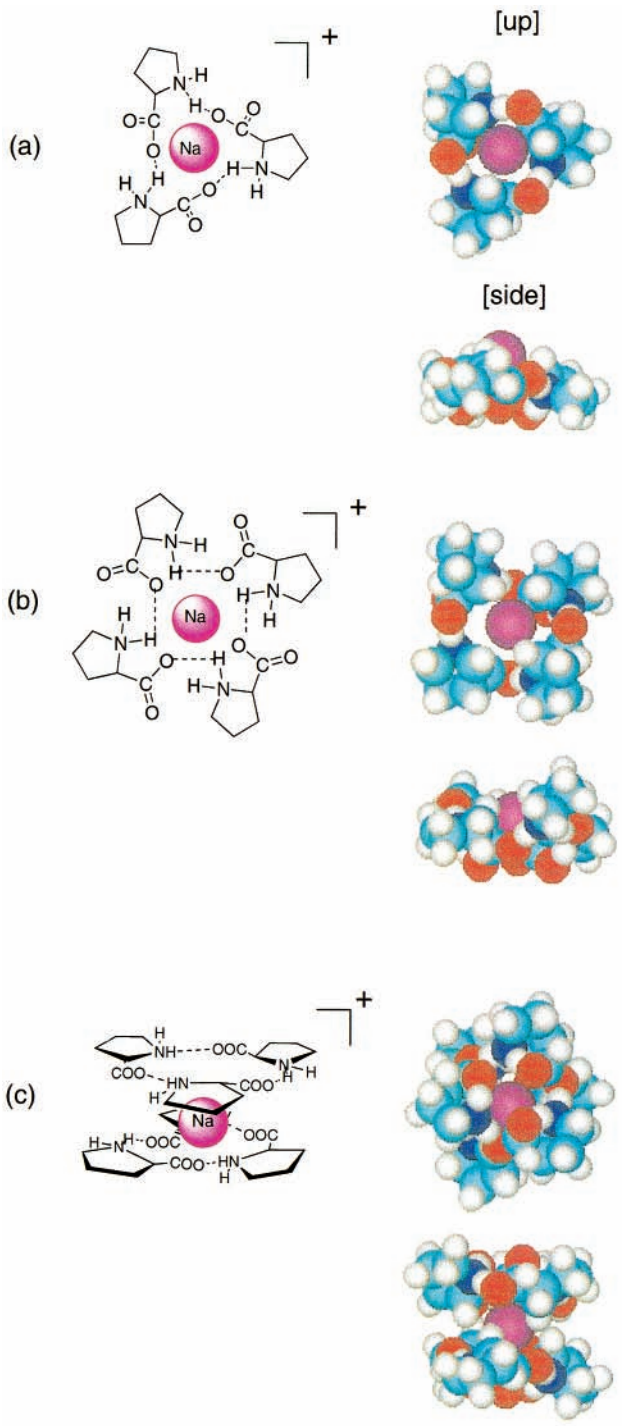

Figure 3. Possible structures of the clusters (a) trimeric (3Pro + Na), (b) tetrameric (4Pro + Na), and (c) bitrimeric (3Pro + Na + 3Pro), obtained from semiempirical MO calculations: Na, purple; C, blue; N, dark blue; O, orange; H, white.

$[8Pro + K]^+$, $[8Pro + Rb]^+$, and $[8Pro + Cs]^+$, for KCl, RbCl, and CsCl, respectively, in each spectrum, except in the case of the sodium ion, for which no octamer peak was

(21) The protonated molecule $[Pro + H]^+$ and/or sodium adduct $[Pro + Na]^+$ were mainly observed by means of conventional ESI-MS measurement.

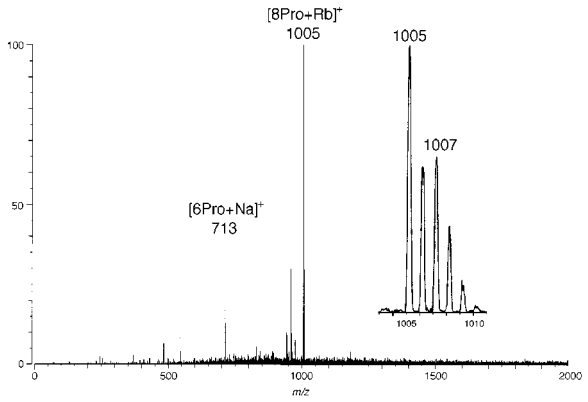

Figure 4. CSI mass spectrum of L-proline + RbCl. Measurement conditions: acceleration voltage, 5.0 kV; needle voltage, 2.4 kV; orifice voltage, 0 V.

observed. It is concluded that formation of proline clusters might be quite strongly dependent upon the nature of the added metal.[16]

A tandem mass spectrometric study confirmed this type of aggregation in the cases of the potassium, rubidium, and cesium adducts. A major fragment ion of $[4Pro + Rb]^+$ (m/z = 545) was clearly observed in the MS/MS spectrum of the rubidium adduct (Figure 5) when $[8Pro + Rb]^+$ (m/z =1005) was selected as the precursor ion.

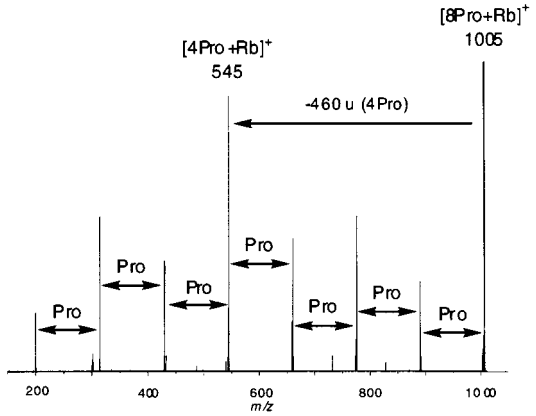

Figure 5. Product ion spectrum of the rubidium adduct of L-proline. The precursor ion was selected as $[8Pro + Rb]^+$ (m/z = 1005).

Similar results were obtained with the potassium and cesium adducts. These experiments may indicate that the octamer is composed of two of tetrameric aggregates, which face each other across the central Rb^+ ion (Figure 6). Sodium, a smaller metal ion,[22] induces formation of $[3Pro + metal]^+$,

(22) Interaction with L-proline was not observed in the case of lithium, presumably because the ion radius is much smaller than the cavity of the trimeric L-proline cluster.

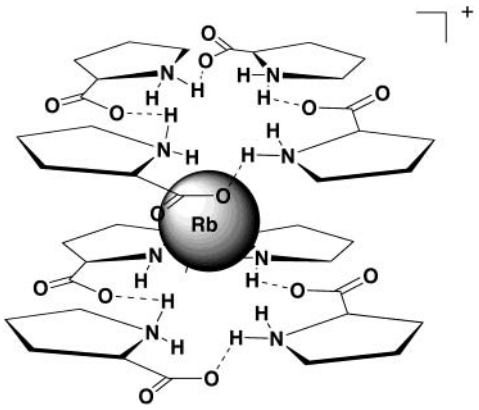

Figure 6. Possible structure of [4Pro + Rb + 4Pro]$^+$.

[3Pro + metal + 3Pro]$^+$, and [4Pro + metal]$^+$, while larger metal ions such as potassium, rubidium, and cesium induce [4Pro + metal + 4Pro]$^+$.

In summary, we have shown that L-proline aggregates to form cyclic clusters in solution in the presence of alkali metal ions. The major cyclic clusters are suggested to be constructed from trimeric and tetrameric subunits. Cyclic cluster induction by specific metal ions as well as large-scale-aggregated chain structure in solution could have very important consequences in the biochemistry of amino acids.

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Supporting Information Available: CSI and ESI mass spectra of amino acids and metal cation adducts of L-proline including MS/MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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