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Serine octamer metaclusters: formation, structure elucidation and implications for homochiral polymerization

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Multiply charged serine metaclusters (composed of two or more homochiral octameric units) are generated by electrospray ionization, and their unique fused structures (hydrogen-bonded through the sticky ends of the drum-shaped octameric units) have been elucidated using tandem mass spectrometry experiments and molecular mechanics calculations.

The formation of non-covalent supramolecular assemblies held together by electrostatic and hydrophobic forces and hydrogen bonding, is at the center of many biological processes including stabilization (*e.g.* DNA base pairing), recognition (enzyme–substrate, antibody–antigen, drug–target), and regulation (activation, deactivation, signaling).[1] Motivated by this, we are engaged in a systematic study of the clustering of the natural amino acids using both positive and negative mode electrospray ionization mass spectrometry, and have published on the clustering of arginine[2] and serine[3] in detail and reported briefly on phenylalanine, cysteine and glutamic acid.[2,4] Serine displays a magic number cluster consisting of eight monomers assembled in a drum-shaped structure consisting of carboxylic acid dimers that link the top and bottom faces.[3] There is also preliminary evidence[3] for the higher-order multiply charged clusters that are the subject of this communication. The remarkable gas-phase stability of the protonated octamer and the chiral dependence of its formation may have implications in biological chirogenesis—the selection of handedness in biological molecules.[5] The unique clustering of serine has also drawn the attention of other authors.[6,7]

Not only does the octamer of serine give rise to prominent magic number clusters in the electrospray mass spectra of concentrated serine solutions, but abundant metaclusters, that is, clusters of clusters, are generated. In addition to the protonated octamer, formula, $[(\mathrm{Ser})_{8n} + n\mathrm{H}]^{n+}$, the positive ion spectrum displays a series of metaclusters $[(\mathrm{Ser})_{8n} + n\mathrm{Na}]^{n+}$ (n = 1–3). These higher clusters are the topic of this communication. The possibility that the symmetry breaking step in homochirogenesis[8] might have occurred in the serine cluster or metacluster rather than in a simpler and more strongly bonded molecule like the monomeric amino acid itself, is a compelling reason for interest in these higher clusters. Attempts to isolate ions in the series $[(\mathrm{Ser})_{8n} + n\mathrm{H}]^{n+}$ and characterize them by collision-induced dissociation were unsuccessful. The stability of the ions was too low, and only the singly charged ion remained trapped to a significant extent. However, the corresponding sodiated ions could be generated, isolated and studied in an ion trap mass spectrometer.

The positive ion electrospray mass spectrum of a 1 : 1 mixture of 10^{-2} M D-serine and 10^{-3} M NaCl, is shown in Fig. 1. The mass spectrum consists of protonated and sodiated dimers and octamers, with the octamers, $[(\mathrm{Ser})_8 + \mathrm{H}]^+$ and $[(\mathrm{Ser})_8 + \mathrm{Na}]^+$, clearly showing such extraordinary intensity relative to the surrounding clusters as to be considered ‘super clusters’.[9] The magnitude of the magic number effect, expressed using the scaling factor,[3] $[(2 \times I_n)/(I_{n-1} + I_{n+1})]$, where I represents signal intensity and n the cluster size, is 20.

The addition of sodium salts to the serine solution results in increased formation of the sodiated serine octamer at the expense of the formation of the protonated serine octamer. The sodium adducts have the same chiral dependence as the protonated serine adducts, *viz.* the absolute intensity is reduced by a factor of five when using the racemic mixture. Use of lithium salts did not lead to observable formation of an oligomeric adduct and potassium octamers were formed but could not be isolated, while addition of Cs, Ca and Mg salts simply reduced the formation of the protonated serine octamer.

Tandem mass spectrometry (MS/MS) was used to further characterize the behavior of the protonated and sodiated serine octamers. Isolation of the octamers was accomplished by setting the ion trap mass spectrometer to trap ions falling within a large (*m*/*z* 20) isolation window. The sodiated monomers, dimers and octamer are all efficiently isolated. The peak at the mass/charge ratio of the sodiated octamer also includes metaclusters with multiples of the same mass and charge, $[(\mathrm{Ser})_{8n} + n\mathrm{Na}]^{n+}$, which were also isolated along with the monomeric cluster. This indicates that these metaclusters are not so weakly bound as to be unable to survive the relatively energetic collisions with helium bath gas that occur during the isolation process. Structural information on the protonated and sodiated serine octamers was obtained by collision-induced dissociation. The MS/MS spectrum of the set of protonated octamer metaclusters (all of *m*/*z* 841) shows preferential formation of the singly charged hexamer $[(\mathrm{Ser})_6 + \mathrm{H}]^+$ with a small contribution of $[(\mathrm{Ser})_4 + \mathrm{H}]^+$ and $[(\mathrm{Ser})_5 + \mathrm{H}]^+$. By comparison, the MS/MS spectrum (Fig. 2) of the set of sodiated octamer metaclusters (all of *m*/*z* 863), shows *inter alia*, the formation of $[(\mathrm{Ser})_{14} + 2\mathrm{Na}]^{2+}$, $[(\mathrm{Ser})_{23} + 3\mathrm{Na}]^{3+}$, $[(\mathrm{Ser})_{22} + 3\mathrm{Na}]^{3+}$, $[(\mathrm{Ser})_{15} + 2\mathrm{Na}]^{2+}$, $[(\mathrm{Ser})_{13} + 2\mathrm{Na}]^{2+}$, $[(\mathrm{Ser})_{12} + 2\mathrm{Na}]^{2+}$, $[(\mathrm{Ser})_{10} + 2\mathrm{Na}]^{2+}$ and $[(\mathrm{Ser})_8 + 2\mathrm{Na}]^{2+}$.

These fragmentations confirm that the signal due to the putative singly charged sodiated octamer includes those for higher order clusters. The direct observation of multiply charged sodiated metaclusters presents two significant questions: what are the structures of these metaclusters and are they homochiral?

A structure for the protonated serine octamer has already been proposed from a combination of tandem mass spectro-

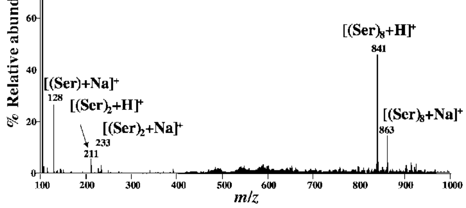

Fig. 1 ESI mass spectrum of 0.01 M D-Ser + 0.001 M NaCl.

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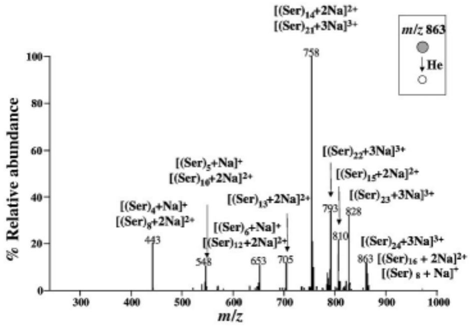

Fig. 2 Mass spectrum: products of CID of $[(\mathrm{Ser})_{8n} + n\mathrm{Na}]^{n+}$.

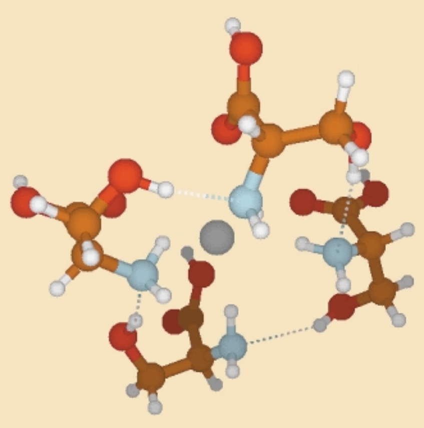

Fig. 3 Calculated position of Na^+ near one face of the serine octamer. Blue atoms are nitrogen, red are oxygen.

metric data and *ab initio* calculations.[3] The protonated octamer is proposed to be formed from four carboxylic acid bound dimers, which assemble through hydrogen bonds between amino and hydroxy groups: each OH forms a hydrogen bond with the NH_2 lone pair. The final drum-shaped structure has incomplete hydrogen-bonding, *i.e.* lone pairs on oxygen and amino hydrogen atoms available for further interactions. The formation of the metaclusters $[(\mathrm{Ser})_{16} + 2\mathrm{H}]^{2+}$ and $[(\mathrm{Ser})_{24} + 3\mathrm{H}]^{3+}$ is predicted to arise as a result of oligomerization of the octamers, held together by the 'sticky ends' present on the top and bottom faces *via* hydrogen bonds between the amino and hydroxy groups. Molecular mechanics calculations (MMFF94) show that two octamers combine to form a higher-order cluster through additional hydrogen bonds between the amino and hydroxy groups. For the sodiated octamer, two energetically similar structures appear to be possible: in the first, the Na^+ lies on the octamer face; in the second, the Na^+ ion lies inside the octamer in a crown-ether-like structure. Molecular mechanics calculations show, however, that the first structure is unstable; it collapses to the second structure during geometry optimization. To evaluate the reliability of the molecular mechanics results, *ab initio* [HF/6-31G(d,p)] calculations were used to estimate the most stable configuration of an ion with Na^+ near the serine octamer. Four serine molecules were placed in positions corresponding to one octamer face (Fig. 3), and then the Na^+ was placed either inside or outside the serine cage. The structure having the Na^+ outside the half octamer was found to be unstable, collapsing to the structure with Na^+ inside the octamer, in agreement with molecular mechanics calculations. Therefore, the presence of a Na^+ does not change the ability of the serine octamer to concatenate face-to-face with other octamers *via* hydrogen bonds, so generating higher metaclusters. Thus, the structure of $(\mathrm{Ser})_{16}\mathrm{Na}_2^{2+}$ is predicted by the calculations to correspond to the fusion of two drum-like $(\mathrm{Ser})_8\mathrm{Na}^+$ clusters, leading to a linear cylindrical structure. Fig. 4 is the calculated structure of $(\mathrm{Ser})_{16}\mathrm{Na}_2^{2+}$ comprised of two drum-shaped $(\mathrm{Ser})_8\mathrm{Na}^+$ clusters, in the form of a linear cylindrical structure. Further $(\mathrm{Ser})_8\mathrm{Na}^+$ units can be added subsequently to form higher members such as $(\mathrm{Ser})_{24}\mathrm{Na}_3^{3+}$.

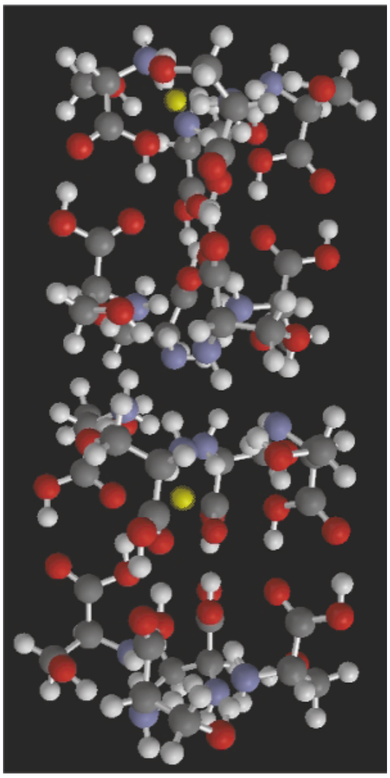

Fig. 4 The calculated structure of $[(\mathrm{Ser})_{16} + 2\mathrm{Na}]^{2+}$. Yellow is sodium, red is oxygen, blue is nitrogen.

These results may be significant for their implications for methods of detecting biologically important non-covalent interactions and for the fact that a new route to homochiral polymerization has been observed. Electrospray ionization from concentrated solutions may be a general means of forming other supramolecular complexes, including those in which conjugates of drugs and other small molecules with their receptors are generated and characterized by mass spectrometry. Homochiral polymerization, a critical step in biochemical evolution, has been proposed to occur *via* enantioselective segregation on the surface of calcite or other inorganic crystals.[10] Our results demonstrate homochiral polymerization of the primitive amino acid serine under mild conditions. This might implicate the serine metaclusters characterized here in biochemical chiral self-assembly.

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