The Prebiotic Role of Serine

Serine Octamer Reactions: Indicators of Prebiotic Relevance**

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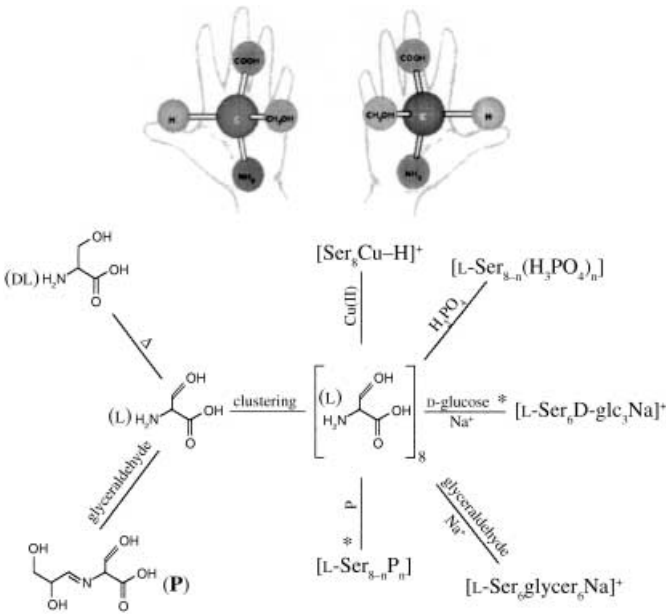
Herein we explore the hypothesis[1,2] that serine played an important role in the prebiotic chemistry that led to living organisms. The principal tool used in this study is sonic-spray ionization (SSI),[3,4] a mild method that facilitates investigation of chemical species present in concentrated aqueous solutions.[5,6] It has been reported that serine, alone among the common α amino acids, forms homochiral magic-number clusters.[1,2,7–9] These clusters incorporate other amino acids of like chirality by substitution for serine[10,11] and they are capable of aggregation.[8] These facts allow a prebiotic scenario in which serine clusters were involved in chiral accumulation and in transmission of chirality to other amino acids. We now report observations that suggest unique connections between serine and other compounds of fundamental importance to biochemistry, including glyceraldehyde, glucose, phosphoric acid, and some transition-metal ions. We also suggest a reaction that might have been the locus for symmetry-breaking in serine.

Serine, the putative product of the reaction between the interstellar molecules glycine and formaldehyde,[12,13] is one of the primitive amino acids.[14] It has not been reported in interstellar space but has been identified in meteorites.[15] Both the strong preference for homochirality and the stability of the magic-number cluster set serine apart from other amino acids.[16,17] Herein we report new experimental facts (Scheme 1) that confirm the uniqueness of serine: 1) Serine clusters with glyceraldehyde, undergo chiro-selective reactions involving the octamer, 2) it forms a chirally dependent magic-number cluster with glucose, 3) it incorporates H_3PO_4 into its homochiral octamer, 4) the magic-number octamer is cationized by Cu^{II} ions, while other-magic number clusters of serine are observed with Fe^{II} and Fe^{III} ions. In all these instances serine reacts uniquely compared to other representative amino acids. Finally, 5) serine is amongst the most easily racemized α amino acids.

The recently proposed mechanism for chiral transfer from serine to other amino acids[10] stimulated the search for a chemical connection between the L-serine and D-sugars of living organisms. The interactions of serine with glyceraldehyde, the simplest aldose, were examined. The two compounds undergo a condensation reaction when a neutral aqueous mixture is heated at approximately 75 °C for 14 h, forming a Schiff base. The SSI mass spectrum shows this serine–glyceraldehyde dehydration product to substitute for one or two serines in the octamer, in a similar fashion to that seen for mixtures of serine with α amino acids.[10,11] Remarkably, substitution into the serine octamer is chirally selective, as confirmed by using purified[18] D-glyceraldehyde and recording the SSI mass spectrum. The L-serine octamer incorporates the condensation product of L-serine and D-glyceraldehyde (8% relative abundance for one substitution, 3% for two) whereas no reaction was detectable for the D-serine/D-glyceraldehyde pair. Other amino acids undergo the same reaction with glyceraldehyde to form analogous Schiff base products. Remarkably, serine and threonine were found to be the only amino acids to incorporate the glyceraldehyde dehydration product into a magic-number cluster (the octamers). It is noteworthy that the chiral selection matches that seen in biological systems (L-amino acids and D-sugars).



Scheme 1. Reactions of serine and its octamer; ⋆ chiro-selective reactions, **P** = serine/glyceraldehyde condensation reaction product.

Under the mild conditions of the SSI process, a solution containing L-serine and DL-glyceraldehyde (Sigma, St. Louis, MO; solid, 90% + purity) exhibits only one prominent product, a pronounced magic-number cluster, $[Ser_6Glyc_6Na]^+$ (m/z 1193). Tandem mass-spectrometry experiments show that $[Ser_6Glyc_6Na]^+$ fragments by loss of $C_6H_{12}O_6$ units which suggests that glyceraldehyde is present in the form of a dimer (hexose or an isomer) in the cluster.[19] Evidence for the structural assignment and information on chiral control of the reaction was obtained by performing experiments with hexose/serine mixtures. The SSI mass spectrum of a L-serine/D-glucose mixture (Figure 1) shows a remarkable magic-number cluster, nominally the same ion seen with glyceraldehyde. MS/MS experiments showed that analogously to the cluster with glyceraldehyde, the loss of one hexose molecule (m/z 1013) yields the dominant fragment. The strong but incomplete chiral preference for the L-serine/

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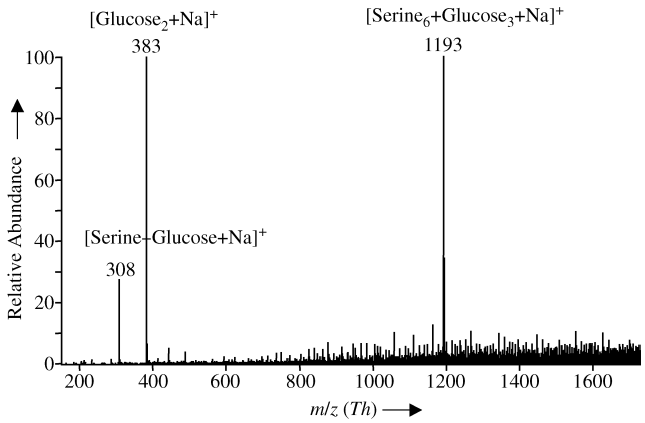

Figure 1. Magic-number cluster at m/z 1193 corresponding to $[Ser_6Glc_3Na]^+$ in the SSI mass spectrum of a L-serine /D-glucose mixture.

D-glucose and D-serine/L-glucose combinations over the homoclusters is evident in both tandem and single-stage mass spectra (Figure 2). Isotopic effects can be ruled out since data taken with 2,3,3-$[D_3]$-L-serine confirmed the chiral preference. In contrast, magic-number heteroclusters (amino acid/hexose) were not observed for other common amino acids, with the exception of threonine, which behaves similarly to serine.

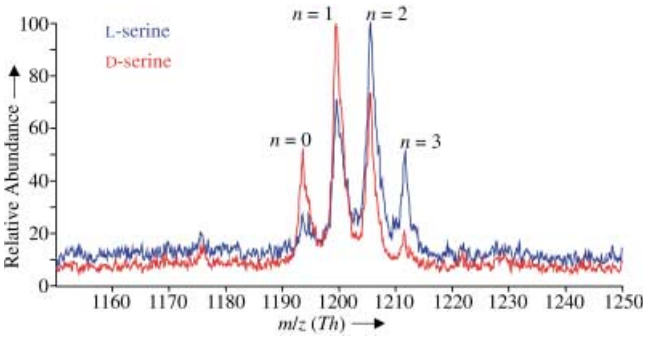

Figure 2. SSI mass spectra for serine/glucose nonameric cluster ions showing the preferential formation of the D-glucose/L-serine and L-glucose/D-serine pairs over their L/L and D/D counterparts using isotopic labeling. Blue: SSI-MS of a solution containing L-serine (5.0 mM), L-glucose (2.5 mM), and D-glucose-U-$^{13}C_6$ (2.5 mM). Red: D-serine (5.0 mM), L-glucose (2.5 mM), and D-glucose-U-$^{13}C_6$ (2.5 mM), where n is the number of D-glucose-U-$^{13}C_6$ in the cluster $[Ser_6Glc_3Na]^+$.

Further tests of the hypothesis that serine played a unique role in prebiotic chemistry involved examining its reactions with phosphoric acid and transition-metal ions. Phosphoric acid incorporates readily into serine clusters by substituting for serine; it gives a series of ions $[Ser_{8-n}+(H_3PO_4)_n+H]^+$,($n=1$–3). Sulfuric acid, by contrast, was not incorporated into clusters of serine under analogous conditions. With other natural amino acids, only single incorporations of phosphoric acid occurred into statistically distributed amino acid clusters, without preferred magic-number effects. The unusual stability of the serine octamer is further indicated by the fact that Cu^{II}, Fe^{II}, and Fe^{III} readily cationize serine clusters. For Cu^{II}, deprotonation is needed for charge balance and Cu^{II} chloride yields the octamer $[Ser_8+Cu\text{-}H]^+$ as well as the analogous tetrameric complex. As expected, Fe^{II} behaves analogously but it also yields an abundant intact adduct $[Ser_{10}+Fe]^{2+}$. Other amino acids generally form dimeric or trimeric clusters with transition-metal ions.

Serine was found to racemize under mild conditions at pH ≈ 6, as shown by a study of 2,3,3-$[D_3]$-L-serine. An aqueous solution heated in a sealed ampoule showed that the 2,3,3-$[D_3]$-L-serine was converted into $[D_2]$-DL-serine. This is evident from the ESI mass spectra in Figure 3, which shows

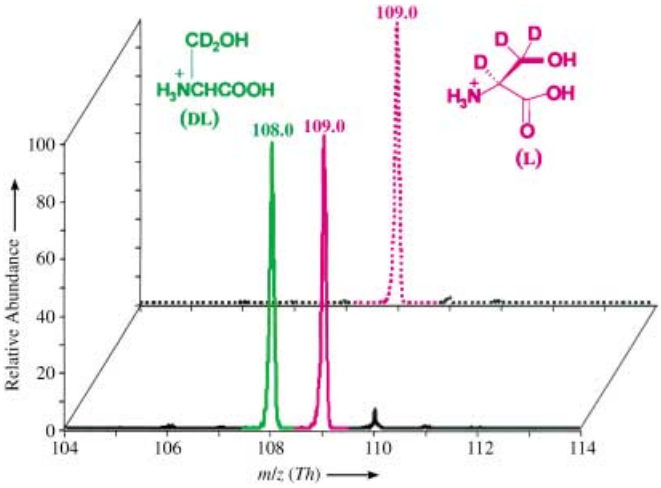

Figure 3. ESI mass spectra of 2,3,3-$[D_3]$-L-serine reference solution (-----; before heating) and a solution of 2,3,3-$[D_3]$-L-serine after heating for 30 h at approximately 160 °C (solid line). Approximately half of the L-serine (——) has racemized (DL, ——), as indicated by the final composition of the sample, about 25 % D- and 75 % L-serine.

peaks arising from the protonated form of 3,3-$[D_2]$-DL-serine at m/z 108 and the remaining protonated 2,3,3-$[D_3]$-L-serine at m/z 109. At shorter times or lower temperatures, conversion was less complete. Confirmation that the product was racemic $[D_2]$-serine was obtained by performing chiral analysis by tandem mass spectrometry[20] and by polarimetry on a L-serine solution heated at 160 °C for 30 h. These experiments showed 24 ± 6 % conversion into D-serine, in agreement with the data of Figure 3. Aspartic acid and threonine were also racemized relatively easily, for example L-threonine showed the formation of 10 ± 3 % of D-threonine under identical conditions. The facile racemization of serine has been previously reported.[21,22] It could be the result of dehydration of the hydroxy group with loss of the hydrogen at the α carbon and formation of a vinylic group. The influence of an external chiral agent upon such a low-energy equilibrium process might have resulted in a preference for D- or L-serine, so allowing symmetry breaking.

Significant features related to modern biochemical systems are displayed uniquely by serine. The other amino acids examined fail to show these types of reactions or do so to a smaller extent and without the tell-tale chiral preferences.

Serine might have separated into its homochiral forms in the course of forming the octamer and its higher clusters in concentrated aqueous solutions; this might then have served as a site for essential prebiotic reactions, which include the binding of C_3 sugars and their dimerization, uptake of phosphoric acid in a form suited to controlled phosphorylation, and transition-metal-ion binding and oxidation. The facile racemization of serine offers a specific chemical process in the context of which symmetry breaking might have occurred.

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