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Thermal Formation of Homochiral Serine Clusters and Implications for the Origin of Homochirality

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Abstract: Spontaneous assembly of amino acids into vapor-phase clusters occurs on heating the solid compounds in air. In comparison to the other amino acids, serine forms clusters to an unusual extent, showing a magic number octamer on sublimation; this octamer can be ionized and characterized by mass spectrometry. Two isomers of the vapor-phase serine octamer are generated, the minor one at 130 °C and the major at 220 °C. The higher temperature cluster shows a strong homochiral preference, as confirmed by isotopic labeling experiments. This serine cluster, like that generated earlier from solution in electrospray ionization experiments, undergoes gas-phase enantioselective substitution reactions with other amino acids. These reactions transfer the chirality of serine to the other amino acid through enantioselective incorporation into the octamer. Other serine pyrolysis products include alanine, glycine, ethanolamine, and small dipeptides, and many of these, too, are observed to be incorporated into the thermally formed serine octamers. Chiral chromatographic analysis confirmed that L-serine sublimation produced DL-alanine, glycine, and ethanolamine, while in the presence of hydrogen sulfide, L-serine yielded L-cysteine. The data demonstrate that sublimation of serine under relatively mild conditions yields chirally enriched serine octamers and that the chiral preference of the starting serine can be transferred to other compounds through cluster-forming chemical reactions.

Introduction

Homochirality is a characteristic feature of living systems, exemplified at the molecular level by the preference for one particular enantiomeric configuration, e.g., L-amino acids. Chirality is also expressed at the supramolecular level, in the selective formation of molecular clusters[1−3] and in the enantioselective self-assembly of homochiral supramolecules.[4,5] Noncovalent clusters of amino acids are readily generated[6,7] by electrospraying solutions of amino acids. The resulting ionic clusters are characterized by electrospray ionization (ESI) mass spectrometry[8] and by tandem mass spectrometry (MS/MS).[9] In the particular case of serine, a self-assembled magic number cluster composed of eight serine molecules is readily generated by electrospray ionization.[6,7,10−19] Studies on the serine octamer have been motivated to some extent by the possibility that serine played a role in the origin of homochirality, a topic that has been reviewed.[20]

The present study focuses on the formation of serine octamers by an alternative method, that of sublimation. It deals with the chiral preferences displayed during this process and with the enantioselective reactions that accompany serine sublimation. The study follows up on a preliminary communication[21] that introduced two methods to generate serine octamers (sublimation of solid serine and dropping an aqueous solution of serine onto a hot surface). The formation of serine octamer by sublimation demonstrates a route to octamer formation that is not associated with spray ionization.

To provide a context for the discussion of the results of the present study, background information on solution-phase spray

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ionization experiments is first reviewed. These experiments used both ESI and the gentler method of sonic spray ionization (SSI).[22,23] Protonated serine octamers are readily generated by ESI of a 0.01 M serine solution. The special stability of the noncovalently bound octamer[6] is evidenced by a strong magic number effect.[10] Another feature that distinguishes this self-assembled cluster from other amino acid clusters is its remarkable preference for homochirality.[6] In addition, (i) chiroselective formation of both positively[6] and negatively[11] charged serine octamers occurs; (ii) two isomeric structures of the protonated octamer have been observed by H/D exchange experiments, the homochiral isomer being the more compact structure;[15] (iii) chiral enrichment accompanies the formation and dissociation of the octamer;[12] and (iv) enantioselective incorporation of other biomolecules into serine octamer occurs[17,18] and provides a mechanism for chiral transmission from serine to other molecules. Multiple analytical techniques have been used in the still incomplete characterization of the homochiral cluster, including isotopic labeling experiments,[7] hydrogen/deuterium (H/D) exchange experiments,[13,14] ion mobility spectrometry (IMS),[15,16] and computations,[15,24,25] as well as a general explanation that invokes entropic driving forces as the source of preferred homochiral aggregation.[19]

Two conformations of the protonated serine octamer are revealed by H/D exchange reaction rates and are designated A and B for convenience.[13,14] Only conformer A displays chiral effects[13] and only this form survives under the conditions of the IMS experiments.[15] On the basis of the H/D exchange data, it has been found that conformer B is more capable of forming metaclusters (namely, higher order clusters-of-clusters, $[Ser_{16}+2H]^{2+}$, $[Ser_{24}+3H]^{3+}$, etc.) than is conformer A.[13] Experimental measurements of the cross-sections of serine clusters show conformer A to have a compact structure with a cross-section of ca. 190 Å.[215] A negatively charged serine octamer, the chloride adduct $[Ser_8+2Cl]^{2-}$, like the protonated analogue, $[Ser_8+H]^+$, shows homochiral preference, cluster stability, and magic-number character, suggesting that the unusual chemical properties of the octamer are intrinsic to the neutral serine cluster.[20]

Two additional striking features of serine have emerged from previous experiments: (i) Cycles of formation and dissociation of the serine octamer have been shown experimentally to lead to enantioenriched serine from nonracemic solutions of serine.[12] Chiral enrichment was demonstrated using tandem mass spectrometry in a purely gas-phase experiment as well as in an ion soft-landing experiment. The increase in chiral purity observed during cycles of octamerization and declustering is proposed to be a simple statistical consequence of the enantioselectivity of serine octamer formation. (ii) Selective transmission of the chirality of serine to other biomolecules occurs during enantioselective incorporation/substitution reactions of other biomolecules into the serine octamer; examples include other amino acids[17,23] and simple sugars, such as glyceraldehydes and hexoses.[18] These characteristic features of serine octamer chemistry have led to the suggestion that its chiroselective self-assembly may have played a role in biochemical evolution.[6,17,18,20,24]

To provide a context for consideration of the sublimation of serine, a systemic study of the thermal clustering of each of the amino acids has been carried out. These studies confirm the special stability of the serine octamer. The protonated serine octamer formed by sublimation was investigated and its behavior was found to parallel that of the corresponding cluster ions generated by spray ionization. The important characteristics of the protonated serine octamer, for instance, the stability, the homochirality, and reaction with chiroselective incorporation of other amino acids, were all confirmed. As in the spray methods of octamer formation, two isomers of the protonated serine octamer were observed (at different sublimation temperatures) and differentiated by their H/D exchange behavior. Their homochiral preferences were investigated through comparisons between enantiomeric and racemic samples and through isotopic labeling experiments. Their substitution reactions with other amino acids were investigated as well. Additional products from pyrolysis of serine were seen, including previously reported[26,27] alanine, glycine, and ethanolamine, while L-cysteine was formed from sublimation of serine when the experiment was performed in the presence of hydrogen sulfide.

Experimental Section

Materials. All amino acids, including arginine, histidine, and lysine in the hydrochloride forms, were purchased from Aldrich-Sigma (St Louis, MO) and powdered using a mortar and pestle before conducting the heating experiments. Isotopically labeled L-serine-2,3,3-d_3 (indicated here as L*-serine or L*-Ser) with a nominal 98% isotopic purity was purchased from Cambridge Isotope Laboratories (Andover, MA). Samples used in isotope experiments were prepared by dissolving, evaporating, drying, and powdering equimolar amounts of L*-serine and L- or D-serine. Samples prepared for studying the incorporation of other amino acids into the serine octamer were physical mixtures of the L-amino acid with L- or D-serine in the range of mole ratio amounts from 1:1 to 1:20. For the ESI and SSI experiments, 0.01 M amino acid solutions were prepared using a methanol/water/acetic acid (50/50/1) mixture as the solvent.

Thermal Formation of Amino Acid Clusters. Thermal formation of amino acid clusters was achieved using a soldering gun with a flat tip as a small hot plate from which the amino acid was sublimed (Figure 1a). The flat tip was 1 cm from a corona discharge needle placed near the inlet of an LTQ mass spectrometer (Thermo Electron Inc., San Jose, CA). In each experiment, 0.02 g of sample was loaded onto the flat tip and then the temperature was increased at a rate of 2 °C/s from 30 to 300 °C (except for those experiments involving the investigation of the formation of the two isomers as a function of heating rate). The sublimation products were ionized by corona discharge and then analyzed using the linear ion-trap mass spectrometer. Yields of ions measured from the ion current reaching the mass spectrometer on heating solid serine were 0.1% of the theoretical maximum. The corona discharge atmospheric pressure chemical ionization and thermal sublimation system is abbreviated as sublimation/APCI in this paper. The temperature of the hot plate was monitored by a microprocessor thermometer (model HH23, Omega Engineering Inc. Stamford, CT) with a T-type thermocouple attached. The instrumental settings employed included values of the discharge voltage (5.0 kV) and the heated capillary temperature (50 °C) optimized for maximum ion abundance of the serine octamer from pure L-serine. This temperature

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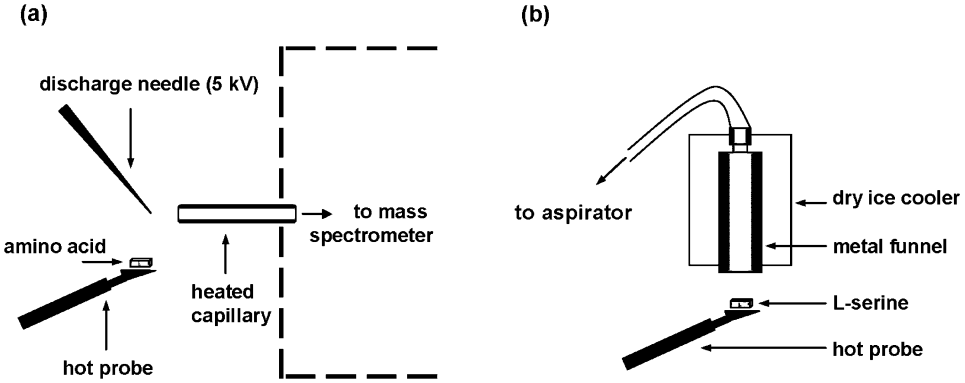

Figure 1. Apparatus used for (a) corona-discharge atmospheric pressure chemical ionization (sublimation/APCI) and (b) sublimate collection.

was raised from 50 to 150 °C to examine the stability of the formed clusters. Cysteine formation experiments used hydrogen sulfide gas flowing at 10 mL/min through a metal tube placed about 3 mm above the hot plate.

Formation of Amino Acid Clusters Using Electrospray and Sonic Spray. A conventional electrospray ionization (ESI) source[28,29] and a homemade sonic spray ionization (SSI) source[23] were used as spray methods to form amino acid clusters from solution for comparison with those generated from the solid amino acids by sublimation/APCI. Figure s1 of the Supporting Information shows schematic diagrams of these sources. All instrumental parameters were optimized for maximum ion abundance of the protonated serine octamer and then used in the cases of all the other amino acids. Typically, a heated capillary temperature of 100 °C and a sample flow rate of 1 μL/min were used for both ESI and SSI. An ionization voltage of 4.5 kV was used for ESI, and a nitrogen nebulizing gas pressure of 1.5×10^4 Pa was used for ESI and 1.2×10^6 Pa was used for SSI.

Collection and Analysis of Sublimate. As shown in Figure 1b, a cooling funnel was set up to collect sublimation products, using a gas flow adapter (parts for a check valve, SSI-02-0129, Scientific Systems, Inc./LabAlliance, State College, PA) as a metal gas trap and a plastic cup as a dry ice reservoir. The flat tip of the soldering gun was 1 cm below the bottom of the cooling funnel (1.0 cm through hole). The top of the gas flow adaptor (0.2 cm through hole) was connected to a water pump to draw the sublimation products into the cooling funnel. Each collection process included 20 cycles of cleaning the hot plate, loading 0.02 g of sample, heating from 30 to 250 °C, and then cooling the probe back down to room temperature. During the entire process of sample collection (typically 90 min), dry ice was added as needed into the plastic cooler to trap the sublimation products efficiently on the inner surface of the metal funnel. The collected sublimate was washed from the metal funnel with 0.5 mL of distilled water three times for GC−MS analysis. The same apparatus and procedures were used to produce cysteine by the reaction of serine with hydrogen sulfide gas.

The collected solution was evaporated to dryness in a stream of nitrogen gas at room temperature. The trapped amino acids were converted to their *N*-(*O*)-pentafluoropropionyl(PFP)-2-propyl esters by treatment with 2.5 M HCl in 2-propanol (0.8 mL) followed by acylation with pentafluoropropionic anhydride (0.1 mL) in CH_2Cl_2 (0.6 mL). Excess reagents and solvents were removed using a stream of nitrogen, CH_2Cl_2 (0.2 mL) was added, and 0.1-μL aliquots of the samples were analyzed by GC−MS.[30]

A GCQ ion-trap mass spectrometer (Thermo Electron Inc., San Jose, CA) was used for these analyses. Chromatographic data and mass spectra were acquired using Xcalibur 1.0 software (Thermo Finnigan). The silica column installed originally was replaced by a CP-Chirasil-L-Val fused silica capillary column (25 m × 0.25 mm i.d.; Varian, Walnut Creek, CA). The carrier gas was helium at an inlet pressure of 80 psi. The injector temperature was 220 °C and the detector temperature was 250 °C. The temperature and pressure programs were optimized for chiral separations of racemic serine and other amino acids. The acquisition of the mass spectrum was set to a delay of 7 min for the solvent elution.

Results and Discussion

Thermal Formation of Amino Acid Clusters. Sublimation of amino acids in air was examined by ionization of the vapor-phase sublimate using corona-discharge atmospheric pressure chemical ionization (sublimation/APCI) (Figure 1a). The temperature was ramped from 30 to 300 °C in 130 s to examine the sublimation of powdered amino acids by mass spectrometry. Typical mass spectra acquired from the sublimation of threonine, proline, and isoleucine at 230, 200, and 210 °C, respectively, are shown in Figure 2, illustrating that cluster formation dominates the observed products of sublimation. The absence of doubly charged odd-mass ions and the unit *m*/*z* distance between the isotope peaks indicate that all peaks in these mass spectra represent singly charged ions. These initially formed clusters, at least in the cases of serine, threonine, alanine, and isoleucine, are neutral because the associated protonated ions only appear in the mass spectra when the voltage on the corona-discharge needle is switched on. Data showing the relative ion abundances for all the amino acid clusters are listed in Table 1.

Almost all amino acids form noncovalent clusters in the sublimation/APCI process. More than half the amino acids form trimers or larger clusters and only a few thermally fragile amino acids (e.g., arginine and glutamic acid) are not observed to form noncovalent clusters. Moreover, the highest ion abundances in the mass spectra are usually associated with the multimers, not the monomers. For instance, the protonated forms of Thr_8, Pro_4, and Ile_4 are particularly abundant in Figure 2. It was also observed that the optimum clustering temperatures for all amino acids fall in a narrow range near 200 °C, while their melting points (listed in Table 1) are higher and cover a much wider range of ~100 °C.[31] The fact that amino acids have such similar clustering temperatures suggests that physical mixtures might coassemble to form mixed molecular clusters as is discussed further below.

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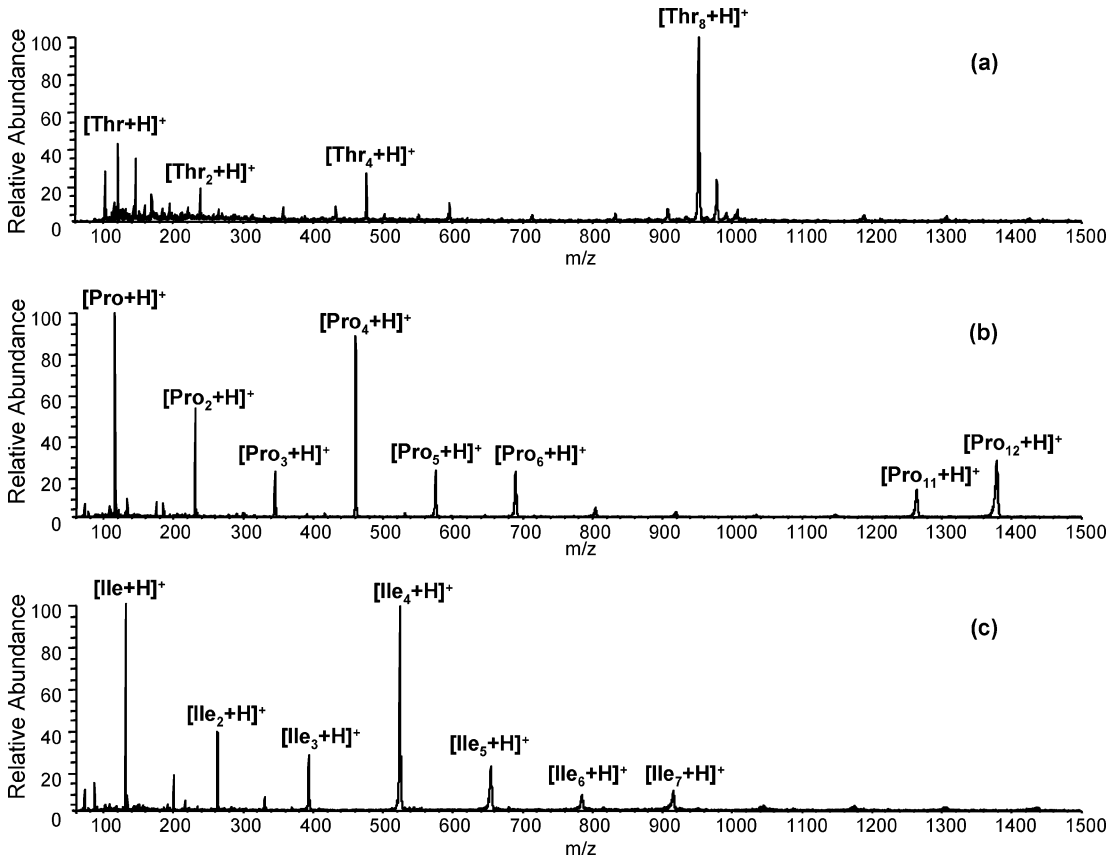

Figure 2. Sublimation/APCI mass spectra of (a) L-threonine at 230 °C, (b) L-proline at 200 °C, and (c) L-isoleucine at 210 °C, respectively.

Table 1. Clustering of Amino Acids via Sublimation/APCI (Magic Number Clusters Are Italicized)

			relative abundance of protonated clusters observed showing size *n*											
amino acids	optimum clustering temp (°C)	melting/dec temp (°C)	1	2	3	4	5	6	7	8	9	10	11	12
Gly	205	240	5	100	23									
L-Ala	200	295−300	57	100	63	35	19							
L-Val	205	295−300	100	81	29	*77*	11							
L-Leu	210	>300	100	63	14	19								
L-Ile	210	288	100	38	27	*99*	22	8	10					
L-Met	230	284	100	44	7	*31*								
L-Phe	200	270−275	100	59		6								
L-Trp	240	280−285	100	27										
L-Pro	200	228	100	53	22	*89*	23	22	5				13	*28*
L-Ser	220	222	38	50						*100*				
L-Thr	230	256	42	18	8	*26*	10			*100*				
L-Cys	200	220	100	56	5									
L-Tyr	230	>300	100	14										
L-Asn	205	235	100	38	16									
L-Gln	200	185	100											
L-Lys	210	215	100	42	29	29								
L-Arg	−	222												
L-His	220	282	100	9										
L-Asp	240	>300	100	8										
L-Glu	−	205												

Magic Number Clusters. Mass analysis of the sublimation products of each of the 20 amino acids confirmed the ready thermal formation of noncovalent clusters. Of even more interest are clusters of unusual stability, which can be expressed using the magic number factor, $(I_n^2)/(I_{n-1}I_{n+1})$, where I represents the signal intensity and n represents the number of components. Experimental results[10] and molecular dynamics calculations[32] for other systems make it clear that large magic number factors are associated with clusters of greater stability than neighboring clusters.

The ion abundances of positively charged clusters of each of the coding amino acids are listed in Table 1 and all magic number clusters are italicized. In this analysis, magic number clusters are defined by magic number factors of 4 or more.[33] Only nine of the 68 clusters are magic number clusters by this

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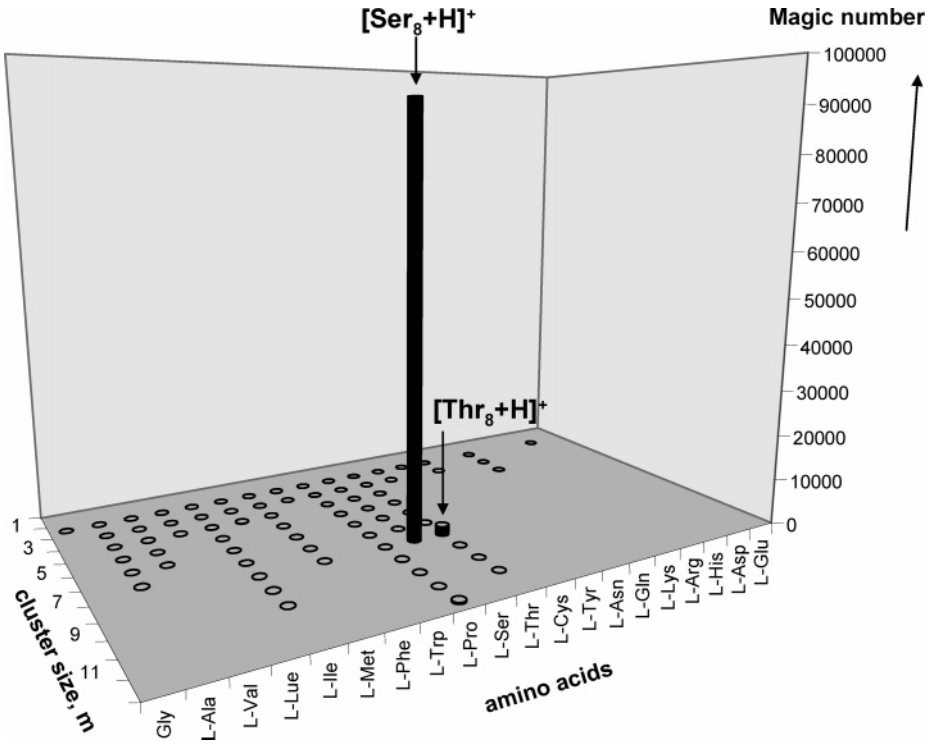

Figure 3. Magic number factor for amino acid clusters formed by sublimation. [All amino acid clusters are plotted as their magic numbers corresponding to $I_n^2/(I_{n-1}I_{n+1})$, where I is the intensity of the cluster and n is the cluster size.]

definition. They are all even-number clusters, either dimers, tetramers, octamers, or dodecamers, suggesting that the dimer might often be the basic unit used to build up magic number amino acid clusters.[6] Among the nine magic number clusters, the serine octamer is in a class by itself due to its extremely high magic number factor, as shown in Figure 3, in which the magic numbers of all the amino acid clusters are plotted. The extraordinary stability of the serine octamer in the gas phase is not only demonstrated by the very large magic number factor (91 080, although the exact numerical value is a strong function of experimental conditions), but it is also evidenced by the fact that this is the only amino acid cluster that does not decrease greatly in relative and absolute ion abundance when the heated capillary temperature in the MS interface is raised from 50 to 150 °C (Table s1 and Figure s2 of the Supporting Information, respectively).

As the natural amino acid most closely related to serine, threonine shows similar features to serine when heated, although these are displayed to a much smaller extent. The sublimation/APCI mass spectra (Figures 2a and 4c) are similar and the threonine octamer also displays a strong magic number factor (2030). This octamer could not be observed under normal ESI conditions but it was readily seen under the milder conditions of sonic spray ionization (SSI, an alterative to ESI).[22,23] The observation of octameric clusters in the course of sublimation of serine and threonine, both β-hydroxyl amino acids, reflects the fact that self-assembly of amino acids occurs before or during the sublimation process and is associated with particular molecular structures. Serine represents an extreme case among the smaller amino acids in that it has four groups capable of hydrogen bonding. It is not surprising that it is the most "sticky" of all amino acids, as shown by its strong clustering behavior. Proline is another amino acid for which interesting magic number clustering has been reported by ESI. Significantly, nanoscale clusters of proline have been shown to display chiral selectivity, with enantiopure solutions yielding elongated organized structures,[34] while proline dodecamers have been suggested to have stable dodecahedral structures.[35] Under the conditions of our ESI and sublimation experiments, proline does not cluster to the extent that serine does although conditions can be found in which the 12-mer dominates the spectrum of the sublimate.

Two Isomers of Serine Octamer. As seen in Figure 3, the very high magic number factor of the serine octamer suggests that it is highly stable in the gas phase. Figure 4 shows thermal self-assembly of serine recorded by examining mass spectra at 190, 200, and 220 °C during a single temperature ramp. Clearly, protonated serine clusters, including the monomer, dimer, and octamer, are dominant features over quite a large temperature window, viz. from at least 190 to 220 °C. Indeed, the serine octamer is still visible even above 240 °C. Surprisingly, at such high temperatures while products of pyrolysis occur (the decomposition point of serine is 222 °C[31]) the ionized sample is still dominated by serine clusters. As shown in Figure 4c, the protonated serine octamer contributes more than half the total observed ion current when serine is sublimed and ionized in air at ca. 220 °C. The remarkable fact is that, under these nonequilibrium conditions, serine has a strong and increasing tendency to associate (or remain associated) rather than to dissociate with increasing temperature, as evidenced by the increased absolute abundance of serine octamer as well as its increased relative abundance.

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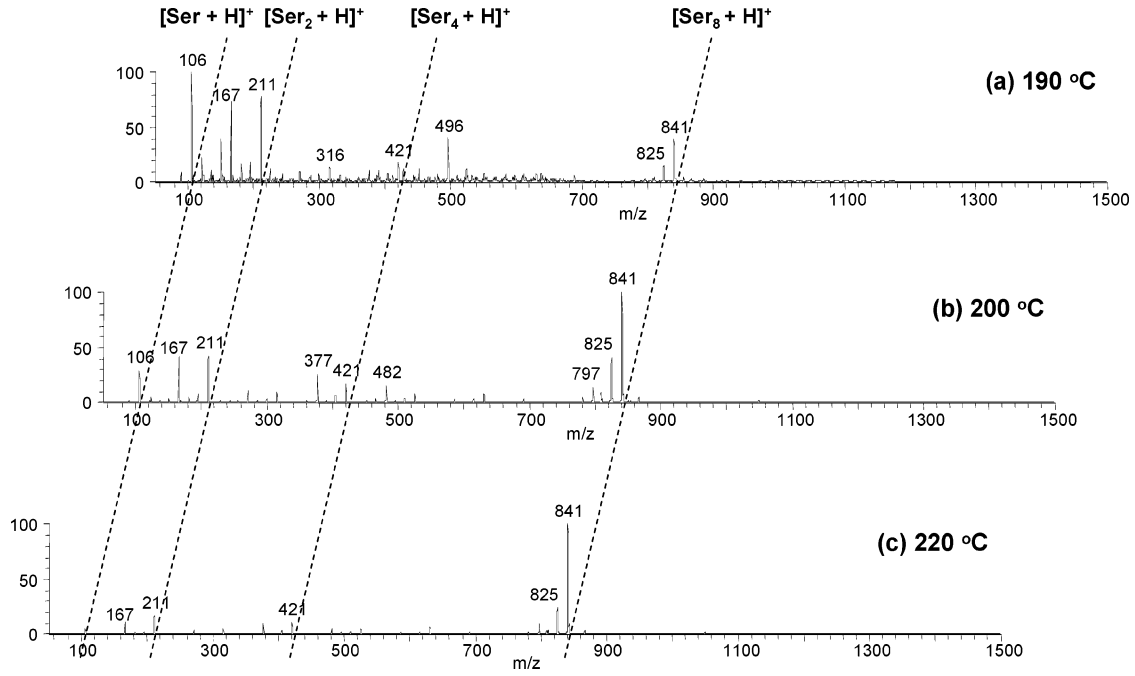

Figure 4. Sublimation/APCI mass spectra of L-serine at (a) 190 °C, (b) 200 °C, and (c) 220 °C.

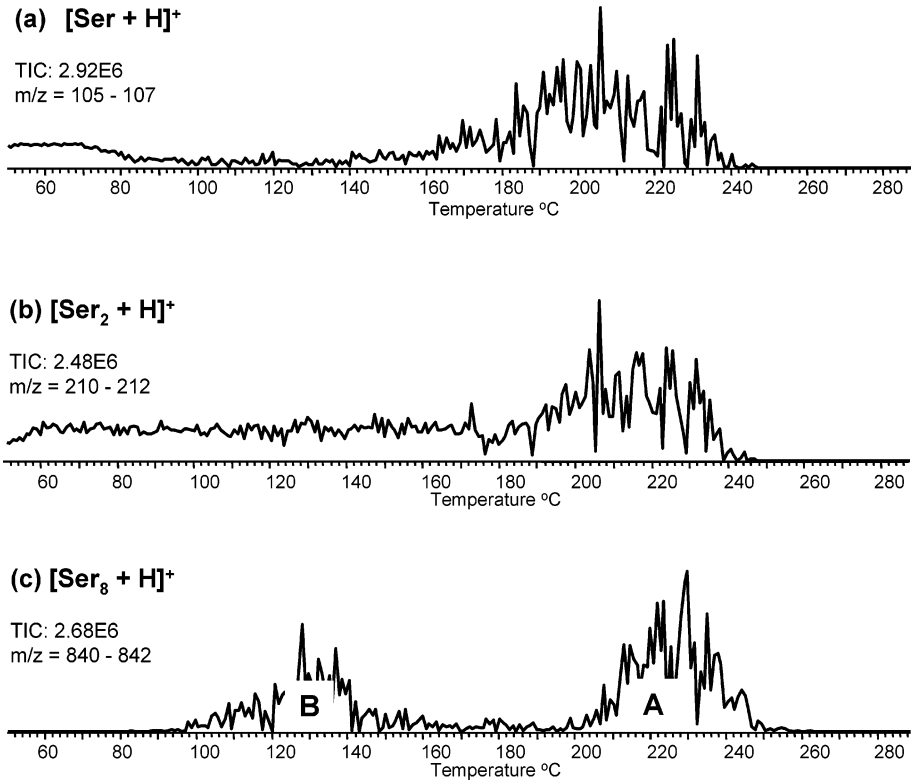

Figure 5. Ion chronogram of the protonated serine clusters: (a) serine monomer ($[Ser+H]^+$), (b) serine dimer ($[Ser_2+H]^+$), and (c) serine octamer ($[Ser_8+H]^+$), generated by sublimation of L-serine.

As shown in Figure 4, the relative ion abundances of the three protonated serine clusters (monomer, dimer, and octamers) vary with temperature. The abundances of particular clusters are plotted as a function of the hot plate temperature in Figure 5. It is noteworthy that two populations of serine octamer, denoted as population A and B, appear in nonoverlapping temperature ranges. While population B appears and reaches its maximum at quite a low temperature, ~130 °C, population A appears at a relatively high temperature ~180 °C, and reaches its maximum at ca. 220 °C. The relative amounts of the two isomers are dependent on the heating rate. As shown in Figure s3, isomer B only appears when using heating rates from 1.0 to 2.5 °C/s; it is not observed at lower or higher heating rates. When the heated serine solids were reheated after cooling, serine octamers were not generally observed unless larger samples were employed.

The two populations of the serine octamer appear to correspond to the two populations formed during the spray process,

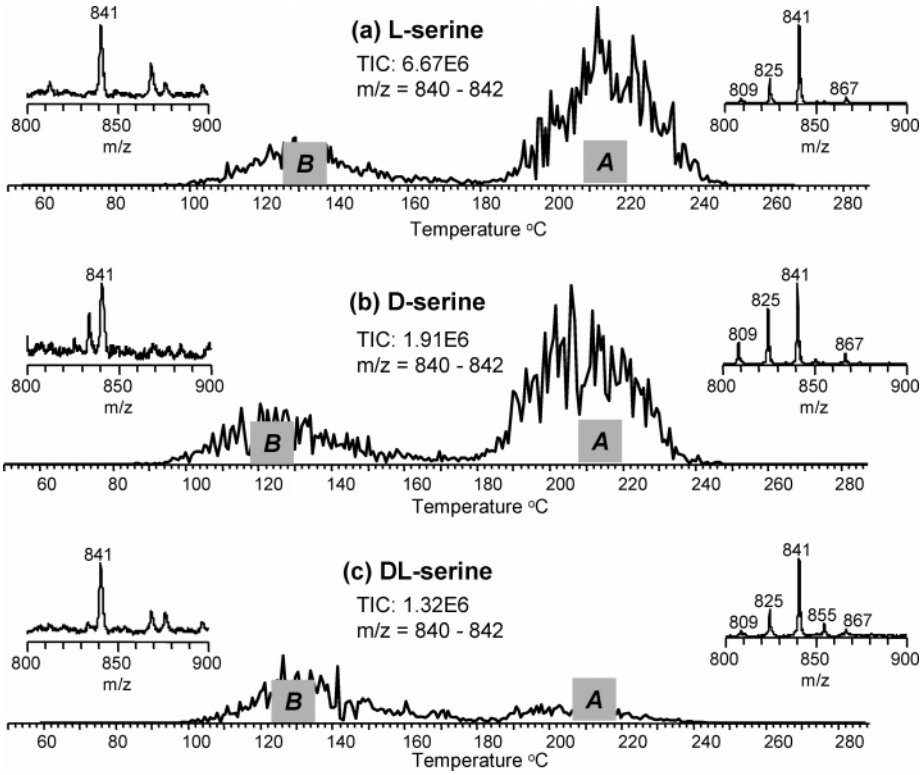

Figure 6. Ion chronogram of serine octamer, $[Ser_8+H]^+$, generated from (a) L-serine, (b) D-serine, and (c) DL-serine, and the corresponding mass spectra of each population.

as inferred from H/D exchange results; this suggests that one of the two structures (A) is comprised of a homochiral collection of serine molecules.[13,36] The examination of the behavior of enantiomeric serine and racemic serine shows that two populations with similar temperature profiles are indeed generated from enantiopure samples of D- and L-serine (Figure 6). However, the racemic sample forms the higher temperature octamer (A) to a much smaller extent. Population A is much more favored by the enantiopure samples, while formation of population B occurs approximately independently of the sample chirality. The tentative conclusion is that population A is a cluster of homochiral serine molecules, and this is confirmed by the additional experiments described below.

The inset spectra included in Figure 6 show the mass spectrum of each population of octamers. In the mass spectra of population A, several adjacent peaks appear consistently along with the peak due to the serine octamer (*m*/*z* 841). These ions are derived from serine octamers with one or more serine molecules being substituted by a pyrolysis product (the formation of which will be discussed further in the pyrolysis reaction section). By contrast, no such substitution peaks are shown in mass spectra of population B. It seems clear that the two populations do indeed represent serine octamers with different structures and that the high-temperature form, population A, is homochiral and can undergo molecular substitution reactions.

Further evidence regarding the structures of the two octamer populations was obtained from isotopic labeling experiments. L*-Ser was mixed with an equimolar amount of L-serine or D-serine, followed by dissolving, evaporating, drying, and powdering to prepare partially isotopically labeled enantiomeric or racemic serine samples. Figure 7 shows the ion chronograms for these labeled samples recorded over the range of *m*/*z* 835−870, which covers all isotopic forms of the octamers. Mass spectra of each population are shown in Figure 7 as well, revealing the combination patterns of mixed serine samples. Consistent with the observation in Figure 6, different behaviors of the two populations were observed in the ion chronograms, and again the formation of the high-temperature octamer A is strongly favored from the labeled homochiral sample. The zoom-in mass spectra of population A show that the homochiral mixture of L-Ser/L*-Ser produces a roughly Gaussian distribution of labeled octamers, while the heterochiral mixture of D-Ser/L*-Ser gives serine octamers in population A as a bimodal distribution. The bimodal distribution is a result of the predominant formation of two homochiral octamers, one unlabeled and centered on *m*/*z* 841 and the other labeled and centered at *m*/*z* 865. The symmetrical distribution matches the statistical binomial prediction.[12] Whether generated from a homochiral or heterochiral mixture, population B gives a bell-shaped distribution of ion abundances in the inset mass spectra in Figure 7, demonstrating the lack of chiral preference during the assembly of eight serine molecules into this particular cluster. This behavior confirms the assertion that the two populations represent two structurally different serine octamers. Both racemic and, to a much greater extent, isotopically labeled serine yield the high-temperature octamer (octamer A), which by inspection of the isotopic distribution consists of a mixture of predominantly D- and predominantly L-octamers in equal amounts. The high enantiomeric purity of these octamers is

(36) Mazurek, U.; McFarland, M. A.; Marshall, A. G.; Lifshitz, C. *Eur. J. Mass Spectrom.* **2004**, *10*, 755−758.

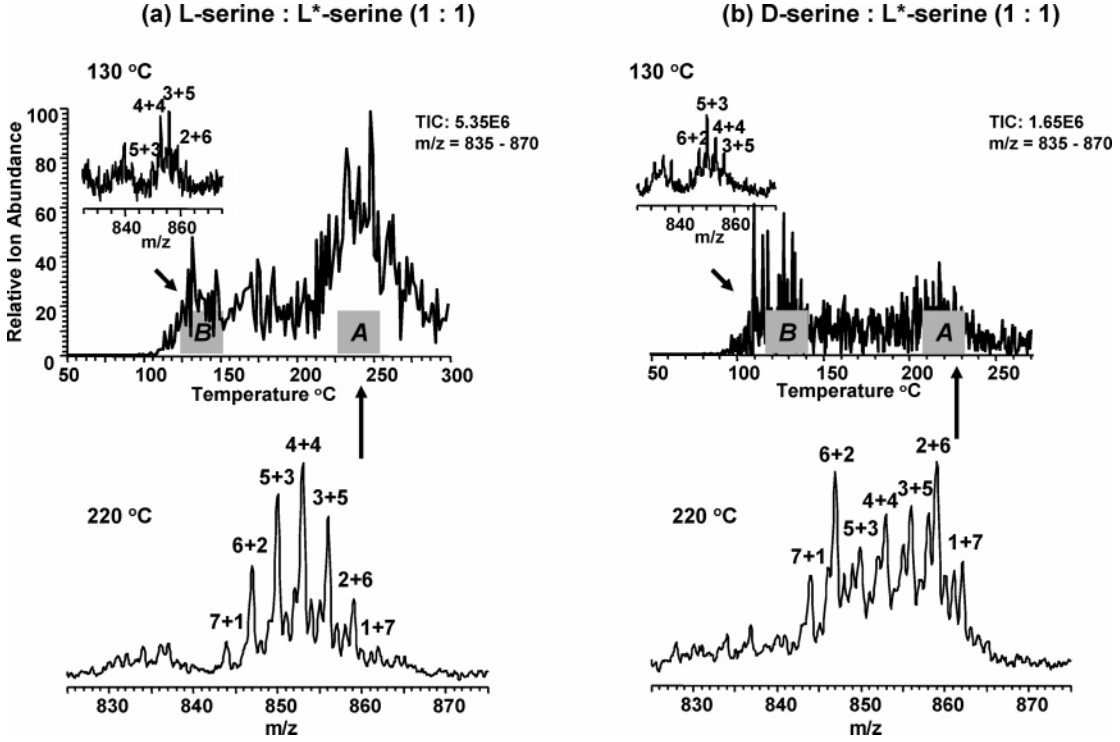

Figure 7. Ion chronogram of serine octamer (*m*/*z* window 835−870), generated from (a) the homochiral mixture (L-Ser/L*-Ser) and (b) the heterochiral mixture (D-Ser/L*-Ser), and the corresponding mass spectra. The clusters are labeled by their component units (no. of L/D-serine + no. of L*-serine). (Note: L*-Ser is isotope labeled L-Ser-2,3,3-d_3.).

shown by comparison with calculated distributions reported in ref 12.

The homochiral population A corresponds to the homochiral isomer A of the earlier spray experiments,[13] where the chiral structure A was found to undergo slower H/D exchange and to be more compact. The earlier work also showed it to be this isomer that undergoes chiroselective reactions,[17,18] and the present results confirm this. The correspondence in temperature ranges over which octamer A, the monomer, and the dimer are formed (Figure 5) suggests two possible mechanisms for formation of serine octamer A. Either it is a product of crystal disintegration into clusters of random sizes, the most favorable of which survive the high-temperature conditions of their formation, or it is the result of coalescence of smaller units at this temperature. The former explanation is in agreement with earlier suggestions regarding serine cluster formation from the crystal.[7] It is also preferred, since there is good evidence that isomer A is zwitterionic and it is well-known that amino acids exist as zwitterions in the solid state.[37−40] The ionic bonds take more energy to break and so isomer A appears at the high-temperature range. By contrast, population B might be composed of nonzwitterionic ("neutral") units, which is consistent with their H/D exchange reactions,[13,14] and be released directly from the crystal at a low temperature, but we have little evidence on this point. (The observation of neutral forms of simple α-amino acids in the solid state has been reported recently at a low temperature.[41])

Amino Acid Chiral Incorporation into the Serine Octamer. Chiral transmission is a process whereby the chirality of one molecule is transmitted through chemical reaction to other molecules. Previous studies on the serine octamer have provided direct evidence from ESI-MS[6] and SSI-MS[23] that several biologically important molecules can be incorporated into the octameric structure via molecular substitution reactions.

In an attempt to gain more insight into the chiral selectivity of the reactions of serine octamers, we studied the incorporation of other amino acids into the serine octamers in the sublimation process. Solid samples of D- or L-serine and another L-amino acid were physically mixed to avoid the more intimate mixing that might occur when drying a solution before sublimation. Of the 19 chiral coding amino acids, threonine is the best candidate for substitution reactions with the octamer, due to its structural similarity to serine. Following the same organization used in Figure 7, Figure 8 shows the ion chronograms of the protonated serine/threonine octamer, $[Ser_4Thr_4+H]^+$, from the homochiral mixture of L-Ser/L-Thr and the heterochiral mixture of D-Ser/L-Thr and their responding mass spectra. Interestingly, only one population appears in the ion chronograms, the temperature window of which matches that of the homochiral serine octamer, isomer A. Such correspondence in the ion chronograms with a single population implies that the mixed cluster, $[Ser_4Thr_4+H]^+$, may arise from a facile substitution reaction between serine octamer (A) and threonine; the same conclusion can be reached from the near-absence of isomer A in the ion chronograms of serine octamer, $[Ser_8+H]^+$ (Figure s4 of the Supporting Information). The mass spectra in the region of the octamer ions also show strong homochiral preference in the formation of serine/threonine octameric

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(38) Moreno, V.; Dittmer, K.; Quagliano, J. V. *Spectrochim. Acta* **1960**, *16*, 1368−1381.
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(41) Gomez-Zavaglia, A.; Fausto, R. *Phys. Chem. Chem. Phys* **2003**, *5*, 3154−3161.

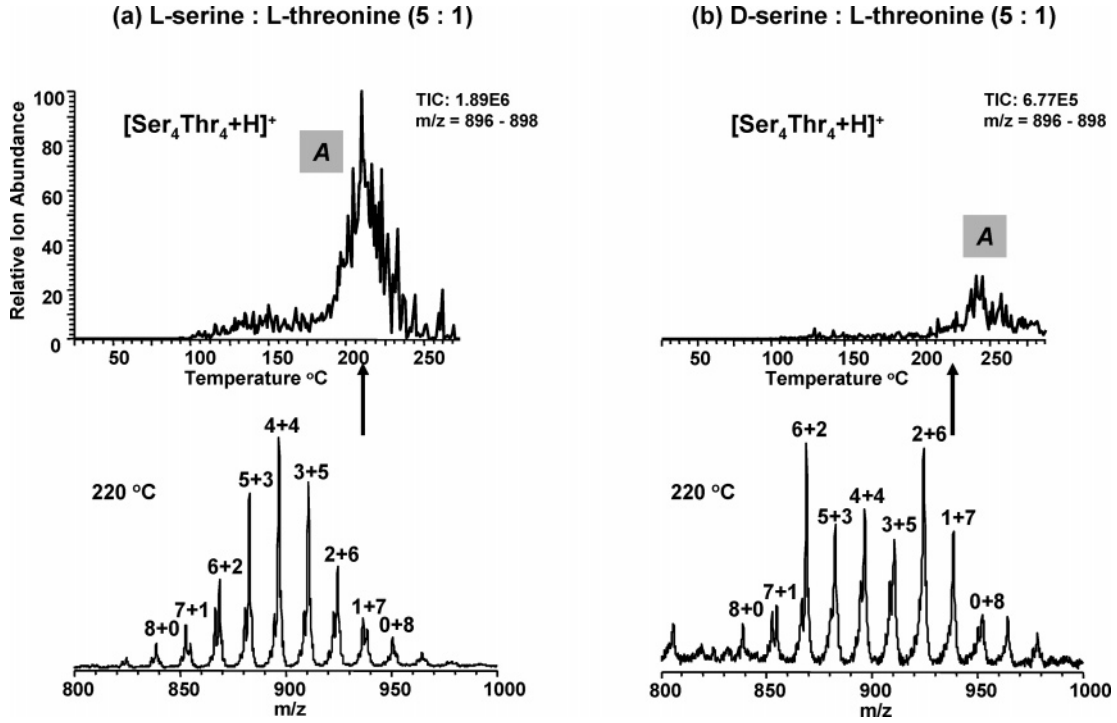


Figure 8. Ion chronogram of the serine/threonine octamer, $[Ser_4Thr_4+H]^+$, generated from (a) homochiral mixture (L-Ser/L-Thr) and (b) heterochiral mixture (D-Ser/L-Thr), and the corresponding mass spectra. The clusters are labeled by their component units (no. of L/D-serine + no. of L-threonine).

clusters. The mass spectrum in Figure 8a shows that threonine with the same chirality is incorporated very efficiently into serine isomer A, in agreement with the statistically binomial prediction. By contrast, the heterochiral mixture shows little tendency to generate the $[Ser_4Thr_4+H]^+$ ion, and even when this does occur, the mass spectrum shows the bimodal distribution characteristic of two enantiomerically homogeneous populations. By contrast, for cluster B, the identical mass spectra (Figure s4) recorded for the homochiral and heterochiral mixtures illustrate that there is no chiral preference for serine to construct B-octamers with threonine. The results imply not only that homochirality dominates the self-assembly of serines to form isomer A but also that the incorporation of threonine into serine ocatmers is chiroslective and limited to isomer A. The same conclusion can be drawn from the results of experiments with cysteine and tryptophan (see Figures s5 and s6 of the Supporting Information).

Investigation of the incorporation of other amino acids into the homochiral serine isomer A was undertaken, and the results are summarized in Table 2.[42] In total, 13 of the 19 amino acids examined could be incorporated into the homochiral serine octamer by this simple heating experiment. Even some amino acids with large side chains are readily incorporated, including phenylalanine and tryptophan. Of the 13 amino acids (nine of which besides serine are considered primitive amino acids), the six amino acids italicized in Table 2 (leucine, isoleucine, tryptophan, threonine, cysteine, and lysine) show the clearest examples of chiral preference on coassembly with serine into an octamer. The poor incorporation observed in the cases of arginine, glutamine, and glutamic acid (all relatively fragile or highly polar amino acids as indicated by their low decomposition temperatures, Table 1) can be explained by their thermally induced decomposition before or during the formation of serine octamer rather than any inefficient coassembly with serine into octamers.

Pyrolysis Reactions. It has been noted that formation of octamers of serine is the dominant process observed when the solid is heated gently. However, some pyrolysis reactions accompany the formation of isomer A as the temperature approaches the decomposition point of serine (222 °C[31]). Several pyrolysis products of L-serine are evident in Figure 9a at 220 °C, including glycine, dehydroserine, alanine, and ethanolamine (EA). They were collected using the apparatus shown in Figure 1b and identified by GC−MS analysis (Figures s7 and s8 of the Supporting Information). It was previously known that glycine and alanine can be generated thermally from serine.[26,27]

As shown in Figure 9b, pyrolysis products are incorporated into isomer A by substitution for one or two serine molecules; these products include $[Ser_6Gly_2+H]^+$ with *m*/*z* 781, $[Ser_7EA_1+H]^+$ with *m*/*z* 797, $[Ser_6Ala_2+H]^+$ with *m*/*z* 809, and $[Ser_7Ala_1+H]^+$ with *m*/*z* 825. Note that although most of the observed alanine was present in these octameric assemblies with serine, a small amount occurred in the form of the monomer. This could be either due to the very efficient substitution of alanine into the serine octamer A, after alanine was generated from serine, or to chemical reactions of serine octamer transforming serine units to alanine. The former explanation seems improbable, because alanine forms clusters at a lower temperature than serine octamer does (Table 1). In addition, mixtures of alanine and serine form homoclusters (Figure s9 of the Supporting Information) and the fact that similar amounts of alanine and serine are produced on serine sublimation (Figure s7 of the Supporting Information) suggests that such mixed clusters should occur from the separate amino acids. Therefore, it is highly likely that

(42) The mole ratio between serine and amino acid was varied from low to high (1:1, 5:1, 10:11, and 20:11) to achieve the protonated serine octamer and substituted octamer peaks clearly and simultaneously.

Table 2. Relative Abundances of Substituted L- and D-Serine Octamer Ions

	no. of incorporations																	
	into L-serine octamer									into D-serine octamer								
amino acid[e]	0	1	2	3	4	5	6	7	8	0	1	2	3	4	5	6	7	8
Gly[b,f]*	100	56	39	12														
L-Ala[a]*	40	82	100	50	16					59	98	100	56	22				
L-Val[a]*	11	34	100	32	16					24	49	100	36	15				
L-*Leu*[a]*	34	53	61	70	100	26	17			40	99	100	19	10				
L-*Ile*[a]*	20	34	49	60	100	57	31	22	18	100	25	52	40	55	16	16	8	
L-Met[a]	4	17	100	64	38	8				10	24	100	51	49	7			
L-Phe[a]	100	71	35							100	80	37						
L-*Trp*[d]	100	72	18							100	20							
L-Pro[a]*	6	22	100	27	5					6	16	100	21	17				
L-*Thr*[d]*	6	13	18	25	64	100	91	54	28	19	36	100	29	32	16	16	9	6
L-*Cys*[a]	100	56	18							100	19							
L-Tyr[d]	100									100								
L-Asn[d]*	100	11								100	24							
L-Gln[b]	100									100								
L-*Lys*[d]*	100	35	10							100	9							
L-Arg[d]	100									100								
L-His[c]	100									100								
L-Asp[c]*	100									100								
L-Glu[b]*	100									100								

[a–d] The molar ratio between serine and amino acid is [a]20:1, [b]10:1, [c]5:1, and [d]1:1. [e] Italic amino acids show chiral recognition on assembly with serine. [f] The primitive amino acids are labeled with an asterisk.

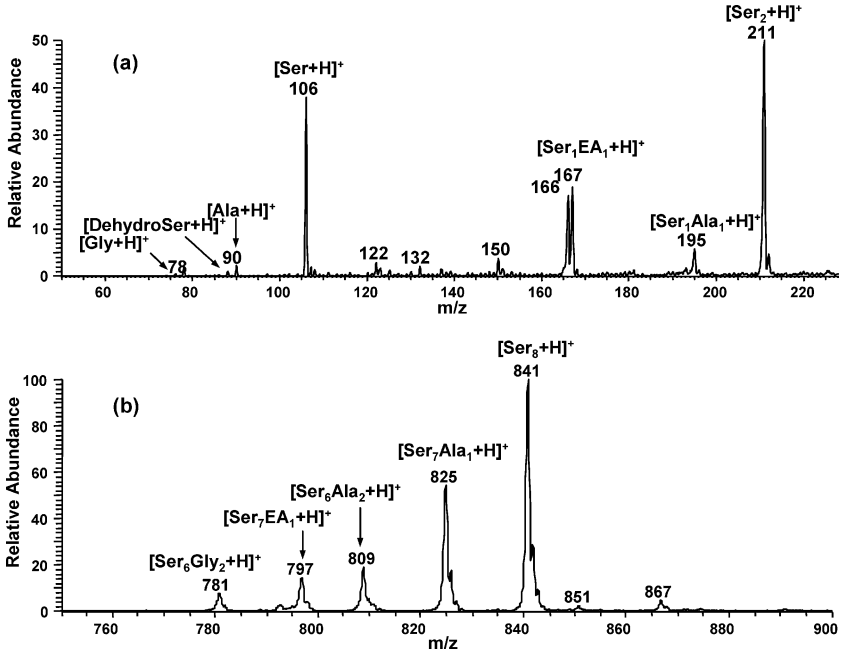


Figure 9. Sublimation/APCI mass spectra at 220 °C of L-serine: (a) low *m*/*z* range (b) high *m*/*z* range (EA represents ethanolamine).

alanine was generated within the serine octamer with the preservation of the octameric structure.

If isomer A indeed plays a key role in the transformation of chirality from serine to other amino acids, the chirality of the generated amino acid may be controlled by that of the serine octamer. This is the case at least when hydrogen sulfide gas (H_2S) was present during the sublimation of serine, and cysteine was formed.[43] The data in Figure 10a show that cysteine was generated and is incorporated into a mixed octamer with serine, just like alanine. GC−MS data confirmed the chiral specificity of the chemical reactions involved, showing that pure L-cysteine is produced in this reaction (Figure 10b). The serine octamer mediates the course of gaseous chemical reactions occurring during serine sublimation and controls the chirality of the products of serine sublimation. However, it is not known why the generated cysteine is enantiopure while the generated alanine is racemic.

The conditions used to effect sublimation also favor dehydration, the occurrence of which is evident from the fact that peptides are generated in the course of L-serine sublimation (Figure 11). Dehydration reactions between nonracemic serine (L-serine more than D-serine) and racemic alanine yield four dipeptides, D-Ser-D-Ala, L-Ser-D-Ala, D-Ser-L-Ala, and L-Ser-L-Ala. These compounds appear with increasing gas chromatographic retention times and give identical mass spectra.

(43) Wiebers, J. L.; Garner, H. R. *J. Biol. Chem.* **1967**, *242*, 5644−5649.

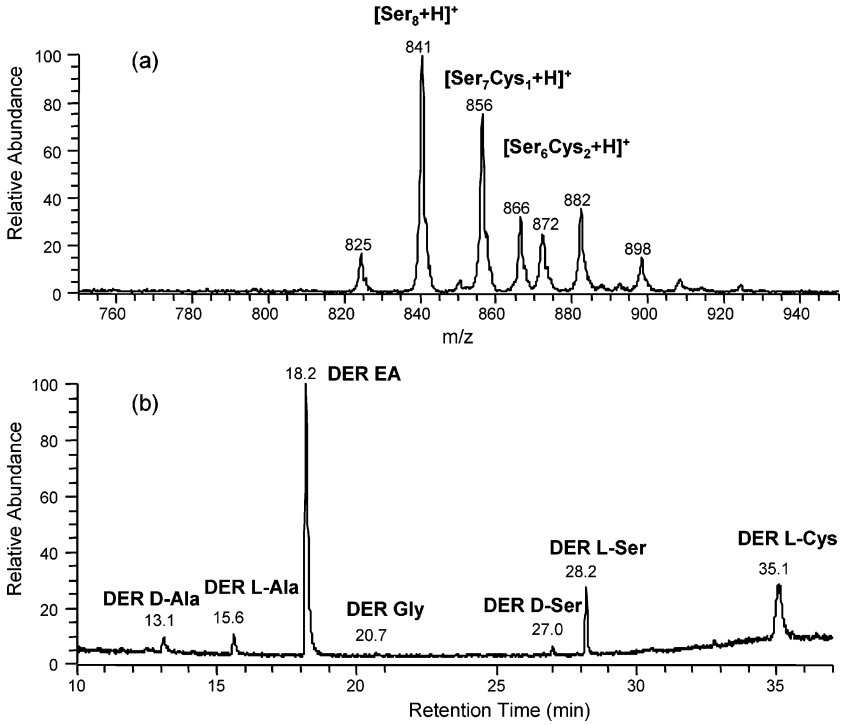


Figure 10. (a) Sublimation/APCI mass spectrum at 220 °C of L-serine with H_2S gas flow and (b) gas chromatograph of the derivatized pyrolysis products from the thermal sublimation of L-serine in the presence of H_2S. DER refers to the *N*-*O*(pentafluoropropionyl-2-propyl) ester derivative

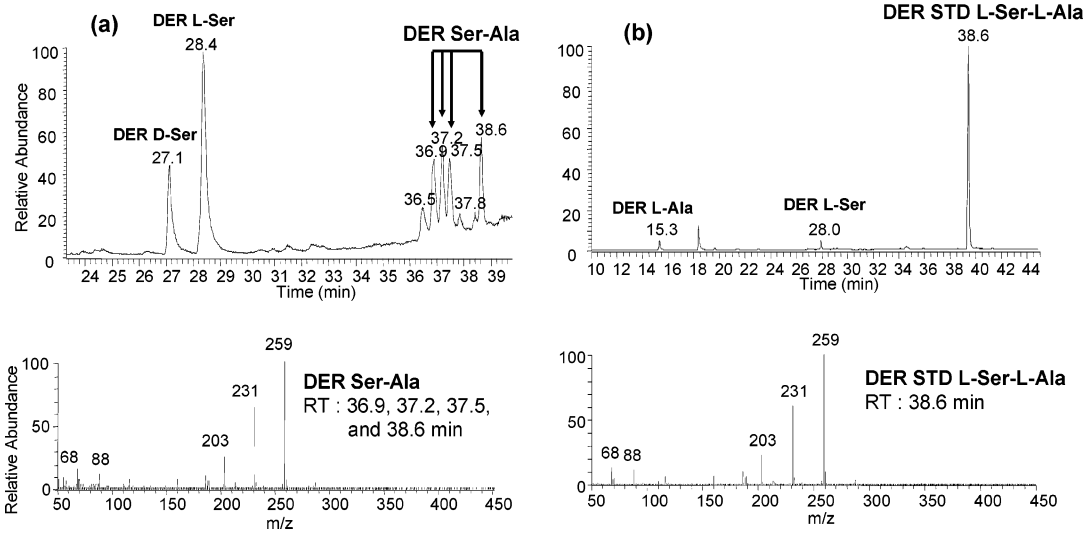


Figure 11. Gas chromatography and mass spectra of (a) derivatized pyrolysis products from the thermal sublimation of L-serine and (b) derivatized standard dipeptide (L-Ser-L-Ala). The generated Ser-Ala dipeptides are D-Ser-D-Ala, L-Ser-D-Ala, D-Ser-L-Ala, and L-Ser-L-Ala, respectively, from left to right with the increment of retention time. DER refers to the *N*-*O*(pentafluoropropionyl-2-propyl) ester derivative.

Comparison to Solution (Spray) Formation of Noncovalent Clusters. The study of the serine octamer started with electrospraying 10^{-2} M serine solution[6,15,24,44] and the discovery of its large magic number factor and unique homochirality. The experimental results from sonic spray[18,23] confirmed that these characteristics of serine octamer are instrument-independent. The characterization of thermally formed serine octamer reconfirms this result.

A brief summary in Table 3 compares the characteristics of serine octamers formed by the spray and thermal methods. It is clear that each of the main features of the serine octamer revealed in ESI and SSI experiments is also observed in the sublimation experiments. Moreover, the thermal method has several advantages over the spray methods, for instance, the enhanced magic number effect, the separation of the two isomers by their formation temperatures, and the thermal transformation from serine to yield alanine and other amino acids. These facts make it possible that formation of serine octamers by spray ionization and sublimation are closely related processes. In particular, sublimation of serine octamers might occur from heated serine microcrystals generated by nebulization in the electrospray experiments assisted by the heat applied in the atmospheric interface of the mass spectrometer.

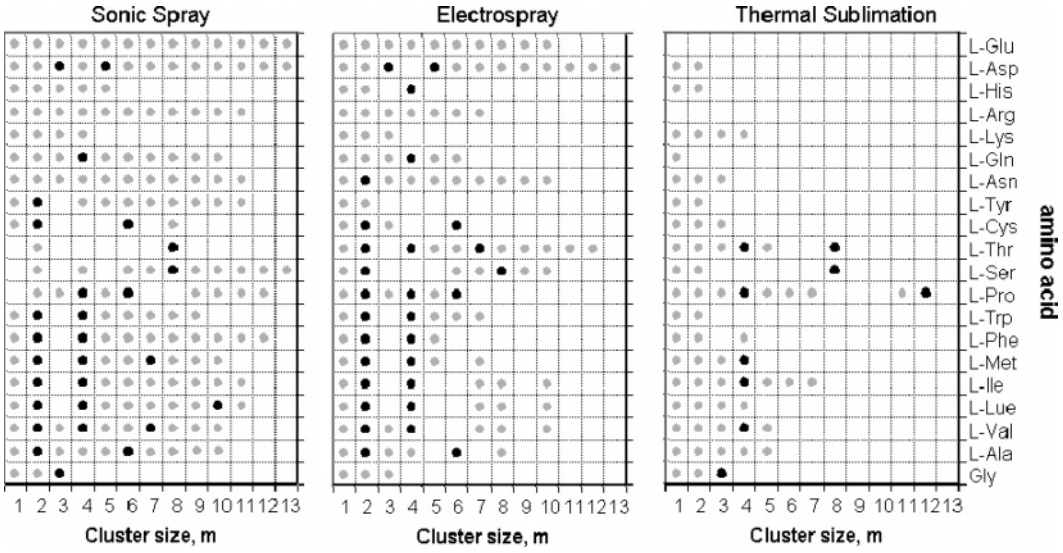
(44) Hodyss, R.; Julian, R. R.; Beauchamp, J. L. *Chirality* **2001**, *13*, 703−706.

Table 3. Features of Serine Octamer Generated from Sublimation of the Solid and Spray Ionization of the Solution

	method		
		solution spray	
features	thermal formulation	electrospray	sonic spray
cluster ions	$[Ser_8+H]^+$	$[Ser_8+H]^+$, $[Ser_8+Na]^+$	$[Ser_8+H]^+$, $[Ser_8+Na]^+$
magic number value[a]	91080[b]	87[b]	107[b]
generation of two isomers	isomer A at 220 °C isomer B at 140 °C	isomers A and B discriminated from H/D exchange experiment	
dissociation	loss of serine dimer	loss of serine dimer	
homochirality	yes, one of two isomers	yes, one of two isomers	
chiral transmission to other amino acids	yes	yes	
chemical reactions	self-assembly coassembly with other amino acids pyrolysis reaction formation of other amino acids and peptides	self-assembly coassembly with other amino acids	

[a] Defined as $(I_n^2)/(I_{n-1}I_{n+1})$, where I represents the signal intensity and n represents the number of components. [b] Strongly dependent on experimental conditions.

Table 4. Self-Assembly of Noncovalent Amino Acid Clusters Generated by Solution Spray and Thermal Sublimation[a]



[a] Magic number clusters given by dark symbols.

A global comparison of self-assembly of amino acids by different methods is summarized in Table 4. All singly charged clusters with 5% or higher ion abundance in their mass spectra are included in the chart, and magic number clusters (magic number factor[3] 4) are given by dark symbols. Briefly, the spray methods form more noncovalent magic number clusters than does sublimation, which may be explained by the decomposition of the relatively more fragile clusters under the conditions of the thermal treatment. This factor alone accounts for the fact that sonic spray shows the best performance in forming bigger clusters, even macrosize clusters.[16] The achievement of noncovalent clusters, especially magic number clusters, by the thermal method may display the chiral selectivity in the cluster assembly process more straightforwardly.

Conclusion

Sublimation of amino acids in air yields clusters that can be studied by mass spectrometry under protonating conditions. In addition to noncovalent clusters, pyrolysis products including small peptides are observed. Serine, in particular, is a sticky molecule and gives stable clusters, especially octamers, upon sublimation in air. The temperature profile of octamer sublimation reveals that two octamers are generated; the higher temperature isomer, A, is generated in an enantioselective fashion and corresponds to the serine octamer generated by ESI. It has the smaller cross section and slower H/D exchange rate.[13,14,36] All the other unique features of the serine octamer discovered by spray ionization experiments are identified in the heating experiments, including its strong magic number property, homochirality, and enantioselective substitution reactions. These similarities suggest that formation of the octamers occurs by the same underlying process of sublimation in both sets of experiments and that the sublimation of the octamers from dried microcrystallites is the basis for octamer formation in the ESI experiments. In addition, sublimation of serine gives pyrolysis products of interest in the study of prebiotic chemistry, including other amino acids and small peptides (e.g., Ser-Ala). The connection between the chirality of the peptides and that of serine will be addressed in a future study.

There remain features of serine sublimation/pyrolysis that are incompletely understood. These include (a) the detailed structures of these octameric clusters, most significantly the enantioselective cluster A; (b) the mechanisms that lead to formation

of enantiopure L-cysteine (but racemic alanine) during L-serine sublimation; (c) the mechanism of small peptide (e.g., Ser-Ala) generation from serine; and (d) the chirality of each residue in the peptides generated from the dehydration and other reactions of L-serine.

The most important result of this study is that mild heating of serine in air produces the homochiral serine octamer as a magic number (highly favored) cluster. The sublimate is chirally enriched in the major enantiomer and is able to transfer this chirality to other simple biomolecules through clustering reactions. Other important conclusions are that (i) the observed clusters likely are derived from units of the serine crystal rather than being assembled in the vapor phase and (ii) spontaneous chiral resolution of racemic serine occurs on heating to generate the isotopically labeled forms of the predominantly homo-L- and homo-D-serine octamers.

Acknowledgment. This work was supported by the National Science Foundation (CHE-0412782).

Supporting Information Available: Additional experiments, mass spectra, and GC−MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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