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Chiroselective Self-Directed Octamerization of Serine: Implications for Homochirogenesis

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Serine undergoes chiroselective self-directed oligomerization to form a singly protonated octamer under positive ion electrospray conditions, as identified by ion trap tandem mass spectrometry. The experiments also show a series of higher-order clusters (metaclusters) corresponding to $[(Ser_8H)_n]^{n+}$, $n = 1, 2, 3$. There is a magic number effect favoring formation of the protonated octamer over its homologues and also a strong preference for octamer formation from homochiral serine molecules. Collision-induced dissociation suggests that the protonated octamer is composed of four hydrogen-bonded dimers, stabilized by further extensive hydrogen bonding. Density functional calculations support this model and show that the protonated homochiral octamer is energetically stabilized relative to its possible fragments (dimer plus protonated hexamer, etc). The calculations also show that heterochiral octamers are less stable than homochiral octamers (e.g., the protonated 7:1 cluster is 2.1 kcal/mol less stable than the 8:0 analogue). The implications of these results for the origin of homochirality are discussed.

Three key steps can be distinguished along the pathway that led to living organisms: (i) the formation of organic molecules from inorganic compounds, (ii) a symmetry-breaking step or symmetry-transfer step needed to generate homochiral organic compounds,[1,2] and (iii) the elaboration of simple chiral organic molecules into complex covalently and noncovalently bonded forms. As a characteristic feature of biological systems, chirality accounts for highly selective recognition, while chiral expression at higher levels of complexity[3,4] is thought to hold the key not only for molecular recognition, transformation and translocation, but also for the self-assembly and the enormous information content of living species. The amino acids play a crucial role in the evolution of life,[5] and it has been suggested that the chirality of amino acids is a possible indicator of life.[6]

This study deals with the clustering of the primitive amino acid serine, and the chiral-selective formation of its stable octamer—an instance of homochiral assembly. It is shown that specific noncovalent interactions[7]—which commonly form the basis of much of supramolecular chemistry[3]—are responsible for serine clustering. It is also suggested that symmetry breaking in the course of biomolecule evolution may have occurred in this noncovalent clustering step. Clustering of serine is studied using electrospray ionization (ESI)[8] in combination with tandem mass spectrometry (MS/MS).[9] This procedure (compare refs 10 and 11) allows intrinsic interactions to be examined in the absence of solvent, although the clusters are generated from aqueous alcohol solution and examined as the corresponding protonated species. While L-amino acids are essential in eukaryotic organisms, it is interesting to note that serine is one of the few amino acids in which both enantiomers are biologically active. D-Serine functions as an important neurotransmitter within mammalian brains.[12−14]

The experimental results reported here have been presented in preliminary form,[15] although the structural interpretation has been changed. This study forms part of a larger scale investigation of clustering of amino acids using electrospray ionization, based on the expectation that clustering can be facilitated in the highly concentrated solutions encountered during the evaporation of microdroplets in ESI. The first finding of this series of studies was that arginine forms particularly stable singly protonated

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tetramers and doubly protonated 12-mer clusters, suggested to be structural analogues of 3 × 3 × 1 and 3 × 3 × 3 salt microcrystallites.[16] In addition, phenylalanine shows a doubly protonated 11-mer and glutaric and aspartic acid form doubly, triply, and quadruply deprotonated clusters.[15] There is strong current interest in several groups in the use of ESI to generate and study cluster ions of biological significance.[17−20] Particularly apposite are two studies on serine clustering of which we have learned since submission of the original version of the manuscript. These investigations, by the groups of Beauchamp[21] and Clemmer,[22] confirm the unusual abundance of the serine octamer reported in ref 15 but propose structures quite different from those we suggest in the present paper. Both also provide evidence for the metaclusters discussed in this paper and in more detail in another paper.[23]

EXPERIMENTAL SECTION

All experiments were performed using a Finnigan LCQ (ThermoFinnigan, San Jose, CA) ion trap mass spectrometer, equipped with an electrospray ionization source and a syringe pump. Operating conditions were spray voltage 4.5 kV, capillary voltage 3.0 V, heated capillary temperature 160 °C, nitrogen sheath gas 0.75 L/min (1.05 L/min for loop injection), with no auxiliary gas (1.5 L/min for loop injection). The samples were either directly infused using the syringe pump at a flow rate of 1 μL/min or introduced via a 5-μL injection loop with a mobile-phase flow rate of 0.2 mL/min using a Bioanalytical Systems (West Lafayette, IN) HPLC pump. Samples were examined in both positive and negative ion modes. Data are presented in thomson (Th): 1 Th = 1 dalton/unit charge.[24]

Charge states of cluster ions were determined by mass/charge ratio measurements and confirmed by higher resolution experiments and tandem mass spectrometry. Isolation of the ions of interest was achieved using a notched waveform to effect broadband excitation and ejection of the undesirable ions. Dissociation of precursor ions was achieved through collisional activation of the isolated cluster ions by collisions with helium buffer gas in the ion trap. The collision energy is optimized for each experiment and expressed in terms of the manufacturer's nominal relative collision energy (%), where the range from 0 to 100% corresponds to a resonance excitation ac signal of 5 V (peak to peak) at the secular frequency of the ion of interest. Spectra shown represent an average of at least 20 scans. Higher resolution was achieved by scanning at a slower rate (1/20 the normal scan rate), a standard method employed in ion trap mass spectrometry.[25]

Serine (D, L, DL), threonine (D, L, DL), cysteine (D, L, DL), (*S*)-(+)-2-aminobutyric acid, and (*R*)-(−)-2-amino-1-propanol were all obtained from Aldrich (Milwaukee, WI), while methanol was purchased from Fisher Scientific (Fair Lawn, NJ). L-Serine-1-^{13}C, nominal 99% isotopic purity, was obtained from Aldrich Chemical Co. (Milwaukee, WI). Solutions were prepared as a 1:1 (by volume) mixture of methanol and water, which was also used as the mobile phase for experiments using loop injections. Experimental parameters examined included the effect of pH (measured using a pH meter) on the clustering of 5 mM serine: in the pH 3−9 range there was little effect on clustering. The effect of serine concentration was investigated and the onset of protonated octamer formation occurred at 0.2 mM. The octamer signal was approximately linear for concentrations up to 20 mM. The dimer was also observable over a similar concentration range. The sequence of appearance of the serine clusters with increasing concentration was 8 ≅ 2, 6, 7, 3, 4, 5, and 9. Odd-*n* clusters were always much less abundant than their even-*n* counterparts.

CALCULATIONS

All calculations were performed without constraints on structure using the GAMESS program.[26] The maximum and rms gradient values were set to 0.0003 and 0.0001, respectively, due to the floppy nature of the species calculated. The structures were visualized using the Molden program.[27]

RESULTS AND DISCUSSION

The mass spectrum of a 10^{-2} M L-serine solution, recorded by positive ion electrospray ionization, is shown in Figure 1. It is obvious that the protonated serine octamer, $[(Ser)_8 + H]^+$, is a magic number cluster, by virtue of its large relative abundance. No other magic number cluster is observed at higher mass (up to cluster size 18), and the magic number effect, expressed using the scaling factor,[28] $(2 \times I_n)/(I_{n-1} + I_{n+1})$, where I represents signal intensity, is >20. This large value indicates that this cluster has greatly enhanced stability over neighboring clusters. The dimer is also relatively stable. Under different experimental conditions which transfer more internal energy, minor ion series are observed corresponding to the singly protonated serine clusters, $[(Ser)_n + H]^+$, where n represents the number of serine molecules in the cluster, as well as sodiated serine clusters and doubly protonated clusters (Table 1).

The protonated L-serine cluster ion was examined by tandem mass spectrometry to obtain structural (connectivity) information from its dissociation behavior. Since the ion might be loosely bound, isolation efficiencies of the protonated serine clusters were first investigated in the absence of collision-induced dissociation. These efficiencies should provide an indication of the relative stability of the selected ions, as shown in previous work on sodium chloride and arginine clusters.[16,29] Using a large (20 Th) isolation

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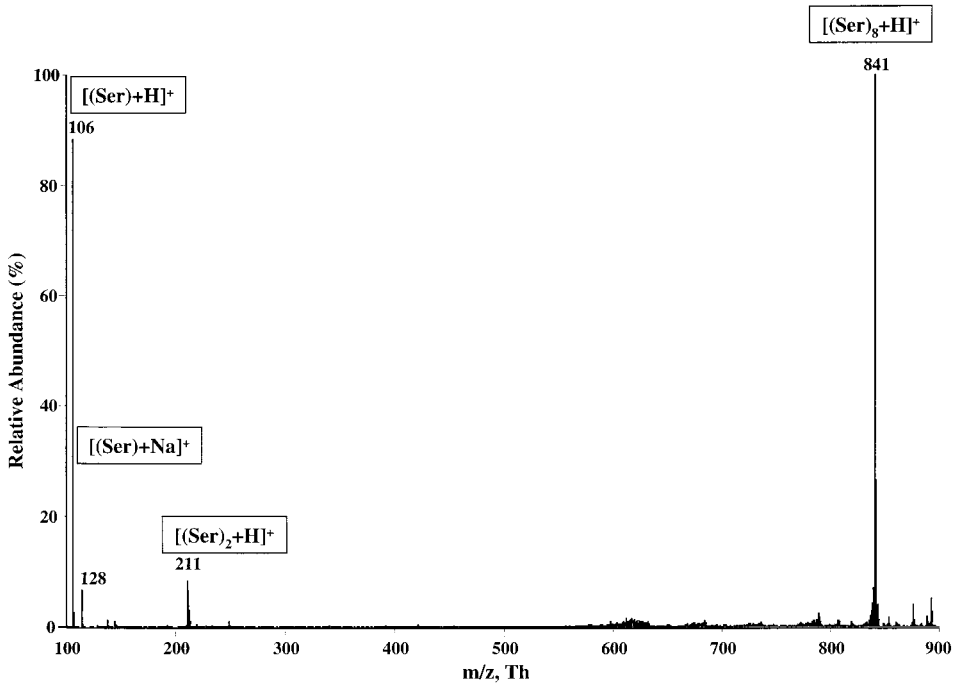

Figure 1. Mass spectrum of a 0.01 M L-serine solution prepared in a methanol−water (1:1) mixture and recorded by positive ion ESI/MS using a 5-μL loop injection.

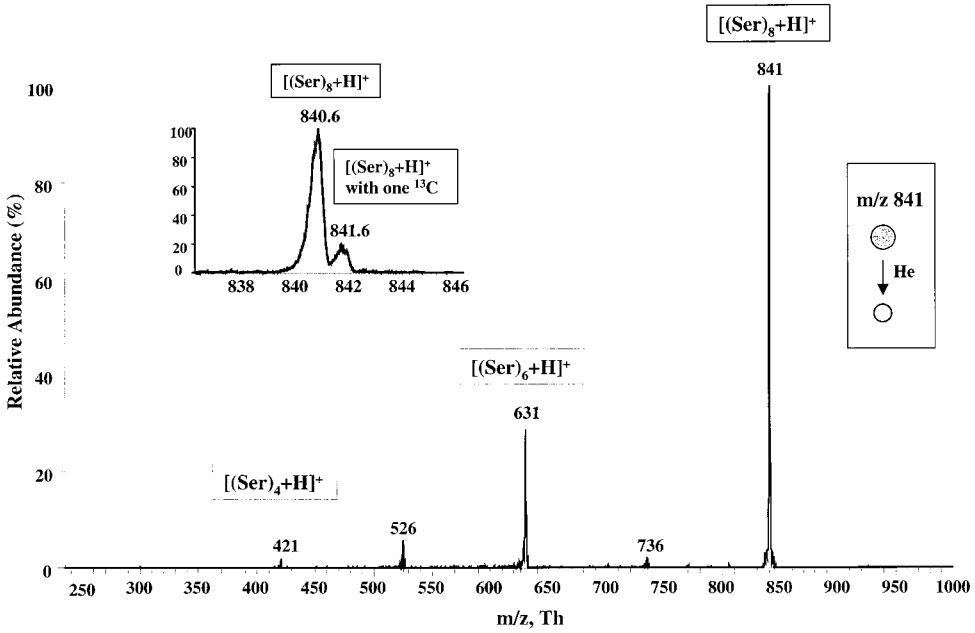

Figure 2. Product ion MS/MS spectrum of protonated serine octamer generated using an isolation width of 20 Th and helium CID with an ac activation potential of 525 mV for 30 ms. Inset: high-resolution mass spectrum of protonated serine octamer, obtained using a 1/20 scan rate, showing that the major charge state of this cluster is +1. (Note that isotopic abundances are not accurate at high resolution.)

window, it was observed that only the protonated dimer and protonated octamer could be efficiently isolated. The other clusters, although present in the mass spectrum, are apparently much less stable and do not survive the relatively energetic collisions with helium bath gas that occur during the isolation process. After isolation and upon activation by collision-induced dissociation,[30] the protonated octamer preferentially yields the protonated hexamer, with small contributions of the pentamer, heptamer, and tetramer (Figure 2). The absence of significant abundances of higher m/z clusters in the MS/MS product ion spectrum—clusters that can only be produced by charge separation from multiply charged clusters—suggests that the mass-selected ion (m/z 841) is mainly composed of the *singly protonated* octamer. This conclusion is supported by the ^{13}C isotopic peak information obtained from high-resolution experiments (inset in Figure 2) which shows that the isotopic peak separation is a whole mass/charge unit rather than a fraction. As shown below, the

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Table 1. Clusters Observed in Electrospray Ionization Mass Spectrum of L-Serine[a,b]

m/*z*	cluster species[c]	rel abund (%)
106	$[Ser + H]^+$	42
128	$[Ser + Na]^+$	3
211	$[(Ser)_2 + H]^+$	68
233	$[(Ser)_2 + Na]^+$	12
316	$[(Ser)_3 + H]^+$	5
338	$[(Ser)_3 + Na]^+$	3
421	$[(Ser)_4 + H]^+$	8
443	$[(Ser)_4 + Na]^+$	6
526	$[(Ser)_5 + H]^+$	6
548	$[(Ser)_5 + Na]^+$	3
631	$[(Ser)_6 + H]^+$	18
653	$[(Ser)_6 + Na]^+$	5
736	$[(Ser)_7 + H]^+$	10
788	$[(Ser)_{15} + 2H]^{+2}$	16
841	$[(Ser)_8 + H]^+$	100
894	$[(Ser)_{17} + 2H]^{+2}$	11
946	$[(Ser)_9 + H]^+$	17

[a] Experimental conditions for this spectrum not identical to those used to record Figure 1. [b] Depending on the sodium cation content of the sample, sodiated cluster ions appear in greater or smaller relative abundance. Cluster species of higher *m*/*z* values than the octamer have much lower abundances in the mass spectrum and are not listed explicitly. [c] The assignments do not exclude the possibility of multiply charged species.

corresponding conclusion does *not* apply to the racemic mixture.

Collision energy-dependent fragmentation of the protonated L-serine octamer is displayed in Figure 3a. This breakdown curve shows that dimer loss is the lowest energy and the most significant channel of dissociation across the collision energy range examined and that loss of odd-number monomer molecules is not favored. This result suggests that the dimer is likely a stable building unit of the octamer and that internal bonding in the dimer must be relatively strong. A further stage of mass selection, dissociation, and mass analysis (MS^3 experiment), carried out on the major dissociation product of the octamer, the protonated hexamer, shows that it too preferentially loses a dimer upon dissociation to yield the protonated tetramer (Figure 3b). These MS^3 results again suggest that the octamer is composed of four dimeric units. Since symmetrical structures are often characteristic of magic number clusters, the protonated octamer is most likely a symmetrical structure. Note that serine has three functional groups that could serve as either hydrogen bond donors or acceptors and it is likely that hydrogen bond networks are responsible for the exceptional stability of the octamer in the gas phase.

The most interesting discovery about the protonated octamer is its chiral dependence: the relative abundance of the protonated serine octamer depends on the enantiomeric composition of the sample being electrosprayed (at constant total serine concentration). The octamer from D-serine shows the same magic number factor (~20) as that for L-serine, but under identical conditions, the protonated octamer was not observed in significant abundance from the racemic serine solution. The mass spectra recorded for solutions of pure L- and pure D-serine are identical (compare Figure 1) while the racemate showed little preference for octamer formation (e.g., octamer/hexamer ratio was >50 for D-serine, >50 for L-serine, but only 3 in the racemic mixture). The strong preference of the protonated octamer to be composed of monomers of identical chirality implies that monomers of the same

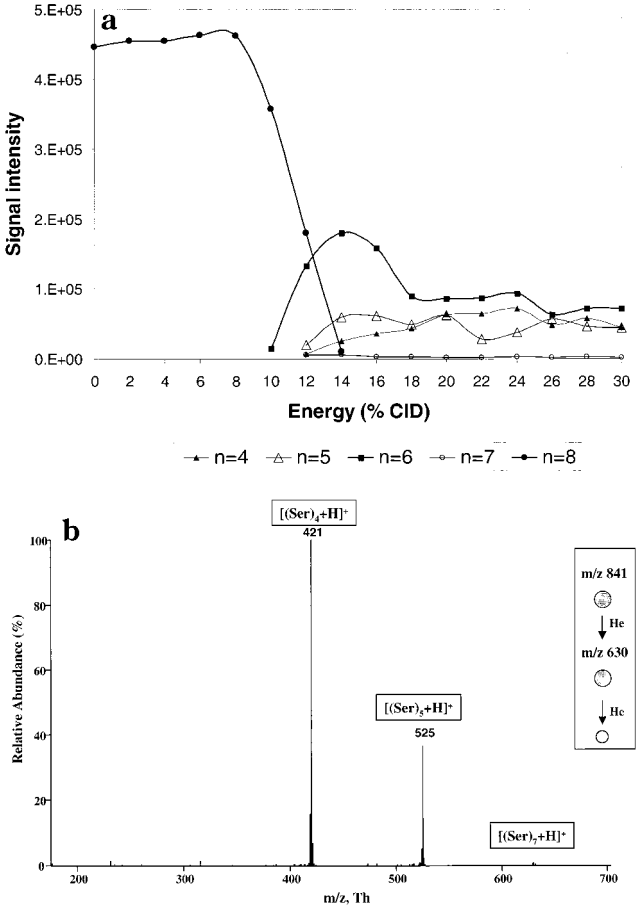

Figure 3. (a) Fragments from dissociation of the protonated serine octamer as a function of CID energy. Isolation of the octamer was achieved using an isolation window of 20 Th, and CID was performed by employing an ac activation potential of varying amplitude. (b) Triple-stage (MS^3) experiment showing the sequential CID fragmentation of protonated serine octamer by successive losses of serine dimer.

configuration bond favorably. Note that no such preference exists for the dimer (*m*/*z* 211), for which the observed relative abundance is independent of the enantiomeric composition of the sample. The protonated serine clusters ($n = 4$ and $n = 6$) show the same behavior as the dimer although the results are not shown explicitly.

To further characterize the protonated serine octamer, the effect of the enantiomeric composition of the serine solution on cluster formation was investigated. The results from this study are shown in Figure 4, where the signal due to the protonated octamer is plotted as a function of the enantiomeric composition of the serine sample. The chiral dependence of octamer formation is clearly evident from this plot, which is discussed further below. In contrast to the chiral dependence shown by the octamer, the abundance of the protonated hexamer shows no chiral dependence—its abundance relative to that of the octamer generated from L-serine is approximately constant, varying within 6% of the average value. In addition, signals due to other clusters are unaffected by enantiomeric composition.

If one assumes that the octamers are randomly constructed by assembling individual amino acids but that only homochiral (all-L and all-D) serine octamers are stable, comparisons of predicted and experimental clustering behavior are possible. The chirality-dependent serine distribution predicted for the protonated

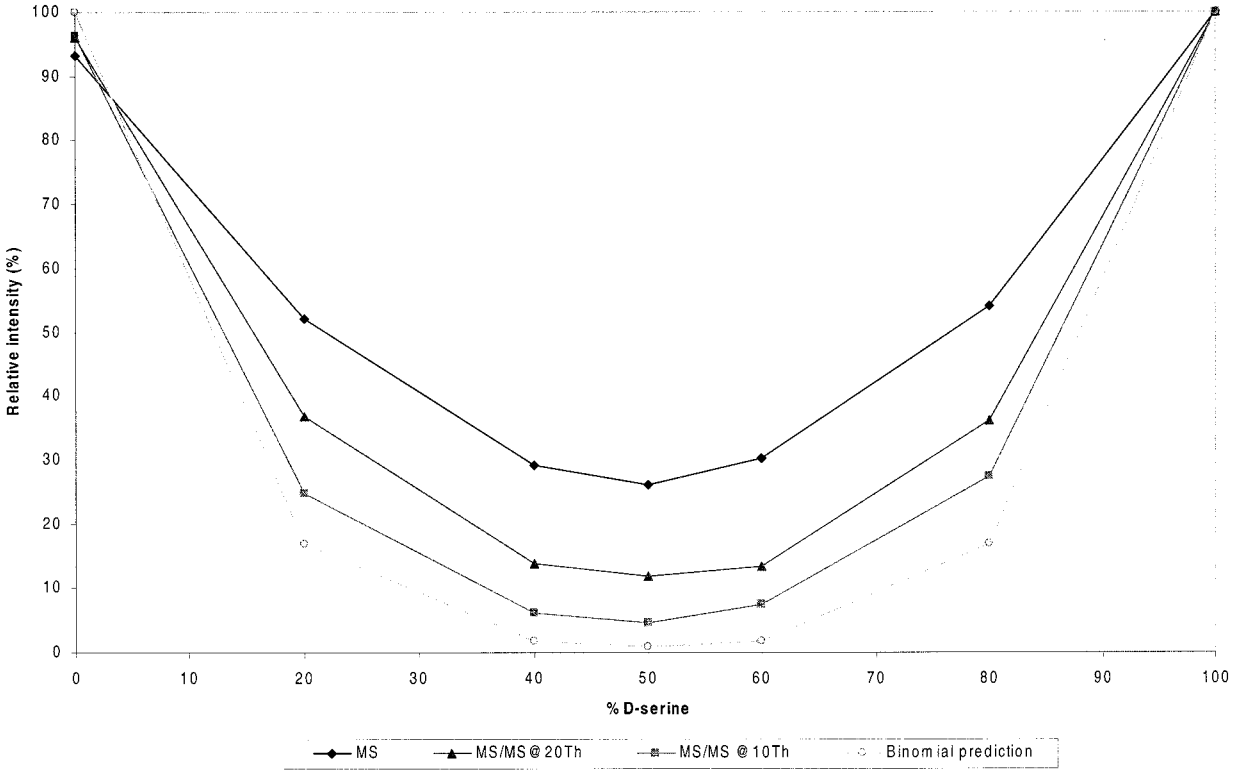

Figure 4. Intensity distributions of *m*/*z* 841 (protonated serine octamer, doubly protoned dimer of serine octamer, etc.) as a function of the enantiomeric composition of the serine sample: (◆) recorded from the mass spectra, (▲) recorded using 20 Th isolation width, and (■) recorded using a 10 Th isolation width and (○) predicted using a binomial function for the chirally pure octamer. The theoretical data are only slightly changed when one also considers those 4D,4L octamers in which chirally identical serines are grouped together (cf. Figure 6 below).

octamer is plotted against the enantiomeric composition of the sample in Figure 4. The calculated data are normalized to the average abundances of the pure D- and L-serine protonated octamers. The shapes of both curves agree, and this constitutes good evidence that there is a strong preference for formation of the homochiral octameric clusters. However, the experimental and calculated results clearly differ and these differences are most marked when the composition of the sample approaches the racemate. One possible reason is that there are contributions from ions of *m*/*z* 841 other than the protonated octamer and these contributions account for the elevated abundance at *m*/*z* 841 when the sample is not enantiomerically pure. Interferences are not unexpected since the predicted abundance of the homochiral octamer at this composition (the pure racemate) is <1%. Interfering ions do exist and are due in part to multiply protonated clusters as discussed further below.

Because of Coulombic repulsion, multiply charged clusters are often inherently less stable than singly charged clusters and this fact can be used to differentiate the multiply charged from the singly charged species. As demonstrated in previous work,[30] the isolation window used in the ion trap provides a means to effect this discrimination. Hence, the effect of enantiomeric composition was reexamined by recording the abundance of the protonated cluster ion at *m*/*z* 841 using different isolation widths (10 and 20 Th) for comparison with the full mass spectrum. The results are shown in Figure 4, along with those obtained from the conventional mass spectrum and the binomial prediction. Direct evidence for a doubly charged ion contribution was obtained by comparing the isotopic peaks associated with different window widths: a peak at *m*/*z* 841.5 is detected in the 20 Th window experiment but not in the 10 Th experiment. It is also obvious that a narrower isolation width causes the experimental distribution to match the prediction more closely. In other words, the data obtained from the mass spectra appear to represent a limit where the ions of *m*/*z* 841 are composed of clusters of a number of different charge states and narrower isolation widths exclude most multiply charged cluster ions or other less stable ions of *m*/*z* 841. As shown in the next section, the additional contributions to the abundance of the protonated octamer in the optically impure samples include contributions from the mixed clusters (e.g., protonated D-Ser_7:L-Ser_1) as well as the multiply charged serine clusters.

Test of Cluster Structure Using Isotopic and Chiral Labeling. An apparently straightforward test of the homochirality of the octameric cluster is to isotopically label either the D- or the L-amino acid and to look for the characteristic signatures of the two separate homochiral compounds. This experiment was performed using [$^{13}C_1$]-L-serine, labeled at the carboxylate carbon, but the very low abundance predicted by the binary theorem for the singly charged homooctamer meant that it was not possible to recognize the 1:1 homochiral $^{13}C_8$ and $^{13}C_0$ octamers. The magnitude of the interference is evident in the mass spectrum of a 1:1 mixture of D-serine and ^{13}C-labeled L-serine, shown in Figure 5. The contributions from doubly and triply charged ions (charge states are indicted as *n*, *m*, and *l* for the singly, doubly, and triply charged series, respectively) are evident. The widths of the isotopic peaks in this spectrum confirm the assignments shown: the isotopic envelopes for the doubly and triply charged ions are half and a third as wide as those for the singly charged ions. Even

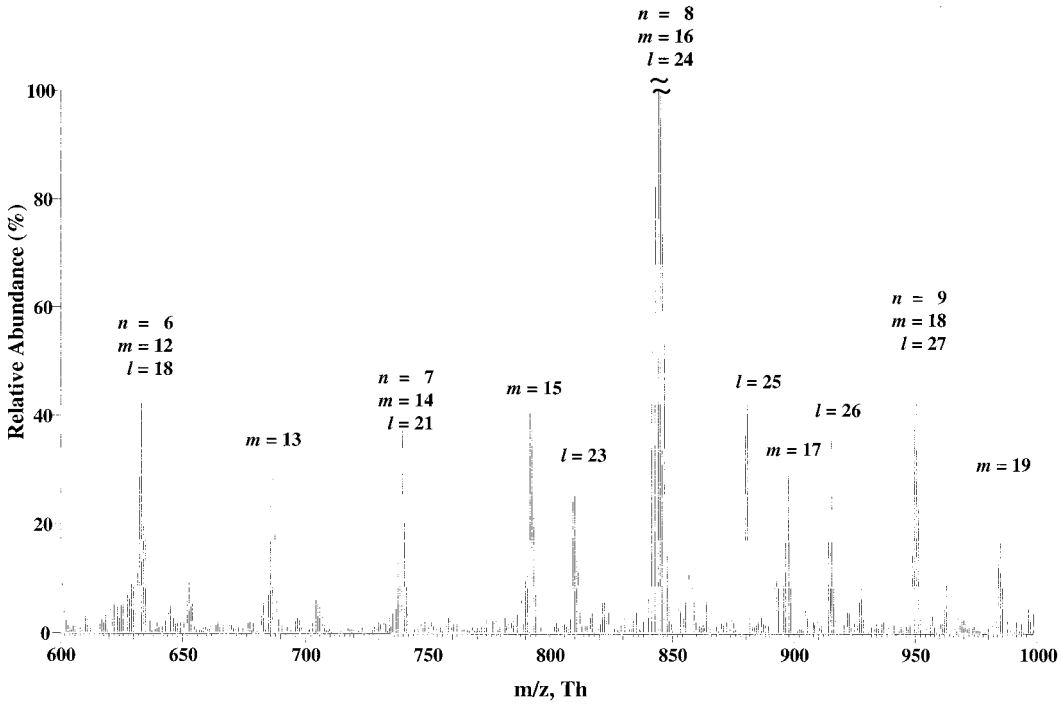

Figure 5. Partial ESI-MS spectrum of a 1:1 mixture of D-serine and $^{13}C_1$-labeled L-serine (99%). Peak widths reflect information on the charge state of the cluster ions, which are labeled as *n*, *m,* and *l* to represent singly, doubly, and triply protonated species. Note that the peaks at *m*/*z* 841 and 842 are off-scale.

Table 2. Molecular Ion Region of Mixtures of Labeled and Unlabeled Serine

	m/*z* (rel abund, %)
D-serine/L-[$^{13}C_1$]serine (7:1)	841 (100), 842 (77), 843 (43), 840 (12), 844 (11)
	849 (100), 848(73), 850 (17), 847 (16), 845 (7)

more direct evidence for the contributions of the mixed clusters comes from careful measurements of ion abundances in the molecular ion region of the protonated octamer. A 7:1 mixture of D-serine and [^{13}C]-L-serine and a 7:1 mixture of [^{13}C]-L-serine and -D-serine shows the abundances given in Table 2. These data, including the enhanced abundances of the M+1 ions compared to the expected 30% value for the natural ^{13}C contribution, clearly demonstrate (i) that the homochiral octamer is more stable than the heterochiral octamer and (ii) that the 7:1 heterocluster is generated in measurable abundance.

In principle, another form of labeling for chirality is possible: one can examine a mixture of serine and an analogue. When a L-serine/L-homoserine mixture is examined, the protonated octamer is formed in 5 times greater abundance relative to the higher homologue of the cluster (*m*/*z* 841 vs 855) than from D-serine/L-homoserine examined under identical conditions. This result, which will be presented in detail elsewhere,[31] confirms that the homocluster is more stable than the heterocluster. Taken together with the earlier results shown in this section, it also confirms that the deviation between experiment and calculation in Figure 4 is due to both mixed clusters and multiply charged clusters. The result also suggests a mechanism of transfer of chirality from serine to other amino acids.

The noncovalent serine octamer is generated in an aqueous environment, and although the exact site of formation of the octamer is not known, gas-phase clustering is extremely unlikely, while the linear dependence of the cluster ion abundance on serine concentration in solution suggests that its formation takes place in the microdroplets generated during electospray. This raises the question of how serine, which exists as the zwitterionic form in aqueous solution,[32] is converted into the neutral form in the gas-phase octamer. Thermodynamically, we calculate that neutral serine is more stable than zwitterionic serine by 24 kcal/mol while the neutral dimer is 37 kcal/mol more stable than the zwitterionic dimer. This suggests that the neutral form is increasingly favored as the concentration of water is reduced in the evaporating microdroplets. In fact, there is evidence that the transition in stability from the zwitterionic form of the monomer to the neutral form has a low barrier and that the latter is the absolute energy minimum.[32]

Ab Initio Calculations. A systematic investigation of the clusters of serine was carried out theoretically, including various cluster sizes, the protonated and neutral clusters, and chirally homo- as well as heterogeneous clusters.

To evaluate the forces involved in the serine clusters,[33] three dimers representing the hydrogen bonds possibly involved in the serine clusters were examined (Chart 1). Note that the zwitterionic dimer is not included, since it was found to be >30 kcal/mol less stable than these forms. The calculations (Table 3) show that the most favorable interaction is between the carboxyl groups (COOH,

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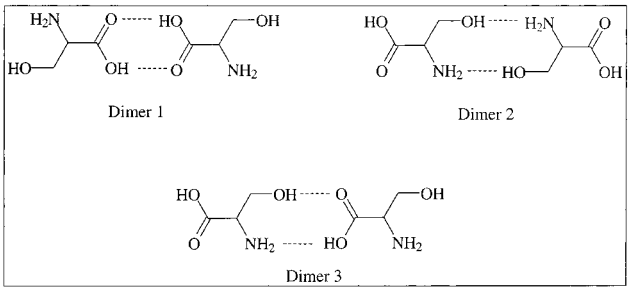
Table 3. Electronic Energies of Possible Serine Dimers[a]

dimer	HF/6-31G (hartree)	rel energy (kcal/mol)	HF/6−31 g(d,p) (hartree)	rel energy (kcal/mol)	B3LYP/6−31 g(d,p) (hartree)	rel energy (kcal/mol)
1	−793.101 412	0	−793.503 865	0	−797.964 902	0
2	−793.091 648	6.1	−793.492 228	7.2	−797.976 225	7.1
3	−793.091 884	5.9	−793.496 483	4.6	−797.957 586	4.5

[a] The zwitterionic dimer is 37 kcal/mol less stable than dimer 1 so is not considered further.

Chart 1. Three Possible Dimers Made from Two Neutral Serine Molecules



dimer 1), followed by those involving NH_2/OH and COOH groups (dimer 3) and then those involving OH and NH_2 groups (dimer 2). The results in Table 3 also show that, despite the lack of polarization functions, the HF/6-31G relative energies are in agreement with those of higher level calculations, making the HF/6-31G a relatively inexpensive but reliable level for the large systems investigated herein. The type of hydrogen bond shown as dimer 1 has been observed in acetic acid vapors.[34,35] The stability of the dimer explains why even-numbered clusters are comparatively more stable than odd-numbered clusters in the mass and MS/MS spectra. For higher-order clusters, the most favorable associations are those in which carboxyl-bounded dimers are held together by H-bonds between their OH and NH_2 groups. The electronic energies of the relevant species are listed in Table 4, including the proposed drum-shaped protonated octamer (Figure 6).

On the basis of these results, relative energies of possible configurations containing eight serine molecules and one proton were calculated (Table 5). Note that all configurations in Table 5, except the one composed of three neutral dimers and a protonated dimer, have 16 hydrogen bonds—eight between COOH groups and eight between NH_2 and OH groups. The higher stability of the protonated octamer is due to a better spatial arrangement that yields stronger hydrogen bonds. The proposed octamer structure is also in agreement with the experimental results on D- and L-serine. For instance, the calculations show that the replacement of one serine in a homochiral octamer by its enantiomer weakens at least one NH_2/OH hydrogen bond, increasing the energy of the system by 2.1 kcal/mol.

The closed, symmetrical structure of the octamer also provides an explanation for the virtual absence of higher singly charged homologues in the ESI spectrum; the addition of an extra dimer to the octamer breaks the alignment of the NH_2 and OH groups making their hydrogen bonds weaker. The octamer structure is also consistent with its magic number behavior.

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Table 4. Electronic Energies of Proposed Serine Clusters Calculated at HF/6-31G

species	electronic energy (hartree)
dimer 1	−793.101 412 358
N atom-protonated dimer 1	−793.480 619 570
tetramer	−1586.221 218 2
N atom-protonated tetramer	−1586.629 411 9
N atom-protonated hexamer	−2379.748 630 8
N atom-protonated octamer	−3172.870 048 7

The formation of the higher charge-state clusters $[(Ser)_{16} + 2H]^{2+}$ and $[(Ser)_{24} + 3H]^{3+}$ is also possible from the proposed octamer structure, since additional NH_2/OH hydrogen bonds can be formed between the "faces" of two octamers. Evidence for this will be presented elsewhere,[31] as will evidence for mixed clusters in which one or two molecules of other amino acids substitute for serine in the octamers.[23]

Multiply Charged Clusters of Serine. To investigate further the multiply charged metaclusters suggested to be present by the isolation and high-sensitivity studies summarized in Figures 4 and 5 and discussed above, the dissociation behavior of the mass-selected clusters at m/z 841, especially that formed from racemic serine, was examined. The product ion spectrum of the mass-selected protonated octamer, generated from a sample of racemic serine and selected using an isolation width of 20 Th, is shown in Figure 7. This figure demonstrates that a significant fraction of the signal *recorded under these gentle isolation conditions* is due to doubly or triply protonated serine clusters. The product ions having even higher m/z than the parent ion must arise by charge separation dissociations of multiply charged ions of 841 Th. The m/z values of all the product ions observed indicate that they are composed of serine molecules only. The lower m/z ions include some due apparently to the loss of half or one-third of the mass of serine, proving the presence of doubly and triply protonated serine clusters of formulas $[(Ser)_{16} + 2H]^{2+}$ and $[(Ser)_{24} + 3H]^{3+}$ in the selected parent ion population. Comparison of these data with those of Figure 5 make it clear that a variety of doubly and triply charged cluster ions occur.

Clustering of Related Compounds. Experiments were carried out to characterize the underlying structural factors leading to the formation of the protonated serine octamer. Using identical conditions, the structurally related amino acids threonine and cysteine, which differ from serine by an additional CH_2 unit and the substitution of an SH by OH, respectively, as well as (*S*)-(+)-2-aminobutyric acid and (*R*)-(−)-2-amino-1-propanol, were examined. None of these compounds except cysteine, which gives a hexamer, showed clusters other than the dimer in their ESI mass spectra. The reasons for this might be complex: for example, the CH_3 group in threonine can impose steric constraints. Similarly,

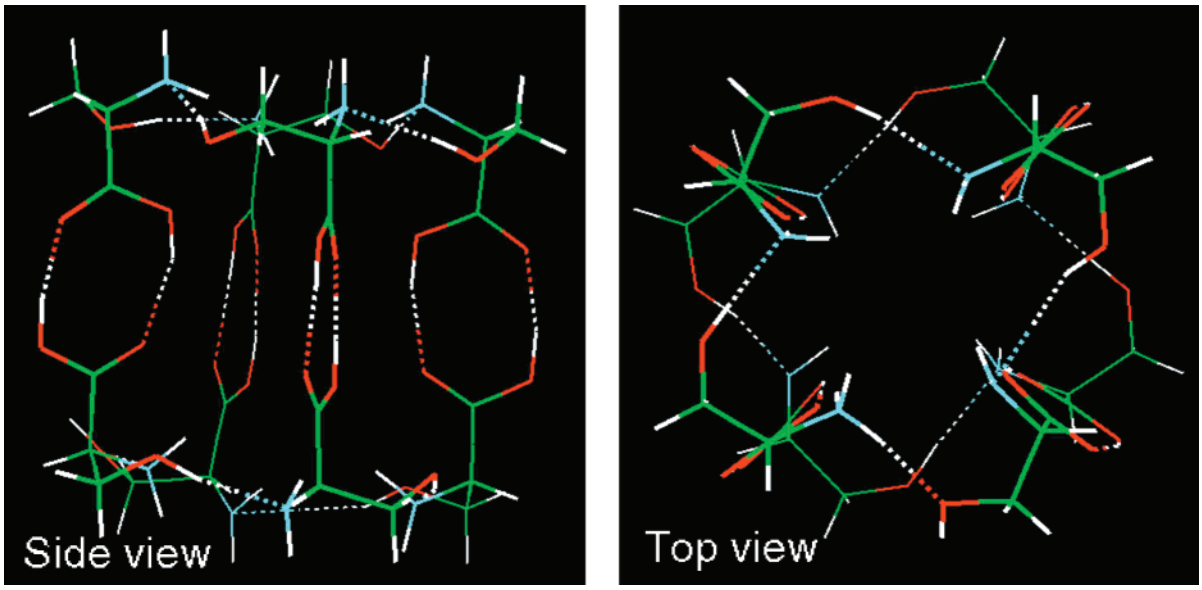

Figure 6. Calculated HF/6-31G structure (two views) of the protonated serine octamer: left, side view; right, the top-down view. Red, oxygen; green, carbon; blue, nitrogen; and white, hydrogen.

Table 5. Relative Energies of Structures Containing Eight Serine Molecules and One Proton

supramolecular structure	description	hydrogen bonds	rel energy (kcal/mol)
1	protonated octamer	8 COOH/COOH + 8 NH_2/OH	0
2	tetramer + N-atom protonated tetramer	8 COOH/COOH + 8 NH_2/OH	12.2
3	dimer + N-atom protonated hexamer	8 COOH/COOH + 8 NH_2/OH	12.6
4	3 Dimers + N-atom protonated Dimer	8 COOH/COOH	53.4

the SH group in cysteine is not as a good proton donor as the OH group, while both aminobutyric acid and aminopropanol have fewer hydrogen bond donors or acceptors than serine and so their clusters would have fewer or weaker hydrogen bonds. These results support the proposed structure of the serine octamer in which all three functional groups play important roles in stabilizing the structure. It has already been noted that magic number clusters are observed in some other amino acids, including $n = 4$ for arginine[16] and $m = 11$ for phenylalanine,[15] although the structure of the phenylalanine cluster has not been elucidated. However, none of these clusters shows a dependence on the enantiomeric composition as large as that seen in serine.

In contrast to the positive ion results, examination of serine in the negative ion electrospray mode displayed signals due to the deprotonated monomer and dimer but failed to yield the corresponding deprotonated serine octamer. Attempts to generate clusters using cations other than the proton gave sodium and potassium cationized octamers as well as the corresponding doubly and, in the case of sodium, triply charged ions.[23] These ions were present as magic number peaks; e.g., when serine was examined from an aqueous alcohol solution containing 10^{-4} M sodium chloride, the major ions in the mass spectrum corresponded to the sodiated and protonated forms of the monomeric, dimeric, and octameric ions.

Implications for Homochirality in Living Systems. There is a consensus that homochirality of the essential biomolecules[36,37] is imperative for the evolution of life.[38,39] It has also been argued that homochirality does not have a direct biogenic origin[40,41] although biological activity can transfer chirality between molecules and increase the chiral purity of abiotically generated chiral samples. Existing hypotheses for the generation of homochirality can be categorized as involving either a spontaneous or an evolutionary process. In the former case, such known processes as the enantioselective crystallization of a very limited number of inorganic molecules[42−44] in the absence of a chiral force or agent are invoked. However, most authors favor an evolutionary process based on initiation by an external chiral force or chiral agent. This latter argument requires that the evolution of life from a racemic environment, as existed on the primitive earth, required a critical symmetry-breaking step.[38,39,41,45]

Several feasible physical processes, for example, enantioselective photolysis by circularly polarized light[46−48] or magne-

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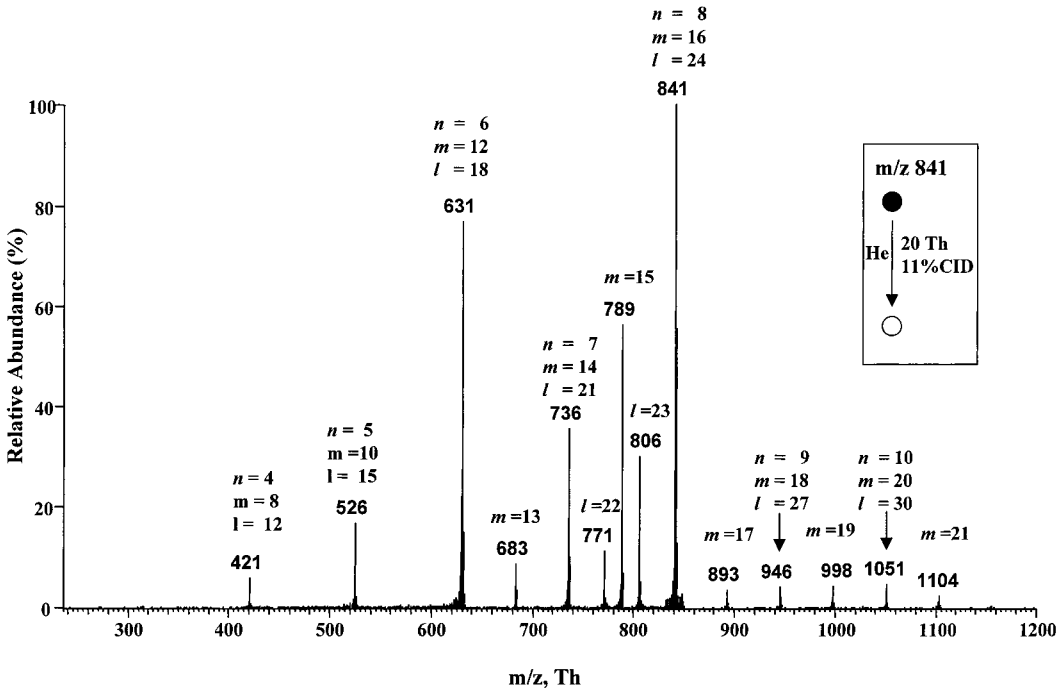

Figure 7. Product ion spectrum of *m*/*z* 841 generated from a racemic serine sample and examined after parent ion isolation using a window of 20 Th and 550-mV (11%) ac activation energy for collision-induced dissociation.

tochiral dichroism,[49] have been shown to induce enantiomeric imbalances that could eventually lead to homochirality. However, it is also realized that a powerful amplification mechanism is necessary for the generation of homochirality via these processes because these fundamental effects generate only a very small enantiomeric excess in a sample.

The discovery of the serine octamer may have implications for the evolution of homochirality. First, the octameric cluster adopts a chirality that is dependent on that of the monomeric amino acid, serine. A prior example of symmetry breaking at the supramolecular level[50] is the transfer of chirality from a chiral barbiturate to a noncovalent assembly of nonchiral molecules and subsequent "chiral memory" retention after removal of the barbiturate. Second, and more importantly, the present results make it is possible to consider new mechanisms for symmetry breaking that operate at the *molecular cluster level*, rather than at the individual molecule level. Both evolutionary and spontaneous mechanisms might operate at this level and they might involve lower energy and hence more likely processes. As one posssible example, chiroselectivity might occur during the formation of the serine octamers and symmetry breaking might be a consequence of a Zeeman effect on the bending deformation required to complete the construction of the octamer by hydrogen bonding in the upper or lower rings of the structure shown in Figure 6. An example of the spontaneous mechanism that is worthy of further testing is that a serine or serine octamer of appropriate chirality is selectively adsorbed onto the chiral surface of a naturally chiral mineral, specifically quartz or calcite. Asymmetric adsorption onto quartz and clays has long been known.[2] The selective absorption of the serine organic cluster onto a chiral inorganic surface may represent the symmetry-transfer (rather than symmetry-breaking) step needed to convert chirality from the inorganic to the organic and subsequently the biological world.

The proposal that chirogenesis is associated with supramolecular clusters, together with the fact that one or two serine molecules in the octameric cluster can be substituted by other molecules to generate new mixed octameric clusters,[31] provides a mechanism for transmission of chirality between different amino acids.

CONCLUSIONS

An unusually strong magic number cluster of serine, observed as the protonated or sodiated or potassiated octamer, is identified using electrospray ionization mass spectrometry. The effect of enantiomeric composition demonstrates that the octamer is preferentially homochiral in nature, and tandem mass spectrometry results suggest—and ab initio calculations support—the fact that the octamer is composed of four dimeric units. The individual dimers are bound through the carboxylic acid groups while additional hydrogen bonding involving the hydroxyl and amino groups generates the final drum-shaped structure. The lowest energy forms of the neutral and protonated octamers are suggested to be homochiral structures consisting of four dimers stabilized by hydrogen-bonding networks. Furthermore, the calculations show that protonation is not important in binding the four dimeric constituents of the octamer together; viz., $[(Ser)_8 + H]^+$ is simply a surrogate for the neutral cluster $[(Ser)_8]$.

The magic number effects show that clustering reflects the influence of the thermochemistry, although the mixture experiments indicate that full thermodynamic equilibrium is not achieved in the time available. Otherwise, homochiral octamers would have been observed, even from racemic serine. It is interesting to estimate the equilibrium constant for interconversion between the

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hetero 7:1 and the homo 8:0 forms of the protonated octamer: assuming that the calculated energy difference (2.1 kcal/mol) is also the free energy difference, and a temperature of 300 K, the value is 34. This number should be compared to the ratio of ~2 observed from the mass spectra of a sample of a 7:1 mixture of D-serine and $^{13}C_1$-labeled L-serine, which is summarized in Table 2. The experiment clearly occurs under kinetic control.

The spontaneous formation of chiral supramolecular clusters may have implications for the evolution of homochirality of amino acids in living organisms, a topic that has been debated in recent years.[38,49,51,52] These data provide a mechanism for self-assembly of homochiral amino acids. Among the many suggestions for this process is the recent postulate that chiral selection of amino acids might occur on calcite surfaces.[53] A hypothesis is advanced in which the symmetry-breaking step in chirogenesis occurs in the course of the formation of the L-octamer structure, i.e., in a molecular cluster. Chiral transmission from serine to other amino acids might be effected by their substitution for serine in these clusters.

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