Spontaneous Anti-Resolution in Heterochiral Clusters of Serine

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The spontaneous resolution of racemic mixtures is rarely observed in the natural world.[1] Macroscopically, resolution occurs when homochiral crystals evolve from racemic solutions.[2] Although serine does not spontaneously resolve into homochiral crystals macroscopically, recent work has demonstrated that the protonated serine octamer undergoes spontaneous resolution.[3] This suggests that different size domains can exhibit opposite chiral preferences. However, little is known about the behavior of small-molecular clusters with respect to chirality. It remains undetermined whether spontaneous resolution in this size regime is common. Here, we provide evidence that chiral selection is potentially a general phenomenon for small clusters. In addition to $[8Ser+H]^+$ we find strong evidence for chiral selectivity in clusters containing 6, 9, 10, and 11 serines, with other larger clusters exhibiting smaller preferences.

Although clusters of amino acids have been studied previously, chiral preferences have been found in only a few.[3,4] Established techniques for examining chiral selectivity in cluster formation (e.g., isotopic labeling of one enantiomer in a racemic mixture) are only suitable when the cluster size is small and contributions from overlapping isobaric peaks from larger clusters having higher-charge states are minimal.[3] Herein, we describe an experimental method that makes it possible to observe chiral preferences in larger clusters and clusters with overlapping masses. The experiment employs electrospray ionization (ESI)[5] to generate clusters, which are then analyzed by a combined ion mobility and mass spectrometry approach.[6,7] The instrument has been described previously.[8] This combination of mobility and mass-to-charge separation allows multiply charged multimers (having identical mass-to-charge ratios) to be resolved.[9] The chiral preference of a cluster is determined by measuring its abundance relative to $[Ser+H]^+$ as the composition of the solution is varied from enantiomerically pure to racemic.

Figure 1 shows data illustrating this approach. We start by acquiring ESI spectra for seven 0.01 M serine solutions comprised of L/D compositions of 100:0, 83:17, 63:37, 50:50, 37:63, 17:83, and 0:100, respectively. Two sample mass spectra are given in a and b of Figure 1 for the isolated +2 charge state. The intensity of each cluster can then be compared to the monomer ($[Ser+H]^+$), which cannot exhibit a chiral preference. Results for the singly protonated octamer are shown in Figure 1d. The octamer is observed to be much more abundant when sampled from enantiomerically pure solutions, resulting in a "V"-shaped distribution. This type of distribution is indicative of a preference for homochirality, which is in agreement with previous results obtained for the octamer.[3]

It is straightforward to extend this approach to other cluster sizes and charge states. Several changes in ion intensity can be observed from the raw data in Figure 1. The most apparent change in abundance is for the peak corresponding to $[10Ser+2H]^{2+}$, which is much more intense when sampled from a racemic solution. The relative abundances of each of these ions with respect to $[Ser+H]^+$ are shown in Figure 1c. The intensity profiles for doubly protonated clusters containing 8, 9, 10, and 11 serines are shaped like an
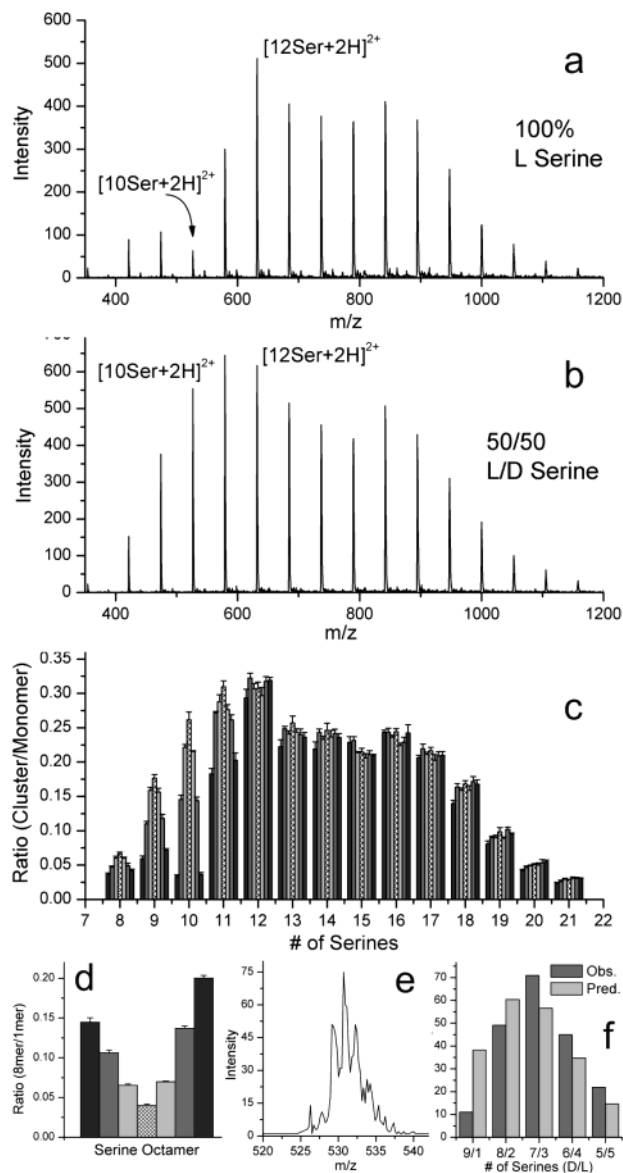

Figure 1. Mass spectra of the isolated +2 charge state serine clusters acquired from a solution of L-serine (a), and 50/50 L/D-serine (b). Note the dramatic shift in relative intensity for the 10mer. (c) The ratios represent relative cluster intensity for solution compositions varying from pure D- to pure L-serine as described in the text. Four data sets have been averaged. Error bars represent ± one standard deviation. (d) The octamer exhibits a strong homochiral preference by the same analysis, as expected. (e) Raw spectrum for a 74:26 D/L (d3)-serine mixture. (f) Comparison of the peak intensities observed in (e) to those predicted by a binomial distribution for $[10Ser+2H]^{2+}$. A heterochiral preference is observed.

"inverted V". All of the other cluster sizes display flat intensity profiles as the composition of the solution is varied.
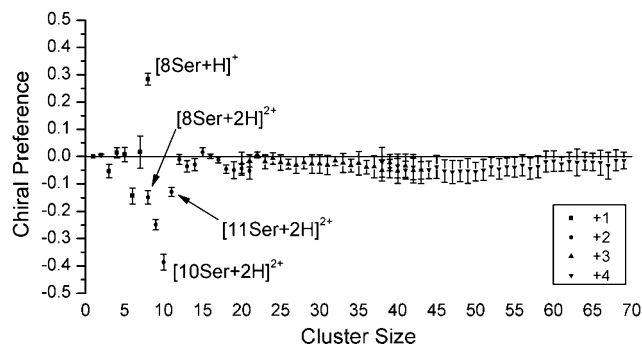
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Figure 2. Chiral preference for each cluster (1−69) versus predicted distribution. Positive numbers indicate a preference for homochirality, negative numbers, a preference for heterochirality. Each point is the average deviation of cluster from the 50:50 and 100:0 L/D data sets. Error bars represent ± one standard deviation.

These results suggest that molecular clusters exist in one of three possible states of chiral selectivity: homochiral, heterochiral, and statistical. Clusters with a flat distribution in Figure 1c exhibit no preference for chirality, resulting in a statistical distribution of intensities. Clusters with a "V"-shaped distribution are preferentially homochiral, while clusters with an "inverted V" distribution favor heterochiral cluster formation. This type of "anti-resolution" has not been observed previously for clusters of amino acids. Nevertheless, a heterochiral preference can also be observed (with greater difficulty) for $[10Ser+2H]^{2+}$ using the isotope-labeling technique as shown in e and f of Figure 1.

The results for all charge states are summarized in Figure 2, which illustrates chiral preference on a scale from +0.5 for purely homochiral clusters to −0.5 for purely heterochiral clusters. On this scale, each point represents the average deviation of the observed cluster distribution from the predicted distribution for a cluster with no chiral preference. In other words, if a cluster exhibits a flat distribution in Figure 1, then the magnitude of its chiral preference in Figure 2 will be zero. Furthermore, the relative intensity of each cluster in Figure 2 has been normalized so that the chiral selectivity of each cluster can be compared, regardless of the abundance of the cluster. Surprisingly, this plot shows that $[10Ser+2H]^{2+}$ exhibits the strongest preference for chirality, with the singly protonated octamer demonstrating the second largest preference.

Interestingly, the doubly protonated octamer exhibits a heterochiral preference, which contrasts the strong homochiral preference of the singly protonated octamer. Two explanations could account for this behavior. (1) If the attachment of charges influences the final structure of the ion, then the number of charges could dictate the chiral preference, or (2) if the structure of the ion determines its final charge state, then a more elongated structure with a heterochiral preference might attach two charges, while a more compact homochiral structure might attach only one.

Moreover, it is clear that the addition of a single serine can greatly influence the chiral preference of a cluster. For example, $[7Ser+H]^{+}$ has no chiral preference, while $[8Ser+H]^{+}$ demonstrates a strong homochiral preference. This suggests that the chirality of each serine is communicated via specific diastereomeric interactions to the remaining molecules in the cluster and that a single serine can alter the preferred composition of the final cluster.

Furthermore, as the cluster size increases, chiral preferences fall into periodic trends where several clusters are slightly heterochiral, followed by several that are not, etc. This observation may be related

to the crystal structure of D/L-serine, which grows in lamellar epitaxial twin sheets, or sheets of D- and L-serine which alternate.[10] If the clusters observed in the present experiments also grow in alternating sheets, then the observed periodicity of chiral preference would be expected.

A preference for chirality indicates the existence of a regular or preferred structure. By this reasoning, our results suggest that even very large structures containing over 40 serines assemble into a single structure or series of closely related structures that are energetically favorable. This observation is remarkable, given the enormous number of possible structures that can be formed by assembling over 40 serines together.

In conclusion, it is clear that chirality plays an important role in formation of small molecular clusters. The spontaneous resolution observed in the octamer is balanced by several highly abundant clusters which disfavor resolution, including $[10Ser+2H]^{2+}$. Therefore, when all of the serine data are considered, the macroscopic observation of racemic crystal formation is in agreement with the data obtained in the molecular cluster regime. Furthermore, the spontaneous resolution observed in the serine octamer has received considerable attention, but our results suggest that the counteracting anti-resolution of $[10Ser+2H]^{2+}$ might effectively prevent a racemic solution from spontaneously resolving. In fact, during the process of crystallization, the presence of the $[10Ser+2H]^{2+}$ cluster would drive a slightly asymmetric solution to form a racemic crystal. We are currently exploring possible structures for this cluster in addition to testing the chiral selectivity of noncovalent aggregates for a variety of other chiral molecules.

Supporting Information Available: Full experimental details and complete data sets (such as those shown in Figure 1c and 1d) for all charge states. This material is available free of charge via the Internet at http://pubs.acs.org.

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