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Evidence for Spontaneous Resolution of Icosahedral Proline

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While examining noncovalent interactions in amino acid clusters we have found evidence for a remarkably stable cluster, $[12Pro+H]^+$, that undergoes spontaneous chiral resolution.[1] The origin of this behavior appears to be the formation of an icosahedron having each of the prolines positioned at 12 equally spaced vertices. In this configuration the rigid pyrrolidine rings wrap around the surface with either left- or right-handed symmetry. The tightly packed cage that is formed houses a single proton in the center cavity.

Although the spontaneous resolution of racemic mixtures is rarely observed in nature,[2] recent work has shown that a few amino acids resolve as small clusters because of unique structural characteristics.[3−8] The most striking of these is the serine octamer that is believed to form a stable cube,[5,9] in which each serine occupies the vertex of a cube; not only does this system resolve,[5,3] but it also selectively substitutes other amino acids into the structure.[5,11,12] The data presented below suggest that the homochiral clustering properties of the serine octamer may be general to other systems through different sizes and geometries. In related work, we have recently reported a family of larger proline clusters produced by electrospray ionization (ESI)[13] that form extended rodlike geometries that show evidence for spontaneous enantiomeric enrichment.[14]

Experimental evidence for the unique nature of $[12Pro+H]^+$ is obtained from the data provided in Figure 1. We began by recording two-dimensional ion mobility-mass spectrometry (IM-MS)[15] data for different amino acids (15 in total, including two non-natural amino acids). The IM-MS approach makes it possible to isolate different charge states into families[16] according to cluster sizes and geometries.[14,17,18] Figure 1 shows ESI mass spectra for the +1 family of ions for 0.01 M enantiopure and racemic proline solutions. Relevant experimental parameters are provided in the Supporting Information. Typical of many amino acids, the racemic solution leads to formation of several small clusters, in the case of proline containing one to six monomer units. The solution of 100% D (or L, data not shown) proline also produces these clusters; however, at higher *m*/*z* values, signals for $[11Pro+H]^+$ and $[12Pro+H]^+$ are also observed. No signals of significant intensity are observed for clusters with more than 12 prolines in the +1 family from the enantiomerically pure solutions. Signals for larger clusters including $[12Pro+H]^+$ can also be detected from racemic solutions. However, these have much lower intensities; from multiple datasets recorded for identical times we determine that $[12Pro+H]^+$ formation is about 20 times less efficient from racemic compared with enantiopure solutions. The IM analysis of $[12Pro+H]^+$ formed from an enantiopure solution shows a single peak that can be represented accurately by simulations of a single structure having a cross section of 280 ± 4 Å^2 from the transport equation;[19] on the other hand, the IM peak associated with the lower-intensity $[12Pro+H]^+$ formed from racemic solutions indicates that these ions have slightly lower mobilities than the enantiopure form and show evidence for multiple structures as a low-intensity tail that extends to longer drift times.[20]

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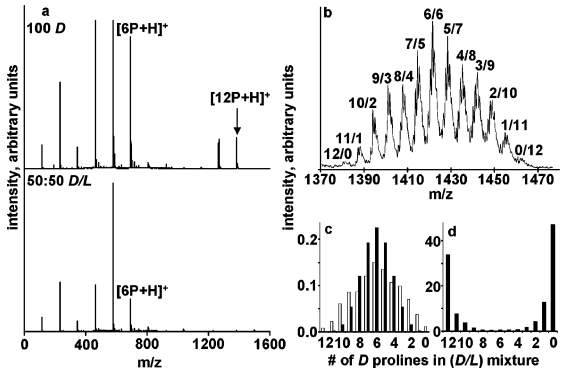

Figure 1. Mass spectra extracted from the +1 charge-state proline clusters acquired from a solution of 100% D and 50:50 D/L proline: (a) note the changes in relative intensity for the $[12P+H]^+$; (b) raw spectrum obtained for a 1:1 D-proline/L-proline ($HN(CD_2)_3CD)COOH$) mixture; (c) comparison of the peak intensities observed in spectra b to those predicted by a statistical distribution for $[12P+H]^+$. White and black bars represent observed and predicted intensities, respectively; (d) ratio of the observed to predicted intensities as shown in panel c. A strong homochiral preference is observed for the $[12P+H]^+$ cluster size.

The data for $[11Pro+H]^+$ and $[12Pro+H]^+$ from an enantiopure solution are noteworthy. The cluster distribution is not continuous. Smaller clusters ($[7Pro+H]^+$ to $[10Pro+H]^+$) or larger clusters (with more than 12 prolines) are not favored, indicating that $[11Pro+H]^+$ and $[12Pro+H]^+$ are not simply random aggregates. The absence of larger cluster sizes indicates that the $[12Pro+H]^+$ may have a closed structure that inhibits its interaction with additional monomer units. Additionally, the preferred formation of $[11Pro+H]^+$ and $[12Pro+H]^+$ from enantiopure solutions indicates a strong chiral effect.

Figure 1b shows a narrow region of the mass spectral data recorded by electrospraying a 0.01 M racemic proline solution in which the L-proline was deuterated ($HN(CD_2)_3CD)COOH$). In these clusters, the incorporation of each deuterated proline increases the measured *m*/*z* value by 7 u. The recorded intensities for incorporation of specific numbers of D and L prolines can be compared with what would be expected from random (statistical) association (Figures 1c and 1d). This comparison confirms the occurrence of spontaneous enantiomer enrichment and resolution in the formation of $[12Pro+H]^+$. For example, a mixed cluster (such as the 6/6 D:L assembly) is disfavored relative to the intensity that is expected statistically (in this case by a factor of ∼0.7); on the other hand, nearly homochiral peaks are substantially enhanced. The heavily enriched 11/1 or 1/11 clusters show intensities that are enhanced by about an order of magnitude relative to what is expected from a statistical assembly. The completely homochiral peaks are ∼40 times more abundant than expected from random formation. This is a remarkable enhancement, even greater than the factor of ∼10 reported for the octameric state of serine.[5]

To understand the origin of the enantiomeric enrichment and discontinuity of cluster sizes, we carried out a series of computer

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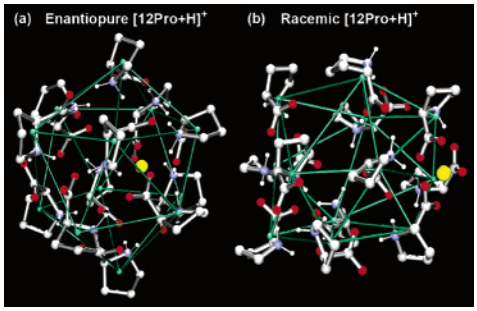

Figure 2. Optimized structures obtained for the (a) enantiopure and (b) racemic $[12Pro+H]^+$ cluster size. The green solids represent center of mass of each proline units in the 12mer and are overlaid on the structure to show the icosahedron and prismatic geometries obtained for the enantiopure and racemic systems, respectively. The yellow circle represents the proton (H^+) in each structure.

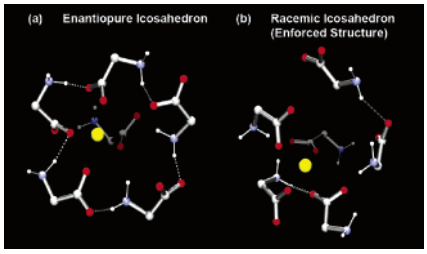

Figure 3. Hydrogen bonding in (a) the enantiopure icosahedron and (b) the hypothetical racemic icosahedron. The top half of the icosahedron and the propylene moiety of each proline were removed for simplicity.

simulations for $[12Pro+H]^+$ using molecular mechanics force fields for the zwitterionic form of proline that were derived and tested in our previous study.[14,20] A structure that emerged in our simulations as most stable for 12 enantiopure prolines is an icosahedron, which is at least 10 kcal/mol more stable than alternative prismatic structures. Considering the number of different cluster sizes and geometries that are possible during clustering, the observation of a symmetric icosahedron is surprising; however, like the cube,[5,9] once the icosahedral shell closes, it is remarkably stable, consistent with the dearth of larger clusters. Calculation of trajectory cross sections[21] for 10 representative average structures from molecular dynamics simulations gives values ranging from 269 to 288 Å^2, averaging to 281 ± 5 Å^2, in agreement with the experimental cross section.[22] In the enantiopure icosahedral cluster, there is a network of 41 hydrogen bonds that support structural integrity. To understand why the racemic cluster cannot behave in the same fashion, we exchanged six prolines in the icosahedron with their enantiomers and reoptimized the hydrogen-bonding network of the hypothetical racemic icosahedron by fixing the position of the α-carbon and using simulated annealing techniques.[20] The best structure possible in a racemic cluster contains only 32 hydrogen bonds, losing 9 hydrogen bonds compared to the enantiopure icosahedron. Figure 3 illustrates how the misalignment of the proline building blocks disrupts the hydrogen-bonding network. A full geometry optimization without constraints gave a prismatic cluster (Figure 2b) where the 32 hydrogen bonds are maintained, but the cavity that was present in the icosahedron collapsed in order to increase the hydrophobic contacts between prolines, which results in additional stabilization through van der Waals contacts, reducing the energy difference to 10 kcal/mol, as mentioned above. In this process, the proton is located on the surface. This prismatic structure can be envisioned as a general structural motif that can accommodate any number of prolines.

The highly symmetric and chiral nature of both the surface and interior cavity of the $[12Pro+H]^+$ icosahedral geometry (Figure 2) are remarkable. Our calculations indicate that the proton adds to the stability of the cavity introducing an intrinsic asymmetry, as it interacts with four prolines strongly at any given instant. Although trapped inside, we note that the proton is not centered in Figure 2a. As a result, the proline that is farthest away from the proton is less strongly bound and can be removed without causing structural collapse. These computational results are consistent with the experimental observation that the enantiopure $[11Pro+H]^+$ species is also detectable.

Finally, it is interesting to consider the properties of the chiral surface and interior associated with the formation of a left- or right-handed icosahedron. Unlike a helical motif, which propagates along a line, the side chains of the icosahedron turn along the surface of the sphere.

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Supporting Information Available: Complete description of the experimental parameters, computational details, Cartesian coordinates of all structures and all energy components. This material is available free of charge via the Internet at http://pubs.acs.org.

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