Do Homochiral Aggregates Have an Entropic Advantage?

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The present work seeks to illuminate the underlying principles which control the aggregation of chiral building blocks into larger aggregates by examining the role that entropy plays in this process. Entropic effects are first examined within the confines of a simple model system, and the results are then compared to experimental data on clusters of amino acids. The model system predicts that the formation of a specific structure is more likely to occur from an enantiopure solution because forming a particular structure from a racemic solution is hindered by significant entropic barriers. These predictions are in good agreement with the experimental results. In our examination of clusters of all of the amino acids, clusters which are unusually abundant are found only when enantiopure solutions are sampled. Furthermore, the majority of all clusters exhibit no preference for chiral composition, suggesting that entropic effects negate any changes in enthalpy. Although the experimental data are not comprehensive, our results strongly suggest that specificity in homochiral clusters is entropically advantageous compared to specificity in racemic clusters.

Introduction

Enantiomers are chemically identical in achiral environments. They also have identical physical characteristics such as melting points and densities. The same is not true for racemates, which often have different melting points and densities than their enantiopure counterparts. Similarly, chiral composition can have a dramatic influence on the properties of small molecular clusters. In this area recent work has concentrated primarily on the unusually abundant protonated serine octamer,[1−5] which is much more abundant when sampled from an enantiopure solution.[1,2] Despite the best efforts of several groups employing both theoretical[3] and experimental[4,5] methodologies, the structure for the protonated serine octamer remains a subject for debate. Similarly, the underlying cause for the strong homochiral preference of the serine octamer is still unknown. Until these questions are answered, the serine octamer will remain a subject of interest.

We have recently reported that several other serine clusters also demonstrate sensitivity to chiral composition.[6] Interestingly, these doubly charged clusters ($[n\text{Ser}+2\text{H}]^{2+}$ where $n = 8-11$) are more abundant when sampled from a racemic solution. All other serine clusters appear to have no preference for chiral composition, or at least the relative intensities do not change as a function of enantiomeric excess.[6] This same indifference toward chiral composition has also been observed for small clusters of arginine[2] and cysteine,[7] but the chiral selectivity of the remaining amino acids remains largely untested. Although it is likely that each individual structure will play an important role in determining the preferred chiral composition of a cluster, it is also important to identify any underlying thermodynamic or kinetic forces which might govern the assembly of all such clusters.

Each of the clusters mentioned above can be generated by electrospray ionization[8] (ESI), but the exact mechanism by which the clusters are formed is not entirely understood. For example, it is not known whether the clusters are generated in the bulk solution, or at some time in the process of droplet evaporation during ESI. Unfortunately, this is an important point, because clusters that are merely sampled from solution might reasonably be assumed to be under thermodynamic control, whereas clusters formed in the process of electrospray itself may be controlled to some extent by kinetics. In either case, it has been documented that changes in concentration can have a notable effect on the observed cluster distributions.[2,9] Furthermore, different instruments with varying source conditions can produce spectra which are somewhat dissimilar in cluster intensities and in the sizes of clusters that are observed.[10] These factors must be considered when comparing data taken with different instruments or under differing experimental conditions.

Herein, we seek to examine the underlying forces that control cluster aggregation by examining the role that entropy plays in the assembly of chiral building blocks into larger aggregates. We will first examine entropic effects in a completely general sense, and then apply the results to our experimental data on clusters of amino acids. It is found in our model system that the formation of a specific structure is more likely to occur from an enantiopure solution. The formation of a particular structure from a racemic solution is hindered by significant entropic barriers. These predictions are in good agreement with the experimental results. In our examination of clusters of all of the amino acids, clusters which are unusually abundant are found only when sampled from enantiopure solutions. Additionally, the majority of clusters do not exhibit a preference for chirality, which suggests that entropic effects negate any changes in enthalpy as the chiral composition of a cluster is varied. Although more experimental data is required, our initial results strongly suggest that specificity is more likely to occur in homochiral clusters formed from homochiral solutions due to entropic constraints placed on racemic clusters formed from racemic solutions.

Experimental Methodology

Electrospray ionization was used to generate protonated noncovalent clusters of amino acids, which were then analyzed

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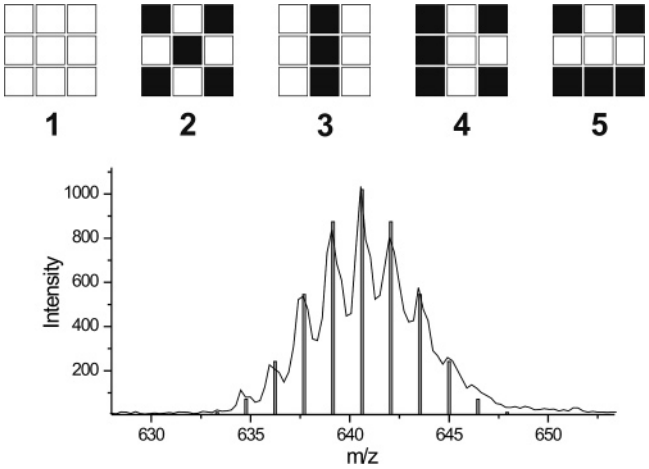

Figure 1. Mass spectrum for [12Ser+2H]2+ taken from a solution comprised of ~50/ 50 d_3-L-serine and D-serine. The expected intensities for a binomial distribution are overlaid in black. The experimental and predicted values are in good agreement.

using a hybrid ion mobility mass spectrometer.[11] The ion mobility separation allows different clusters with identical mass-to-charge (m/z) ratios to be evaluated individually on the basis of their different mobilities through a buffer gas. For example, the singly protonated octamer is easily separated from the doubly protonated 16mer, which would appear at the same nominal m/z in a mass spectrometer. This ability to separate the clusters is important for accurately determining the intensities of each individual cluster. Chiral effects on cluster formation and stability were determined utilizing a technique which has been recently described.[6] Briefly, the relative intensity of an ion is measured versus the intensity of the monomer as the enantiomeric excess (ee) of the solution is varied. Clusters which prefer a particular chiral composition will manifest a change in intensity as the ee is modified. All of the chiral naturally occurring amino acids and several closely related molecules were evaluated as homogeneous protonated clusters.

Results and Discussion

A Simple Model. We will begin by evaluating entropic effects for a simple, generalized model system. This model will exist in two dimensions where the "molecules" will be represented by squares. Dark squares and light squares will represent enantiomeric pairs, and clusters will be represented by collections of squares. We arbitrarily choose a cluster of nine squares for our analysis, but the results are generally applicable to other clusters sizes. In this system the "lowest energy" structure for a cluster is assumed to be a square or the closest approximation to a square, with all squares having similar enthalpic terms regardless of chirality. Thus the sole structure for a 9mer formed from an enantiopure solution is shown as structure **1**. If the same cluster is formed from a racemic solution, combinations of the two enantiomers will lead to 2^9 (or 512) possible structures. The compositions of the racemic clusters will vary according to a binomial distribution, with 1(9L), 9(8D/1L), 36(7L/2D), 84(6L/3D), 126(5L/4D), 126(4L/5D), 84(3L/6D), 36(2L/7D), 9(1L/8D), and 1(9D) possible clusters, respectively. This distribution can be observed experimentally by using isotopic labels as shown in Figure 1 for a cluster of 12 serines. Structures **2**, **3**, and **4** are representative racemic structures which have mixing multiplicities of 1, 2, and 4, respectively. In other words, structure **4** can also be generated by simple rotation of structure **5** or two other structures. The largest set of structures will have a multiplicity of 4; therefore, a more conservative estimate of the number of unique racemic structures is 512/4, or 128.

TABLE 1: Estimated Entropic Penalties for Model Clusters

no. of molecules	entropic penalty (kJ/mol)
4	4.4
5[a]	5.2
6	6.9
7	8.6
8	10.3
9	12.0
10	13.7
11	15.5
12	17.2
13	18.9
14	20.6
15	22.3
16	24.0

[a] The number of states for clusters of 5 molecules and higher is estimated by $2^n/4$.

Up to this point, we have ignored the entropy related to the number of permutations that lead to equivalent structures by exchanging nondistinguishable molecules. For a homochiral structure, there will be $N!$ states, where N is the number of molecules. For a racemic system, it can be shown that there will also be $N!$ equivalent states for a cluster comprising N molecules (see Supporting Information). Therefore, the total number of additional states available to the racemic system is ~128, which come entirely from the mixing of the two enantiomers. According to the Boltzmann equation, $S = k \ln(W)$, where S is entropy, k is the Boltzmann constant, and W is the number of available states, the entropic penalty for forming a particular racemic structure instead of 128 different structures is ~12 kJ/mol at 298 K. This is a substantial barrier which can be overcome only by favorable enthalpic contributions which are greater in magnitude. Estimated entropic penalties based on a similar analysis for other cluster sizes are given in Table 1. It is clear from the results given in Table 1 that entropic effects will become more pronounced with increasing cluster size.

The situation is clearly more complicated in an actual cluster, making quantitative predictions more difficult. First, enthalpic contributions are not likely to be equal for all clusters regardless of chiral composition. Second, even a homochiral cluster can adopt a very large number of different conformational structures, as illustrated by the different structures which have been proposed for the serine octamer.[3] If multiple conformations are similar energetically, then calculating the entropic contributions becomes more complicated. However, it is reasonable to suggest that heterochiral clusters could adopt even more potential structures than homochiral clusters, which would make entropic effects even more pronounced. Despite the increased complexity in a real cluster, entropic effects are likely to be important in a manner similar to those predicted by our model system. Finally, we note that due to the natural log term in the Boltzmann equation, even large inaccuracies in the number of potential states will only lead to small differences in the calculated entropic penalties. Nevertheless, predictions are valuable only if they are supported by experimental results, which we examine below.

Clusters of Amino Acids Generated Experimentally. To assess the predictions made above, clusters which are known to have specific structures must be examined. Although it is difficult to prove experimentally that a cluster has a particular

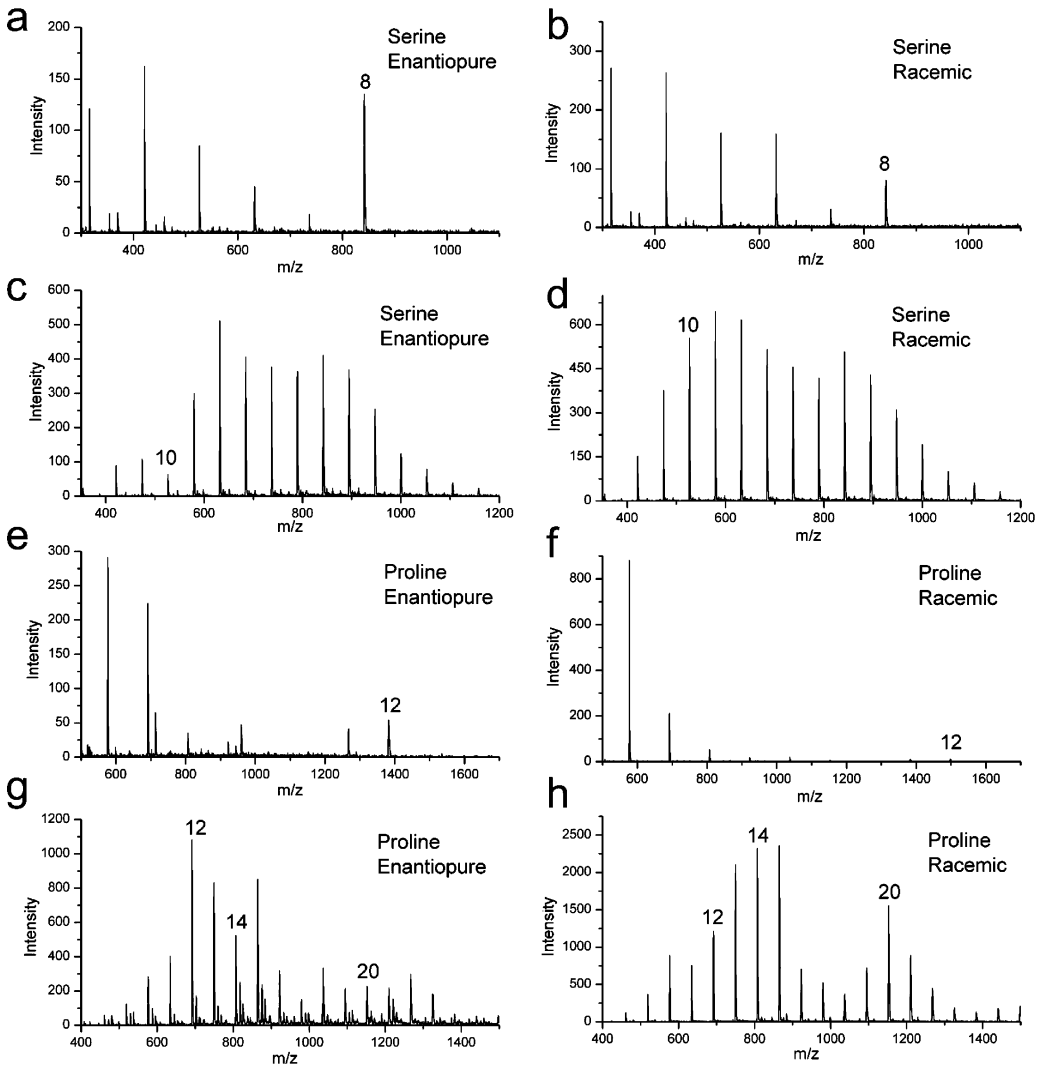

Figure 2. Serine and proline cluster distributions obtained by electrospray ionization of enantiopure (left-hand column) and racemic (right-hand column) solutions. Note the dramatic changes in relative cluster intensity for select clusters as enantiomeric composition of the solution is changed. Clusters of interest are labeled according to the number of amino acids in the cluster. (a) Singly protonated clusters of serine. The octamer is a magic number cluster. (b) The octamer is much less magic when sampled from a racemic solution. (c) Doubly protonated clusters of serine. The 9mer, 10mer, and 11mer are all suppressed, or anti-magic when sampled from an enantiopure solution. (d) The intensities of the 9mer, 10mer, and 11mer become part of a normal distribution when sampled from a racemic solution. (e) Similarly, the singly protonated proline 12mer is a magic number cluster. (f) The 12mer nearly disappears when sampled from a racemic solution. (g) Several doubly protonated proline clusters are also interesting. The 12mer is magic, while the 14mer and 20mer are both anti-magic. (h) A bimodal distribution of clusters is observed for the racemic solution, with all clusters fitting in with the adjacent cluster intensities. Parts c and d are reproduced with permission from ref 6. Copyright 2004 American Chemical Society.

structure, certain characteristics can strongly suggest that this is the case. For example, unusually abundant or "magic" clusters,[12] defined as those clusters which are much more intense than the accompanying $n + 1$ or $n - 1$ clusters, typically derive their unusual abundance from specific structural or chemical characteristics. In the present work, we also observe clusters with unusually low abundance, which we refer to as "anti-magic" clusters. The abnormally low intensity of these clusters suggests that they are not energetically stable. The unusual energetics which lead to magic and anti-magic clusters make them excellent targets for studying the effects of chirality on structural specificity.

We have examined all of the chiral amino acids and several related molecules under identical operating conditions. Under our experimental conditions, we observe only two strongly magic clusters—the protonated serine octamer (Figure 2a) and the protonated proline 12mer (Figure 2e).[13] Most of the intensity for the accompanying $[11Pro+H]^+$ peak comes from dissociation of $[12Pro+H]^+$ at the back of the drift tube, indicating that the proline 12mer is actually more magic than is apparent in the spectrum. Additionally, the doubly protonated proline 12mer forms a less intense magic number cluster (Figure 2g). There are also several anti-magic clusters. Figure 2c shows that doubly protonated clusters with nine, ten, and eleven serines are suppressed. In Figure 2g, doubly protonated proline clusters with 14 and 20 prolines are suppressed. All of the magic and anti-magic clusters that we observe are generated from enantiopure solutions, suggesting that homochiral clusters are more likely to form specific structures which are in turn more likely to exhibit unusual energetic stabilities.

In Figure 3, the percent change in the relative intensity of a cluster (referenced to the intensity of the monomer) is tracked as a function of the ee of the solution from which it was sampled. As the ee is varied, cluster stability can increase, decrease, or remain the same. Most clusters do not exhibit any change in relative intensity as the ee is varied, and they exhibit results similar to those shown for the doubly protonated cluster $[16Ser+2H]^{2+}$ in Figure 3a. More interestingly, both the serine

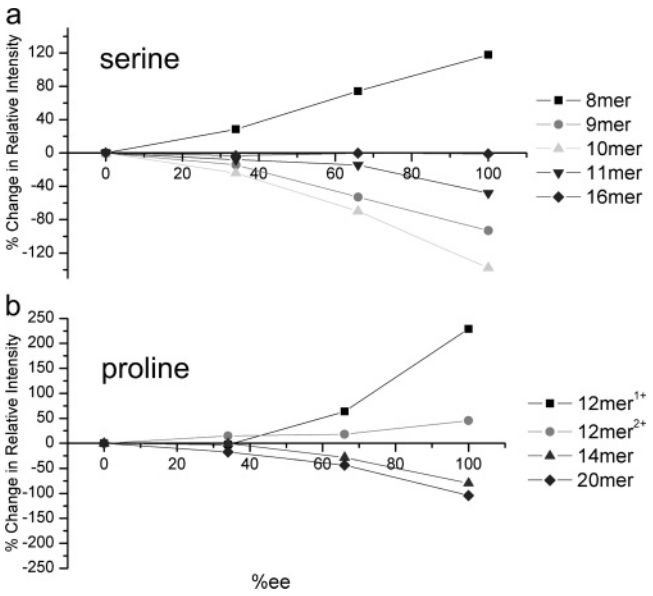

Figure 3. The percent change in the relative intensity for each cluster is shown as a function of the enantiomeric excess (ee) of the solution from which it was sampled. (a) The serine 16mer, which is representative of most of the observed clusters, does not vary in intensity with changing ee. The serine octamer becomes more abundant with increasing ee, while other peaks are suppressed. (b) The proline 12mer intensity rises abruptly near 100% ee, suggesting larger entropic effects than those influencing aggregation of the serine octamer.

octamer and the proline 12mer display dramatic changes in relative intensity as the ee is varied. As explained above, entropic penalties are predicted to become more important with increasing cluster size, indicating that prodigious assembly of the homochiral proline 12mer will become unfeasible more quickly as the ee is lowered than it will for the serine octamer. This prediction is reflected in the data. The abundance of the serine octamer rises steadily with ee in Figure 3b, while the relative intensity for the proline 12mer rises more abruptly, and only when the ee is nearly homochiral.

Figure 3 shows that increasing the ee leads to reduced relative intensity for anti-magic clusters. Thus, it would appear that the energetic stability of these clusters decreases as their composition becomes homochiral. Although the results do not suggest an exclusive explanation for this observation, one possibility is that the homochiral structures for anti-magic clusters are unusually unstable and therefore dissociate into smaller clusters. If this is proves to be true, then as the composition of the solution becomes more racemic, a wider range of possible structures will become available. As these structures become increasingly populated, the overall observed cluster intensity will rise because the channel leading to dissociation is shut off. The anti-magic clusters are observed only in enantiopure systems, because the variety of possible racemic structures makes the probability unlikely that all of them are unusually unstable. Therefore, it would appear that anti-magic clusters are controlled by both entropic and enthalpic contributions which lead to enhanced abundance for the racemic clusters.

The experimental results presented thus far, though limited in scope, are in complete agreement with the predictions made by our simple model. However, up to this point we have neglected most of the data. The vast majority of clusters of all the amino acids do not exhibit any preference for a particular chiral composition. Can this observation be explained by entropic barriers as well? If entropic penalties are generally larger than changes in enthalpy as the chiral composition or structure of the cluster is restricted, then no chiral effects will be observed. This is true whether the more stable structure is homochiral or racemic; entropy will always disfavor the formation of one structure when many are possible. This suggests that the ΔH for inserting the wrong enantiomer into the serine octamer or proline 12mer must be positive and quite high.

Conclusions

Entropy is predicted to play an important role in the assembly of chiral aggregates. The formation of specific structures from racemic solutions is hindered by significant entropic penalties. By contrast, homochiral aggregates assembled from enantiopure solutions are more likely to form specific structures and display unusual energetic stabilities. These entropic effects become more important as the size of the aggregate increases. Limited data obtained from experiments on clusters of amino acids in the gas phase are in complete agreement with these predictions. Magic clusters are observed only from enantiopure solutions. In addition, most clusters exhibit no preference for chirality, suggesting that entropic effects mediate any changes in enthalpy as the chiral composition of the cluster is modified. Additionally, we note that the same thermodynamic arguments presented here can be applied to any binary (or multiple component) system, and are not restricted to the case of two enantiomers.

If these principles prove to be general in nature, then there are several possible implications related to the origin of life[14−16] and the process of crystal nucleation.[17,18] First, these results suggest that homochirality is a mechanism for reducing entropic barriers to the formation of large organized structures such as those that were necessary for the emergence of life. However, these results do not shed any light on the mechanism by which homochirality may have emerged. Second, the underlying reasons for the predominance of racemates over conglomerates in the crystallization of chiral compounds are still elusive.[17,18] If the nucleation aggregates that lead to the growth of crystal structures are controlled by the same entropic considerations examined presently, then the lack of conglomerates might be explained by a paucity of homochiral nuclei. In both cases, further studies will be required to test the validity of these possibilities.

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Supporting Information Available: Statistics for estimating the number of available states are explained in detail. This material is available free of charge via the Internet at http://pubs.acs.org.

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