Diversity and Selection in Self-Assembled Tetrameric Capsules

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Received February 11, 2000

Self-assembling systems can be used to generate molecular variance through the reversible association of a few components into a large number of multicomponent species.[1,2] The result is a dynamic library of assemblies whose composition is thermodynamically controlled—the relative populations of its components are determined by noncovalent interactions. If these assemblies are also receptors, the library distribution will be biased toward those members that interact most favorably with added target molecules. Here, we report the generation of large numbers of self-assembled molecular capsules from relatively few reversibly associating components. This library of capsules displays spontaneous selection of the strongest receptors for the binding of guest molecules.

Molecular capsules emerge when subunits displaying appropriate curvature and self-complementary hydrogen bonding sites self-assemble.[3] These capsules assemble only in the presence of a guest of suitable size and shape, empty capsules are not formed. Compound **1** (Figure 1a) has been shown to assemble into a tetrameric capsule that encapsulates a variety of cyclic guest molecules.[4] The assembly arises from the preferential hydrogen bonding of the cyclic sulfamide donor and glycoluril acceptor that results in a head-to-tail arrangement of monomers (Figure 1b,c). Substituents on the central aromatic spacer do not disrupt the forces responsible for the assembly of the capsule but do have effects on the size, shape and chemical surface of the cavity.[5] Either attractive hydrogen-bonding interactions (R = OH, Figure 1e)[5] or repulsive steric interactions (R = OMe, Figure 1f) result from the proximal positioning of substituents on neighboring subunits in the assembled state. In short, each self-assembled capsule may show different affinities for a given guest molecule.

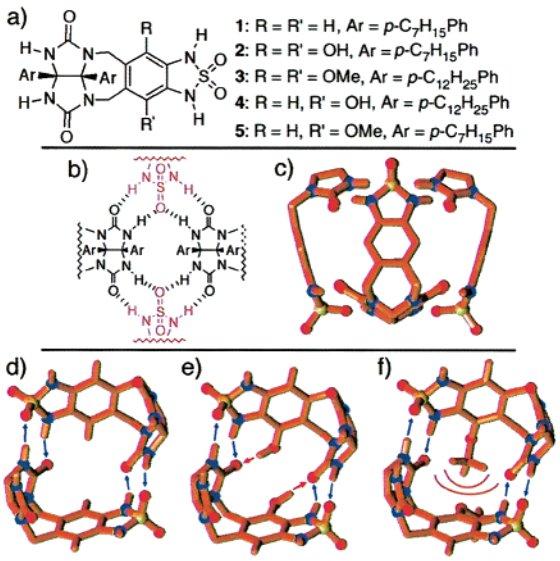

Figure 1. (a) Structures of compounds **1**−**5**. Both **4** and **5** are chiral and are each present as racemates. (b) The seam of eight hydrogen bonds at each end of the capsule. (c) Structure of capsule **1:1:1:1**.[10] (d) A model[10] of two neighboring subunits from the tetrameric capsule made up of compound **1**. (e) The attractive hydrogen bonding interaction due to hydroxyl groups on the central ring (as in **2** and **4**). (f) The repulsive steric interactions due to methoxyl groups on the central ring (as in **3** and **5**). Some substituents and hydrogen atoms have been omitted for clarity.

We expected that an equilibrating system of mixed-composition tetrameric capsules would result from the combination of subunits **1**−**5**.[6] Due to the complexity of the mixture ^{1}H NMR is of limited utility in analysis. Instead, electrospray mass spectrometry[7] (ESMS) was used to determine the composition of the mixture through mass coding. The peripheral alkyl groups of the monomers were selected such that each of the possible *tetramers* has a unique mass. Encapsulation of a charged guest species in an organic solvent gives ionic species, their relative abundances and energies are qualitatively reflected in the gas phase.[7a]

The combination of two monomers (**1** and **2**) can produce six structurally different tetrameric capsules, having five different masses. An equimolar solution of these subunits in the presence of ethyltrimethylammonium cation (**6**)[8] gave a nonstatistical mixture of all five observable receptor-guest complexes in the dynamic library as determined by ESMS. There is clearly not one favored species in this limited dynamic library. There is, however, a significant thermodynamic bias for the binding of the guest **6**: the measured composition of 22:32:31:13:2 (by percentage, from lowest to highest mass) is significantly different from the statistically predicted 6:25:38:25:6.[9] Equilibration of equimolar amounts of **1** and **2** with methylquinuclidinium cation (**7**) as a guest results in a very different profile. The mass spectrum shows one capsule (**7@1:1:1:1**) in a greater than 5-fold excess over any other combination. Capsule **7@1:1:1:1** represents 69% of the observed tetrameric capsules, or 11x the amount predicted by

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(4) Martín, T.; Obst, U.; Rebek, J., Jr. *Science*, **1998**, *281*, 1842.

(5) Nuckolls, C.; Hof, F.; Martín, T.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 10281.

(6) Compounds **2**, **3**, and **5** were synthesized by routes analogous to those previously published for compounds **1**[4] and **4**.[5]

(7) For the use of ESMS to study the parent tetrameric capsule, see (a) Schalley, C. A.; Martín, T.; Obst, U.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 2133. (b) For application to other self-assembled systems, see: Schalley, C. A. *Int. J. Mass. Spec.* **2000**, *194*, 11 and references therein.

(8) All cationic guests were added as the tetrafluoroborate salts.

(9) It is impossible to measure the distribution of capsules formed in the absence of a guest's thermodynamic bias, due to the inability of the capsules to form without a guest. Thus, comparison to the mixture predicted by statistics is the only means of examining the thermodynamic bias imparted by the guests.

10.1021/ja0005136 CCC: $19.00
Published on Web 04/14/2000

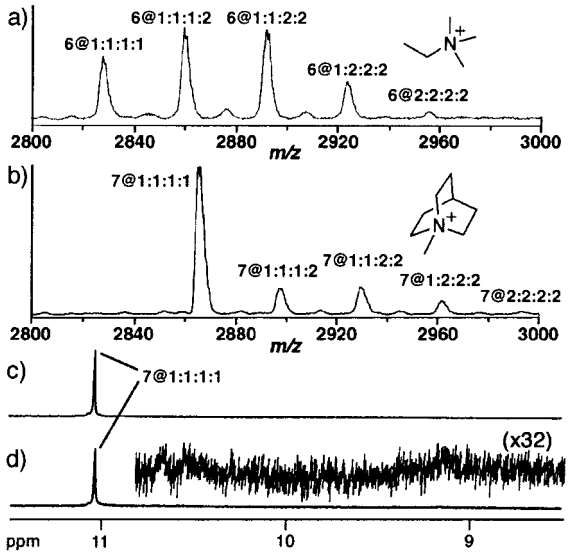

Figure 2. Mass spectra resulting from the equilibration of equimolar mixtures of **1** and **2** in CH_2Cl_2 with (a) guest **6** and (b) guest **7**.[8,13] (c) Downfield region of the ^{1}H NMR spectrum of **7** and **1** in CD_2Cl_2, showing the sulfamide protons of the assembled capsule. (d) Downfield region of the ^{1}H NMR spectrum arising from an equimolar mixture of **1** and **2** equilibrated with **7** in CD_2Cl_2. Region corresponding to unassignable sulfamide signals of mixed tetramers is shown magnified 32×.

statistics (6.25%). The same mixture at a 10-fold higher concentration allows a comparison of the gas and solution phase properties by ^{1}H NMR analysis. The structure of the most favored capsule in solution can be unambiguously assigned to that of the known capsule **7@1:1:1:1** (Figure 2c). By integration, the lower limit for the amount of **7@1:1:1:1** in solution can be set at 75% of the tetrameric capsules formed.

The smaller and somewhat flexible **6** (110 ± 3 Å^3)[10] shows little preference for receptors, whereas **7** (138 ± 3 Å^3), a larger, rigid guest displays a clear preference for those receptors possessing a larger cavity. Capsule **1:1:1:1** is calculated to enclose a volume of 169 ± 3 Å^3, while capsule **2:2:2:2** is reduced in size to 157 ± 3 Å^3 through the participation of the phenols in additional attractive hydrogen bonding interactions[5] (Figure 1e). The receptor that best fits the structure and electronics of the targeted guest molecule *spontaneously emerges* to become the predominant species in solution.[11]

These libraries were expanded through the use of equimolar mixtures of monomers **1**−**5** equilibrated in the presence of each of the two guests (Figure3). The mixture of these 7 monomers (including both enantiomers of **4** and **5**) features capsules of 70 distinct compositions. The total number of structurally distinct receptors possible (including enantiomers) is a ferocious 613. As in the smaller library, target molecule **6** gave rise to a broader range of species, showing less preference for any given receptor. Still, of the 70 possible different receptors (by composition) only

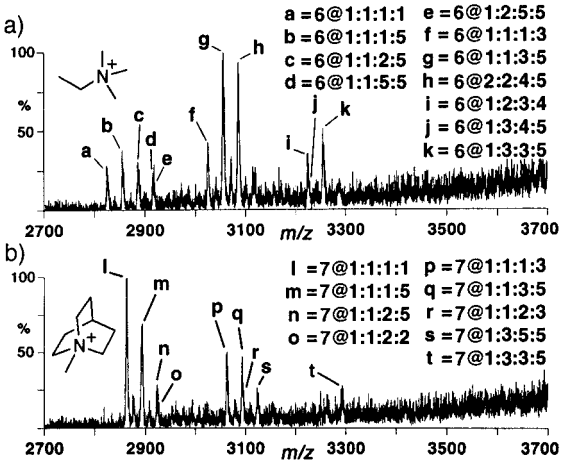

Figure 3. Mass spectra resulting from the equilibration of equimolar mixtures of **1**−**5** with (a) guest **6** and (b) guest **7**,[8] showing the entire mass range of possible tetrameric species.[14]

11 are formed in any significant amount. Target molecule **7** showed a more defined preference for a smaller number of receptors; again, the best receptor for **7** is capsule **1:1:1:1**. Even when the guest molecule shows little overall preference for a given member of the library (Figure 2a), the distribution of host−guest complexes is nonstatistical.

Combinatorial chemistry builds diversity through the simultaneous synthesis of a large number of molecules, and creates libraries in which the composition is kinetically controlled by the formation of covalent bonds.[12] Dynamic libraries as described here start with diversity, select the best receptors, and produce the winners in significant concentrations. The present system is able to explore the entire available diversity space at once and arrive at the best *global* structure. It is hoped that this system can be exploited for the discovery of other emergent properties.

Acknowledgment. The authors thank Dr. Gary Siuzdak, Kenneth Harris, William Webb, and Dr. Steven Bark for helpful discussions regarding the ESMS measurements. Dr. Ed Wintner of Neogenesis kindly helped in the design of the mass coding scheme. F.H. thanks NSERC for fellowship support, and C.N. thanks the NIH for fellowship support. The authors thank the Skaggs Institute for Chemical Biology and the National Institutes of Health for financial support.

Supporting Information Available: Experimental procedures for the syntheses of **2**, **3**, and **5**, procedures for MS and NMR measurements, and tabulation of masses and structures for all possible tetramers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0005136

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(11) The high occupancy factor (70−80%) is explained by attractive interactions between the capsule and the charged guests. See ref 7a and Mecozzi, S.; Rebek, J., Jr. *Chem. Eur. J.* **1998**, *4*, 1016.

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(13) The abundance of ions in the gas phase depends on the initial solution concentration, the ionization efficiency, and the kinetic stability of each species. These factors can be assumed to vary slightly for different tetrameric capsules, and not to have a great effect on the relative abundance observed in the gas phase. This is supported by previous MS studies[7a] and the agreement observed between ESMS and NMR measurements.

(14) The response of the detector varies as the inverse of the square root of the mass. For this library, (∼2700−3700), the mass discrimination is expected to result in a decrease in signal intensity of ∼15% for the heaviest species relative to the lightest. Over the smaller mass range of the libraries in Figure 2a, b these effects can be ignored.