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A Synthetic Mimic of Protein Inner Space: Buried Polar Interactions in a Deep Water-Soluble Host

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Cavitands are open-ended vase-like molecules that temporarily trap complementary guests within a limited space. While isolated from bulk solvent, guests are held in a fixed solvent "sphere" that comprises eight benzene rings—a structured, hydrophobic environment. Water-soluble versions[1,2] create a hydrophobic pocket which may resemble the environment of protein interiors. This research was undertaken to evaluate the effect of the cavitand's environment on polar interactions shielded from the aqueous solution outside. We have fixed an "introverted" carboxylic acid functional group on a water-soluble cavitand which folds around cationic guests. This synthetic structure reproduces the ability of natural hosts such as enzymes, ribozymes, and antibodies to (1) fold around a target molecule, (2) present it with specific recognition functionality, (3) isolate it from the aqueous medium, and (4) fix it in a structured hydrophobic environment. The results are consistent with quantitative studies of buried polar interactions in proteins.

Acid cavitand (±)-**1** was prepared from known Boc-protected resorcinarene[2] following the procedures previously described for (±)-**3** (see Supporting Information).[3] Deprotection with HCl gave (±)-**2**, which presents an inwardly directed carboxylic acid substituent on one cavitand wall and is rendered water-soluble with charged ammonium "feet" (Figure 1). The nonfunctionalized, water-soluble octa-amide cavitand **4** was prepared as described earlier[2] and was used as a control receptor.

Binding of quinuclidine **5** inside (±)-**1** in $CDCl_3$ was evident from the ^{1}H NMR spectra, where guest resonances appear in the far-upfield region (Figure 2a). The association constant was larger than

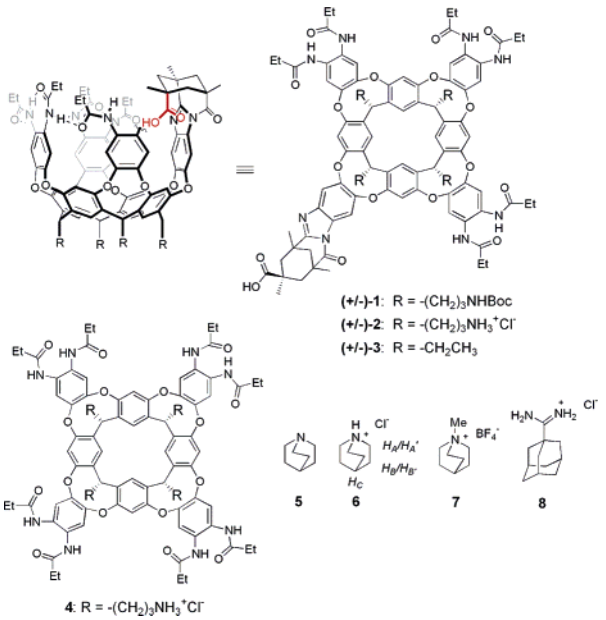

Figure 1. Cavitand hosts and guests.

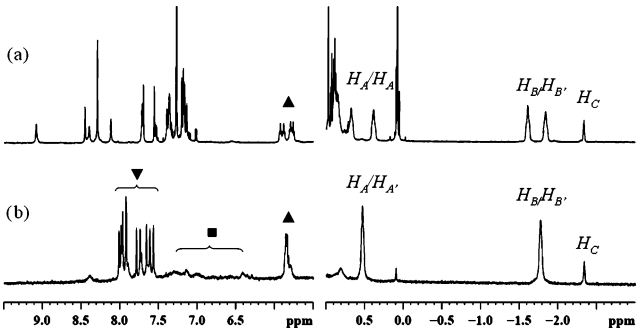

Figure 2. ^{1}H NMR spectra (600 MHz, 300 K) of complexes: (a) (±)-**1·5** in $CDCl_3$; (b) (±)-**2·6** in D_2O (▲ = methine protons for the vase conformation; ▼ = aromatic protons for the cavitand vase; ■ = remnants of the unstructured form of (±)-**2**).

what could be determined by NMR (Table 1). The chiral environment of host (±)-**1** led to splitting of the guest proton resonances of **5**. In $CDCl_3$, one orientation (cyclodiastereomer) of the amide rim provides more hydrogen bonding interactions and is energetically favored (Figure 3, structure I). This amplifies the intrinsic asymmetric magnetic environment provided by the acyl benzimidazole function.

In D_2O, (±)-**2** with guest **6** exhibits methine proton resonances in the region of ~5.7 ppm, characteristic of the vase conformation (Figure 2b).[4] Resonances for **6** in the cavity were also in the far-upfield region of the spectrum (Figure 2). Unexpectedly, the signals for geminal protons of guest **6** were coalesced at 300 K (Figure 2b). While we cannot conclusively explain the collapse of the signals in D_2O, we suspect that water molecules participate in the hydrogen bonding seam[1a] and lead to fewer differences between the two cyclodiastereomers and may aid their rapid interconversion (Figure 3). Consistent with this, collapse of the signals of **5** bound to (±)-**1** occurred in water-saturated $CDCl_3$ (see Supporting Information). Upon cooling the (±)-**2·6** complex to 275 K, the influence of the chiral upper rim of the host on **6** did become apparent in D_2O, and splitting of the $H_A/H_{A'}$ protons which reside closer to the upper rim was observed.

The ^{1}H NMR of (±)-**2** in the absence of guests shows a poorly resolved and complicated spectra, and methine resonances appear in the region of 4.1 ppm, indicating the open kite conformation.[4] A dimeric kite conformation has also been observed for **4** in the absence of guest.[2] The addition of **6** to (±)-**2** led to sharpening of the host proton NMR peaks in a vase conformation. The cavitand folds around the ammonium guest through an induced-fit process.

The binding constant for complex (±)-**2·6** was 1300 M^{-1}, whereas a binding constant of 12 M^{-1} was determined for the **4·6** complex in D_2O (Table 1). The introverted carboxylate, which presumably forms an ionic hydrogen bond with **6**,[5] enhances the affinity by −2.7 kcal/mol (Table 1). A significantly weaker interaction was observed between (±)-**2** and **7** ($\Delta\Delta G_{binding} = -0.6$

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Table 1. Thermodynamic and Kinetic Parameters for Host−Guest Complexation at 300 K[a]

complex host·**X**	solvent	K_{assoc} M^{-1}	$\Delta G_{binding}$ kcal/mol	$\Delta\Delta G_{binding}$[b] kcal/mol	$\Delta G_{diss}^{\ddagger}$ kcal/mol
1·5	$CDCl_3$	$>10^4$	n.d.	n.d.	19
2·6	D_2O	1300	−4.2(0.1)	−2.7	19
4·6	D_2O	12	−1.5(0.1)		17
2·7	D_2O	18	−1.7(0.1)	−0.6	n.d.
4·7	D_2O[c]	4	−0.8(0.2)		n.d.
4·7	D_2O	6	−1.1(0.3)		n.d.
2·8	D_2O	50	−2.3(0.2)	−1.0	n.d.
4·8	D_2O	9	−1.3(0.1)		n.d.

[a] Experiments in D_2O were performed in 10 mM sodium phosphate buffer, pH 5.25. [b] $\Delta\Delta G_{binding}$ with guest **X** was calculated according to $\Delta G_{binding}$((±)-**2·X**) − $\Delta G_{binding}$(**4·X**). [c] At pH 1.4.

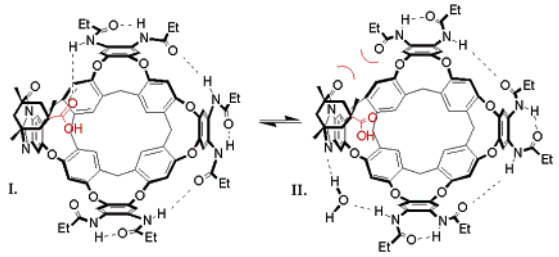

Figure 3. Proposed equilibrium of (±)-**2** in D_2O.

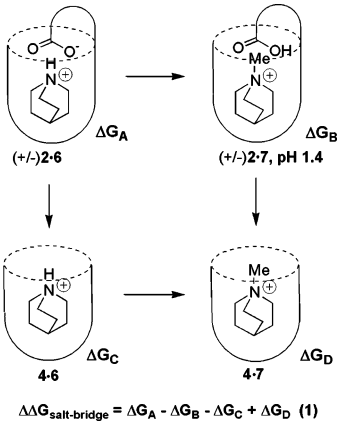

Figure 4. Free energy cycle.

kcal/mol), which likely reflects a loss of a buried hydrogen bond relative to (±)-**2·6**. Amidinium guest **8** also showed only a weak interaction with (±)-**2** (Table 1). Molecular modeling indicates an ideal fit of **6** within the cavity, whereas **8** poses some steric problems.

A more rigorous calculation of the buried interaction in (±)-**2·6** was performed using a free energy cycle (Figure 4).[6] Complex (±)-**2·7** was used as one extreme; at pH 1.4, the acid is protonated and prevents charge interactions. The salt-bridge interaction in (±)-**2·6** was isolated according to eq 1 (Figure 4) and was determined to be worth −3 kcal/mol.

The salt bridge in complex (±)-**2·6** is significantly larger than one exposed to aqueous solvent, where estimates are in the range of −0.2 to −1.5 kcal/mol.[7] Buried charge−charge interactions can vary,[8] from energetically repulsive[9] to stabilizing by as much as −3.5 kcal/mol.[10] A buried Asp−Arg in Barnase is worth −3.3 kcal/mol,[10b] in good correlation with the present case.

The influence of the introverted acid on the guest exchange kinetics was investigated by exchange spectroscopy (EXSY).[11] Complex **4·6** has a dissociation barrier, $\Delta G_{diss}^{\ddagger}$, of 17 kcal/mol in D_2O (Table), which is consistent with $\Delta G_{diss}^{\ddagger}$ for complexes of guests with unfunctionalized cavitands in organic solvents.[12] This suggests that the dissociation mechanism in host **4** follows the mechanism in organic solvents: a vase to kite conformational shift (10−12 kcal/mol) and rupture of the upper rim hydrogen bonds (5−7 kcal/mol).[12] Both (±)-**2·6** in D_2O and (±)-**1·5** in $CDCl_3$ show $\Delta G_{diss}^{\ddagger}$ values of 19 kcal/mol (Table 1). The 2 kcal/mol increase in $\Delta G_{diss}^{\ddagger}$ for the introverted acid receptors may reflect the energy required to break the ionic interaction, as well as increased physical constraints imparted on the guest by the introverted acid.

Many water-soluble hosts incorporate charged substituents on the solvent-exposed host surfaces,[13] where solvent screening is high, and there are few examples that use the receptor backbone to shield polar groups from water.[14] Buried salt bridges and hydrogen bonds are important regulators of structural specificity in proteins[15] and are often stronger in the interior than at the surfaces.[16] In (±)-**2**, the deep host aromatic walls provide a hydrophobic environment, and the secondary amides of the upper rim resemble peptide bonds of protein backbones. Accordingly, (±)-**2** folds around a cationic guest, temporarily locks it in a defined environment, and stabilizes a buried polar interaction similar to the favorable cases measured in proteins.

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Supporting Information Available: Synthetic procedures, experimental details, and 1- and 2-D NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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