J|A|C|S
COMMUNICATIONS

Published on Web 11/02/2002

A Synthetic Receptor for Choline and Carnitine

Pablo Ballester, Alexander Shivanyuk, Adel Rafai Far, and Julius Rebek, Jr*

The Skaggs Institute for Chemical Biology, The Scripps Research Institute, MB-26, 10550 North Torrey Pines Road, La Jolla, California 92037

Received August 23, 2002

Molecular recognition arises from intermolecular forces acting on complementary surfaces. Biology offers several combinatorial algorithms to generate complements to a given target, for example, phage display, antibodies, and aptamers, but chemical methods rely on synthesis to position functionality, engineer molecular curvature, and fill space. We describe here a receptor for choline and carnitine that features a deep, concave binding site. The cavity is of the appropriate shape and size for alkylated, roughly spherical trimethylammonium ions yet resists occupancy by larger ions. Choline and carnitine are bound with high affinity even though no complementarity charges are involved. The system overcomes the lack of selectivity encountered with other synthetic receptors.

Choline **1a**$^+$ and carnitine **1b** (Figure 1) are common biological agents that pose uncommon problems for molecular recognition. The positive charge of choline and the roughly spherical shape of its trimethylammonium group beg for negative charges on concave surfaces. Their quaternized nitrogens, although charged, are not capable of salt-bridging or hydrogen-bonding. Instead, aromatic surfaces are frequently found as complements in naturally occurring receptors,[1] and in several synthetic receptors. An early model is the calixarene tetrasulfonate **2**.[2] Affinity for choline in water is very good (log $K_a = 4.7$) but selectivity is not: tetraethylammonium ions **1c**$^+$ bind even better (log $K_a = 5.6$) and tetrapropylammonium ions **1d**$^+$ bind nearly as well (log $K_a = 4.5$). The crystal structures of these complexes reveal the reason: the concavity of the receptors is modest, and they surround only a small fraction of the target molecules.[2,3] The receptor lacks complementarity in size or shape. X-ray evidence[4] exists for cation$-\pi$ interactions between a neutral macrocyclic receptor **3** and acetylcholine **1h**$^+$, but receptor **3** is even shallower than **2.** Consequently, the tetraanions of **3** can recognize little more than the positive charge.[5] Recently, π-basic, neutral macrocycles were shown to bind weakly to acetylcholine and tetramethylammonium cation in $CDCl_3$.[6] Selectivity for other quaternary salts was not reported, but the binding was dependent on the counterion present. Deeper cavitands[7] of the proper size can offer interactions with increasingly larger surface areas of small, convex molecular targets. We report here such a receptor and, although it bears no anions, it recognizes choline and carnitine. Complementarity of *size and shape* provides high affinity and selectivity without recourse to charge/charge attractions.

The synthesis of the new receptors begins with the reaction of resorcinarene **3** and 4 equiv of 1,2-difluoro-4,5-dinitrobenzene to give octanitro compound **4** in high yield.[8,9] Reduction with $SnCl_2$ in EtOH/ HCl gave the hydrochloride salt of **5** in pure form as it precipitated from the reaction mixture. Trituration of this salt with ammonium hydroxide and extraction with EtOAc gave octaamine **5** as a yellowish solid, which is surprisingly stable to air oxidation.

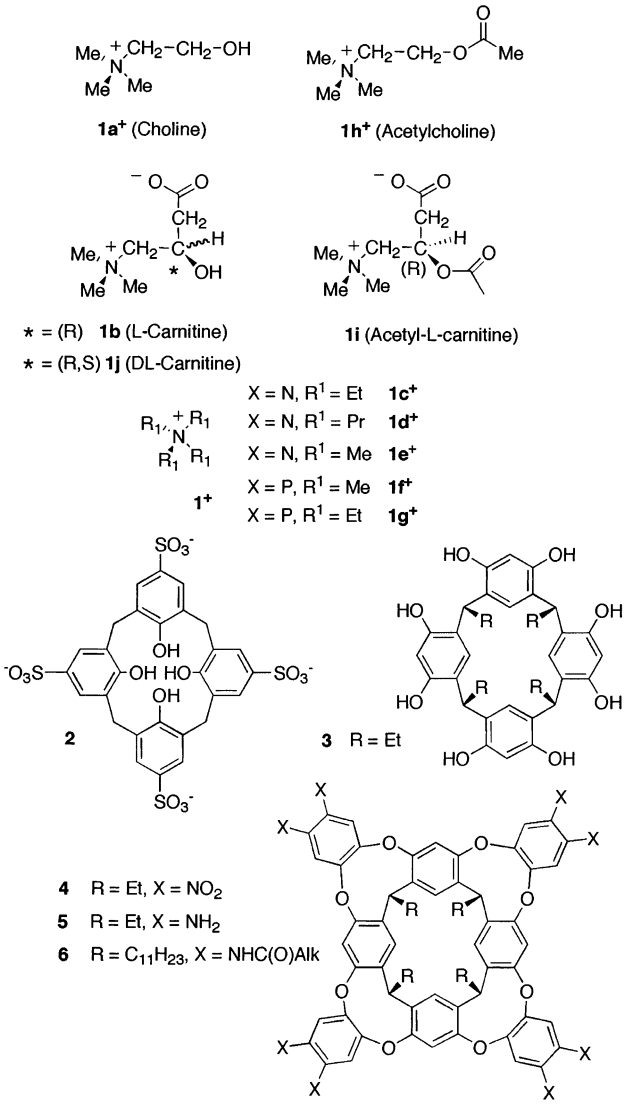

Figure 1. Structures of guests **1** and hosts **2**−**6**.

When a seam of hydrogen bonds exists at the upper rim of deep cavitands, the "vase" conformation is stabilized, and inclusion complexes are formed with neutral and cationic organic guests.[10] An especially beautiful example has been recently described by Atwood[11] for surrounding Me_4N^+. The octaamine has no such seam of hydrogen bonds (Figure 2, top) but can be provided with one by appropriate solvent molecules. Specifically, DMSO can accept hydrogen bonds from two donors at each of the cavitand corners as modeled in Figure 2, center. No conclusive evidence supports this structure, but modeling and binding are consistent with it.[12]

* Address correspondence to this author. E-Mail: jrebek@scripps.edu.

10.1021/ja0282689 CCC: $22.00

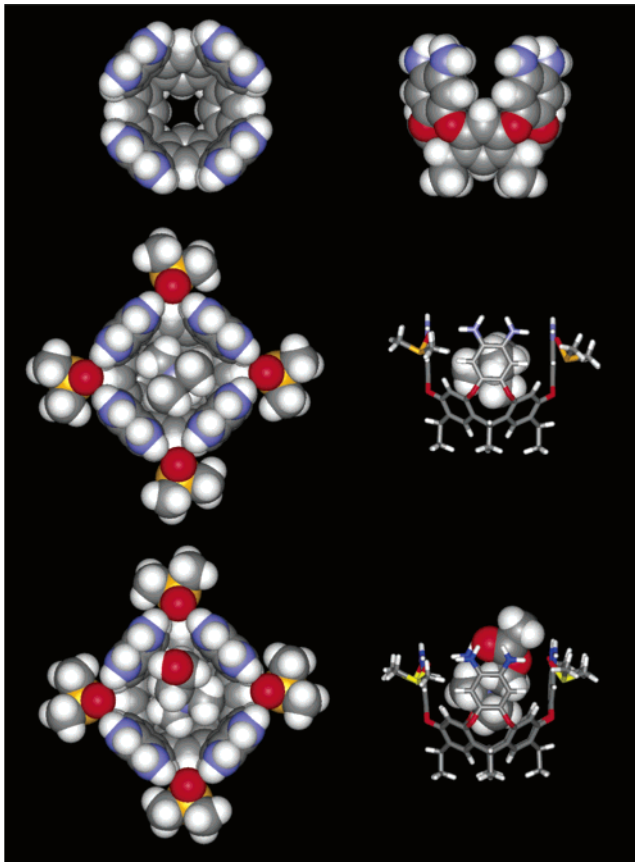

Figure 2. Molecular mechanics-optimized [AMBER force field[14]] structures; peripheral alkyl groups are truncated to methyls for clarity. (Top) Views of the receptor **5**. (Middle) **5**·4 DMSO with tetramethylammonium ion (**1e**$^+$) in the cavity. Left, the four DMSO molecules provide the oxygen acceptors (red) that complete the seam of hydrogen bonds. On the right, the receptor is represented as a bond framework while the guest is rendered as a space-filling model. (Bottom left) **5**·4 DMSO with choline (**1a**$^+$). (Bottom right) **5**·4 DMSO with acetyl choline (**1h**$^+$).

The ^{1}H NMR spectrum of octaamine **5** in DMSO-d_6 (Figure 3a) showed resonances characteristic of the "vase" conformation.[13] The signals are broadened by dynamic effects, probably involving exchange equilibration with the alternative "kite" conformation. The 16 protons of the amino groups emerge as a broadened singlet at 4.50 ppm. The addition of tetramethylammonium chloride **1e**$^+$·Cl$^-$ to the solution of **5** in DMSO-d_6 resulted in a new set of signals that emerge at the expense of the original set as the salt concentration increases (Figure 3b). A sharp singlet appears at −0.5 ppm, corresponding to the protons of the encapsulated tetramethylammonium cation. The large upfield chemical shift ($\Delta\delta = -3.6$ ppm) places the detained cation in a cavity surrounded by aromatic rings. Titrations established the stability constant of this complex as 2.4 × 10^4 M^{-1} at 298 K (Table 1). The 2D NOESY and ROESY spectra revealed intermolecular NOEs between the protons of encapsulated **1e**$^+$ and all aromatic protons of **5**, indicating close host/guest contacts. The molecular mechanics (MM) minimized structure of complex **5**·**1e**$^+$ shown in Figure 2 (center) predicts multiple polarized CH−π interactions.

The larger tetraethylammonium **1c**$^+$ is also bound by the cavity of **5**, but at a price. The binding constant is nearly 100-fold smaller than that of **1e**$^+$ (Table 1). No evidence of binding to tetrapropylammonium cation **1d**$^+$ was observed.

Choline **1a**$^+$ is also included into the cavity of **5** with K_{ass} of 1.2 × 10^4 M^{-1}. The resonance of protons of the trimethylamino fragment in the complex is shifted upfield by 3.8 ppm (Figure 3c),

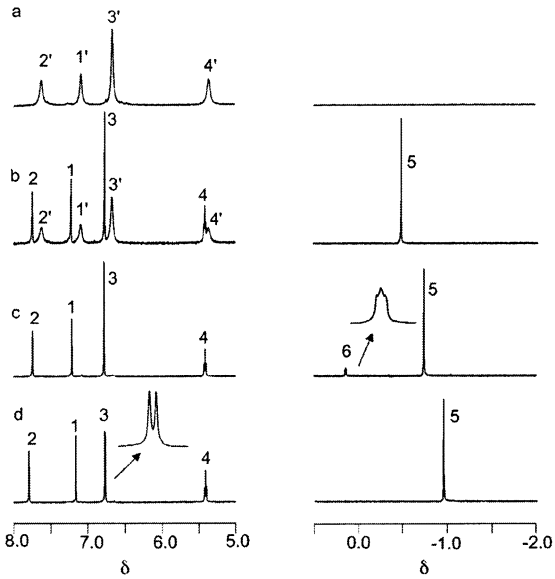

Figure 3. ^{1}H NMR spectra of inclusion complexes of **5** with ammonium salts (DMSO-d_6, 298 K, [**5**] = 2.7 mM, 600 MHz). Signals labeled 1 and 2 represent protons of the resorcinol rings; 3 are the protons of the cavitand's walls; 4 are methine protons of the bridges; Primed numbers indicate the signals of free **5**; 5 represents the *N*-methyl protons of encapsulated guest; 6 represents the NCH_2 protons of encapsulated **1h**$^+$. **(**a) **5**; (b) **5** + 0.6 equiv **1e**$^+$ Cl$^-$; (c) **5** + 1.5 equiv **1a**$^+$ Cl$^-$; (d) **5** + 3 equiv **1b**; the arrow indicates the expansions of the signals.

Table 1. Stability Constants (K_{ass}, M^{-1}), Selectivities of Binding Relatively to Choline and Free Energies of Formation (ΔG_0, kcal/mol) for 1:1 Complexes of **5** with Ammonium Salts in DMSO-d_6 at 298 K

guest	K_{ass}	K_{ass} (choline)/ K_{ass} (guest)[b]	ΔG_0
Me_4N^+·Cl$^-$	22000 ± 4000 [a]	0.5	5.9 ± 0.1
Me_4P^+·Br$^-$	1500 ± 300 [a]	13	4.3 ± 0.1
Et_4N^+·Cl$^-$	260 ± 30 [a]	-	3.3 ± 0.1
choline·Cl$^-$	12000 ± 2400	1	5.5 ± 0.1
acetylcholine·Cl$^-$	4000 ± 800	3	4.9 ± 0.1
L-carnitine	15000 ± 3000	0.8	5.6 ± 0.1
DL-carnitine·HCl	2500 ± 500	5	4.6 ± 0.1
acetylcarnitine·HCl	30± 6	-	2.0 ± 0.1

[a] These values are thermodynamic and reflect the higher symmetries of these guests; for direct comparison with, e.g., choline the statistically corrected (intrinsic) association constants should be used and are 1/4 of the values shown. [b] Determined from direct competition experiments with choline.

while the N−CH_2 protons experience slightly weaker shielding ($\Delta\delta = -3.2$ ppm). The methyl protons are maximally exposed to π-systems of the aromatic rings, while the 2-hydroxyethyl fragment is nearer the upper rim of the vase (Figure 2, bottom left). MM calculation suggests a hydrogen bond forms between the hydroxy group of the guest and an amino group of the host. The complexation of acetylcholine **1h**$^+$ is 0.6 kcal/mol weaker than that of choline (Table 1). The difference can be due to the loss of this admittedly weak hydrogen bond, or it may be steric in origin. The negligible change of chemical shift for the methyl protons of the acetyl group ($\Delta\delta = -0.08$ ppm) indicates that this group extends beyond the cavity (Figure 2, bottom right). The changes in chemical shifts for other resonances were comparable to those of choline (Figure 3c,d).

The exchange rates of guests into and out of the complexes **5**·**1b** and **5**·**1h**$^+$ were determined to be 1.5 ± 0.2 s^{-1} and correspond to an activation barrier $\Delta G^‡$ of about 17 kcal/mol at 298 K. Comparable values have been observed for the complexes of

octaamides **6** with adamantane derivatives[15] in $CDCl_3$ ($\Delta G^{\ddagger} = 16.9$ kcal/mol). The release of the guest from any of these cavities involves the vase-to-kite activation barrier of 10−12 kcal/mol[16] and requires the disruption of several hydrogen bonds.

Strong binding of L-carnitine **1b** (Table 1) is also apparent. The ^{1}H NMR spectrum of the complex reflects the diastereotopic protons of the cavitand's walls (Figure 3d) when this asymmetric guest is in the receptor. The signals of the diastereotopic N-CH_2 hydrogens are shifted upfield by 2.1 and 2.2 ppm, respectively. DL-Carnitine hydrochloride shows a reduced affinity toward receptor **5** compared to **1b**. Size and shape selectivity appears dramatically during the complexation of L-acetylcarnitine hydrochloride. It showed a very weak affinity, some 2.6 kcal/mol less than DL-carnitine hydrochloride. Loss of hydrogen bonding or acetylation of the primary hydroxyl group as inferred from the observed choline/acetyl choline selectivity cannot account for more than 0.6 kcal/mol. In this particular case the branching of the structure at the acetoxy group must create steric clashes with the walls of the receptor. Small perturbations have large consequences when a guest is closely surrounded in a deep cavity.

The high symmetry of tetraalkylammonium salts imparts a statistical advantage; four ways exist for tetramethylammonium ion to fit into the cavity where only one way is allowed for carnitine, choline, or their acetyl derivatives. Accordingly, the intrinsic (statistically corrected) value for the association constant of tetramethylammonium cation is only $^1/_4$ that shown in Table 1. Given this correction, carnitine is the best guest of those tested. Its carboxyl and hydroxyl functions are well-positioned for hydrogen bonding to the amino groups at the rim of the host.

Considerably weaker complexation is seen for tetramethylphosphonium bromide $\mathbf{1f}^+ \cdot Br^-$ ($K_{ass} = 1.5 \times 10^3\ M^{-1}$). This is unlikely to result from a bad fit, since the size of the ion is scarcely larger than its nitrogen counterpart. The lower electronegativity of phosphorus versus the nitrogen leads to smaller partial positive charges on the methyl groups of $\mathbf{1f}^+$ and is reflected in weaker C−H/π interactions. The inclusion of $\mathbf{1f}^+$ by **5** is also slow on the ^{31}P NMR time scale; the phosphorus atom of $\mathbf{1f}^+$ becomes more shielded in the cavity ($\Delta\delta = -7.3$ ppm). The tetraethylphosphonium cation $\mathbf{1g}^+$ showed a 20-fold lower affinity than its tetramethyl counterpart.

The octaamine **5** recognizes alkylated derivatives of the trimethylammonium ion. The size and shape of this functional "knob" complements the lining and dimensions of the cavity and are the source of the unprecedented binding selectivity. Specific cation−π attractions[17] between the positive charge of the guest and the electron-rich aromatic surfaces of the host result in the formation of complexes with highly kinetic and thermodynamic stability. They are more than adequate alternatives to the charge/charge interactions featured by other synthetic receptors.

Acknowledgment. We are grateful to the Skaggs Foundation and the National Institutes of Health (GM 27932) for financial support. A.S. and A.R.F. are Skaggs Postdoctoral Fellows. P.B. thanks SEEyU (Spain) PR2001-0274 for partial financial support.

Supporting Information Available: Experimental details for the titrations and analysis of the data for the calculation of the association constants (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Zacharias, N.; Dougherty, D. A. *Trends Phramacol. Sci.* **2002**, *23*, 281.
(2) Lehn, J.-M.; Meric, R.; Vingeron, J. P.; Cesario, M.; Guilheim, J.; Pascard, C.; Asfari, Z.; Vicens, J. *Supramol. Chem.* **1995**, *5*, 97.
(3) Atwood, J. L.; Barbour, L. J.; Junk, P. C.; Orr, W. *Supramol. Chem.* **1995**, *5*, 105.
(4) Schneider, H.-J.; Schneider, U. *J. Org. Chem.* **1987**, *52*, 1613.
(5) Murayama, K.; Aoki, K. *Chem. Commun.* **1997,** 119.
(6) Bartoli, S.; Roelens, S. *J. Am. Chem. Soc.* **2002**, *124*, 8307.
(7) (a) Cram, D. J.; Cram, J. M. In *Container Molecules and Their Guests;* Stoddart, F., Ed.; The Royal Society of Chemistry: Cambridge,1994. (b) Timmerman, P.; Verboom, W.; Reinhoudt, D. N. *Tetrahedron* **1996**, *52*, 2663. (c) Rudkevich, D. M.; Rebek, J., Jr. *Eur. J. Org. Chem.* **1999**, 1991. (d) *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Saadioui, M., Eds.; Kluwer Academic Publishers: Dordrecht, Boston, London, 2001.
(8) Rudkevich, D. M.; Hilmersson, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 12216.
(9) Amrhein, P.; Shivanyuk, A.; Johnson, D. W.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2002**, *124*, 10349.
(10) (a) Haino, T.; Rudkevich; D. M.; Shivanyuk, A.; Rissanen, K.; Rebek, J., Jr. *Chem. Eur. J.* **2000**, *6*, 3797. (b) Saito, S.; Nuckolls, C.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2000**, *122*, 9628. (c) Rafai Far, A.; Shivanyuk, A.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2002**, *124*, 2854.
(11) Atwood, J. L.; Szumna, A. *J. Am. Chem. Soc.* **2002**, *124*, 10646.
(12) Kinetically stable encapsulation complexes were also observed with **5** in DMF-d_7 and solvent mixtures containing DMSO-d_6 with $CDCl_3$ or D_2O. In 41% D_2O the K_a for choline was calculated to be 200 M^{-1}. Higher contents of D_2O induce precipitation.
(13) Moran, J. R.; Ericson, J. L.; Dalcanale, E.; Bryant, J. A.; Knobler, C. B.; Cram, D. J. *J. Am. Chem. Soc.* **1991**, *113*, 5707.
(14) *Hyperchem*, release 7; Hypercube Inc., 2002.
(15) These values were obtained from a 2D EXSY experiment ($t_m = 300$ ms). For a recent review on dynamic NMR studies of supramolecular complexes, see: Pons, M.; Millet, O. *Prog. Nucl. Magn. Reson. Spectrosc.* **2001**, 267.
(16) Cram, D. J.; Choi, H.-J.; Bryant, J. A.; Knobler, C. B. *J. Am. Chem. Soc.* **1992**, *114*, 7748.
(17) Dougherty, D. A. *Science* **1996** *217*, 1303.

JA0282689