Remarkable Effect of Receptor Size in the Binding of Acetylcholine and Related Ammonium Ions to Water-Soluble Cryptophanes

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The factors that govern the binding of acetylcholine and structurally related species to natural or artificial receptors are of current interest.[1] The assumption that acetylcholine esterases (AChEs) contain an anionic site binding the Me_3N^+ head electrostatically has recently been challenged[2] both by experiments on artificial model receptors[1,3-5] and by the elucidation of the structure[6] of the presumed binding pocket of acetylcholine in AChE, a narrow gorge surrounded by 14 aromatic residues and containing only a few acidic residues. In artificial receptors of acetylcholine which also contain a combination of aromatic rings and carboxylic acid groups,[1,2] the latter are generally remote from the cavity and their electrostatic contribution to the binding is considered to be secondary.[7] According to recent views,[2] it is the existence of cation-π interactions between the Me_3N^+ group and the electron rich aromatic groups of the artificial as well as natural receptors that would represent the main driving force for the binding.[8] The experiments that we report here suggest that, besides cation-π interactions and other driving forces related to hydrophobic effects, the *degree of freedom* of the bound substrate may also play a significant role. A relatively loose association appears to be more favorable than a tight lock and key pairing to achieve a strong binding of Me_3N^+R species in water. This hypothesis is based on the observation of a considerable difference between the affinities of a series of ammonium substrates to the water-soulble cryptophanes[9] **3** and **4**, which differ by ca. 9% in the van der Waals diameter of their quasi-spherical cavities.[10]

Cryptophanes **3** and **4** were synthesized from cryptophane-E (**1**)[11,12] and cryptophane-O (**2**)[13] as described previously in a similar case.[13] Stirred suspensions of **3** or **4** in D_2O were titrated by addition of 1 M NaOD until four of the six acidic groups were ionized, to give ca. 10^{-2} M solutions (pH 6.5 ± 0.5).[14] An ammonium salt (picrate or iodide, 0.95 equiv) was then added, and the host-guest interactions were studied at 300 K by ^{1}H-NMR (200 MHz for **3**, 500 MHz for **4**). In all case we observed a reversible binding under slow-exchange conditions on the NMR time scale. Figure 1 shows a plot of the free enthalpy of binding $\Delta G°$ as a function of the van der Waals volumes of the guests, for the two series of experiments, together with data[15] on the binding of the same substrates to **1** (same cavity size as **3**) in an organic solvent, $(CDCl_2)_2$.

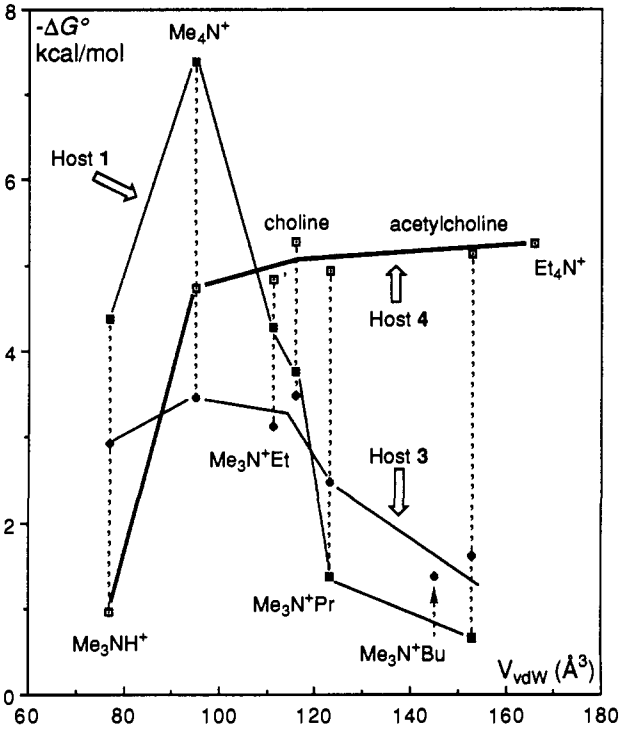
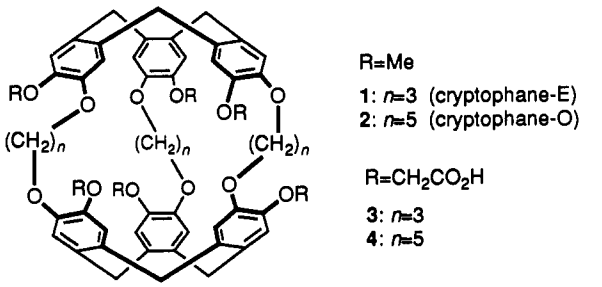


Figure 1. Free energy of binding $\Delta G°$ at 300 K as a function of guest size for host **1** in $(CDCl_2)_2$ and hosts **3** and **4** in D_2O, pH 6.5.



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(7) Strong electrostatic binding of Me_3N^+R substrates may take place with receptors bearing charges on the inside of their cavity, such as macrocyclic polyphenolates; see ref 5.

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(10) The host geometries were derived from the crystal structures of **1** and **2**; for **1**, see: Canceill, J.; Cesario, M.; Collet, A.; Guilhem, J.; Lacombe, L.; Lozach, B.; Pascard, C. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1246-1248. The structure of **2** was established by A. Aubry (unpublished work). The van der Waals dimensions of the quasi-spherical cavities (5.33 Å in diameter and 89 Å^3 in volume for **1** and **3**, vs 6.03 Å and 115 Å^3 for **2** and **4**, respectively) were estimated from the X-ray data as described in the following: Garel, L.; Dutasta, J.-P.; Collet, A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1169-1171.

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(13) Canceill, J.; Lacombe, L.; Collet, A. *J. Chem. Soc., Chem. Commun.* **1987**, 219-221. Selective demethylation of the MeO groups in **1** (82%) and **2** (66%) was achieved by reaction with Ph_2PLi in dry THF (24 h, room temperature). The resulting hexaphenols were O-alkylated by reaction with methyl bromoacetate and Cs_2CO_3 in DMF (56% and 89%, respectively). Then, alkaline hydrolysis (NaOH, H_2O, MeOH) provided the hexaacids **3** and **4**, which were fully characterized (^{1}H- and ^{13}C-NMR, FABMS, elemental analyses).

(14) We did not observe any significant effect of the pH on the binding in this range; no hydrolysis of acetylcholine to choline took place under these conditions.

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For host **1** in $(CDCl_2)_2$, Me_4N^+ is the best substrate, and the very large and unprecedented binding constant (K = 225 000 M^{-1}, $\Delta G°$ = –7.4 kcal/mol) indicates that this spherical cation prefers to be surrounded by aromatic rings than by chloroalkane molecules. The effect of replacing one of its Me groups by a longer R tail (R = Et, Pr, CH_2CH_2OH, Bu, and CH_2CH_2OAc) is a considerable drop of the complex stabilities, with a weak binding of acetylcholine ($\Delta G°$ = –0.6 kcal/mol) and no detectable binding of Me_3N^+Bu. The same trend is observed with **3** in D_2O; Me_4N^+ still prefers the aromatic host to water; however, its $\Delta G°$ is reduced to –3.5 kcal/mol, presumably because this cation is strongly solvated in water. For the Me_3N^+R substrates with R = Pr and larger groups, there is a moderate increase of the complex stabilities with respect to the organic solvent, which may be due to the hydrophobic character of the tail.

On going from **3** to the larger cryptophane **4**, there is a dramatic *increase* of the binding constants (except for Me_3NH^+, which is too small). All substrates in the range of size Me_4N^+ to Et_4N^+ show K's of the same order of magnitude (2700–6400 M^{-1}). The small stability increase on going from Me_4N^+ to acetylcholine and Et_4N^+ in **4** may be due to the optimization of attractive van der Waals interactions, or to the fact that larger substrates may release more water molecules on complexation. The high affinities of choline ($\Delta G°$ = –5.3 kcal/mol) and acetylcholine (–5.2 kcal/mol) to **4** are now comparable to those reported for acetylcholine esterases[16] and for the best artificial hosts (having cyclophane[1] and bis(ethanoanthracene)[2] structures) under similar conditions (neutral pH). As both the substrate–solvent interactions and the possible effects of the external ionized or un-ionized carboxylic groups of the hosts are comparable for the two sets of experiments, this change should be ascribed to small differences in the conformations and degree of solvation of the hosts prior to complexation (the importance of these effects is difficult to appreciate), and to differences in the host–substrate interactions.

Comparison of the ^{1}H-NMR induced shifts ($\Delta\delta$'s) provides evidence that the host–substrate interactions may indeed be significantly affected by small changes in the size of the host cavity. With **3**, the magnitudes of $\Delta\delta$ for Me_4N^+ (3.39 ppm) and for the Me_3N^+ head of the other substrates (3.20–3.35 ppm) indicate that the latter is located at the center of the cavity, and the R tails are turned toward the exterior and even pointing outside, across one of the host windows, for the acetylcholine complex (*downfield shift* of –0.17 ppm for the AcO group). Except for the spherical Me_4N^+, which may rotate freely in the cavity of **3**, the Me_3N^+R complexes are therefore probably highly anisotropic and ordered; molecular modeling and CPK models support this view and show that a tight lock and key fit is achieved in these complexes. By contrast, with the larger host **4**, which binds Me_4N^+ with a $\Delta\delta$ of 2.39 ppm, the upfield shifts of the Me_3N^+R substrates are more averaged between the head and the tail (Figure 2), suggesting that these complexes are more isotropic and disordered than those of **3** and **1**.

Me_3N^+——CH_2——CH_2——R

3	3.35	3.21	1.58		R=H
4	1.90	2.00	1.88		R=H
3	3.16	3.27		-0.17	R=OAc
4	2.16	2.66	2.73	0.12	R=OAc
3	3.20	2.98	1.97	0.30	R=Me
4	2.01	2.38	1.91	0.84	R=Me

Figure 2. ^{1}H-NMR $\Delta\delta$'s (ppm) for trimethylammonium cations bound to **3** and **4**.

The above experiments support the idea that, besides other factors, a *loose association* is required to achieve a strong binding of quaternary ammonium species to their artificial or natural receptors in water. This requirement may also mean that the "soft" cation–π interactions, which are supposed to provide a favorable enthalpic contribution to the $\Delta G°$, are not strong enough to compensate the defavorable entropy decrease which would follow the formation of an *ordered complex* at 300 K. These findings are also in line with related experiments on the binding of aromatic guests to α- and β-cyclodextrins[17] and, more generally, underline the contradiction that often exists between organization and stability in supramolecular assemblies.[18]

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