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Acetylcholine Binding by a Synthetic Receptor: Implications for Biological Recognition

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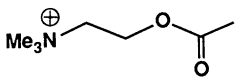
The neurotransmitter acetylcholine (ACh) is bound with 50-micromolar affinity by a completely synthetic receptor (host) comprising primarily aromatic rings. The host provided an overall hydrophobic binding site, but one that could recognize the positive charge of the quaternary ammonium group of ACh through a stabilizing interaction with the electron-rich π systems of the aromatic rings (cation-π interaction). Similar interactions may be involved in biological recognition of ACh and other choline derivatives.

ACETYLCHOLINE (ACh) AND RELATED quaternary ammonium compounds (R_4N^+, where R is an alkyl or aryl group) interact with a wide range of biological binding sites, including those in acetylcholine esterases (AChE), ACh receptors (AChR), and voltage-gated potassium channels. We report that the synthetic, designed receptor (host) **1** (Fig. 1) shows a

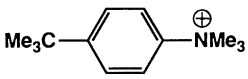


ACh

strong affinity for ACh. At 295 K in aqueous buffer, **1** binds ACh with a dissociation constant K_d = 50 μM, a value comparable to those of the biological recognition sites (*1–5*). Previous studies (*6, 7*) have established that **1** is, in fact, a general receptor for quaternary ammonium compounds. The primary binding force is a cation-π interaction (*8*)—the stabilizing attraction between a quaternary ammonium group and the π electrons of electron-rich aromatic systems. These findings lead us to propose a novel model for biological binding sites for choline and its derivatives, in which aromatic amino acids (Phe and especially the electron-rich Tyr and Trp) are critical for ACh binding through the cation-π interaction, with anionic residues playing a secondary role.

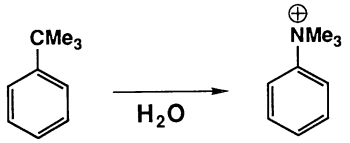
The tight binding of ACh was presaged by our extensive studies of **1** and related hosts with a variety of substrates (guests) (*6*). The binding region of **1** (Fig. 1) is quite hydrophobic, and in aqueous media, nonpolar organic molecules are driven into the cavity by the hydrophobic effect. Superimposed on this is a specific attraction (*9*) between **1** and organic cations, including ammonium and immonium (such as alkylated pyridines and quinolines) (*6*), sulfonium (R_3S^+) (*10*), and alkylated guanidinium (*11*) compounds. It has been established that aromatic π systems in the host, but not analogous aliphatic structures, are important for cation recognition. In organic media, the neutral host **2** also shows a significant preference for cationic guests (*6*). This finding and others (*6*) establish that the remote anionic groups of **1**, which are included to induce water solubility, are at best a secondary factor in cation binding. Other synthetic receptors bind quaternary ammonium compounds (*12, 13*), but charge-charge interactions between anionic groups on the host and cationic guests are much more important in these systems. For example, one system (*12*) binds ACh (K_d = 2 mM), but RNH_3^+ structures are bound more effectively than $RNMe_3^+$ (Me, methyl), in direct contrast to hosts **1** and **2** and to the biological receptor sites. In another case (*13*) *tert*-butyl analogs do not bind at all, establishing the dominant role of charge interactions.

To appreciate the potential magnitude of the cation-π attraction, consider guest **3**.



3

Previous nuclear magnetic resonance (NMR) studies (*6*) established that the ammonium group ($-NMe_3^+$) of **3** is bound in the cavity of **1**, in preference to the *tert*-butyl ($-CMe_3$). This effect occurs even though the ionic ammonium group must be much better solvated by water than the neutral *tert*-butyl, and binding must involve a considerable desolvation. We have evaluated the potential magnitude of this effect by calculating the differential solvation energy (ΔDG_{sol}) in water for the species in Eq. 1.



$\Delta\Delta G^\circ_{sol}$ = −65 kcal/mol (1)

The calculations involved statistical perturbation theory with full Monte Carlo simulations, following a standard protocol (*14–19*). Similar results were obtained for the conversion of CMe_4 to NMe_4^+. Clearly, the ammonium ion is not completely desolvated on binding in the cavity of **1**, but these results do suggest that the magnitude of the cation-π interaction can be quite substantial in aqueous media.

We propose that similar forces are important in a variety of biological choline binding sites. Detailed structural data are sparse, and so evidence concerning important binding interactions is mostly circumstantial. Still, many observations can be rationalized by invoking cation-π interactions between the ammonium ion of ACh and π systems at the binding site. We present here a necessarily selective overview of this broad area to provide support for the model. We emphasize from the start that our goal is not to rule out any possible role for anionic groups at the binding sites, but simply to demonstrate that cation-π forces are likely to be important in the binding and that, in some cases, all available data can be accounted for without invoking an anionic site.

The assumption that AChEs contain an anionic site that is responsible for binding the quaternary ammonium ion is widespread (*20*). However, neutral analogs (that is, $RCMe_3$ versus $RNMe_3^+$) are effective inhibitors of AChE, and they can bind at the same site as ACh (*2*). This result is certainly inconsistent with a model invoking a charge-charge interaction as the primary

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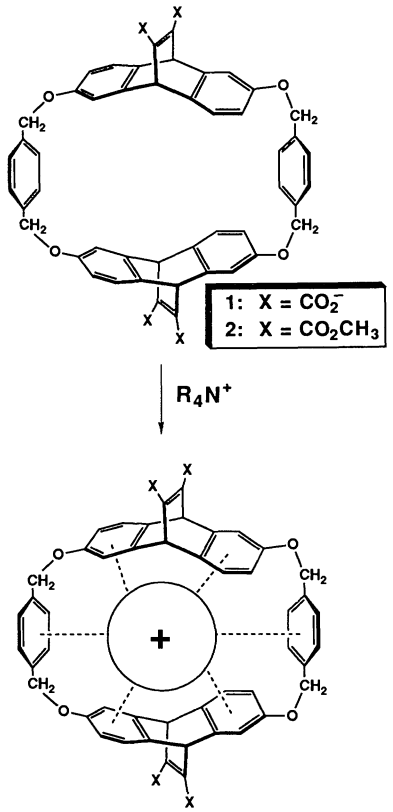

Fig. 1. Structure of hosts **1** and **2** and a schematic of their interaction with a general quaternary ammonium compound (R_4N^+). The stabilizing, cation-π interaction is symbolized by dotted lines between the positive charge of the ammonium and the π faces of the aromatic rings that define the interior of the host.

force for binding. Often, a positive charge contributes only a factor of 10 or less to the binding (*2*). Observations such as these led to the conclusion that there is "no cogent evidence for a negative charge" at the binding site of AChE (*2*). Instead, it was proposed that long-range electrostatic interactions with the overall negative charge of the enzyme are responsible for preferential binding of cations. However, a binding site containing aromatic rings would provide a hydrophobic environment that would bind neutral analogs ($RCMe_3$), but would still show a preference for ions such as ACh because of the cation-π interaction. Consistent with this speculation, a photoaffinity labeling study of *Electrophorus* AChE found a Gly-Ser-X-Phe sequence at the proposed binding site, where the X residue was labeled but could not be identified (*21*). An analogous sequence has been found in *Torpedo* (Gly-Ser-Phe-Phe) and *Drosophila* (Gly-Thr-Tyr-Phe) (*22*), suggesting that the labeled residue was aromatic. We do not rule out the existence of an anionic unit in the binding region (*23*), but the available data can be explained without invoking one.

Similar conclusions can be reached based n what is known about the nicotinic acetylcholine receptor (nAChR), for which the assumption of an anionic binding region is again widespread (*24*). However, a region around Cys^{192} and Cys^{193} in the α subunit of *T. californica* nAChR has been established to form part of the ACh binding site, and this region is particularly rich in aromatic residues (*25*). A photoaffinity labeling study of the *T. marmorata* nAChR identified several residues in this ACh binding region (*26*). Along with the two cysteines (192 and 193), all other labeling was of aromatic residues (Trp^{149}, Tyr^{190}, and possibly Tyr^{151} and Tyr^{198}). No anionic residues were labeled. All labeled residues are conserved in the α subunits of muscle nAChR from all species examined to date. The authors proposed that the lone pairs on the oxygen of Tyr, the nitrogen of Trp, and the sulfur of Cys may stabilize the charge of ACh. Recently, the same group has identified another aromatic residue (Tyr^{93} and possibly Trp^{86}) in the binding region (*27*), leading to a proposal that aromatic rings "may directly contribute" (*27*) to the binding. Of course, our results support the more recent interpretation.

Another suggestive recent result comes from work on an especially effective, synthetic ion channel that can be incorporated into phosphatidylcholine vesicles (*28*). It was found that a successful "flux-promoting" compound must contain three essential units: a hydrocarbon tail that embeds itself in the membrane; a polyether moiety to facilitate cation transport; and a phenyl ring that was proposed (*28*) to associate with the trimethylammonium groups of the phosphatidylcholines through a cation-π interaction. The outer surfaces of cells are rich in choline-containing phospholipids (*29*), and it is interesting to speculate that cation-π interactions may be important in certain aspects of cell surface recognition (*30*).

The only choline binding site for which detailed structural information is available is the phosphocholine (PCh)–binding immunoglobulin Fab McPC603, a structure that has been much discussed (*3, 31*). Of course, the phosphate group of PCh contributes substantially to the binding of the hapten through various hydrogen bonds, most notably to Arg^{52H}. Nevertheless, it is instructive to examine the cation-binding region of this Fab. In McPC603 there are two anionic residues (Asp^{97L} and Glu^{61H}) near the ammonium. However, much closer contacts are made with Trp^{107H}, Tyr^{33H}, and Tyr^{100L} (Fig. 2) (*32*). The interaction with Trp^{107H} is especially striking, and has been noted by other workers (*31*). The trimethylammonium of PCh is surrounded by three aromatic walls, which constitute a first "solvation shell." The anionic residues appear more as a second solvation layer. It is a difficult matter to partition the total binding energy into specific interactions, although attempts have been made (*31*). Certainly the charge-charge interactions are longer range than the cation-π interactions, and Asp^{97L} is clearly a factor in cation binding. Nevertheless, the large number of *N*-methyl–aromatic contacts is striking.

Although these are only a few examples, they reflect an emerging trend that aromatic residues are found frequently at ACh binding sites. As such, we propose a binding mode in which the positively charged quaternary ammonium ion is surrounded by aromatic π systems, with the more electron-rich Tyr and Trp residues being especially effective. This motif produces a generally hydrophobic binding site, but one that can

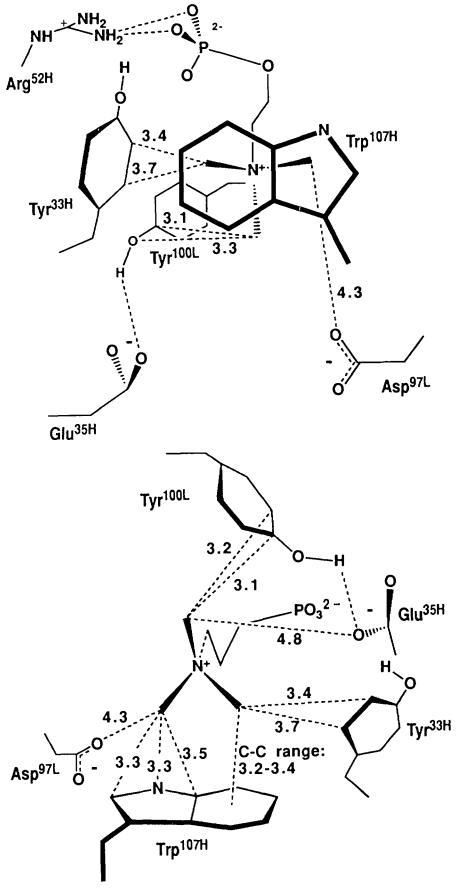

Fig. 2. Two views of the binding of phosphocholine to McPC603. Close contacts (in angstroms) between the carbons of the *N*-methyl groups (*32*) and other heavy atoms are shown, along with selected hydrogen bonds. A large number of contacts <3.5 Å are made to the aromatic residues Tyr^{33H}, Tyr^{100L}, and especially Trp^{107H}. One of the *N*-methyl groups lies almost directly over the center of the benzene ring of Trp^{107H}, with six C–C contacts in the 3.2 to 3.4 Å range (lower drawing). Data are from the published structure (*3*), as deposited in the Brookhaven Protein Data Bank (file 2MCP).

discriminate the cationic ACh from analogous neutral molecules through a stabilizing cation-π interaction.

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1. Acetylcholine binds to esterases with K_d of ~1 mM (*2*). At the McPC603 binding site, K_d is ~10 μM for phosphocholine, and 1 mM for choline (*3*). Binding to the ACh receptors is complex, involving two positively cooperative, nonequivalent sites, and at least four functional states in the nicotinic AChR. The value of K_d obtained depends on the type of measurement being made. Dose-response curves characterize a low-affinity conformation of the nAChR (50 to 100 μM) associated with the resting state. Equilibrium binding studies identify a high-affinity conformation (~3 nM) associated with desensitized states. See (*4*) for further discussions.
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Crystal Structure of Cobra-Venom Phospholipase A_2 in a Complex with a Transition-State Analogue

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The crystal structure of a complex between a phosphonate transition-state analogue and the phospholipase A_2 (PLA_2) from *Naja naja atra* venom has been solved and refined to a resolution of 2.0 angstroms. The identical stereochemistry of the two complexes that comprise the crystal's asymmetric unit indicates both the manner in which the transition state is stabilized and how the hydrophobic fatty acyl chains of the substrate are accommodated by the enzyme during interfacial catalysis. The critical features that suggest the chemistry of binding and catalysis are the same as those seen in the crystal structure of a similar complex formed with the evolutionarily distant bee-venom PLA_2.

PHOSPHOLIPASE A_2 (PLA_2) HYDROlyzes the *sn-2* ester of phospholipids, preferably in lamellar or micellar aggregates. Special interest in the mechanism of PLA_2 action stems from its role as a paradigm for understanding calcium-mediated enzymatic events at the surface of membranes, especially those that release arachidonate and other second messengers (*1*).

In this report, we describe the crystal structure of a complex formed by PLA_2 from the venom of an elapid snake (*N. n. atra*) and a transition-state analogue (Fig. 1). The analogue, *L*-1-*O*-octyl-2-heptylphosphonyl-*sn*-glycero-3-phosphoethanolamine [figure 1 of (*2*)], was designed to emulate the tetrahedral transition state formed in the hydrolysis of dioctanoyl phosphatidylethanolamine and therefore is designated diC_8(2Ph)PE.

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The two molecules in the crystallographic asymmetric unit chosen for refinement form a poorly stabilized dimer related by a rotation of 179.6° and a translation of 0.6 Å along the rotation axis. The bound transition-state analogue does not contribute in any obvious way to the stability of the dimer. Moreover, from the orientation of the inhibitor's *sn-1* and *sn-2* substituents, it is highly unlikely that both of this dimer's active sites can simultaneously interact with the same substrate aggregate. Thus, the architecture of this dimer provides no support for the notion that substrate-induced enzyme aggregation plays a role in PLA_2 catalysis (*3*).

When the α-carbon backbone trace of the PLA_2 from the venom of *N. n. atra* is contrasted with those of the enzymes from bovine pancreas (monomeric) (*4*) and the venom of *Agkistridon piscivorus piscivorus* (dimeric) (*5*), the elapid enzyme shows strong conservation of the homologous core [root-mean-square (rms) differences of 0.99 Å and 1.04 Å, respectively] (*6*). The conformation of the noncore backbone of the Class I *N. n.*