Acetylcholinesterase: structure and use as a model for specific cation–protein interactions

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Acetylcholinesterase is an α/β protein with an overall fold very similar to several hydrolytic enzymes of widely differing phylogenetic origin and catalytic function. The structure reveals, for the first time, a binding pocket for the neurotransmitter acetylcholine. The catalytic triad lies near the bottom of a deep and narrow gorge, lined with aromatic amino acids. The positive quaternary group in acetylcholine and other cholinergic ligands makes close contact with aromatic residues; thus, the π electrons of aromatic rings play a key role in cholinergic ligand binding.

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Introduction

The principal biological role of acetylcholinesterase (AChE, acetylcholine hydrolase, EC 3.1.1.7) is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) to yield acetic acid and choline [1]. In keeping with its function, AChE possesses a remarkably high specific activity, especially for a serine hydrolase (for a review, see [2]), functioning at a rate approaching that of a diffusion-controlled reaction [3]. The acute toxicity of organophosphorus poisons (as well as of carbamates and sulfonyl halides, which function in an analogous manner) arises primarily because they are potent inhibitors of AChE [4]. These compounds inhibit AChE by forming a covalent bond to a serine residue in the active site [2]. AChE inhibitors are used in the treatment of various disorders such as myasthenia gravis and glaucoma [5], and their use has been proposed as a possible therapeutic approach in the management of Alzheimer's disease [6]. Knowledge of the three-dimensional structure of AChE is, therefore, essential for understanding its remarkable catalytic efficacy, for rational drug design, and for developing therapeutic approaches to organophosphate poisoning. Furthermore, information about the ACh-binding site of AChE may help us to understand the molecular basis for the recognition of ACh by other ACh-binding proteins such as the various ACh receptors [7••].

Kinetic studies indicated that the active site of AChE contained two subsites, the 'esteratic' and 'anionic' sites, corresponding to the catalytic machinery and the choline-binding pocket, respectively [2]. Consequently, the esteratic subsite was believed to be similar to the catalytic subsite in other serine hydrolases. Thus, it had already been shown that diisopropyl phosphorofluoridate labeled a single serine residue, and that chemical modification identified a histidine within the active site [2]. The anionic subsite, which binds the quaternary group of ACh and of quaternary ligands that serve as competitive inhibitors, such as edrophonium [8] and N-methylacridinium [9], has been suggested, on the basis of the ionic strength dependence of the 'on' rates of quaternary ligands [10], to contain six to eight negative charges. It has also been claimed, however, on the basis of studies employing charged and uncharged isosteric substrates and inhibitors [11], that the anionic site is uncharged and lipophilic. Both chemical modification and spectroscopic studies supported the presence of aromatic residues within the active site (for literature, see [12••]).

In addition to the two subsites of the catalytic center, AChE possesses one or more additional binding sites for ACh and other quaternary ligands. Such 'peripheral' anionic binding sites (PASs) are clearly distinct from the choline-binding pocket of the active site. They were initially proposed on the basis of kinetic studies using, in particular, bisquaternary ligands, which are believed to derive their potency from the fact that they span the peripheral and active-site anionic sites (for a review, see [13]). The existence of the PAS was firmly established by spectroscopic studies employing fluorescent probes [9,14]. It was recently suggested that the PAS is involved in the substrate inhibition that is characteristic of AChE [15].

Three-dimensional structure

Acetylcholinesterase has an ellipsoidal shape with the dimensions 45 × 60 × 65 Å, and belongs to the class of

Abbreviations

ACh—acetylcholine; **AChE**—acetylcholinesterase; **nAChR**—nicotinic acetylcholine receptor; **BuCh**—butyrylcholine; **BuChE**—butyrylcholinesterase; **CPW**—wheat carboxypeptidase II; **DDF**—p-N,N-dimethylaminobenzene diazonium fluoride; **DLH**—dienelactone hydrolase; **GLP**—*Geotrichum candidum* lipase; **PAS**–peripheral anionic site; **TEA**—tetraethylammonium.

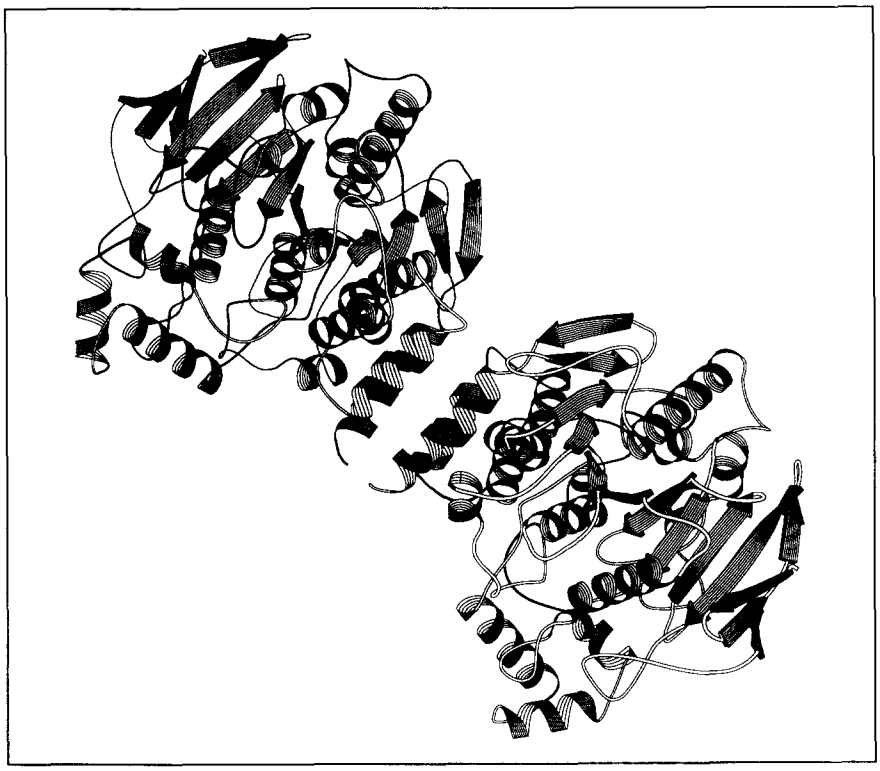

Fig. 1. Schematic ribbon diagram of the three-dimensional fold of the *Torpedo californica* acetylcholinesterase (AChE) dimer. The amino-termini are located at the top left and bottom right of the figure, and the carboxyl-termini are found in the center, at the site of the four-helix bundle, which holds the two monomers together. The monomer fold consists of an 11-stranded central mixed β-sheet surrounded by 15 α-helices, and a short three-stranded β-sheet at the amino terminus which is not hydrogen-bonded to the central sheet. This description of the secondary structure of AChE, i.e. the separation of the central β-sheet from the short amino-terminal β-sheet is slightly different from the original description [12••], after carefully comparing its three-dimensional structure with that of *Geotrichum candidum* lipase [26•].

α/β proteins [16] (Fig. 1). The AChE homodimer, whose subunits are related by a crystallographic twofold axis, is linked by a disulfide bond between the two carboxy-terminal residues. In addition, the subunits appear to be held together by a four-helix bundle composed of two helices from each subunit [12••,17]. This kind of motif is often found at the junction between homodimers, e.g. in the small RNA-binding protein Rop [18], or between two very similar structures, e.g. the L- and M-subunits of the photosynthetic reaction center from the purple bacterium, *Rhodopseudomonas viridis* [19].

One of the most surprising results that emerged soon after the structure determination of AChE was the realization that it is a member of a new protein structural family characterized by the α/β hydrolase fold, which is shared by several hydrolytic enzymes of widely differing phylogenetic origin and catalytic function, all of whose structures have been solved only recently [20••]. These enzymes include AChE from *Torpedo californica* [12••], wheat carboxypeptidase II (CPW) [21], dienelactone hydrolase (DLH) from *Pseudomonas sp.* B13 [22], haloalkane dehalogenase from *Xanthobacter autotrophicus* [23•], and a lipase from *Geotrichum candidum* (GLP) [24••]. They have very different sequences, substrates and physical properties except for AChE and GLP, which share 25% amino acid identity [25,26•].

The core of each enzyme is similar: an α/β-sheet (not barrel) of eight β-strands connected by α-helices (Fig. 2). These enzymes have evidently diverged from a common ancestor so as to preserve the active-site geometry but not the binding site. They all have a catalytic triad, the elements of which are borne on loops which are the best conserved structural features in the fold. Only the histidine in the nucleophile-histidine-acid catalytic triad is completely conserved; the nucleophile and acid loops accommodate more than one type of amino acid. There are now four groups of enzymes that contain catalytic triads and are related by convergent evolution towards a stable, useful active site: the eukaryotic serine proteases, the cysteine proteases, the subtilisins and the newly discovered α/β-hydrolase-fold enzymes. A more thorough discussion of the fascinating similarity of these hydrolase-fold enzymes has been presented recently [20••].

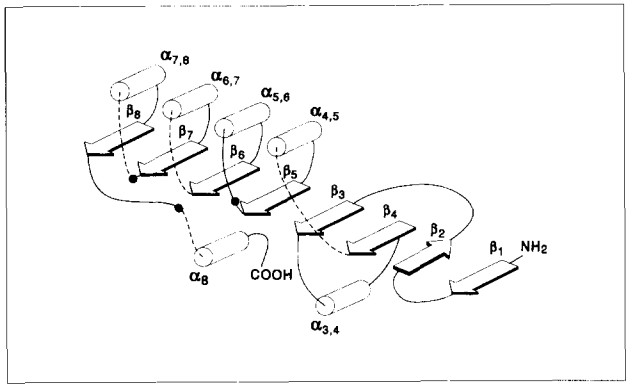

Fig. 2. A schematic drawing of the α/β hydrolase fold [20••], which consists of an eight-stranded β-sheet surrounded by α-helices. The residues of the catalytic triad are indicated with black dots. The broken lines indicate places where some members of the α/β-hydrolase-fold protein family have additional excursions.

On the basis of the three-dimensional superposition of AChE and GLP, an improved alignment of a collection of 32 related sequences of other esterases, lipases and related proteins has been produced [26•]. According to this alignment, 24 residues are invariant in all of the sequences and an additional 70 are well conserved. A detailed comparison of the X-ray structures of AChE and

GLP made it possible to find a clear structural basis for the preservation of many of these residues.

As already mentioned, the mechanism of action of AChE was believed to resemble that of other serine hydrolases, involving the formation of an acetyl-enzyme intermediate [27]:

$$CH_3COOCH_2CH_2N^+(CH_3)_3 + AChE$$
$$\downarrow$$
$$CH_3CO\text{–}AChE + HOCH_2CH_2N^+(CH_3)_3$$
$$\downarrow$$
$$CH_3COO^- + H^+ + AChE$$

and chemical evidence had been presented for the presence of serine and histidine in the active site. The existence, however, of a catalytic triad in AChE similar to that found in chymotrypsin and subtilisin has been the subject of controversy [2]. The use of the proton inventory technique has led Quinn and coworkers [28•] to suggest that, under physiological conditions, AChE-catalyzed hydrolysis of ACh occurs via a single-proton mechanism, suggesting that a charge-relay system is not functional in AChE in such a situation. The earlier identification of Ser200 as the active-site serine of *T. californica* AChE [29] has recently been supplemented by the designation of His440 as the catalytic histidine residue on the basis of sequence comparison [30] and site-directed mutagenesis [31]. The three-dimensional structure of AChE structure clearly supports this assignment by placing His440 close to Ser200, as well as close to Glu327 [12••]. The three residues thus form a planar array which resembles the catalytic triad of chymotrypsin and other serine proteases [32].

There are, however, two important differences. First, AChE together with the above mentioned GLP [24••] are, to the best of our knowledge, the first published cases of glutamate occurring instead of aspartate in a catalytic triad. Second, as in CPW, DLH and the neutral lipases, which have a similar fold to AChE, this triad is of the opposite 'handedness' to that of chymotrypsin [12••], and resembles more closely the configuration of papain [33], as was already observed for the GLP active-site triad [24••]. This results in a change in the direction of the polypeptide backbone around the histidine and serine residues. This suggests that the contribution to the oxyanion hole, which comes from the amide NH of the active-site serine in the serine proteases, would instead come from the amide NH of the following residue in AChE, Ala201, as appears to be the case for human pancreatic lipase [34] and for the other structurally related hydrolases [20••]. All three residues of the triad occur within highly conserved regions of the sequence [12••] and, as is typical of active sites in α/β proteins [35], are in loops following the carboxyl termini of β-strands.

The structural evidence, which clearly demonstrates the presence of a catalytic triad in *Torpedo* AChE, is not necessarily in conflict with the kinetic data of Quinn and coworkers [28•] referred to above. Thus, Quinn himself has pointed out that, rather than participating in a charge relay system, "E327 may stabilize the H440 [His440] tautomer required for general acid-base function and/or electrostatically stabilize the incipient H440 imidazolium cation during catalysis". Such roles have been suggested by others for the acid residue in the catalytic triad of serine proteases [36].

Active-site gorge

The most remarkable feature of the AChE structure is a deep and narrow gorge, ~20 Å long, which penetrates halfway into the enzyme and widens out close to its base. This cavity has been named the 'active site gorge' [12••] because it contains the AChE catalytic triad (Figs 3 and 4). The O^γ of Ser200, which can be seen from the surface of the enzyme, is ~4 Å above the base of the gorge. Fourteen aromatic residues line a substantial portion (40%) of the surface of the gorge (Tyr70, Trp84, Trp114, Tyr121, Tyr130, Trp233, Trp279, Phe288, Phe290, Phe330, Phe331, Tyr334, Trp432 and Tyr442). These residues and their flanking sequences are highly conserved in AChEs from widely different species [12••], and are located primarily in loops between β-strands. It should be noted that the gorge contains only a few acidic residues, including Asp285 and Glu273 at the very top, Asp72 (hydrogen-bonded to Tyr334), about half way down, and Glu199 near the bottom of the gorge.

One of the most interesting features of the active-site gorge is, of course, the large number of aromatic rings that contribute to its lining. We have little idea, as yet, of the structural and/or functional roles of most of these residues, but we know that two, at least, contribute significantly to quaternary ligand binding. Thus, docking studies have suggested that the quaternary group of ACh makes direct contact with the indole ring of Trp84, as was earlier suggested by affinity labelling [37••]. The crystal structure of the two ligand–AChE complexes that we have studied (edrophonium–AChE and tacrine–AChE) offers direct evidence that the quaternary group, or protonated heterocycle in the case of tacrine, nestles against this same indole ring (Fig 5; M Harel *et al.*, abstract, 36th Oholo Conference, Multidisciplinary Approaches to Cholinesterase Functions, 1992). In both these complexes, however, it is also apparent that the benzene ring of an additional aromatic amino acid, Phe330, undergoes a significant conformational change to make an aromatic–aromatic interaction with the bound ligand. These latter crystallographic data are in good agreement with the findings of Hirth, Goeldner and coworkers (L Ehret-Sabatier *et al.*, abstract, 36th Oholo Conference, Multidisciplinary Approaches to Cholinesterase Functions, 1992), who used the photoaffinity label p-N,N-dimethylaminobenzene diazonium fluoride (DDF) as a topographical probe for residues in the immediate environment of the quaternary-ligand-binding site, and found that the principal residue labeled was Phe330. Figure 6 contrasts schematically the early view of the active site of the AChE, derived from studies in solution, with our present understanding, which is based on X-ray crystallography.

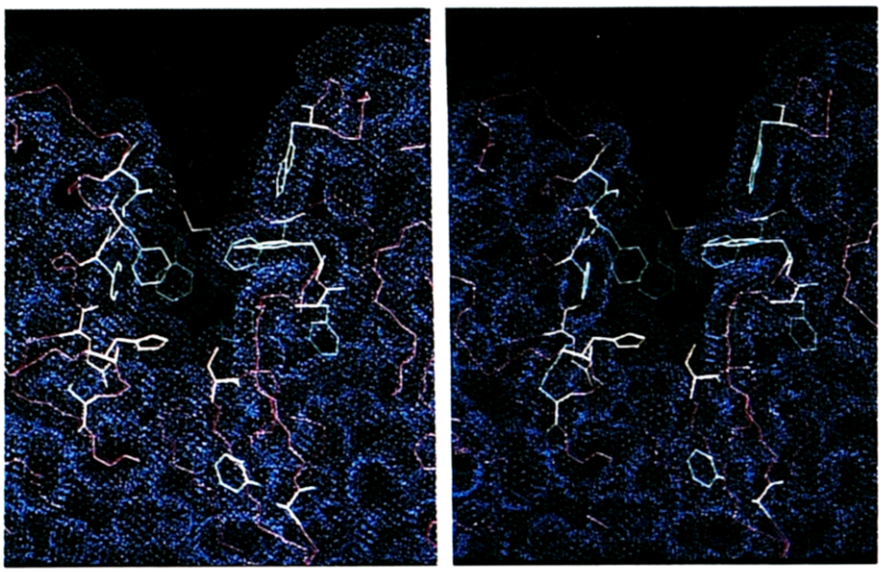

Fig. 3. Stereo drawing of a thin slice through the acetylcholinesterase monomer. The blue-dot van der Waals surface shows the narrow gorge leading down to the active site. The backbone is pink, the aromatic groups are light green, and the catalytic triad is white.

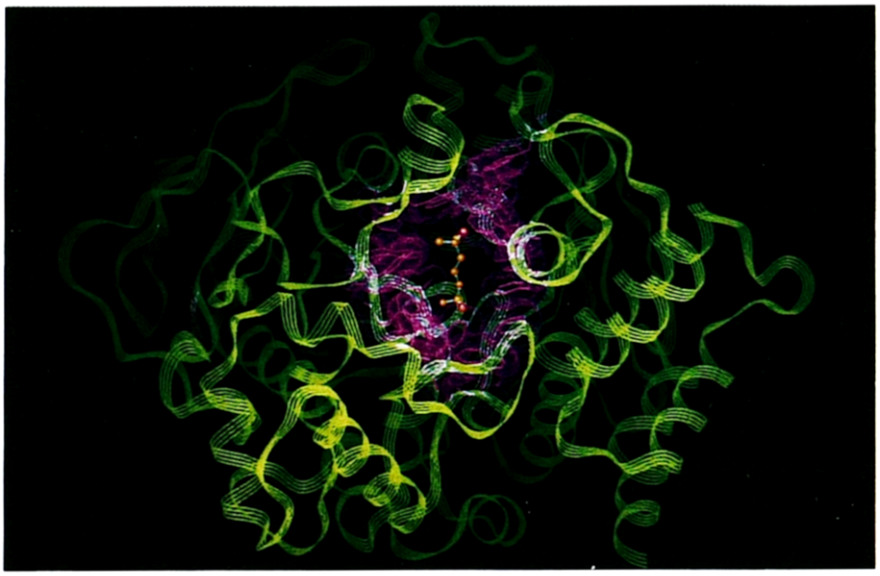

Fig. 4. Schematic ribbon diagram of the structure of *Torpedo californica* acetylcholinesterase. Green represents the 537-amino-acid polypeptide of the enzyme monomer, pink corresponds to the 14 aromatic residues that line the deep aromatic gorge leading to the active site, and gold and blue represent a model of its natural substrate, the neuotransmitter acetycholine, docked in the active site.

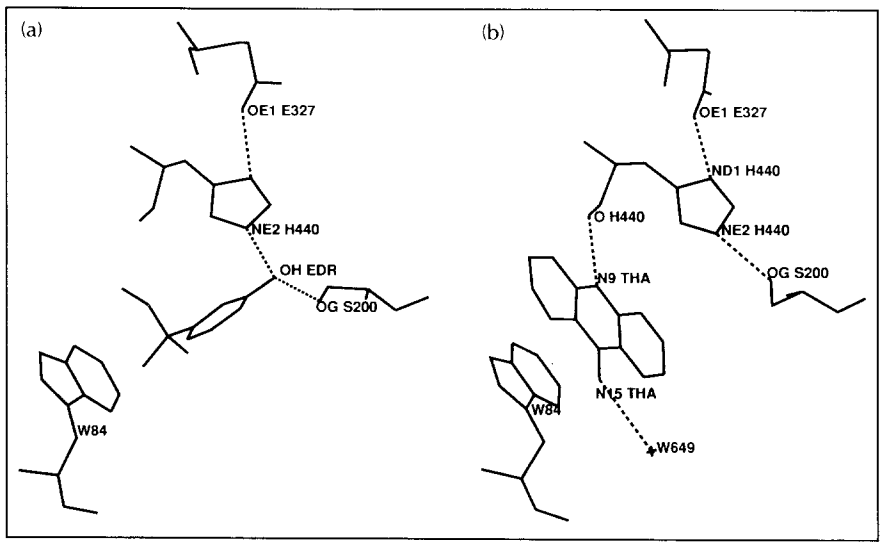

Fig. 5. Close-up views, in the vicinity of the active site, of two drug complexes of acetylcholinesterase (AChE): (a) edrophonium–AChE and (b) tacrine–AChE.

The significance of the aromatic residues that line the active-site gorge can be seen when comparing *Torpedo* AChE with human butyrylcholinesterase (BuChE). Although they clearly differ in substrate specificity and

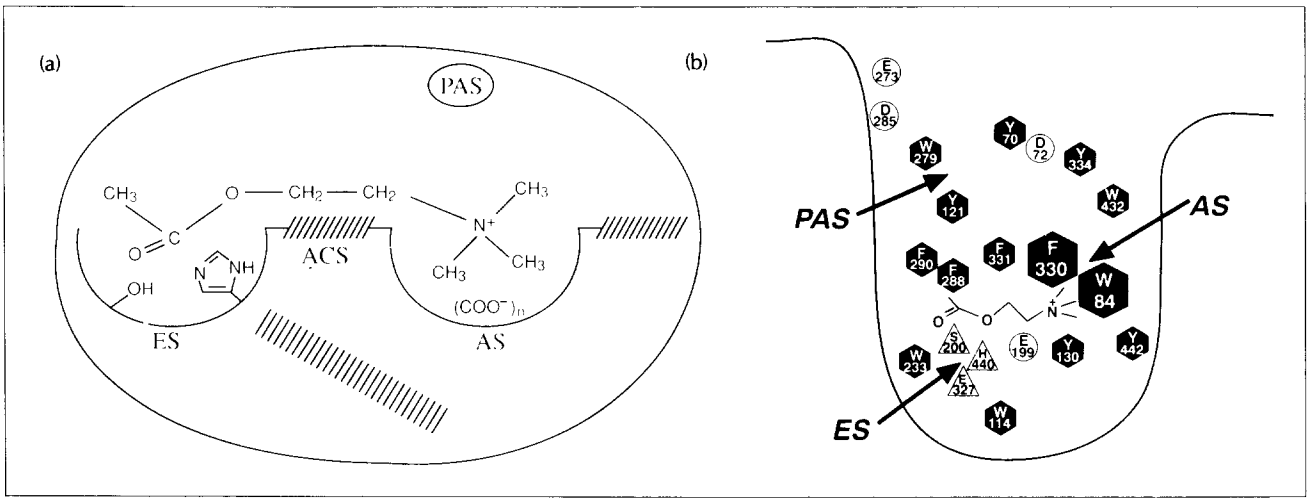

Fig. 6. Cartoons of the acetylcholine (ACh) binding site(s) on acetylcholinesterase (AChE). (a) Before X-ray structure determination, the binding sites were identified by kinetic, spectroscopic and chemical modification studies [ES, esteratic site; AS, anionic substrate-binding site; ACS, active-site-selective aromatic cation-binding site; PAS, 'peripheral' anionic site(s)]. The hatched areas represent putative hydrophobic binding regions. The ACh molecule is shown spanning the ES and the PAS of the catalytic center. Imidazole and hydroxyl side chains of histidine and serine are shown in the ES. Within the AS, $(COO^-)_n$ represents six to nine putative negative charges. (b) The X-ray structure reveals that the catalytic triad (shaded triangles) of the ES lies near the bottom of a deep gorge, which contains a few acidic residues (circles) but is lined primarily with aromatic residues (black hexagons), two of which (W84 and F330, using the one-letter amino acid code) contribute directly to the AS. One aromatic residue, W279, near the top of the gorge, serves as part of the PAS.

sensitivity to various inhibitors, these two enzymes possess 53% sequence homology [30]. This has permitted modeling of BuChE on the basis of the three-dimensional structure of *Torpedo* AChE [38•]. The modeled BuChE structure closely resembled that of the AChE in overall features. Six aromatic residues, however, which are conserved in AChE and are known to line the gorge leading to the active site, are absent in BuChE. Modeling has showed that two such residues, Phe288 and Phe290, which are replaced in BuChE by leucine and valine, respectively, may prevent butyrylcholine (BCh) from fitting into the acyl-binding pocket. Their mutation to leucine and valine in *Torpedo* AChE, by site-directed mutagenesis, produced a double mutant which hydrolyzed BCh almost as well as acetylthiocholine. The mutated enzyme was also inhibited well by the bulky BuChE-selective organophosphate inhibitor isoOMPA (tetraisopropylpyrophosphoramide). Trp279, at the entrance of the active-site gorge in *Torpedo* AChE, is absent in BuChE. Our modeling designated this residue as part of the PAS, which is absent in BuChE. The mutant Trp279Ala displayed strongly reduced inhibition by the PAS-specific ligand propidium, relative to the wild-type *Torpedo* AChE, whereas its inhibition by the catalytic-site inhibitor edrophonium was unaffected [38•].

Although a functional role for some of the aromatic residues in the active-site gorge can be suggested, the purpose of the rest remains to be elucidated. We have suggested that some of them, at least, may be involved in facilitating the rapid translation of the quaternary substrate, ACh, down the gorge to the active site, by what we have called 'aromatic guidance' [12••]. Thus, not only does the quaternary group of ACh interact favorably with Trp84 within the substrate-binding site itself, but it may also use the aromatic surface of the gorge to slide down to the bottom via a series of low-affinity sites. This aromatic guidance is somewhat similar in concept to the 'electrostatic guidance' [39••] proposed for another rapid enzyme, superoxide dismutase, in which it was suggested that a positive electrostatic field guided the negative superoxide ion towards the active site. It should be noted that earlier, Rosenberry and Neumann [40] suggested that an array of negative charges might similarly facilitate two-dimensional diffusion to the active site on the surface of the AChE molecule, and later suggested that the cationic substrate might be attracted towards the active site by a cluster of negative charges within the anionic site (see above) [10]. On the basis of the three-dimensional structure of the enzyme [12••], Ripoll and Faerman have indeed demonstrated that a powerful electrostatic field extends out from the mouth of the active-site gorge, which might serve to suck the ACh into it (D Ripoll and C Faerman, personal communication). It is possible that a dual mechanism operates, i.e. the electric field provides the driving force to bring the ACh into the gorge, and that the aromatic groups facilitate its subsequent translation, as discussed above.

Structural relationship to acetylcholine receptors

An important question which arises is whether the specific case of AChE tells us anything about other ACh-binding proteins, such as the nicotinic and muscarinic ACh receptors. Much research has been and is being devoted to structure–function relationships in these receptors, both at the fundamental level and in relation to drug design, but little direct evidence is available concerning their ligand-binding site topography. In a recent study which combined theoretical considerations with construction of model hosts for quaternary guests, Dougherty and Stauffer [7••] showed that a completely synthetic receptor (host) containing primarily aromatic rings in its annulus binds quaternary ligands substantially better than

isosteric non-charged compounds. They suggested that this was the result of a cation–π electron interaction between the quaternary ion and the rings of aromatic amino acids (see also the earlier works [41,42]). This proposal is also consistent with crystallographic studies on the interaction of water with benzene rings [43], as well as with the observation that aromatic rings often play a role as hydrogen-bond acceptors in protein structures [44]. Furthermore, crystallographic evidence reveals close interactions of positively charged groups with aromatic side chains [45,46••].

A number of groups have presented evidence, based primarily on chemical-labelling techniques, that aromatic groups may be involved in the ACh-binding site of the nicotinic acetylcholine receptor (nAChR), although there appears to be no significant sequence similarity between AChE and nAChR [47]. Photoaffinity labelling studies, using the same probe, DDF, that was used to identify Phe330 in the substrate-binding site of *Torpedo* AChE, implicated several aromatic rings in the binding site of the nAChR [48]. Some of these residues were labelled more strongly in the desensitized state, suggesting that they might play an important role in the putative conformational changes involved in regulation of ligand-gated ion channels [49•]. Aromatic residues in the nAChR are also labeled, chemically and photochemically, respectively, by lophotoxin [50] and by nicotine [51]. Of particular interest is a recent study by Cohen *et al.* [52•], who used [^{3}H]ACh mustard to label specifically Tyr93 in the *Torpedo* nAChR. This mustard produces an aziridinium ion as the reactive species, and, as pointed out by the authors, this reactive quaternary species must be in direct contact with the phenol ring. This labelling is, therefore, quite analogous to the labelling of Trp84 in *Torpedo* AChE by phenylaziridinium [37••]. As noted by Galzi *et al.* [48], Tyr93 is conserved in the α-subunit of all functional muscle and neuronal anchors identified so far.

Fraenkel, Gershoni and Navon [53] have used sophisticated NMR techniques, i.e. transferred nuclear Overhauser effects, to demonstrate that the quaternary group is located less than 5 Å from the indole ring of Trp84 of the α-subunit. Evidence that aromatic groups contribute substantially to the ACh-binding pocket of the nAChR has also come from approaches based on molecular biology techniques. Thus, site-directed mutagenesis suggested a role for a number of aromatic amino acids in a neuronal nicotinic receptor [54]. Fuchs and coworkers [55] have cloned the nAChRs for the venomous snakes and for their enemy the mongoose in order to elucidate the structural factors governing the resistance of these creatures to the snake venom toxin α-bungarotoxin. Various differences were observed in amino acid sequences corresponding to the putative toxin-binding site which might account for the resistance, including certain aromatic residues. As these authors pointed out, however, these receptors still retain their cholinergic characteristics and, indeed, all the aromatic residues known to be labeled by DDF in the studies of Galzi *et al.* [48] are conserved in the α-subunit of both the snake and the mongoose nAChRs.

The emphasis placed on the participation of aromatic residues in the recognition sites for quaternary ligands should not allow us to lose sight of the fact that such ligands are, of course, positively charged and capable of interacting with negative charges. Indeed, nitrogen mustards have been shown to label carboxylates in the non-competitive agonist site of the nAChR (SE Pedersen and JB Cohen, abstract, *Biophys J* 1990, 57:126) and in the muscarinic ACh receptor [56]. The fact that it has been reported that muscarinic receptors are sensitive to tacrine [57] suggests, however, that aromatic residues might also be present in their agonist-binding pocket.

In connection with this, a recent site-directed mutagenesis study of McPC603 by Glockshuber *et al.* [58] is noteworthy. They showed that it is extremely difficult to assess the relative contributions of negative charges and aromatic rings to the binding of the quaternary moiety of the phosphorylcholine ligand.

Aromatic residues in cation channels

Recent research on voltage-gated K^+-channels has raised the possibility that aromatic amino acid residues may be involved in interactions with pore-blocking agents and, perhaps more intriguing, may contribute to the lining of the ion channel itself. Hartmann *et al.* [59] have provided evidence that an 18-amino acid sequence, SS1-SS2, provides the lining of the pore. It has been suggested that four such sequences, from four identical subunits, each traverse the membrane twice in a hairpin configuration. It has long been known that tetraethylammonium (TEA) and other quaternary ions can block K^+ channels by blocking the pore on either the extracellular or intracellular side. MacKinnon and coworkers [60], using site-directed mutagenesis to study cloned K^+ channels from *Drosophila*, have markedly enhanced sensitivity to TEA by mutating to tyrosine the threonine residue immediately following the SS1-SS2 sequence. They consider their findings to be consistent with a TEA-binding site formed by a bracelet of pore-lining aromatic residues, which would bind TEA through a cation–π orbital interaction in a manner analogous to that observed in the model host-guest systems discussed above.

Although tacrine is a strong competitive inhibitor of AChE, it exhibits a variety of other pharmacological effects [61], such as the blockage of various K^+ channels, including the voltage-dependent ones. In the model of the voltage-dependent K^+ channel mentioned above, residues 3–5 of the SS1-SS2 sequence (Phe-Trp-Trp) lie on one arm of the hairpin, and site-directed mutagenesis of the phenylalanine residue affects pore selectivity [62]. This motif would provide a plausible site for interaction with tacrine.

As pointed out by Heginbotham and MacKinnon [63••], the threonine residue which they mutate to tyrosine does not appear to be a critical site for ion conduction in the K^+ channel. They do, however, speculate that cation–π-orbital interactions are important for inorganic permeant ions in a narrower region of the pore. A recent publication has considered the possibility that the SS1-SS2 sequence, with four polypeptides each traversing the membrane four times, might form a β-barrel which would serve as the pore for K^+ [64]. In this

model, the possibility is considered that interaction of the alkali metal ion with π-orbital electrons of one of the two tryptophan residues mentioned above, four of which would be arranged around the walls of the pore, would reduce the energy barrier that appears to exist if such an interaction is not taken into consideration. It should be noted that experimental justification for the interaction of alkali metal ions with aromatic rings can be found in the physicochemical literature [65,66]. It is also worth pointing out that the aromatic residues within the active-site gorge of AChE, which we propose facilitate translation of ACh down the gorge, may be viewed as providing the lining of a channel through which the cationic substrate is driven.

Conclusions

The solution of the three-dimensional structure of AChE has permitted direct access to some features of the mechanism of action and specificity of this rapid synaptic enzyme. AChE has been shown to belong to a new family of hydrolases — the α/β-hydrolase-fold proteins — which share a common scaffolding on which the catalytic machinery is mounted, but differ greatly in substrate specificity as well as in their rate of enzymic activity.

The enzymic characteristics of AChE are embodied in a unique feature, the active-site gorge, which extends 20 Å into the protein and is lined with the rings of 14 highly conserved aromatic amino acids. AChE possesses a catalytic triad similar to that of other serine hydrolase, and this is located at the bottom of the gorge. The role of the aromatic residues seems to be substrate recognition by dipole–dipole interaction between the π electrons of the aromatic rings and the quaternary nitrogen of the ACh. Recognition is not merely confined to the substrate-binding pocket but seems also to provide an 'aromatic guidance' mechanism for rapid translation of the substrate from the surface to the active site. However, why the active site of a particularly rapid enzyme should be located near the bottom of a deep cavity remains a mystery.

The role(s) of the aromatic residues within the active site gorge of AChE may thus serve as a valuable model for understanding the forces and specificity that operate in other ACh-binding proteins, such as the ACh receptors, and may also shed light on the putative roles that have recently been proposed for aromatic residues in cation channels.

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- • of special interest
- •• of outstanding interest

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