

Three-dimensional structure of a complex of E2020 with acetylcholinesterase from *Torpedo californica*

Gitay Kryger^a, Israel Silman^b, Joel L. Sussman^{a, c}

Departments of ^aStructural Biology and ^bNeurobiology, Weizmann Institute of Science, Rehovoth 76100, Israel
^cBiology Department, Brookhaven National Laboratory, Upton, NY 11973, USA

Abstract — The 3D structure of a complex of the anti-Alzheimer drug, E2020, also known as Aricept[®], with *Torpedo californica* acetylcholinesterase is reported. The X-ray structure, at 2.5 Å resolution, shows that the elongated E2020 molecule spans the entire length of the active-site gorge of the enzyme. It thus interacts with both the ‘anionic’ subsite, at the bottom of the gorge, and with the peripheral anionic site, near its entrance, via aromatic stacking interactions with conserved aromatic residues. It does not interact directly with either the catalytic triad or with the ‘oxyanion hole’. Although E2020 is a chiral molecule, and both the S and R enantiomers have similar affinity for the enzyme, only the R enantiomer is bound within the active-site gorge when the racemate is soaked into the crystal. The selectivity of E2020 for acetylcholinesterase, relative to butyrylcholinesterase, can be ascribed primarily to its interactions with Trp279 and Phe330, which are absent in the latter. (©Elsevier, Paris)

Résumé — Structure d’un complexe de E2020 avec l’acétylcholinestérase de *Torpedo californica*. La structure 3D d’un complexe formé par une drogue anti-Alzheimer E2020 aussi connue sous le nom de Aricept[®] avec l’acétylcholinestérase de *Torpedo californica* est décrite. La structure aux rayons X, à la résolution de 2.5 Å, montre que la molécule allongée E2020 s’étire sur l’entière longueur de la gorge catalytique de l’enzyme. Elle interagit donc à la fois avec le sous-site anionique, au fond de la gorge et avec le site périphérique anionique, près de l’entrée par les interactions avec les résidus conservés aromatiques empilés. (©Elsevier, Paris)

Alzheimer’s disease / drug design / peripheral site

1. Introduction

Acetylcholinesterase (AChE) terminates synaptic transmission at cholinergic synapses by rapid hydrolysis of acetylcholine (ACh) [15]. Anticholinesterase agents are used in the treatment of various disorders [21], and have been proposed as therapeutic agents for the management of Alzheimer’s disease [7]. Two such anticholinesterase agents, both of which act as reversible inhibitors of AChE, have been licensed by the FDA: tacrine (1,2,3,4-tetrahydroacridine) [6], under the trade name Cognex[®], and, more recently, E2020 ((R,S)-1-benzyl-4-[5,6-dimethoxy-1-indanon-2-yl]methylpiperidine) [18], under the trade name Aricept[®]. Tacrine and E2020 share the same target, but, whereas tacrine must be administered up to four times a day, and displays hepatotoxic side effects, E2020 may be administered once daily, and has fewer side effects. Furthermore, E2020 displays very high selectivity, ~1000-fold, for AChE relative to butyrylcholinesterase (BChE), whereas THA has similar affinity for the two enzymes. This may be important, since it has been suggested that inhibition of human plasma BChE may cause potentiating side effects [23].

The active site of AChE contains a catalytic subsite, and a so-called ‘anionic’ subsite, which binds the quaternary group of ACh [15]. A second, ‘pe-

ripheral’, anionic site is so named since it is distant from the active site [22]. Bisquaternary inhibitors of AChE derive their enhanced potency, relative to homologous monoquaternary ligands [13], from their ability to span these two ‘anionic’ sites, which are ca. 14 Å apart.

The 3D structure of *Torpedo californica* (Tc) AChE [20] reveals that the active site is located at the bottom of a deep and narrow cavity; named the ‘aromatic gorge’, since > 50% of its lining is composed of the rings of 14 conserved amino acids [1, 20]. The peripheral site is located at the entrance to the gorge [8].

X-ray crystallographic studies of complexes of AChE with drugs of pharmacological interest can reveal which amino acid residues are important for binding the drug, and where space might exist for modifying the drug itself, information crucial for structure-based drug design. In the following, we describe the crystallographic structure, at 2.5 Å resolution, of a complex of E2020 with TcAChE.

2. Materials and methods

2.1. Protein preparation and crystallization

TcAChE was purified and crystallized as described previously [16]. E2020, as the hydrochloride salt of the pure racemate, was a generous gift from Dr. B.P. Doctor (Division of

Biochemistry, Walter Reed Army Institute of Research, Washington, DC, USA). *TcAChE* crystals were soaked in ~10 mM (R,S)E2020 for 5 days at 4 °C, and flash cooled to 100°K.

2.2. X-ray data collection and processing

Data were collected 'inhouse', at the Weizmann Institute of Science, on a Rigaku FR300 generator set at 50 mA, 50 kV and 1.54184 Å wavelength (Cu K radiation), equipped with a R-AXI-SII detector.

2.3. Model refinement and analysis

The structure was refined on the basis of the starting model of native *TcAChE* (PDB ID 2ACE), using only the polypeptide and none of the solvent atoms, employing XPLOR [2]. Voids were calculated between the inhibitor molecule as one entity, and the protein and solvent molecules as a second entity [12].

3. Results and discussion

The 3D structure of the complex shows more detail of the AChE structure than the starting native model (2ACE). Primarily, residues 2 and 3, at the N-terminus, and the 484–490 loop, which were not seen in the original model, can be discerned. In addition, it was possible to model the proximal Nacetyl glucosamine (NAG) moiety at four out of five putative glycosylation sites, viz. at residues Asn59, Asn416 (where two NAG moieties could be fitted), Asn457 and Asn533.

3.1. All three segments of E2020 interact with AChE

E2020 binds along the active site gorge. All three major segments of the elongated molecule make specific interactions with the enzyme, and each of these interactions involves discrete watermediated contacts which appear crucial for specificity (*figure 1*). E2020 makes principal interactions with the enzyme through: 1) the benzyl moiety; 2) the piperidine nitrogen; and 3) the dimethoxyindanone moiety. It utilizes the conserved aromatic residues which line the gorge for hydrophobic and stacking interactions, and does not make direct contact with the protein through H-bonds or salt-bridges other than via water molecules.

3.2. Interactions at the bottom of the gorge

Near the bottom of the gorge, one face of the benzyl ring stacks against the six-membered ring of the indole moiety of Trp84, similarly to THA [8]. On the opposite face, it makes a classic aromatic hydrogen bond with a water molecule (WAT1160). This water is held firmly by a hydrogen bond to another water molecule (WAT1161), in the 'oxyanion hole',

and to WAT1159. WAT1161 is another example of a tightly bound water molecule; it hydrogen bonds to residues in the 'oxyanion hole' and to S200O. E2020 does not interact directly either with the catalytic triad or with the 'oxyanion hole'.

3.3. Interactions in the middle of the gorge

In the constricted region, halfway up the gorge, the charged nitrogen of the piperidine ring makes a cation- π interaction [5] with the phenyl ring of Phe330. The ring nitrogen also makes an in-line H-bond with WAT1159. The principal binding site for the quaternary nitrogen of ACh within the active site, and for homologous ligands, is the indole ring of Trp84 [9]. These data suggest that Phe330 may serve as an additional quaternary binding site, of possible functional significance, midway down the gorge, between the peripheral binding site and the anionic subsite of the active site.

3.4. Interactions at the entrance to the gorge

At the top of the gorge, the indanone ring stacks against the 6-membered ring of the indole moiety of Trp279, in the peripheral binding site, in a classic parallel π - π interaction. The fact that the binding of E2020 is strongly dependent on interaction with Trp279 and Phe330, which are absent in BChE, may explain its high relative specificity for AChE versus BChE. The carbonyl function on the indanone is not in direct contact with the protein, but appears to make a water-bridged contact with F288N.

3.5. Only one enantiomer of E2020 is bound to AChE

The reported pharmacological studies on E2020 emphasize that both enantiomers are active, and exhibit similar, but not identical, binding affinities for AChE [11, 19]. Since we used the racemate in our crystallographic study, we expected either to find evidence, in the form of electron density, for the presence of both enantiomers in the crystal structure, or partial disorder which would support the presence of both. Yet, when we attempted to fit a number of plausible inhibitor conformations within the active-site gorge, a unique fit to the experimental electron density was found for one conformation of the R form, which is very similar to our energetically minimized E2020 conformation, with the indanone carbonyl group pointing towards F288N as mentioned above. Thus, *TcAChE* appears to have bound the R form selectively, despite the similarity in binding constants. Selective binding cannot be due to a limitation in the amount of the S form, since the racemate was soaked into the crystal at 10 mM, i.e.,

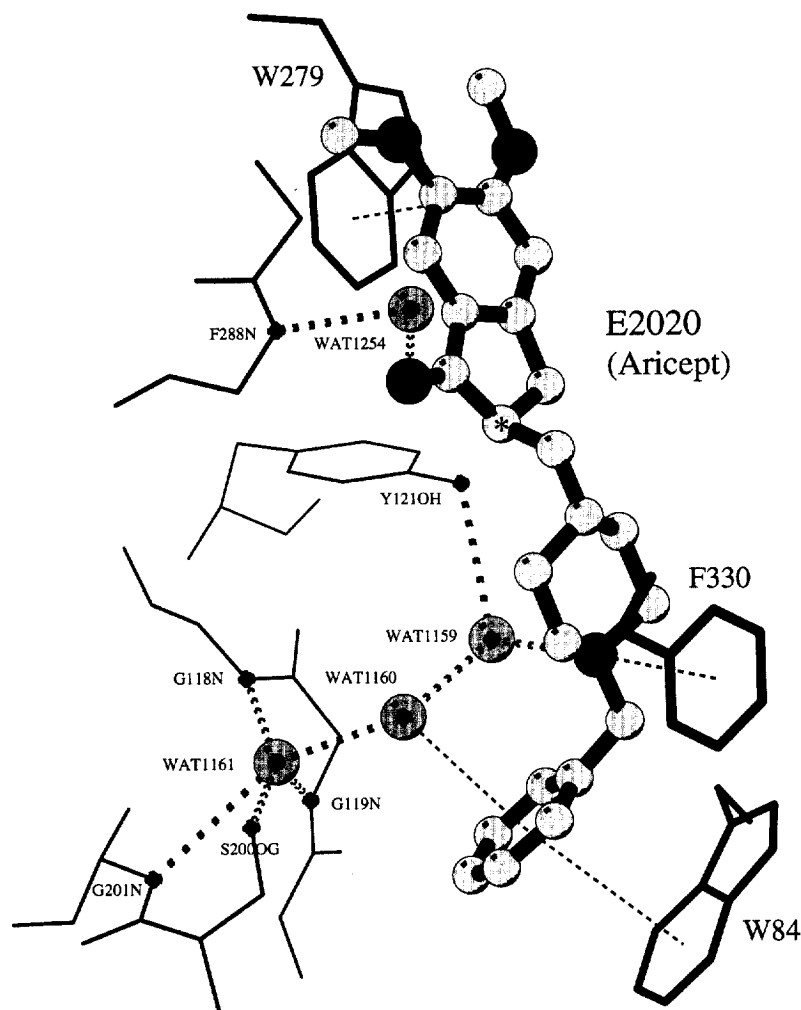


Figure 1. Binding modes of E2020 to *TcAChE*. E2020 are displayed as a ball-and-stick model (chiral center marked with black star); direct binding residues are represented as dark grey sticks; water-mediated binding residues as light grey sticks; water molecules as light grey balls; 'standard' H-bonds as heavy dashed lines; aromatic H-bonds, -cation and -stacking as light dashed lines.

at a great molar excess. Although the two of E2020 interconvert readily in aqueous solution, via a ketoenol intermediate [14], it is not immediately obvious what could cause such a preferential binding of one form, taking into account their similar affinities. It should, however, be borne in mind that not only are the ligand and active site chiral, but also the entire lattice of the crystalline enzyme. Such a chiral environment might cause the stereoisomers to tautomerize at different rates. Based on steric considerations, it appears that both the R and S enantiomers could bind in very similar conformations, with only the position of the carbonyl function on the 5-membered ring of the indanone moiety distinguishing between them. A model which we built displaying a carbonyl on the other side of the indanone,

representing the S form, does not make contact with any protein atom, and thus might be bound less tightly than the observed R form, even though this is inconsistent with the published inhibition data [11]. Furthermore, the 4.0 Å link between the indanone carbonyl and F288N, whether bridged by a water molecule or not, combined with the fact that the carbonyl 'nests' amongst three aromatic systems of residues Phe288, Phe290 and Phe331, might be sufficient to introduce a bias in favor of preferential binding of the R form. As already mentioned, R-S interconversion, via tautomerization, is known to occur, and might indeed take place under the experimental conditions employed. Thus, one explanation for the inhibitory potency of the S form would invoke AChE-induced S-to-R tautomerization.