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Conformational Flexibility in the Peripheral Site of *Torpedo californica* Acetylcholinesterase Revealed by the Complex Structure with a Bifunctional Inhibitor

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X-ray crystallography is a powerful tool that provides time-averaged pictures of biological macromolecules. These seemingly static structures are in reality animated by molecular motions stemming from transitions between substates in a conformational energy landscape.[1] This landscape is characterized by a very large number of conformational substates, the population of which depends on their free energy. A crystallographic structure represents only the predominant substate of a molecule, and minor populations can usually not be assessed. Yet, the understanding of biological function on a molecular level requires knowledge about both structural and dynamical aspects of, e.g., an enzyme. This is particularly obvious for acetylcholinesterase (AChE), the active site of which is accessed by a deep and narrow gorge[2] that makes "breathing" motions essential for traffic of substrates and products to occur.[3] Here, we report the X-ray crystallographic structure of AChE from *Torpedo californica* (*Tc*AChE) in complex with a gorge-spanning inhibitor, NF595[4] (Figure 1). NF595 has been synthesized with a view to developing a new generation of anti-Alzheimer drugs, interacting with both the active and peripheral binding sites of AChEs. Surprisingly, and for the first time in *Tc*AChE, a major conformational change is observed upon complexation in the peripheral substrate-binding site. We suggest that this conformational substate is part of the equilibrium dynamics of the native enzyme and that it has been selected by inhibitor binding.

AChE is one of nature's fastest enzymes. Essential in the process of signal transmission in cholinergic synapses, AChE is responsible for the breakdown of the neurotransmitter acetylcholine into acetate and choline.[5] It is the target of all currently approved anti-Alzheimer drugs, of insecticides, and of chemical warfare agents. The first structure of an AChE revealed the buried nature of the active site and a peripheral substrate-binding site at the entrance of the gorge near the enzyme's surface[2] (Figure 2a). Numerous structures of *Tc*AChE in complex with various inhibitors have been solved, providing insights into inhibitor-induced conformational changes.[5] The conformation of the active-site gorge appears to be highly conserved, and the observed structural changes are small, except for those of Phe330.

Trigonal crystals of *Tc*AChE[2] were soaked into the mother liquor solution containing 2 mM NF595, loop-mounted, and flash-cooled at 100 K; diffraction data were collected on beamline ID14-EH2 at the European Synchrotron Radiation Facility. Structure refinement to a resolution of 2.2 Å included rigid body refinement, simulated annealing, energy minimization, and individual B-factor refinement (PDB accession code 2CEK).

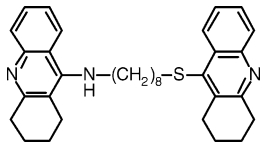

Figure 1. Structure of NF595 (referred to as compound 3h in ref 4). The heterodimer bears a tacrine moiety and a sulfur-containing tetrahydroacridine system connected by a 8-methylene linker.

NF595 spans the gorge, in agreement with previous docking studies,[4] and binds at the peripheral and the active sites (Figure 2b). Both heteroaromatic moieties are engaged in $\pi-\pi$ stacking interactions with two aromatic residues, i.e. with Trp84 and Phe330 in the lower part of the gorge, and with Trp279 and Tyr70 at the peripheral site. To make this parallel stacking mode possible, the side chains of both Phe330 and Trp279 rotate by about 90° with respect to their native positions. Whereas Phe330 is known to be a mobile residue, the conformational change of Trp279 has never been observed before in *Tc*AChE.

Bourne and collaborators recently determined the structure of mouse AChE (mAChE) in complex with a bifunctional inhibitor (*syn1* TZ2PA6) formed by in situ click chemistry.[6] This complex also revealed a 90° rotation of the mAChE Trp286, equivalent to *Tc*AChE Trp279, the key constituent of AChEs peripheral site. In the mAChE complex, Trp286 reorients in such a way that it is exposed to the solvent, whereas in the *Tc*AChE complex, Trp279 draws back toward the inside of the gorge. The two inhibitor-induced conformations of the peripheral-site tryptophan residues are thus 180° apart (Figure 2c). This conformational difference might be due either to the different inhibitors used or to the different packing patterns in crystals of *Tc*AChE and mAChE. The peripheral-site architecture in AChEs from different species, including mAChE, *Tc*AChE, and human AChE, is structurally conserved. Therefore, the inhibitor-induced conformations of Trp279 in *Tc*AChE and of Trp286 in mAChE are most probably part of a common energy landscape. They illustrate the wide range of conformations accessible to a functionally important residue. A smaller movement of Trp279 has also been observed in the crystal structure of *Tc*AChE in complex with DFP, in which a loop comprising this residue undergoes a conformational change.[7]

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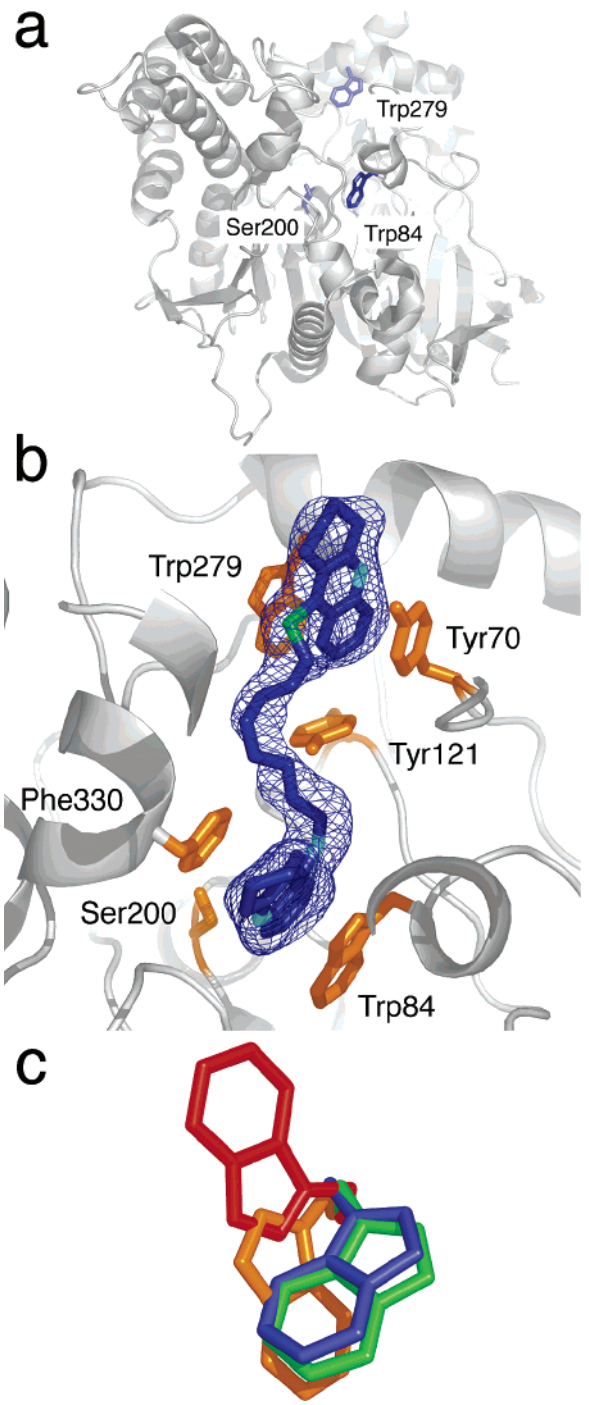

Figure 2. (a) 3D structure of native *Tc*AChE (PDB access code 1EA5), highlighting the catalytic Ser200 and Trp84 in the active and Trp279 at the peripheral substrate binding site. (b) The bifunctional inhibitor NF595 (S: green, N: light-blue, C: dark-blue) spans the gorge of *Tc*AChE. Its orientation was identified with the help of the strongly diffracting sulfur atom. Phe330 is present in two alternate conformations. Only the one close to NF595 is shown. The simulated-annealing omit map is contoured at 4.5 σ. (c) Conformational plasticity in the peripheral-site tryptophan (Trp279 of *Tc*AChE in the native (blue) and the NF595-bound (orange) structure and Trp286[6] of mAChE in the native (green) and an inhibitor-bound (red) structure. The χ values for the peripheral-site Trp side chains are: $\chi_1 = -61°$, $\chi_2 = 92°$ (Trp279 native), $\chi_1 = -116°$, $\chi_2 = -132°$ (Trp279 NF595), $\chi_1 = -60°$, $\chi_2 = 90°$ (Trp286 native) and $\chi_1 = -162°$, $\chi_2 = 52°$ (Trp286 *syn1* TZ2PA6). Panels a−c show the enzyme in very similar, yet not exactly identical orientations.

The AChE peripheral site has been shown to promote the growth and maturation of β-amyloid plates,[8] a process that is thought to be at the origin of neurodegeneration associated with Alzheimer disease. The observed mobility of the peripheral site should be of importance in the elaboration of models that aim at addressing the structural interaction between AChE and β-amyloid structures.

The observation of conformational flexibilities of the peripheral-site tryptophan, Phe330,[9] the catalytic His440,[10] and parts of the enzyme's wall near the acyl-binding pocket[11] draws a dynamic picture of the gorge and the active site in *Tc*AChE. Indeed, a delicate balance between rigidity and flexibility might resolve the apparent paradox between AChE catalytic efficiency and the reduced accessibility of its active site.

We suggest that the NF595 inhibitor selects a minor, yet already existing conformation of the complex energy landscape of the native enzyme. Indeed, experimental[12] and computational[13] evidence indicates that motions required for ligand binding or catalysis are already existing in the equilibrium dynamics of the native state of enzymes. Whether this holds true for Trp279 could be addressed by molecular dynamics simulations. The observed plasticity of functionally relevant structural elements is of importance for structure-based drug-design. Indeed, in the quest for tailor-made drugs, an enzyme should not be considered only as a rigid template, but conformational heterogeneity should be taken into account as well.[11] In this context, X-ray crystallography is a powerful tool that can address both structural and dynamical aspects of biological macromolecules.

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Supporting Information Available: A movie, showing structural changes in the gorge upon binding of NF595, and X-ray data processing and structure refinement statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

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