Regular article

Influence of ligand binding on the conformation of Torpedo californica acetylcholinesterase

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Abstract. With the aim of identifying structural changes in acetylcholinesterase, induced by ligand binding, we use a completely automatic procedure to analyse the differences between the backbone conformation of the free enzyme and those in eight complexes of Torpedo californica acetylcholinesterase, with various quaternary ammonium ligands, and with the protein inhibitor fasciculin. In order to discriminate between structural changes due to ligand binding and those arising from model imprecision, we also examine protein–ligand and protein–water contacts. Except for the peptide flip in the complex with huperzine A, the backbones of other complexes with quaternary ammonium ligands display negligible changes relative to the free enzyme. Another exception is the complex with the bisquaternary ammonium ligand decamethonium, where several loops display above average deformations, but only two, those spanning residues 334–348 and residues 277–304, seem to move as a result of ligand binding. Movement of the ω loop (residues 61–95) is detected only in the complex with the protein fasciculin.

Key words: Structural changes – Acetylcholinesterase – Structure alignment program

1 Introduction

The enzyme acetylcholinesterase (AChE) terminates nerve impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine into choline and acetate acid [1]. Understanding at the atomic level the mechanism whereby various ligands inhibit this enzyme should be of help in designing new therapeutic agents in diseases such as Alzheimer’s disease [2] that involve acetylcholine insufficiencies.

The apparent complexity of AChE inhibition resides primarily in the features of the enzyme 3D structure [3], as revealed by the crystal structure of Torpedo californica AChE (TcAChE) [4] and confirmed by those of variants from other organisms [5, 6]. The active-site triad (S200, E327, H440) is located at the bottom of a long and narrow gorge, whose lining is composed of 40% of aromatic side chains. The bottom of the gorge features an “esteratic” subsite, containing the catalytic apparatus, and a separate “anionic” subsite, which binds the quaternary group of the substrate [7]. Various studies also support the existence of a second “peripheral” anionic site, 14 A from the first, closer to the top of the gorge [8, 9]. According to the type of ligand occupying this site, the enzyme can be either activated or inhibited. Alternatively, the site can serve as an intermediate halt for ligands travelling towards the active pocket [10].

A number of crystallographic studies have been devoted to the analysis of AChE–ligand complexes. They illustrate well the diversity and complexity of the underlying interactions. The complexes studied involve inhibitors as chemically diverse as decamethonium (DEC), edrophonium (EDR), tetrahydroaminoacridine [11], m-(N,N,N-trimethylammonio)trifluoracetophenone (TMTFA) [10], (−)-huperzine A (HUP) [12], polyacrylamide (PAM) and (R,S)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)2-yl]methylpiperidine (E2020) [13] as well as a protein inhibitor, the snake toxin fasciculin (FAS) [14]. In these complexes some of the inhibitors are found to bind in or near the anionic and esteratic active site pockets, whereas others interact more exclusively with the peripheral site. Some bisquaternary cations, such as DEC, are shown to span both sites, a likely reason for their enhanced potency relative to monoquaternary ligands. Many of the ligands analysed are rather bulky, suggesting that the enzyme must display appreciable flexibility to allow them to penetrate the deep gorge and bind to the observed sites. The short time scale of the TcAChE enzymatic reaction [15] suggests that this must involve negligible energy barriers. Identifying the regions

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of the enzyme undergoing movement upon ligand binding has, therefore, been of interest.

The comparison of the complexed and free enzyme crystal structures revealed only very minor differences in the backbone conformations, ranging between 0.3 and 0.4 Å root mean square (rms) [11, 13]. Also, with hardly any detectable backbone changes, these studies focused mainly on commenting reorientation of key side chains such as Phe330 and Trp279 or changes in the pattern of interactions with water molecules [13]. Small changes in the backbone of several loops were also reported for the complex of TcAChE with FAS [14].

Here we report a systematic analysis of the differences in the backbone structures between a large number of available complexed forms of TcAChE, including those with quaternary ligands and the protein FAS, and the corresponding ligand-free enzyme solved at 2.5 Å (2ACE). To this end we use a completely automatic procedure for analysing structural changes in two proteins developed by some of us [16]. This procedure was shown to describe conformational changes in a variety of systems and that even in cases where the structural changes were very limited. It therefore seemed appropriate to use it in order to identify ligand-induced structural changes in the AChE system. However, in addition to the small magnitude of the expected structural changes, the AChE system presents a major difficulty. The atomic models available for most of the complexes have been derived from 2.8–3.0-Å resolution electron density maps before the R-free based refinement became common practice, and are hence not of the highest quality. Thus, a major challenge facing an analysis such as that proposed here is the ability to distinguish amongst the identified structural changes, those caused by ligand binding from those arising from model imprecisions.

To meet this challenge, we first analyse the changes in the three higher-quality structures of the HUP–TcAChE, E2020–TcAChE and EDR–TcAChE complexes, solved at 2.5 and 2.4 Å resolution, respectively. Then the changes identified in these complexes and those detected in the less accurate structures (2.8 Å) of the other TcAChE complexes are compared. Our automatic procedure is used to derive optimal global structure superpositions of the complexes relative to the free enzyme. In addition, it identifies the protein segments that undergo local deformation as well as groups of segments which move as rigid bodies. These analyses are performed for the different complexes, and their relevance to ligand binding is validated by analysing protein contacts with the ligands and with crystallographic solvent molecules.

This yields a consistent picture, which provides useful insight into how the ligands considered may influence the conformation of TcAChE in the crystal. It also illustrates some of the limitations encountered in extracting information on structural flexibility from crystallographic data.

2 Methods

2.1 Structural similarities between two polypeptide chains

The comparison between the 3D structures of two polypeptide chains was performed using the structure alignment program SoFi [17]. This algorithm uses as sole structural similarity to measure the rms deviation (rmsd) of N, Cα, C and O backbone atoms after coordinate superposition and is designed to determine the best match between all short segments of the two chains yielding the low global rmsd. To this end, the procedure first identifies all overlapping segments of a given length with similar backbone conformations in both 3D structures. Conformational similarity is evaluated by the rmsd value. Next, to obtain the global alignment of the two proteins, the pairs of equivalent segments are assembled, in order of decreasing structural similarity, by a multiple-linkage hierarchical clustering algorithm (MLC). This algorithm generates several intertwined clustering trees. From these trees, solutions corresponding to the best alignments are selected. In a final stage, the aligned segments are extended at both ends to include residues outside the initial segment limits.

2.2 Analysing conformational changes between different 3D structures of the same protein

The conformational changes between two different 3D structures of the same protein are detected using a fully automatic procedure [16]. This procedure uses as its sole information the atomic coordinates of the structures and involves two main steps, which can be summarized as follows.

The first step of this method is a variant of the procedure described in the previous section. It operates by first deriving the global 3D structure alignment between the two proteins using a MLC procedure. Since the limits of secondary structures tend to be the same in the two protein structures to be compared, the equivalent chain segments that we cluster correspond to the secondary structure elements (α helices and β strands) and/or loops (defined as all regions outside the secondary structures). The limits of these segments are determined by the DSSP algorithm [18].

In the second step, the resulting intertwined clustering trees are analysed in order to extract information on the rigid static core, defined here as the secondary structure elements, which do not move in the conformational change, and on the movers (segments of secondary structure and loops), whose conformation or spatial positions relative to the static core differs in the two structures. For that purpose, the intertwined trees are automatically scanned to determine the jump-minimizing path. By construction, this path groups the structural elements which move least in the conformational change. It starts at the node with lowest rmsd and travels down the trees through successive nodes, such that when moving from one node to the next, the difference in the rmsd is a minimum. The static core is defined as the node along this path which is separated by a large enough rmsd change from the following node. Once the static core is identified, the remaining fragments, which are added onto it along the jump-minimizing path define the principal movers. The size of the rmsd jump, produced when they are assembled, determines their category as minor or major movers.

3 Results

3.1 Structural changes induced by ligand binding

In the following we present the results obtained with our automatic procedure on the structural changes observed in seven complexes of TcAChE with quaternary ammonium ligands, EDR (PDB code 2ACK), tacrine (PDB code 1ACJ), DEC (PDB code 1ACL) as well as with other ligands, PAM, TMTFA (PDB code 1AMN), HUP (PDB code 1VOT) and E2020 (PDB code 1EVE). The structural changes are analysed relative to the structure of the free enzyme determined at 2.5-Å resolution (PDB code 2ACE). Throughout the remaining sections, all the structures analysed are referred to by their PDB codes or by the abbreviated ligand names. An annotated ribbon drawing of this TcAChE structure is presented in Fig. 1.