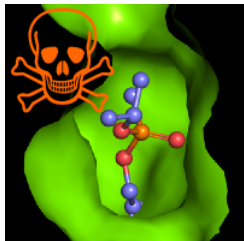


Acetylcholinesterase: A gorge-ous enzyme



It's always said "Don't shoot the messenger", but if the messenger keeps delivering the same message, he quickly becomes irritating! It is the job of the enzyme acetylcholinesterase (AChE) to 'shoot the messenger' at the neuromuscular junction, the point of contact between the nervous system and the muscles. This enzyme splits the neurotransmitter acetylcholine into acetate and choline (fig. 1) in order to prevent its accumulation, which would lead to repeated and uncontrolled muscle stimulation. Acetylcholine was the first neurotransmitter to be identified, earning Dale and Loewi the [1936 Nobel Prize](#) in Physiology or Medicine. AChE is such a key enzyme that it is a target for chemical weapons, pest-control agents, drugs, and even snake venoms.

AChE is an efficient enzyme, catalysing the breakdown of up to 10,000 acetylcholine molecules per second. The first structure of AChE (PDB entry [1ace](#)) was from the electric ray *Torpedo californica*, which has large amounts of AChE in its electric organ. It revealed that the active site is not on the protein's surface but at the bottom of a 20Å deep gorge (view-1) lined with many aromatic residues. Even though the acetylcholine substrate is positively charged (fig. 1), there are few negatively charged residues in the gorge.

Gorge-ous interactions.

Before the structure was solved, it was known that there were two binding sites for the positively charged (cationic) substrate, which were termed anionic sites. The structure showed that these sites both lie within the gorge but neither is actually anionic. They are formed by a group of aromatic residues, predominantly tryptophans. The 'peripheral anionic site' (PAS; view-1) lies at the entrance to the gorge and sequesters acetylcholine. Acetylcholine forms a π -cation interaction with the tryptophan, and the carbonyl of the acetyl group forms a weak hydrogen bond to a tyrosine further down the gorge. Acetylcholine, or the substrate analogue acetylthiocholine (PDB entries [2ha4](#), [2c4h](#), [2c58](#)), is only observed at this site when high concentrations were present in the crystallisation solution, indicating that the PAS is only occupied transiently.

At the base of the gorge, a second tryptophan plays a key role in the 'catalytic anionic site' (CAS). Again, acetylcholine forms a π -cation interaction between its quaternary amine and the tryptophan ring. The acetyl group is bound in the acyl pocket, formed from further aromatic residues lining the base of the gorge.

Between the two anionic sites, the gorge narrows due to a constriction formed by two aromatic sidechains (view-1). The constriction is smaller than acetylcholine, indicating that the protein must undergo considerable conformational changes locally to widen the gorge and let the substrate through.

Triad catalysis

Binding in the CAS and acyl pocket locates acetylcholine at the active site of the enzyme (view-2). Three amino acids, glutamate, histidine and serine, known as a catalytic triad, form

this site which is common in protease enzymes. These residues are arranged in such a way that the histidine and glutamate donate electrons to the serine, making it reactive. The serine is able to form a covalent bond with acetylcholine, reducing its carbon-oxygen double bond (fig 1). This species is termed the tetrahedral intermediate, in which the oxygen is charged. This 'oxyanion' is stabilised by interactions with backbone amide groups in an area termed the 'oxyanion hole'. (view-2). From this intermediate, the acetyl-choline bond is broken, releasing choline and leaving acetyl-serine. The serine-acetate bond is then hydrolysed by water to release acetate and regenerate the enzyme.

Because the active site is so deep within the protein, the substrate is almost totally surrounded by protein, leading to stabilisation of the intermediate and the rapid catalytic rate.

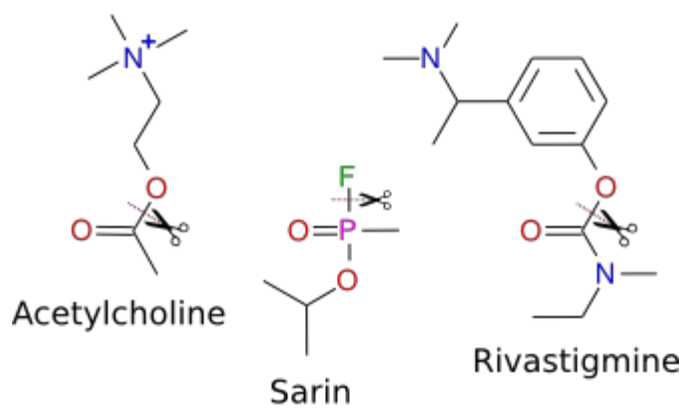


Figure 1. Compounds that react with acetylcholinesterase discussed in the article: acetylcholine (natural substrate), sarin (nerve agent that acts as a suicide inhibitor), and rivastigmine (inhibitor used

therapeutically). The cleaved bonds are indicated with scissors.

Weapon of Mass Destruction

The banned nerve agent sarin (fig. 1) is a potent inhibitor of AChE. It is a highly volatile liquid that reacts rapidly with the active-site serine of AChE (PDB entries [2y2v](#) and [2whp](#)), displacing the fluorine and forming an oxygen-phosphorus bond (view-2) that cannot be hydrolysed. The catalytic serine is thus irreversibly modified and the enzyme inactivated. Sarin and similar organophosphorous nerve agents cause death by asphyxiation as acetylcholine accumulates in the neuromuscular junction and the muscles around the lungs go into spasm.

Some insecticides, such as fenamiphos (e.g., PDB entries [2jgf](#), [2wu3](#), [2wu4](#)) and malathion, are also organophosphates and act in the same way as sarin. These insecticides have varying levels of toxicity to humans; in fact, malathion is used to treat head lice! However, as is the case with sarin, exposure to sublethal doses can lead to permanent neurological problems.

Drugs: Making a little go a long way.

Several drugs which inhibit AChE, some derived from plants, are used clinically to treat the symptoms of dementia. In diseases such as Alzheimer's, certain types of brain cell produce

too little acetylcholine, which they use as a neurotransmitter. Inhibiting AChE means that what little neurotransmitter is produced can deliver its message more times before it is broken down. The side-effects of these drugs include nausea and vomiting (also symptoms of sublethal sarin poisoning) so it is easy to see why they act as a plant defence against herbivores.

Rivastigmine (fig. 1), derived from the Calabar bean alkaloid physostigmine, reacts with the active-site serine in AChE (PDB entry [1gqr](#)) leaving a carbamate group attached (view-3). Unlike sarin, rivastigmine is a relatively slow inhibitor of AChE, so the drug does not kill the patient! The cleaved portion of the drug also remains bound through an aromatic ring-stacking interaction with the CAS tryptophan.

Other drugs block the active site but do not react with the enzyme. Huprine (e.g., PDB entries [1e66](#), [4bdw](#)) is a synthetic derivative of huperzine (PDB entries [1vot](#), [1gpk](#), [4ey5](#)), extracted from a moss which has been used in Chinese medicine for centuries. Galanthamine (e.g., PDB entries [1w6r](#), [4ey6](#)), first isolated from snowdrops (*Galanthus sp*), is a bulky molecule like huperzine. It interacts with both the PAS and the acyl-binding pocket (view-3), competing with acetylcholine for the active site. Galanthamine is also taken to promote lucid dreams and out-of-body experiences, but whether its anti-cholinesterase activity or some other property causes these effects is not known.

Tacrine (PDB entry [1acj](#)) is a synthetic molecule that binds only to the CAS (view-3) and does not interact with residues around the active site. Donepezil (also known as aricept) is bivalent, interacting with both the CAS and PAS tryptophans (view-3) via ring-stacking interactions (PDB entries [4ey7](#), [1eve](#)). Donepezil is a 20-fold more potent inhibitor of AChE than tacrine. In the USA alone, the market for donepezil was \$2.5 billion in 2010.

Snake venom.

The venom from some snakes contains the protein fasciculin, so called because it causes fasciculation (involuntary muscle twitches) in the snake's prey. Fasciculin targets AChE, contributing to immobilisation of the imminent meal. It inhibits AChE by binding at the top of the gorge (e.g., PDB entries [1fss](#), [1b41](#), [2x8b](#)) and plugging it (view-4). Fasciculin is described as a three-fingered protein and two of the fingers hold on to the rim of the gorge. Finger 1 lies on the outer surface of the enzyme while finger 2 interacts with residues of the PAS within the gorge. Whilst there are some interchain hydrogen-bonds, the interaction between the two proteins is largely hydrophobic. Unusually, a methionine sidechain stacks with the tryptophan ring of the PAS (view-4). The surface of fasciculin is complementary in shape to that of AChE, burying over 2000 Å² of surface when the proteins come together. While there are no salt bridges between the two proteins, it has been noted that the dipole moments of the two proteins are approximately aligned ([ref. 1](#)), which may also aid in binding.

AChE: a target of many different molecules

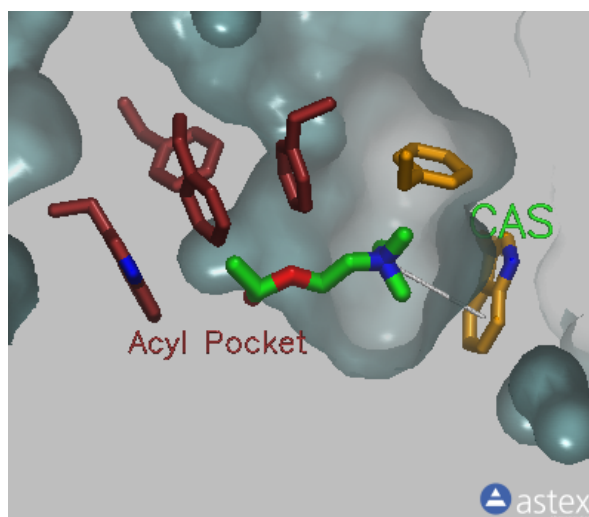
AChE is an essential component of the nervous system. Some snakes have found a way to inhibit this enzyme to help immobilise their prey, and some plants have molecules which inhibit it just enough to discourage animals from eating them. Mankind has targeted AChE in

the treatment of disease, to kill pests, and also to kill other humans. The [2009 IgNobel Prize](#) in Public Health was awarded for a means of avoiding chemical weapons, presumably including sarin which targets AChE, whilst the 2013 [Nobel Peace Prize](#) was awarded to the Organisation for the Prohibition of Chemical Weapons "for its extensive efforts to eliminate chemical weapons."

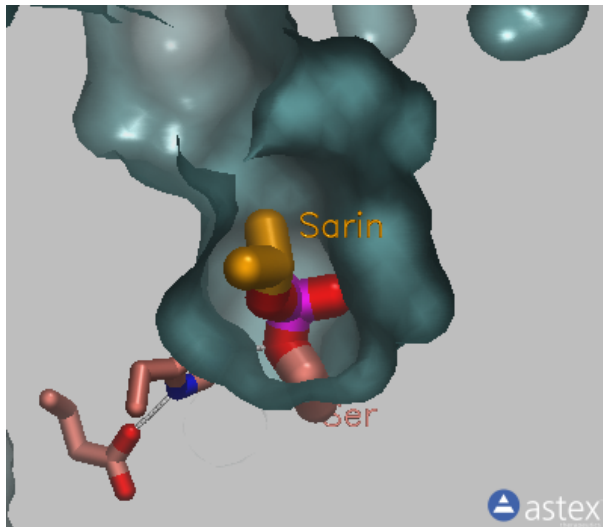
Further exploration

You can explore the many structures of acetylcholinesterase in the PDB archive using the PDBe [Enzyme Browser](#).

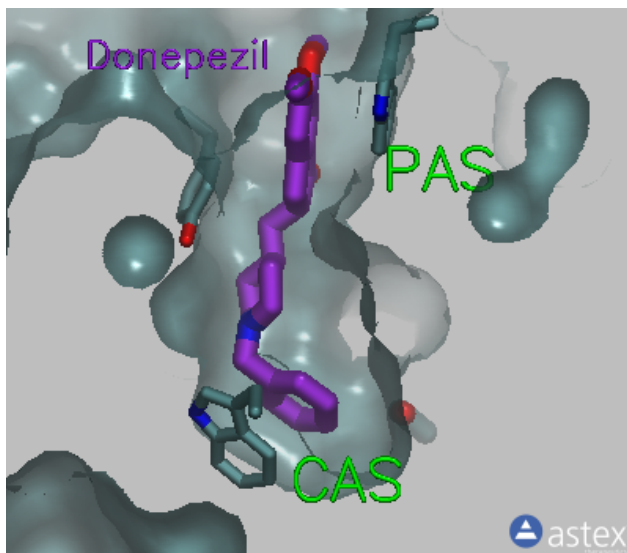
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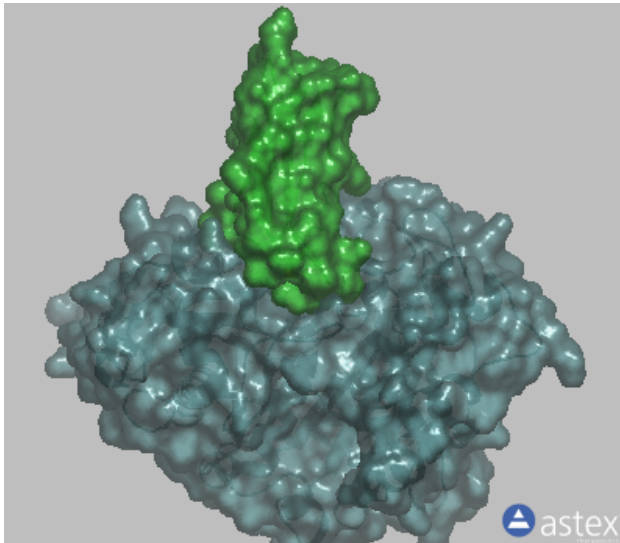
View 1: Ligand-binding sites in acetylcholinesterase. Acetylcholinesterase is initially shown in a cartoon representation. Hydrophobic residues in the gorge are shown as **orange** sticks. The surface of the protein is shown in **cadetblue**. Acetylcholine (**green** sticks) is shown first in the the peripheral and then in the catalytic anionic site (PAS and CAS respectively). π -cation interactions and hydrogen bonds are shown as white dotted lines. The mid-gorge constriction is highlighted. The acyl pocket residues are shown as **brown** sticks.



View 2: The active site. Acetylcholine (green sticks) is shown at the bottom of the active-site gorge (cadetblue surface). The Ser-His-Glu catalytic triad and oxyanion-hole residues are shown as salmon and deeppink sticks respectively. Hydrogen-bonding interactions are shown as dotted white lines and the covalent bond between the active-site serine and the substrate as solid white lines. Finally, the nerve agent sarin (orange sticks) is shown bound to the active-site serine.



View 3: Drugs which target acetylcholinesterase. Drugs which target acetylcholinesterase are shown. The drugs rivastigmine (which reacts with the enzyme, galanthamine, tacrine and donepezil are shown as coral, spring green, orange, and purple sticks respectively. Protein residues that interact with these compounds are also shown.



View 4: Fasciculin blocks the gorge. Acetylcholinesterase (cadetblue surface) is inhibited by fasciculin (limegreen cartoons). The methionine (fasciculin) and tryptophan (AChE) which interact at the peripheral anionic site are shown as sticks. Finally, the surface of fasciculin is shown coloured limegreen.