Modulation of Acetylcholinesterase Dynamics by Ethyl Paraoxon

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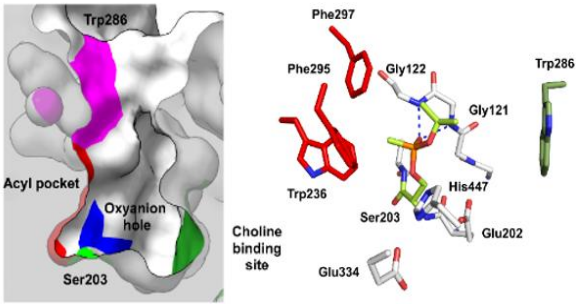

Figure 1. Structural features of AChE

Understanding organophosphate effect on AChE dynamics is vital for the design of novel reactivators. Many organophosphates (OPs) are used as pesticide agents where some, e.g. parathion, generate highly toxic metabolites such as ethyl paraoxon (PE). The toxicity arises due to the inhibition of the cholinergic enzyme acetylcholinesterase (AChE) which is crucial to hydrolyse the neurotransmitter acetylcholine (ACh), which regulates muscle contraction and nerve signalling. Inhibition of AChE leads to accumulation of acetylcholine which in some cases can cause cholinergic crisis with muscle paralysis and death. The inhibition of AChE by OPs occurs by the formation of a covalent bond to Ser203. Due to their easy availability, it is estimated that approximately 200,000 people die every year due to an intoxication by OP pesticides. (Eddleston M, Phillips MR. Self poisoning with pesticides. BMJ 2004; 328: 42–4.) By understanding the modulation of inhibited acetylcholinesterase dynamics, the design of suitable treatments is facilitated.

By combining structural and biophysical approaches such as HDX-MS, Thermal shift assay (TSA) and Cryo-EM, the dynamics of PE-inhibited AChE have been investigated. The binding of PE in the active site of AChE seems to increase the dynamics of the enzyme when inhibited. This can be seen using HDX-MS experiments where PE-inhibited AChE shows large dynamic shifts in several regions of the enzyme, such as the dimeric interface (Figure 2). Furthermore, the TSA displays a significantly smaller ΔT_m for PE than OP nerve agents (Table 1), which could indicate a more dynamic protein complex upon inhibition with the pesticide compared to the nerve agent inhibition. The dynamics of PE-inhibited AChE has also been studied using Cryo-EM. In summary, these experiments show how the inhibition of AChE by PE increases the dynamics of the enzyme and are good examples of the application of integrative structural biology.

Table 1. ΔTm values for various OPNA-enzyme complexes.

OPNA	Acyl pocket	Choline binding site	ΔT_m (ºC)
VX			4.24 (0.05) [6CQZ]
Sarin			4.31 (0.15) [5FPQ, 6WUZ]
Soman (aged)			12.13 (0.01) [6WVC]
Ethylparaoxon			1.31 (0.07) [5HF5, 8DT2]

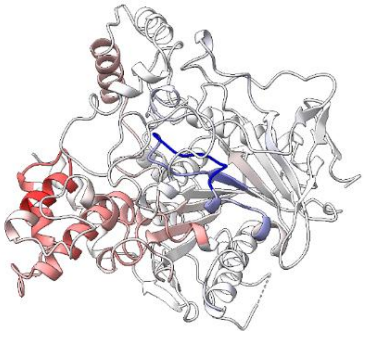

Figure 2. HDX-MS deuteration difference between PE-AChE vs. apo-AChE