Poster

Disentangling the mechanism underlying the covalent methanesulfonyl fluoride acetylcholinesterase adduct formation and evolvement: structural and mechanistic insights into an aged-like inactive complex susceptible to reactivation by a combination of nucleophiles

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Chemical warfare nerve agents and pesticides, known as organophosphorus compounds inactivate cholinesterases (ChEs) by phosphorylating the serine hydroxyl group located at the active site of ChEs. Over the course of time, phosphorylation is follow ed by loss of an organophosphate-leaving group and the bond with ChEs becomes irreversible, a process known as aging. Differently, structurally related irreversible catalytic poisons bearing sulfur instead of phosphorus convert ChEs in its aged form only by covalently binding to the key catalytic serine.

Kinetic and crystallographic studies of the interaction between *Torpedo californica* acetylcholinesterase and a small organosulfonate, methanesulfonyl fluoride (MSF), indeed revealed irreversibly methylsulfonylated serine 200 (Fig.1 **B**), to be isosteric with the bound aged sarin/soman analogues. The potent bulky reversible inhibitor 7-bis-tacrine (BTA) adopts, in the active site of the crystal structure of the MSF-enzyme adduct(Fig. 1**A**), a location and an orientation that closely resemble the one being found in the crystal structure of the BTA-enzyme complex [1]. Remarkably, the presence of BTA accelerates the rate of methanesulfonylation by a factor of two. This unexpected result can be explained on the basis of two facts: i) the steric hindrance exerted by BTA to MSF in accessing the active site and ii) the acceleration of the MSF-enzyme adduct formation as a consequence of the lowering of the rotational and translational degrees of freedom in the proximity of the catalytic serine.

It is well known that pralidoxime alone or in the presence of the substrate acetylcholine cannot reactivate the active site serine of the *Tc*AChE-MSF adduct. We show that the simultaneous presence of pralidoxime and the additional neutral oxime, 2-[(hydroxyimino)methyl]-l-methylimidazol, triggers the reactivation process of *Tc*AChE within the hour timescale.

Overall, our results pave the way toward the likely use of a cocktail of distinctive oximes as a promising recipe for an effective and fast reactivation of aged cholinesterases [2].

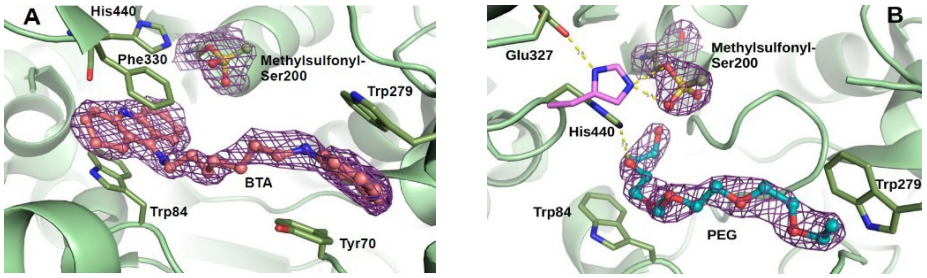

Figure 1. Close-up view of the active sites in the 3D structures of the *Tc*AChE-MSF-BTA (**A**) and *Tc*AChE-MSF (B) complexes respectively. The final $(2|F_o| - |F_c|, \Phi_c)_A$-weighted electron density map is contoured at 1.5 . Methylsulfonyl-Ser200, BTA and PEG are shown as stick and ball models with oxygen, nitrogen and sulfur atoms colored red, blue and yellow, respectively. Selected key protein residues surrounding the ligands are rendered as sticks and labeled appropriately. In panel (**B**) the 3D structure of the *Tc*AChE-MSF binary complex is shown highlighting the superimposed His440 (colored in magenta) of the 3D structure of the *Tc*AChE-MSF-BTA ternary complex, the latter is displayed in panel (**A**). The relevant hydrogen bonding interactions highlight the catalytic triad disruption.

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