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MEETING ABSTRACTS

ROOM-TEMPERATURE CRYSTALLOGRAPHY AND NEUTRON SCATTERING STUDIES OF HUMAN ACETYLCHOLINESTERASE TO INFORM THE DESIGN OF OXIME REACTIVATORS

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Human acetylcholinesterase (hAChE) is responsible for degrading neurotransmitter acetylcholine at synapses of the nervous system. Organophosphate (OP) nerve agents and pesticides inactivate hAChE through chemical modifications of the catalytic serine. The current generation of oxime antidotes is not highly efficient. Insights into the molecular structures of AChEs from various species reveal possible limitations in enhancing reactivation rates, but provide only limited information, because the structures have been obtained at cryo-temperatures. Moreover, X-ray crystallography usually cannot resolve positions of hydrogen atoms involved in proton transfer processes during reactivation. Thus, we use room-temperature X-ray and neutron crystallography to obtain structures at physiological conditions and to visualize hydrogen atoms.

Several X-ray structures of native and VX and POX-conjugated hAChE in complex with oxime reactivators, RS2-170B and RS-194B have been obtained. hAChE crystallized in a unit cell (a=124.3, c=129.1 Å; $P3_1$) amenable to neutron crystallography. For the first time we show how RS2-170B binds in the non-modified and OP-conjugated active site gorge at room temperature. RS-194B is observed with its oxime group pointing away from the catalytic Ser203 and the reactivator is pushed out to bind at the peripheral site in the VX-modified structure. Dynamics of hAChE was probed by neutron vibrational spectroscopy to look at harmonic vibrations. POX binding induces significant changes in the acyl pocket loop conformation expelling the weakly binding RS-194B from the active site gorge completely, and the loop becomes more dynamic. We hypothesize that increased dynamics of the acyl pocket loop contributes to the POX-conjugated hAChE resistance to reactivation.

Keywords: room-temperature crystallography; neutron vibrational spectroscopy; oxime reactivator; protonation state; hydrogen bonding; protein dynamics

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