The Full Mechanistic Tale of Pralidoxime in Acetylcholinesterase: Binding, Reaction, and Release

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The intensive use of organophosphate (OP) pesticides has raised serious health concerns due to their irreversible inhibition of acetylcholinesterase (AChE), leading to human neurotoxicity. Pralidoxime (2-PAM) is an FDA-approved antidote for OP poisoning, yet its complete detoxification mechanism at the atomic level remains unclear. In this study, we investigate the full story of the 2-PAM mode of action and mechanism, from binding to reactivation and product release, within human AChE (HuAChE) using a combination of enhanced molecular simulations. We employed ligand-binding Parallel Cascade Selection Molecular Dynamics (LB-PaCS-MD) to show three distinct binding routes, identifying a dominant in-line entry through the acyl door (63.79% ± 6.83%). Key conformational shifts in residues W86, Y341, Y449, and the Ω-loop were found to regulate door dynamics and binding selectivity. To explore the reaction pathway, we introduced PaCS-Q, a quantum mechanics/molecular mechanics (QM/MM) enhanced sampling method that identifies reaction intermediates and transition states without relying on predefined reaction coordinates. Our integrated approach provides a comprehensive mechanistic understanding of 2-PAM in HuAChE, showing valuable insights for the rational design of more effective antidotes and covalent therapeutics targeting AChE and related enzymes.