Unexpected Acetylcholinesterase Activity of Cocaine Esterases

Supporting Information

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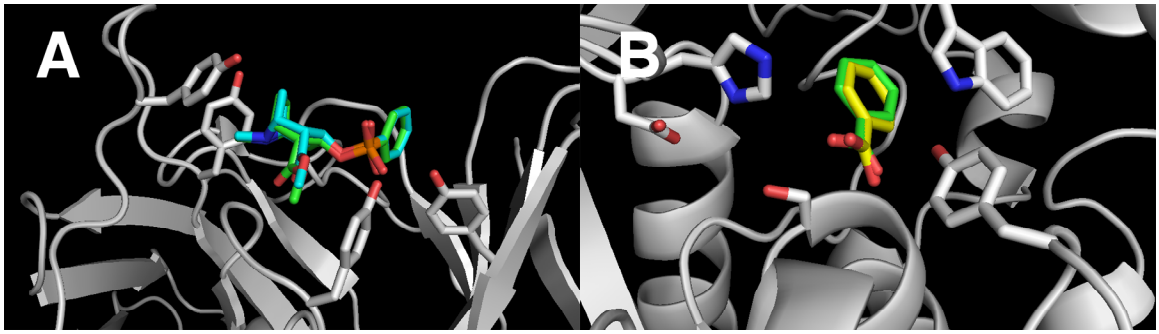
Supporting Figures



Supporting Figure 1. Comparison of the docked ligand to the crystallographic ligand as a validation of the docking model. A) The docked (cyan) versus crystallographic (green) structure of a phosphonate transition state analog of cocaine in the active site of GNL7A1 (RMSD = 0.384 Å). B) The docked (yellow) versus crystallographic (green) structure of benzoic acid bound to cocE (RMSD = 0.475 Å).

Experimental Procedures

Docking Studies. Ligand structures for the transition-state analog of cocaine, ground-state cocaine and benzoic acid were extracted from pdb files, accession codes 2AJX, 2AJV, and 1JU4, respectively. Structures for the transition state analog of acetylcholine (ACh) and ground state ACh were drawn in Cerius2. For all ligands, explicit hydrogens and charges were added (using the qEQ module) in Cerius2. Ligand geometry was minimized using the Dreiding force field[1] in sgb implicit water using MPSim.[2] The proteins were extracted from the pdb file, and explicit hydrogens and CHARMM22 charges were added. The protein geometry was minimized in the same manner as the ligands. Potential ligand binding sites for the proteins were determined using as previously described by Goddard, *et al.* with a buried surface cutoff of 70%.[3] Ligands were docked using MPSim-Dock[3] using the default settings with the exception of the cocaine ligand in cocE in which torsion drive was used to relieve strain. After docking, the five lowest-energy structures were fully minimized in MPSim, as above.

Kinetic Experiments. Kinetic experiments with acetylthiocholine (ATCh) were performed in the manner described by Ellman, *et al*.[4] Recombinant cocaine esterase (cocE) was expressed in *E. coli* and purified by Ni^{2+}-affinity chromatography, as previously described.[5] Protein concentrations were measured by the method of Bradford.[6] For kinetic measurements, a solution of 20 μM antibody or 30 nM cocE and 340 μM 5,5'-dithiobis(2-nitrobenzoic acid) in 100 mM phosphate buffer, pH 8.0 was incubated at 37 ºC. After 5 min, acetylthiocholine iodide (0-8 mM) was added, and the reaction was followed by monitoring the increase of absorbance at 412 nm. All assays were run in triplicate. Initial rates were determined by subtracting the average observed rate from the rate of the nonenzymatic reaction. The kinetic parameters k_{cat} and K_m were determined by fitting plots of v_0 versus [S] to the Michaelis-Menten equation.

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