

Heroin and Cocaine Metabolism in Humans as Revealed in Structural Studies of a Single Promiscuous Drug-processing Enzyme

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Introduction

Mammalian carboxylesterases are promiscuous enzymes responsible for processing a wide variety of drugs and xenobiotics [1]. In particular, carboxylesterases metabolize two dangerous narcotics (heroin and cocaine) and three potent chemical weapon agent (sarin, soman, and tabun). We have elucidated the first structure of a human carboxylesterase and unraveled the detailed role it plays in heroin and cocaine metabolism. SER-CAT beamline 22-ID at the APS played a key role in the success of these studies.

Human carboxylesterase 1 (hCE1) is a broad-spectrum enzyme that acts on ester and amide linkages in numerous structurally distinct substrates. This promiscuous enzyme is expressed largely in the liver, with lesser amounts in the intestines, kidneys, lungs, and testes. We sought to understand how a single enzyme, hCE1, can act on substrates as distinct in structure as heroin and cocaine. X-ray crystallography is an ideal method to examine the manner in which an enzyme binds to and processes different chemicals.

Methods and Materials

We used the structure of a rabbit liver carboxylesterase (rCE), which activates the camptothecin anticancer prodrug Irinotecan (CPT-11) (as determined in our laboratory) [2], to assist in the structural examination of hCE1. We crystallized hCE1 in complexes with the cocaine analogue homatropine and with the heroin analogue naloxone. Then we collected x-ray diffraction data at the SER-CAT beamlines at the APS. The ability to move the charge-coupled device (CCD) x-ray detector at SER-CAT to a location that was 300 mm from the crystal helped us to resolve diffraction from these crystals with large unit-cell dimensions.

Results and Discussion

The results from these structures reveal that hCE1 is in a complex trimer-hexamer equilibrium that can be adjusted by the allosteric binding of substrate molecules

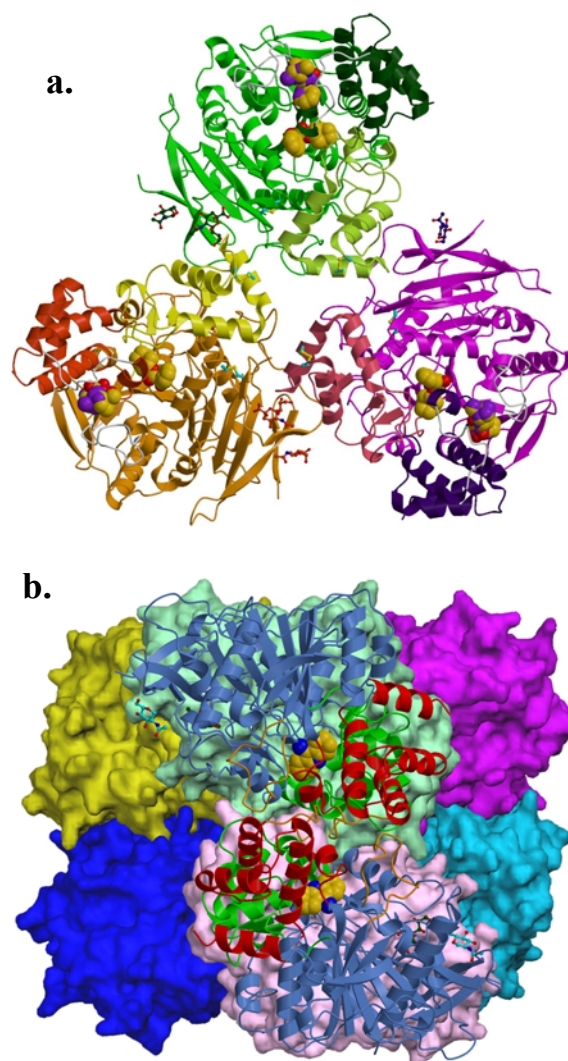


FIG. 1. a. The hCE1 trimer as observed in the homatropine complex. Homatropine molecules bound at the active sites are gold. Homatropines bound at the Z site, between the Ω loops, are gold and purple. b. A molecular surface representation of hCE1 hexamer.

[3] (Fig. 1). Our structures, together with the results from atomic force microscopy (AFM), provide the first evidence of hCE1's trimer-hexamer equilibrium. In addition, we found that a surface-binding site between two hCE1 trimers plays a key role in shifting the equilibrium toward trimer. The positions of bound homatropine (a cocaine analogue) and bound naloxone (a heroin analogue) reveal how hCE1 utilizes its large, flexible pocket promiscuously and how it uses its small, rigid pocket specifically in substrate recognition and alignment (Fig. 2). In this way, the enzyme can place chemical structures with widely distinct characters in the flexible, promiscuous pocket and structures with more specific characters in the rigid pocket.

We have also presented the structure of hCE1 in complexes with an anti-Alzheimer drug, tacrine [4]. The manner in which tacrine binds, and the shape and size of hCE1 substrate-binding gorge, are distinct from the tacrine-bound acetylcholinesterase structure determined previously. Furthermore, tacrine appears in multiple conformations within hCE1's substrate-binding gorge. We conclude that hCE1's ability to bind the same ligand in up to five conformations may contribute to its promiscuity and facilitate its ability to process a variety of endogenous and exogenous compounds.

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References

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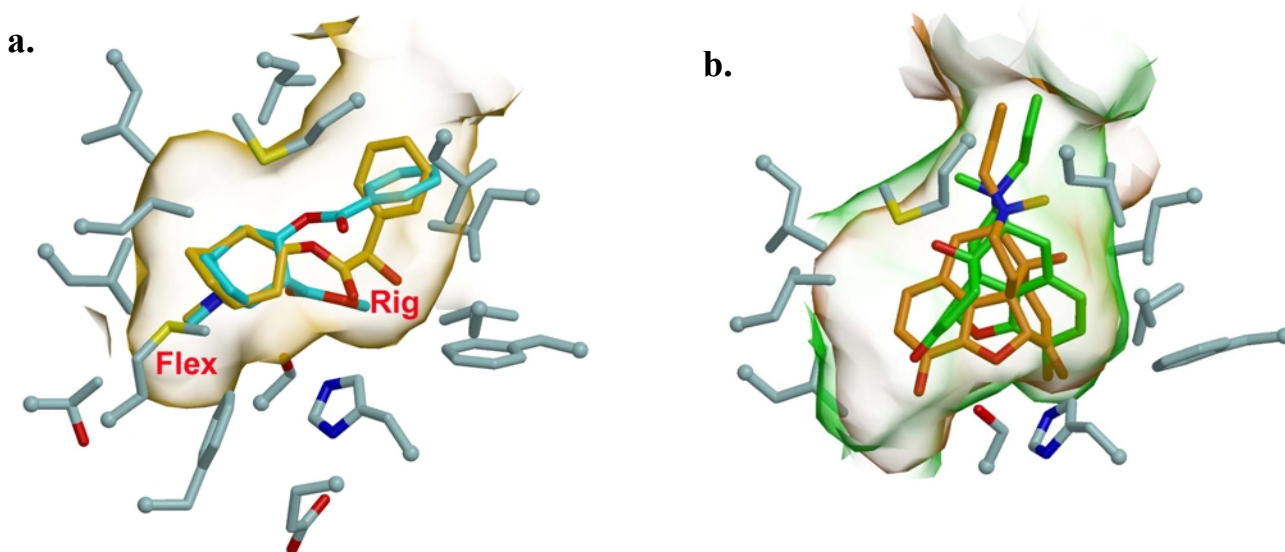


FIG. 2. a. Homatropine (gold) bound within the hCE1 catalytic pocket, along with a proposed binding mode for cocaine (cyan). The molecular surface of the catalytic binding pocket is transparent gold. The catalytic Ser221 is located in between the large, flexible pockets and the small, rigid pockets (Flex and Rig, respectively). b. Two orientations of naloxone methiodide (green and orange) bound within the hCE1 catalytic pocket. The two binding modes of this heroin analog are related by an opposite twofold rotation about the vertical axis. The molecular surfaces of the catalytic binding pocket corresponding to the two orientations of the naloxone ligand, rendered in transparent green and orange, demonstrate the induced-fit property of the substrate-binding pocket.