High Resolution Snapshots for the Complete Reaction Cycle of a Cocaine Catalytic Antibody

Xueyong Zhu[1], Tobin J. Dickerson[2,3], Claude J. Rogers[2,3], Gunnar F. Kaufmann[2,3], Jenny M. Mee[2,3], Kathleen M. McKenzie[2,3], Kim D. Janda[2,3,4,*] and Ian A. Wilson[1,4,*]

Departments of Molecular Biology[1] and Chemistry[2] and Immunology[3], and The Skaggs Institute for Chemical Biology[4], The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

Cocaine is a powerful addictive stimulant that affects the brain, and abuse of cocaine has been a substantial social problem. Unfortunately, no FDA-approved treatments exist for cocaine abuse, addiction, and overdose. Development of effective treatment for cocaine abuse has been frustrated by the complex neurochemistry in inhibiting a blocking agent. Nevertheless, within the past decade, immunotherapy for cocaine abuse has been evaluated in pre-clinical and human clinical trials.

Cocaine-binding antibodies have shown some promise in neutralizing the cocaine toxicity, but they would be saturated by high concentrations of cocaine because of the 1:1 binding stoichiometry. A cocaine-degrading catalytic antibody, such as 7A1, is far more effective in metabolizing the drug, since the antibody would be regenerated with each turnover[1]. Catalytic antibodies have emerged as a powerful tool at the interface of chemistry and biology. In this regard, antibody-catalyzed ester hydrolysis is one of the hallmark reactions. Since cleavage of the benzoate ester of cocaine produces the nonpsychoactive metabolites ecgonine methyl ester and benzoic acid, it is an excellent target for an immunopharmacological strategy. A crystal structure of the mouse monoclonal antibody 7A1 would help to determine the molecular basis for catalysis and provide a foundation for murine antibody humanization and mutagenesis studies to increase the catalytic proficiency for potential therapeutic use.

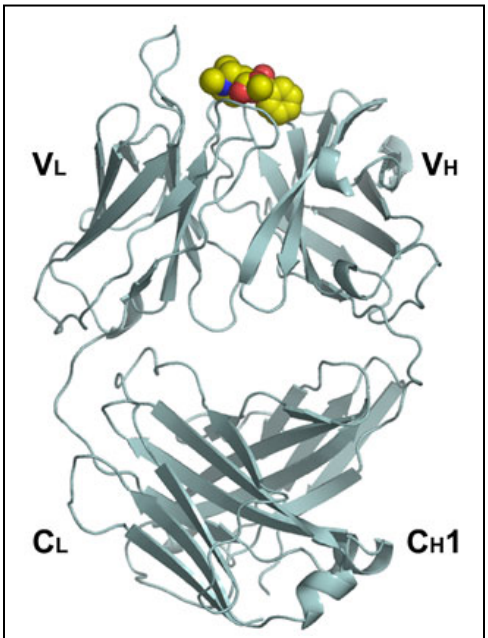

Figure 1 Crystal structure of the 7A1 Fab' cocaine complex with the secondary structure of the antibody light (L) and heavy (H) chains colored in cyan. Substrate cocaine is also shown in spheres with yellow carbons, blue nitrogen, and red oxygens in the active site.

Although it is rarely possible to acquire experimentally-determined structures of each step along an enzyme reaction coordinate, 7A1 Fab' antigen binding fragment was able to be co-crystallized with substrate, a transition state analog (TSA), both products and heptaethylene glycol. Using x-ray diffraction data collected at SSRL beamline 9-2 and the ALS, 7A1 Fab' and six complexes with substrate cocaine (Figure 1 and 2b), TSA, both products (ecgonine methyl ester and benzoate), one product ecgonine methyl ester, and finally the other product benzoate, as well as heptaethylene glycol, were determined at 1.5-2.3 Å resolution (Figure 2). Here, high resolution snapshots are presented for the complete reaction cycle of the cocaine catalytic antibody.

Significant conformational changes were observed along the 7A1-catalyzed cocaine hydrolysis pathway, but are generally limited to some active site key residues and ligands

themselves. Antibody CDR loop movements (up to 2.3 Å) and large side-chain movements (up to 9 Å) alter the antibody active site from "open" to "closed" to "open" (with the approximate size changes from 320 to 500 $Å^3$) for the substrate, transition state and product states, respectively.

In the unliganded apo 7A1, the active site adopts an "open" form with two essential residues TyrL94 and TyrH97 showing flexible side-chains (Figure 2a). In the substrate cocaine-bound state, TyrH97 is fully ordered, TyrL94 displays some partial occupancy to accommodate the two side-chain rotamers of TrpH47, while the active site retains a modified "open" form (Figure 2b). In the transition state analog complex, the active site shows the "closed" form with the CDR loops, particularly H2, moving towards the active site, along with the rearrangements of side chains of ArgH52, ArgH58 and IleH56 by several angstroms (Figure 2c); TyrL94 and TyrH50 now hydrogen bond with the pro-*R* phosphonate oxygen of the TSA, and possibly constitute an oxyanion hole to stabilize the transition state. When the cocaine hydrolysis is achieved, the active site returns to an intermediate "open" with two products initially remain trapped; the side chains of TyrH50, ArgH52, ArgH58 and IleH56 adopt conformations between those found in the transition state and those in the substrate-bound state, and the side chain of TyrL94 has partial stabilization and a different rotamer (Figure 2d). From this comprehensive series of crystal structures, a catalytic mechanism has been proposed, as well as possible mutations that explore how to improve catalytic proficiency.

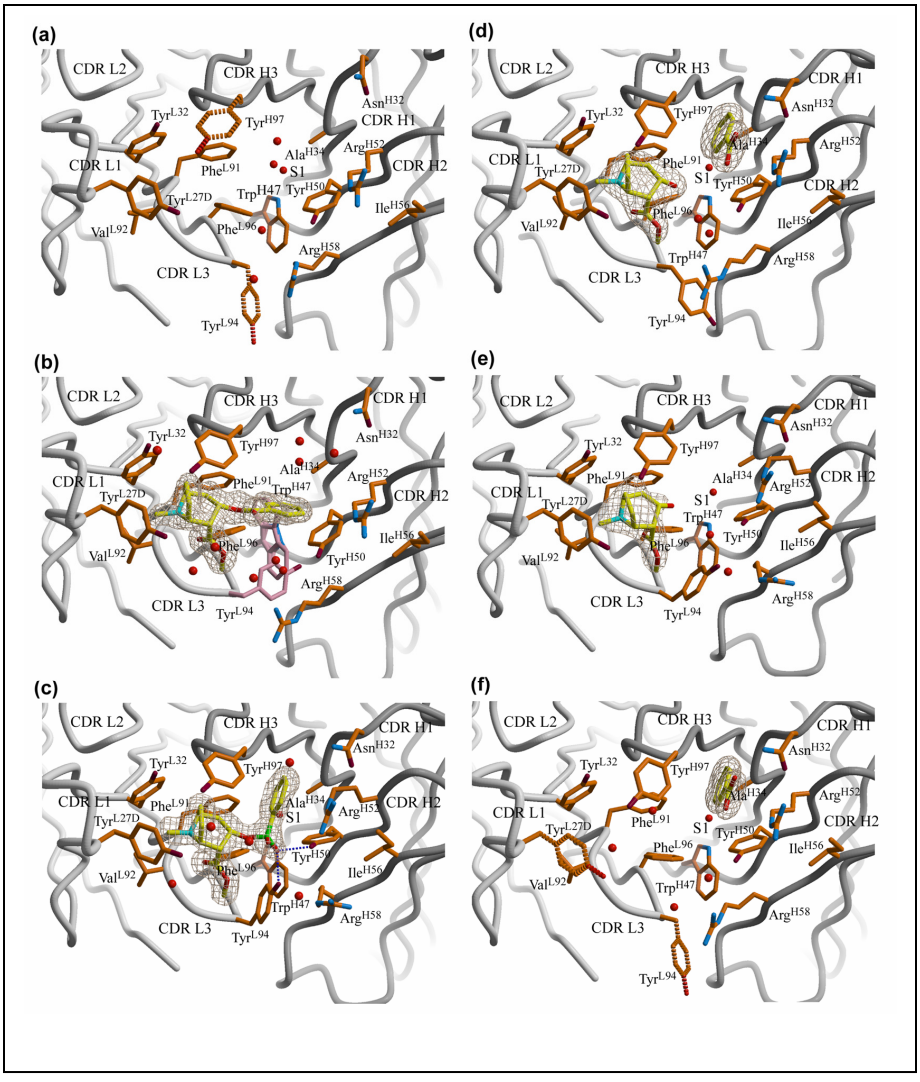

Figure 2 The active site of 7A1 corresponding to different steps in the reaction pathway. (a) Apo form (1.5 Å resolution). (b) Complex with substrate cocaine (1.5 Å). (c) Complex with transition state analog (TSA) (1.85 Å). (d) Complex with both products ecgonine methyl ester and benzoate (2.1 Å). (e) Complex with product ecgonine methyl ester (2.3 Å). (f) Complex with product benzoate (1.85 Å). Disordered side chains are highlighted in dashed bonds. Alternate conformations of TrpH47 and the partially-occupied TyrL94 are rendered with pink side chains in the 7A1 Fab' cocaine complex. The corresponding ligands for each structure are shown with yellow carbons, blue nitrogens, red oxygens and green phosphorus atoms. Water molecules in the active site are shown in red spheres and the conserved water is labeled S1 (From Zhu *et al.*, 2006).

(click on image for larger view)

This work was supported by the National Institutes of Health grants GM38273 (IAW), DA08590 and DA15700 (KDJ), and The Skaggs Institute for Chemical Biology, The Scripps Research Institute (IAW and KDJ).

Primary Citation

Zhu, X., Dickerson, T.J., Rogers, C.J., Kaufmann, G.F., Mee, J.M., McKenzie, K.M., Janda K.D. and Wilson I.A. Complete reaction cycle of a cocaine catalytic antibody at atomic resolution. *Structure*, **14**, 205-216 (2006).

References

1. Matsushita, M., Hoffman, T.Z., Ashley, J.A., Zhou, B., Wirsching, P. and Janda, K.D. Cocaine catalytic antibodies: the primary importance of linker effects. *Bioorg. Med. Chem. Lett.* **11**, 87-90 (2001).

Related Links

Scripps Press Release: http://www.scripps.edu/news/press/020706.html
Wilson Research: http://www.scripps.edu/mb/wilson
Janda Research: http://www.scripps.edu/chem/janda

SSRL is supported by the U.S. Department of Energy, Office of Basic Energy Sciences. The SSRL Structural Molecular Biology Program is supported by the National Institutes of Health, National Center for Research Resources, Biomedical Technology Program and by the U.S. DOE, Office of Biological and Environmental Research.