DRUG METABOLISM

Enigmatic enzyme

DOI:
10.1038/nrd2183

Cytochrome P450 (CYP) 3A4 is the most studied, and yet probably the most challenging, drug-metabolizing enzyme for those involved in drug R&D. Now, a report in *PNAS* sheds further light on the structure and function of CYP3A4 and provides the first evidence of the feasibility of multiple ligand binding. However, their evidence also shows that the enzyme has an extremely flexible ligand-binding site, and might adopt more than one ligand-bound conformation, suggesting that any attempts to model ligand binding to CYP3A4 using computational tools will be challenging in the absence of experimental data.

CYP3A4 is responsible for the metabolism of more than 50% of all marketed drugs, and is also renowned as being the CYP isoform most frequently involved in drug–drug interactions. This is thought to result from its ligand promiscuity and atypical enzyme kinetics, particularly its tendency to show increased or decreased activity in the presence of different substrates, a behaviour known as cooperativity.

Much effort has been spent trying to understand the unconventional mechanism of CYP3A4 ligand binding, and since the first crystal structures of the enzyme were published in 2004 the possibility of using computational tools to model CYP3A4–drug interactions became a reality. However, the initial structures did not provide much insight into how CYP3A4 binds diverse and multiple substrates, because there was no evidence of conformational change or of either ligand being present in a site that would enable catalysis.

Ekroos and Sjögren therefore set out to obtain structures of CYP3A4 in complex with larger ligands to probe the conformation of the enzyme. They report crystal structures of human CYP3A4 complexed with ketoconazole and erythromycin, and reveal that the enzyme forms different conformations with each ligand. Some conformational change was observed with erythromycin, but the structure seems to be non-productive for the most common type of metabolism. However, binding of ketoconazole caused a much more dramatic conformational change, such that the size of the active site increased by 80%. Most strikingly, one of the structures seems to show two molecules of ketoconazole stacked in the active site, at a resolution of 2.8 Å. Although, as the authors note, this could be an artefact of crystallization, it is the first preliminary evidence of multiple ligand binding in the CYP3A4 active site.

The authors' observations with ketoconazole would certainly go some way to explaining the broad substrate specificity and atypical kinetics that have been so widely reported for CYP3A4. However, they caution that understanding the structural basis of CYP3A4 binding for a single ligand does not necessarily provide insights into the binding of other ligands, given the major conformational change observed with ketoconazole. So, although this paper provides fascinating insights into the flexibility of enzyme active sites, it seems that for drug discovery efforts CYP3A4 still remains something of an enigma.

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FURTHER READING Guengerich, F. P. A malleable catalyst dominates the metabolism of drugs. *Proc. Natl Acad. Sci. USA* **103**, 13565–13566 (2006)