CHAPTER 2

Clues from Three-Dimensional Structure Analysis and Molecular Modelling:
New Insights into Cytochrome P450 Mechanisms and Functions

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Abstract

Cytochrome P450 is a focus of attention as it comprises one of the largest superfamilies of enzyme proteins. Metabolization of many drugs is affected by cytochrome P450. It is an attractive drug target, e.g., cytochrome P450s of *Mycobacterium tuberculosis* are promising targets in the fight against tuberculosis. The structure provides new insights for investigation of structure/mechanism of cytochrome P450, and for rational design of inhibitor molecules. We will illustrate how biocomputing and bioinformatical techniques reveal details, functions and further secrets of this exciting molecule. Molecular modelling along with site-directed mutagenesis of P450 2B1 elucidated the molecular determinants of substrate specifity. Regioselectivity of progesterone hydroxylation by cytochrome P450 2B1 was reengineered based on the X-ray structure of cytochrome 2C5. Docking approaches rationalized the regioselectivity of the reengineered cytochrome P450 2B1. Furthermore, by methods of molecular dynamic simulations, routes were identified by which substrates may enter into and products exit from the active site of cytochrome P450.

Introduction

Cytochrome P450 enzymes[1-5] form an ubiquitous heme protein monooxygenase family (EC: 1.14.14.1). They play an important role in the synthesis and degradation of many physiologically important compounds such as steroid hormones, cholesterol, bile acids and in the detoxification of xenobiotics in many species of microorganisms, plants and animals.

P450 are of great medical relevance: Mutations in P450 genes are triggers of human diseases such as primary congenital glaucoma and there are evidences for associations between cytochrome P450 enzyme-polymorphism and cancer. Some P450 enzymes are able to activate procarcinogens to genotoxic intermediates. They play a major role in drug-metabolism, for example the P450 3A family of enzymes are able to metabolize the majority of commercially available drugs such as Codeine (narcotic), Diazepam (Valium), Erythromycin (antibiotic). Drug metabolism polymorphism or interactions with other drugs can cause severe sideeffects in patients.

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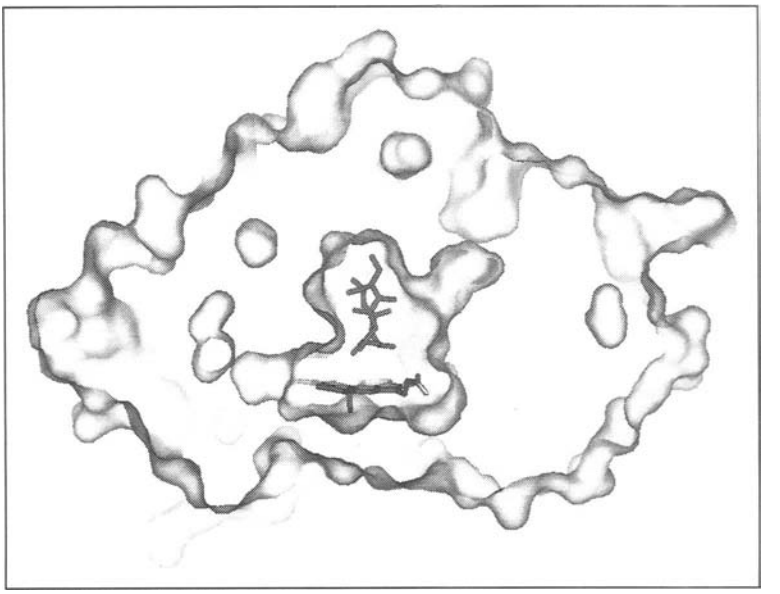

Figure 1. The active site molecular surface and the outer molecular surface of cytochrome P450eryF (CYP107A1) are not connected with each other. A substrate molecule, 6-deoxyerythonolide, is shown in the active site above the heme. The molecular surface was computed with the PyMOL program.[53]

P450 are heme-thiolate containing proteins where the ligand of the heme iron is delivered by a cysteine residue in a highly conserved region of the enzyme. The active site is buried at the center of the enzyme (Fig. 1). They are named P450 for the absorption band at 450 nm of their carbon-monoxide-bound form. The reactions carried out by cytochrome P450 molecules are very diverse and include hydroxylation, N-, O- and S-dealkylation and oxidation of heteroatoms.

According to their sequence similarity P450 enzymes are subdivided into families (sequence identity greater than 40%) and subfamilies (sequence identity greater than 55%). In humans 57 CYP genes are sequenced (and 58 pseudogenes) which are subdivided into 18 families and 43 subfamilies.

In prokaryotes P450 are soluble proteins whereas in eukaryotes P450 are usually membrane-associated within the inner mitochondrial membrane or endoplasmic reticulum.

Because of their physiological importance and medical relevance the P450 enzymes are an emerging field of research. Major unresolved issues are structurefunction relationships such as the understanding of substrate specificity, the catalytic mechanism of multi-step reactions, the dynamical properties that allow substrates to enter the active site and products to leave the active site or the identification of essential determinants of drug metabolism or tolerance. In the following paragraphs methods of computational biology are presented which aid our understanding of this interesting enzyme. However the presented methods are applicable to a variety of biomolecules.

Modelling

The gap between the high number of known protein sequences and the only limited available 3-dimensional protein-structures is increasing rapidly. Molecular modelling techniques are valuable tools to fill this gap.[6,7] In the field of cytochrome P450 research this technique is of high interest. Up to now more than 3700 cytochrom P450 (different named) sequences of different species are known, the determination of all these protein structures is a tedious work, because crystallization of some P450 enzymes, especially of the membrane-associated ones is