Crystal structure of human cytochrome P450 2C9 with bound warfarin

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Abstract:

Cytochrome P450 proteins (CYP450s) are membrane-associated haem proteins that metabolize physiologically important compounds in many species of microorganisms, plants and animals. Mammalian CYP450s recognize and metabolize diverse xenobiotics such as drug molecules, environmental compounds and pollutants. Human CYP450 proteins CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are the major drug-metabolizing isoforms, and contribute to the oxidative metabolism of more than 90% of the drugs in current clinical use. Polymorphic variants have also been reported for some CYP450 isoforms, which has implications for the efficacy of drugs in individuals, and for the co-administration of drugs. The molecular basis of drug recognition by human CYP450s, however, has remained elusive. Here we describe the crystal structure of a human CYP450, CYP2C9, both unliganded and in complex with the anti-coagulant drug warfarin. The structure defines unanticipated interactions between CYP2C9 and warfarin, and reveals a new binding pocket. The binding mode of warfarin suggests that CYP2C9 may undergo an allosteric mechanism during its function. The newly discovered binding pocket also suggests that CYP2C9 may simultaneously accommodate multiple ligands during its biological function, and provides a possible molecular basis for understanding complex drug–drug interactions.

Introduction:

So far the understanding of the molecular basis of drug binding by human CYP450s has been derived largely from the structures of bacterial CYP450s complexed to ligands; however, the low amino acid sequence identity (20–30%) between the human drug-metabolizing and the bacterial CYP450s limits this approach. Furthermore, despite the report of the crystal structure of a mammalian CYP450, CYP2C5 from rabbit, insights into how the human CYP450s are able to recognize structurally diverse ligands remain elusive. In this study we report the crystal structure of a human cytochrome P450, CYP2C9. The structure of the protein has been determined in the absence and presence of a known substrate, the anti-coagulant drug warfarin.

CYP2C9 is a two-domain protein with an overall fold characteristic of the CYP450 family (Fig. 1a). The B–C loop contributes to substrate specificity, and in both the substrate-free and complexed structures of CYP2C9 residues 101–106 in the B–C loop form helix B0 . In addition, residues 212–222 in the F–G loop form helices F0 and G0 , a feature previously not observed in any other CYP450 structure. The haem is located between helices I and L and the iron is pentacoordinated with Cys 435 as the single ligand, and despite the lack of an ordered water molecule in the sixth

coordination position, the spin state of the haem iron in the crystal is unknown, as the environment may change during the experiment. As in other CYP450 structures, a water molecule, located 7 A ° above the haem iron (Supplementary Fig. S1), is hydrogen bonded to a highly conserved threonine, Thr 301, appropriate for its role in the proton-transfer path10. In addition there is some residual electron density located 4 A ° above the haem, running up to and alongside helix I (Supplementary Fig. S1). The features of this electron density make an interpretation ambiguous; however, it does not appear close enough to the haem iron to be a sixth ligand. The haem is stabilized by hydrogen bonds between the propionates and the side chains of residues Trp 120, Arg 124, His 368 and Arg 433. A key residue implicated by mutagenesis studies11, Arg 97, also forms hydrogen bonds to the propionates, as well as the carbonyl oxygen atoms of Val 113 and Pro 367 (Fig. 1b). Thus it would seem that the main role of Arg 97 in CYP2C9 is haem stabilization rather than substrate interaction as previously suggested.

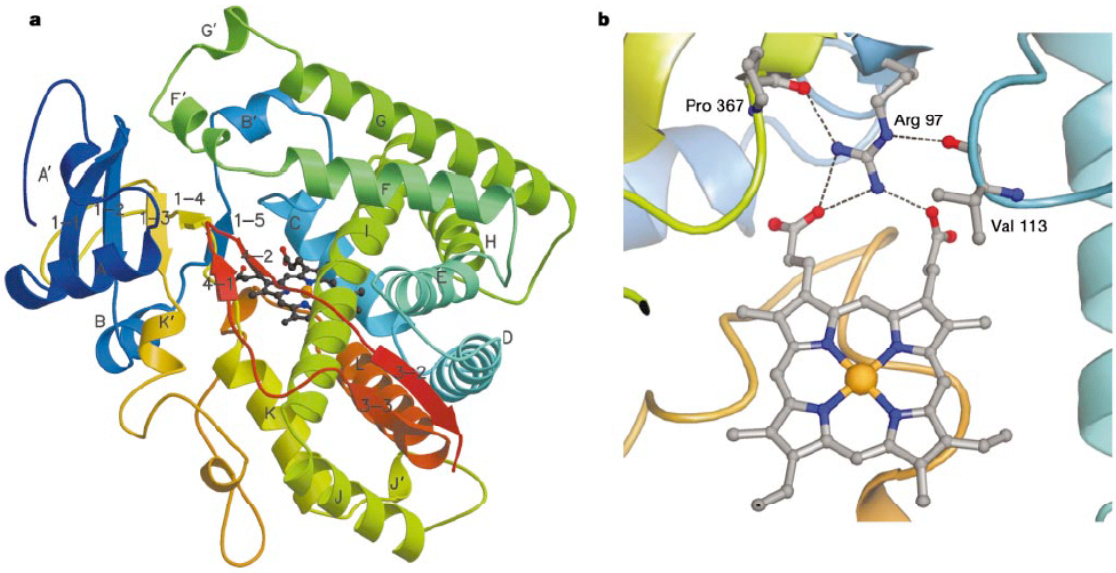

Figure 1
Structure of P450 CYP2C9. a) Overall fold of CYP2C9, coloured from blue at the N terminus to red at the C terminus. The haem group is depicted as a ball-and-stick model in the centre of the molecule, flanked by helices I and L. There is a slight distortion in helixI, close to the haem. The substrate access channel is widely acknowledged to involve the loops between helices B and C, and helices F and G. The figure was produced using Molscript (**http://www.avatar.se/molscript**). b) View of Arg 97 and the haem group (shown at the bottom). Arg 97 is held in position by hydrogen bonds (indicated by dashed lines) to the haem propionates and to the carbonyl oxygen atoms of Val 113 and Pro 367.