BIOCHEMICAL JOURNAL LETTERS

Conservation of a functionally important surface region between two families of the cytochrome *P*-450 superfamily

Although the X-ray-crystallographic structures of a few prokaryotic cytochrome *P*-450 enzymes have been solved (Poulos et al., 1987; Boddupalli et al., 1992), the tertiary structure of eukaryotic cytochromes *P*-450 remains undetermined. Both we (Edwards et al., 1989, 1991a) and others (Nelson and Strobel, 1988; Poulos, 1991) have attempted to produce a general model for the three-dimensional structure of mammalian cytochromes *P*-450, using CYP101 (individual cytochrome *P*-450 enzymes are referred to as 'CYP', followed by a combination of numbers and letters; for an explanation of the nomenclature see Nebert et al., 1991) as a structural template. We have used site-specific anti-peptide antibodies to confirm the predicted surface location of selected regions in this model (Edwards et al., 1991a). The location of pro-inhibitory epitopes, which may be conserved amongst forms of cytochrome *P*-450, is invaluable in such studies.

Recently, Manns et al. (1991) and Gueguen et al. (1991) have independently mapped the region for the binding of human liver and kidney microsomal autoantibodies type-1 (LKM-1 autoantibodies). Such antibodies recognize a single form of cytochrome *P*-450 in man, CYP2D6, in both native and denatured states, and inhibit its catalytic activity (Zanger et al., 1988). The antibodies are thus likely to bind to a continuous epitope on the surface of the protein at a functionally important region. In the study by Manns et al. (1991), of 26 sera tested, 22 were found to bind to short peptides containing the core sequence, DPAQPPRD, corresponding to residues 263–270 of CYP2D6. It was proposed that this sequence is located in a surface loop region corresponding to that between the G and H helices of CYP101. Similarly, Gueguen et al. (1991) showed that this region was important in antibody binding: the sequence corresponding to residues 239–271 of CYP2D6 is recognized by all antisera tested, with the essential part of the epitope comprising the tripeptide TWD. This corresponds to residues 261–263 of CYP2D6, which overlaps the N-terminus of the core peptide identified by Manns et al. (1991) (Figure 1). Thus binding of the LKM-1 antisera, determined in two separate studies, occurs at a consensus core

sequence corresponding to residues 261–270. Further confirmation that this region contains the critical epitope is provided by the fact that LKM-1 antisera bind to rat CYP2D enzymes (Gueguen et al., 1988) which display 100 % sequence identity in this region (Manns et al., 1991).

Both groups suggested that LKM-1 antibodies may be produced as a result of virally induced autoimmunity, such as has been proposed for CYP1A2 (Bourdi et al., 1990), and sequences similar to the predicted LKM-1 epitopes have been found in proteins of hepatitis C virus (Manns et al., 1991), herpes simplex virus type I and human T lymphotropic virus type II (Gueguen et al., 1991). In all cases, the regions of sequence similarity were centred on the consensus core region.

We have previously identified a functionally important surface region of rat CYP1A2, using inhibitory anti-peptide antibodies (Edwards et al., 1990) and have postulated that this site may form part of the binding site for cytochrome *P*-450 reductase. The function of this region is conserved in rat CYP1A1 (Edwards et al., 1991b), even though the primary structure of the two cytochrome *P*-450 enzymes in this region is quite different and antibodies raised against peptides corresponding to these regions were specific for their respective cytochromes *P*-450. LKM-1 antiserum appears to be specific for CYP2D6 among human cytochromes *P*-450, as immunoprecipitation resulted in a single band corresponding to this cytochrome *P*-450 (Zanger et al., 1988). The primary structure of the inhibitory region of CYP2D6 bears little resemblance to the inhibitory regions of rat CYP1A1 and CYP1A2. Thus the inhibitory sites on each of the three cytochrome *P*-450 enzymes are different in their primary structures and are immunologically distinct.

If human CYP2D6 is aligned with the sequences of rat CYP1A1 and CYP1A2 (Figure 1), it can be seen that the region targeted by the inhibitory anti-peptide antibodies to the rat CYP1A proteins does not overlap with the consensus core sequence of CYP2D6 identified by Manns et al. (1991), nor with the epitope determined by Gueguen et al. (1991). Both of these regions lie further towards the N-terminus of the aligned proteins than the region strictly equivalent, by alignment, to the binding site of the inhibitory anti-peptide antibodies.

The difficulty in obtaining a global alignment of cytochrome *P*-450 sequences of different families, which does not result in the use of a large number of spacers, has been discussed previously

CYP2D6	[233]	IPALAGKVLK.FQKAFLTQDELLETHRMETWDPAQPPRDLTAEFLAEMEKAK..GNPESFND
CYP1A1	[247]	LPNSSLDFAFDLNKKFYSFMKKLIKHEHYRTFEKGHI.RDITDSLIEHCQDRRLDENANVQLSD
CYP1A2	[244]	LPNPALKRKFNFNDNFVFLQKTVQEHYQDFNKNISI.QDITGALFKHSENYK..DNGGL.IPQ

Figure 1 Alignment of sequences by primary-structure homology

Aligned sequences of human CYP2D6 (Gonzalez et al., 1988), rat CYP1A1 (Sogawa et al., 1984) and rat CYP1A2 (Sogawa et al., 1985) in the region of interest. Numbers in square brackets refer to the sequence position of the first residue of the fragment, numbered from the N-terminal methionine. Alignment was performed with the program MULTALIN (Corpet, 1988), by using the Dayhoff matrix and a gap penalty of 8. Dots have been inserted in the sequences to obtain the best alignment. The binding peptide immunogens of the anti-CYP1A1 and anti-CYP1A2 antibodies, and the proposed epitope of the LKM-1 antisera in CYP2D6 determined by Manns et al. (1991), are underlined. The epitope determined by Gueguen et al. (1991) is shown in **bold**. The core epitope of the LKM-1 antiserum (Manns et al., 1991) is double-underlined.

Figure 2 Alignment of sequences by predicted secondary structure

The sequences above were aligned on the border of the predicted C-termini of regions of CYP1A and CYP2D6 predicted to be equivalent to the G-helix of CYP101 as described by Edwards et al. (1989). The boxes show the predicted locations of α -helices. The position of the CYP1A H-helix has not been determined. The regions underlined, double-underlined or in bold are the same as those shown in Figure 1.

(Edwards et al., 1989). Instead, it has been found preferable to consider the consensus secondary structure, predicted from sequences aligned within their families (Edwards et al., 1989). This is reinforced when the present sequences are analysed in this way. The assigned structural location of the CYP1A anti-peptide antibody-binding sites lies immediately adjacent to the C-terminus of a region equivalent to the G helix of CYP101 (Edwards et al., 1989, 1990, 1991b). If the regions in CYP2D6 which have been assigned as equivalent to the α -helices of CYP101 are considered (Edwards et al., 1989), the core binding site of the inhibitory LKM-1 antibodies is structurally equivalent to the binding site of the inhibitory anti-peptide antibodies, and the essential part of the CYP2D6 epitope identified by Gueguen et al. (1991) is adjacent to the anti-peptide antibody-binding site (Figure 2). It should be noted that it is particularly difficult to assign a region in the mammalian CYP1A family which is equivalent to the H helix of CYP101, and it has been postulated that this structure may well be absent from this cytochrome P-450 family (Edwards et al., 1989).

It thus appears that the functionally important surface region identified in the members of the rat CYP1A subfamily, distal to the G helix, is conserved in human CYP2D6. This adds further weight to the hypothesis that the three-dimensional structure of cytochromes P-450 is well conserved, albeit at the expense of primary-structure similarity.

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Mechanism of β -carotene degradation

Ever since the observation that β -carotene was metabolized to vitamin A (Moore, 1930), the search has been on to determine the mechanism by which carotenoids with vitamin A activity are converted into vitamin A. The main condition for provitamin A activity is that the carotenoid must possess at least one β -ionone ring residue. Of all the provitamin A carotenoids, β -carotene, the most potent of them all, is known to be half as active as vitamin A. The activity of the other carotenoids decreases according to their chain length and number of conjugated double bonds.

Karrer et al. (1931) first reported the chemical relationship between β -carotene and vitamin A and suggested that the simple addition of a water molecule to the central double bond of β -carotene would yield two molecules of vitamin A. However, subsequent attempts, by other workers, to achieve this conversion failed to yield two molecules of vitamin A. It was then suggested that the cleavage of carotenoids would occur at random, on the assumption that the two ends of the conjugated system of double bonds were equally susceptible to attack.

Zechmeister et al. (1943), based on physicochemical grounds, suggested that, because of resonance, the central double bond was more stable than the terminal ones and therefore was less susceptible to attack. El-Tinay and Chichester (1970) showed that the terminal double bonds were the sites of highest electron density. In our e.s.r. study of retinyl derivatives, we demonstrated, using a spin-trapping technique, that both the secondary and primary sites of these molecules were all vulnerable to attack, more so at the secondary sites (Mordt and Walton, 1990). By analogy, therefore, both these sites on the conjugated system of β -carotene would be vulnerable to attack. The conclusion reached here has been borne out by experiments on the autoxidation of β -carotene, where a series of β -apocarotenals were isolated (Mordt et al., 1993).

Glover and Redfearn (1954) first demonstrated conclusively