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

Scheme 1

the formation of apocarotenals in β -carotene oxidation with H_2O_2/OsO_4 reagent by monitoring the progress of the reaction with time. Recent research on product analysis using the more sophisticated techniques of h.p.l.c. and liquid chromatography/m.s. has shown that these apocarotenals are the major products of β -carotene oxidation with tissue extracts and chemical oxidations (Mordi et al., 1993; Tang et al., 1991; Wang et al., 1991).

The identification and characterization of these apocarotenals led Glover (1960) to propose a mechanism for the degradation of β -carotene by the eccentric cleavage mechanism. In this process, β -carotene is cleaved by a β -mechanism, starting at either end of the conjugated system, producing a series of apocarotenals. When the central 15,15' double bond is reached, further oxidation is blocked because of the C_{13} methyl group which is at a β -position from the central double bond. Various workers have tried to show the viability of this mechanism, and their results have been reviewed by Ganguly and Sastry (1985).

The crucial question, however, is at what point along the conjugated system of double bonds does the initial attack take place? Current thinking is that the whole process involves first isomerization of the all-*trans* to the *cis* isomer, followed by the formation of a diradical, or they may both occur simultaneously and reversibly (see Scheme 1). The involvement of *cis-trans* isomerization in β -carotene oxidation was originally noted by Friend (1958) and has been recently confirmed by us using an h.p.l.c. analytical technique (Mordi et al., 1993). The all-*trans*-*cis* isomerization in β -carotene has recently been implicated from the work of Doering and colleagues (Doering and Kitagawa, 1991; Doering et al., 1991; Doering and Sarma, 1992) on determining the stabilization energies of some semi-rigid three-, five- and seven-double-bond conjugated systems.

Glover (1960), in his proposal of β -oxidation mechanism,

suggested that the formation of 15,15'-*cis*- and 13'-*cis*- β -carotene would lead to shorter lengths of the conjugated double bonds on either side of the *cis* bond where the overlap in the π -electron fields has been weakened. He also suggested, based on biological activity, that the 15,15'-*cis*-isomer was probably not a favoured intermediate. His suggestion, however, does not rule out the formation of this isomer.

Since the products of β -carotene oxidation by molecular oxygen are similar to the products obtained in the singlet oxygen oxidation, then one of the reactants in the former reaction must be activated. The involvement of a radical species would explain the similarity in product formation in the two reactions. If the diradical of β -carotene is formed as suggested, then the singlet diradical is formed when β -carotene is in the *cis*-form, oxygen attack on either side of the *cis* bond is enhanced giving β -carotenyl peroxy radicals, followed by further reactions as shown in Scheme 1. Radical attack followed by homolytic internal substitution gives the epoxides, which appear as initial products. The stable final products, the apocarotenals and apocarotenones, could be formed from peroxy radicals formed by oxygen attack at any position of the diradical. This peroxy radical then undergoes S_{Hi} reaction to a dioxetane, which decomposes to give aldehydes and ketones. That this is so is seen from the fact that all the apocarotenals begin to appear at the same time. Other possible routes to apocarotenals have been suggested by Mordi et al. (1993).

The conclusion that can be drawn from this is that β -carotene is degraded by *cis-trans* isomerization, followed by the formation of a singlet diradical. Although the question of the exact point of attack has not been answered, research is pointing the way, more to a random mode of attack rather than to attack at specific positions on the conjugated system of double bonds. The

implication of this is that we can begin to understand the mechanism by which β -carotene functions in nature, and its antitumour and antioxidant properties. We can also begin to tackle the question, 'is it β -carotene itself or its degradation products that are responsible for the inhibition of certain tumours and photosensitization?' As has been shown, β -carotene is degraded to a series of apocarotenals in both studies *in vitro* and chemical studies. It is therefore necessary to investigate the ability of these apocarotenals to inhibit those afflictions which β -carotene is known to prevent. Vitamin A has already been studied to some extent, and its importance in the aging process and cell development is well known. We are using e.s.r. spectroscopy and a spin-trapping technique to investigate and confirm the presence of the diradical.

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