Cytochrome P-450 Enzyme-specific Control of the Regio- and Enantiofacial Selectivity of the Microsomal Arachidonic Acid Epoxygenase*  

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Jorge H. Capdevila‡§, Armando Karara‡, David J. Waxman¶, Martha V. Martin§, J. R. Falck**, and F. Peter Guenguerich§

From the Departments of Medicine (Nephrology Division) and Biochemistry, Vanderbilt University Medical School, Nashville, Tennessee 37232, the Department of Biological Chemistry and Molecular Pharmacology and Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, and the Department of Molecular Genetics, University of Texas, Southwestern Medical Center, Dallas, Texas 75235

Chiral analysis of the rat liver microsomal arachidonic acid epoxygenase metabolites shows enantioselective formation of 8,9-, 11,12-, and 14,15-cis-epoxycosatetraenoic acids in an approximately 2:1, 4:1, and 2:1 ratio of antipodes, respectively. Animal treatment with the cytochrome P-450 inducer phenobarbital increased the overall enantiofacial selectivity of the microsomal epoxygenase and caused a concomitant inversion in the absolute configurations of its metabolites. These effects of phenobarbital were time-dependent and temporally linked to increases in the concentration of microsomal cytochrome P-450 enzymes. Reconstitution of the epoxygenase reaction utilizing several purified cytochromes P-450 demonstrated that the asymmetry of epoxidation is under cytochrome P-450 enzyme control. These results established that the chirality of the hepatic arachidonic acid epoxygenase is under regulatory control and confirm cytochromes P-450 IIB1 and IIB2 as two of the endogenous epoxygenase induced in vivo by phenobarbital.

The biological significance of arachidonic acid as the precursor for several physiologically important oxygenated metabolites is well established (1, 2). Upon release from cellular glycerolipid stores, the fatty acid is metabolized by cyclooxygenase or lipoxygenases to a variety of lipid mediators of cell and tissue function (1). The initial report by our group (3) that the in vitro catalysis of arachidonic acid oxygenation has catalytic of arachidonic acid oxygenation has stimulated interest in its potential physiological role as a participant in the endogenous arachidonic acid cascade (1, 2, 6). The microsomal cytochrome P-450 arachidonic acid epoxygenase catalyzes the NADPH-dependent epoxidation of the fatty acid to 5,6-, 8,9-, 11,12-, and 14,15-epoxycosatetraenoic acids (EETs) (7). The EETs exhibit several potent in vitro biological activities (1, 2).

Based on the documentation of the EETs as endogenous constituents of rat liver (8, 9) and of rabbit kidney (10) and human urine (11), we proposed the epoxygenase reaction as an additional member of the arachidonate cascade (8). Recently, this view has been substantiated by the high chirality of endogenous hepatic EETs (6). A functional role for P-450 as the endogenous epoxygenase was suggested by induction studies demonstrating a remarkable in vivo control of EET regio- and enantioselectivity by changes in tissue P-450 enzyme composition (6). These studies suggested a hitherto unrecognized function for this ubiquitous and important group of heme proteins in the control of cell and tissue homeostasis. As an important tool for studies dealing with the molecular characterization, regulation, and physiological significance of arachidonic acid epoxygenase, we examined the stereochemical characteristics of its products and demonstrated herein that their chirality is under P-450 enzyme control.

MATERIALS AND METHODS

Microsomal Metabolism—Microsomal fractions were isolated from the livers of adult male Sprague-Dawley rats (250-300 g) as previously described (12). To diminish adventitious cytosolic epoxide hydratase (13), microsomal fractions were resuspended in 0.15 M KCl and centrifuged at 100,000 x g for 60 min (12). For PB induction studies, animals were injected once with PB (intraperitoneally, 40 mg/kg of body weight) and then maintained for variable time periods (1-14 days) with their drinking water replaced by a 0.05% (w/v) solution of the sodium salt of PB. For β-NF induction studies, animals were injected daily (intraperitoneally, 80 mg/kg of body weight) for 4 days with a suspension of β-NF in corn oil (0.15-0.25 ml). Cytochrome P-450, cytochrome b⁶, and protein concentrations were determined as described (12, 14). Microsomal fractions were suspended in 0.1 M Tris-Cl buffer (pH 7.5) containing 0.25 M sucrose (15-25 mg of protein/ml), maintained at 2-5 °C, and discarded after 24 h (12). Microsomal incubations were performed exactly as reported (12). Briefly reaction mixtures containing 50 mM Tris-Cl buffer (pH 7.5), 150 mM KCl, 10 mM MgCl₂, 8 mM sodium isocitrate, 0.5 IU of isocitrate dehydrogenase, 0.5-0.75 mg of microsomal protein/ml, 0.1 mM [1-¹⁴C]arachidonic acid (1.8-1.0 μCi/mmol), and 1 mM NADPH were incubated at 30 °C with continuous mixing. At different time periods, aliquots were withdrawn, and the reaction products were extracted into ethyl ether, dried under an argon stream, and analyzed by HPLC as detailed below.

Purified Cytochrome P-450 Reconstitution Studies—Rat liver P-450 forms IABB, IA2, IIIA, IIIIB, IIB2, and IIC12 were purified according to published procedures (15-18). Rat liver cytochrome b⁶,
and NADPH-cytochrome P-450 reductase were purified to homogeneity as described (16, 19). The metabolism of arachidonic acid by purified P-450s was reconstituted as described (12). Briefly, P-450, NADPH-cytochrome P-450 reductase, and cytochrome b$_5$ (1 pm each, final concentration) were mixed in the presence of sonicated L-a-dilinoleoyl phosphatidylcholine (200 mm) (19) and NADPH (1 mm) (12). The mixture was diluted 5-fold with 50 mm Tris-Cl buffer (pH 7.5) containing 150 mm KCl, 10 mm MgCl$_2$, 8 mm sodium isocitrate, 0.5 mm isocitrate dehydrogenase, and 10% (v/v) glycerol. Addition of [1-14C]arachidonic acid (47 pmol, 50 pm final concentration) was followed by initiation with NADPH (1 mm final concentration). Incubations were done under air with constant mixing at 30 °C. At different time periods, samples of the reaction mixtures were extracted with ethyl ether and the organic solubles products dried under an argon stream.

**EET Purification and Derivatization**—The reaction products generated by microsomal incubations or reconstituted P-450 systems were initially resolved by reverse phase HPLC on a 5-µm Dynamax Microsorb C$_8$ column (4.6 x 250 mm, Rainin Instrument Co., Inc., Woburn, MA) using a linear solvent gradient from H$_2$O/CH$_3$CN/CH$_3$COOH (49.95:49.95:0.10, v/v/v) to CH$_3$CN/CH$_3$COOH (99.9:0.1, v/v) over 40 min at a flow of 1 ml/min (3). Radioactive material with retention times corresponding to those of synthetic 14,15-EET (24.5 min) and a mixture of synthetic 11,12-, 8,9- and 5,6-EET (25.5-27.0 min) were pooled batchwise and dried under argon stream.

The enzymatically formed [1-14C]14,15-EET was mixed with 20 µg of racemic 14,15-EET, methylated as described (8), and purified by reversed phase HPLC as above (14,15-EET-Me. R$_f$: 32 min). The mixture of biosynthetic [1-14C]11,12-, [1-14C]8,9-, and [1-14C]5,6-EET was mixed with racemic 11,12-, 8,9-, and 5,6-EET (20 µg each) and then converted to a mixture of the corresponding EET-PFB esters by reaction with pentafluorobenzyl bromide exactly as described (6). The mixture of EET-PFBS was resolved into individual regioisomers by normal phase HPLC on a µ-Porasil column (10 µm, 4.6 x 300 mm, Baker Chemical Co.) exactly as described (20). Absolute configurations were assigned by chromatographic comparisons with enantiomerically pure 14,15-EET-Me and 11,12- and 8,9-EET-PFB prepared by total chemical synthesis (21, 22). The individual EET derivatives, dissolved in mobile phase, were injected onto the HPLC column and the eluents monitored at 210 nm (20). The optical antipodes of each EET derivative were individually collected from the HPLC column based on their UV elution profile, dried under argon stream, and quantified by liquid scintillation spectrometry. Recovery of the radioactive activity injected onto the Chiralcel columns was in all cases, ≥85% (20).

**RESULTS AND DISCUSSION**

An essential requirement for the delineation of the potential functional significance and regulation of the arachidonic acid epoxygenase is the characterization of those P-450s involved in the bioactivation of the fatty acid. A detailed knowledge of the stereochemistry of the epoxide metabolites would be a unique aid for the characterization of the relevant enzyme forms (23-25). Consequently, we have utilized a simple nondestructive chromatographic method, recently developed in these laboratories, for the enantiomeric resolution of all four regioisomeric EETs (20) to profile the chirality of the epoxide metabolites produced by rat liver microsomes.

For these studies, the incubation conditions favored primary metabolism, i.e. the initial oxidation products do not undergo significant further metabolism (12). With low microsomal protein concentrations (~0.70 mg/ml) and short incubation times (~10 min at 30 °C), enzymatic and/or chemical hydration of 8,9-, 11,12-, and 14,15-EET was minimal and did not significantly alter EET concentration and/or recovery (12, 13). On the other hand, the proximity of the carboxylic acid to the 5,6-oxido ring facilitates spontaneous hydration and lactonization. Consequently, the labile 5,6-EET was recovered in poor and variable yields, and it was not further investigated. In control experiments, it was determined that neither substrate concentration (1-50 µm arachidonic acid) nor incubation time (2-10 min) had significant effects in EET stereochical purity or absolute configuration.

As previously reported, olefin epoxidation accounts for approximately 64% of the total arachidonic acid metabolism by uninduced rat liver microsomal fractions (26) (Table I, Fig. 1). Generation of 11,12-EET, the predominant product of the microsomal epoxygenase, proceeded with high enantiofacial selectivity (81% (11R, 12S)) (26). On the other hand, the microsomal enzyme system displayed lesser stereoselectivity toward the 9,10- (69% (8R, 9S)) and 14,15-olefins (67% (14S, 15R)) (Table I). Significantly, the in vitro stereospecificity of the microsomal system (Table I) was opposite that of the in vivo epoxygenase (6) and that of a purified rat liver P-450 (form IIB1) (25). These results clearly suggested the presence in the microsomal fractions of P-450 enzyme forms with distinct enantiofacial selectivities.

**TABLE I**

| Epoxygenase regioisomer | Rate of formation | Enantiomeric distribution |
|------------------------|------------------|--------------------------|
|                        |                  | R,S                      | S,R                      |
| 5,6-EET                | ±0.01            | ND                       | ND                       |
| 9,10-EET               | 0.38 ± 0.11      | 88 ± 2                   | 19 ± 1                   |
| 11,12-EET              | 0.59 ± 0.02      | 81 ± 1                   | 19 ± 1                   |
| 14,15-EET              | 0.42 ± 0.01      | 33 ± 2                   | 67 ± 2                   |
| Total                  | 1.40 ± 0.10      |                          |                          |
of P-450 enzymes whose inventory is under genetic control and can be experimentally manipulated in vivo, e.g. by induction with either PB or β-NF (27, 28). This heme protein multiplicity influences the regio- and stereoselective properties of the microsomal enzyme system. The P-450 form-specific control of enantioselectivity during the asymmetric epoxidation of aliphatic alkenes or polyenic aromatic hydrocarbons has been demonstrated (24, 29).

Previous work showed that animal treatment with P-450 inducers such as PB or β-NF results in clear changes in reaction rates and in the regioselectivity of the rat liver microsomal arachidonic acid monoxygenase reaction (26, 30). After PB treatment, there was a net increase in catalytic activity, while β-NF treatment resulted in lower rates of microsomal arachidonate metabolism (2.2 ± 0.3, 4.2 ± 0.1, and 1.3 ± 0.1 nmol/min/mg microsomal protein for controls, PB-, and β-NF-treated animals, respectively). Fig. 1 shows a comparison of HPLC chromatograms of the organic soluble products isolated from inmatates containing [1-14C]arachidonic acid, NADPH, and microsomal fractions isolated from the livers of either untreated or β-NF- or PB-treated animals. Microsomal fractions isolated from β-NF-treated animals epoxidized arachidonic acid at a reduced rate (Tables I and II) and showed a concomitant increase in their ability to hydroxylate the fatty acid at C15 through C19 (Fig. 1) (30). Additional evidence for the presence of cytochrome P-450 epoxygenases enzymes with unique enantiofacial selectivities in the endoplasmic reticulum is presented in Tables I and II. When compared with uninduced controls (Table I), β-NF treatment resulted in overall decreases in EET optical purity (Table II). These results suggested a net loss of unique hepatic P-450 epoxygenase enzyme(s) with distinct regio- and stereoselectivities. A similar analysis done with microsomal fractions isolated from the livers of PB-induced animals is illustrated in Fig. 1 and Table III. Parallel to a nearly 1.7-fold increase in the catalytic rate of the arachidonic acid epoxygenase (Tables I and III) (26), PB treatment was accompanied by (a) an overall increase in the optical purity of the epoxygenase products (Tables I and III) and (b) a remarkable inversion in the absolute configurations of 8,9-, 11,12-, and 14,15-EET (Table III). Importantly, the chirality of the EETs formed in vitro by the PB-induced microsomal arachidonic acid epoxygenase (Table III) is similar to that of the EETs present in vivo in the livers of control and PB-induced animals (6).

The coexistence in the microsomal membrane of multiple P-450 forms with unique enantioselective properties is more clearly illustrated when the effect of the inducers is analyzed in terms of the rate of formation of each individual EET enantiomer. While β-NF decreased the rates of formation of both enantiomers of 8,9-, 11,12-, and 14,15-EET to similar extents (Fig. 2, A-C), PB induction selectively increased the rates of formation of single enantiomers of 8,9-, 11,12-, and 14,15-EET, i.e. (8S,9R), (11S,12R), and (14R,15S)-EET, respectively (Fig. 2). Therefore, PB treatment changed the overall stereoselectivity of the microsomal epoxygenase, presumably by increasing the steady state concentrations of one or more P-450 forms with a high and distinct enantiofacial selectivity toward the 8,9-, 11,12-, and 14,15-olifins, while at the same time decreasing the specific content of those forms responsible for the formation of the opposite stereoisomers (Fig. 2, A-C).

To further elucidate the role of different forms of microsomal P-450 on the in vitro chirality of EET formation, adult
rats were treated with PB for 1, 2, 4, and 8 days, and the effects of PB treatment duration on the asymmetry of arachidonic acid epoxidation and on the concentrations of total and of P-450s IIB1 and IIB2 were compared with that of nontreated animals. After an almost linear rise, the specific content of microsomal P-450 reached a maximum at the 4th day of PB treatment and remained essentially constant thereafter (0.9 and 2.4 nmol of P-450/mg of protein for control and PB microsomes, respectively) (Fig. 3A). However, immunoblot analysis showed a rapid increase in the concentration of microsomal P-450s IIB1 and IIB2, the major PB-inducible forms of rat liver P-450 (27), between the first and second days of PB treatment (56-fold increase over control values) (Fig. 3A). Moreover, analysis of the relationship between EET chirality and the duration of PB treatment demonstrated that a) the inversion of EET configuration described in Tables I and II was time-dependent (Fig. 3, B-D), suggesting a correlation with changes in microsomal P-450 enzyme levels (Fig. 3A) rather than a direct effect of the barbiturate on the epoxygenase kinetic properties. b) The time course of the changes in the overall microsomal enantioselectivity was regioselective. Loss of stereoselectivity for 14,15-EET occurs after the first day of PB treatment (Fig. 3D) and between the 3rd and 4th day of treatment for 8,9- and 11,12-EET (Fig. 3, B and C). c) In contrast to the cytochrome P-450 and lipoxygenase members of the arachidonate cascade, the enantioselectivity of the microsomal epoxygenase is variable and highly dependent on regulatory factors that control the inventory of P-450(s) present in the microsomal membrane. Thus, among the enzyme systems of the arachidonate cascade, the P-450 epoxygenase is unique in that its stereochemical selectivity is under regulatory control and can be experimentally altered by animal manipulation in vivo (Fig. 3) (6, 27, 28).

Fig. 4 shows the absolute configurations of the predominant enantiomers of 8,9-, 11,12-, and 14,15-EET generated by incubates containing microsomal fractions isolated from the livers of untreated animals or from animals treated with PB or β-NF. Assuming a folded hairpin conformation (31, 32) for the arachidonic acid molecule and a plane of symmetry established by coplanarity of its carbon atoms, the microsomal enzymes oxygenate the 8,9- and 11,12-olefins with the same sidedness, opposite to that of the 14,15-olefin. Animal treatment with PB changed those topographic features of the enzyme(s) active site critical for substrate binding without altering the above relationship, i.e. there was an apparent 180° rotation of the substrate molecule with respect to the heme-oxygenating locus.

In the last few years considerable advances have been made in the isolation and structural characterization of several forms of rat liver microsomal P-450 (27, 28). Studies at the protein or gene level have documented that structurally distinct macromolecules provide the molecular basis for the catalytic heterogeneity of microsomal P-450 (27, 28). The resolution of the microsomal electron transport chain into defined components that can be reconstituted into catalytically functional systems provides a unique tool for the analysis of the regio- and stereoselective properties of different heme protein forms. Therefore, the following forms of male rat liver P-450 were solubilized and purified from the livers of untreated (forms IIC11 and IIC12), isosafrole (form IA2), β-NF (form IA1), or PB-treated animals (forms IIA1, IIB1 and IIB2). The regioselectivity of these different P-450 forms was then evaluated by reconstituting the arachidonic acid monooxygenase reaction in the presence of NADPH-cytochrome P-450 reductase, cytochrome b5 and NADPH. P-450 form IA1 actively catalyzes the hydroxylation of arachidonic acid at those carbons proximal to the methyl terminus (C15-C20 alcohols, 87% of total products) (30) (Table IV). While P-450 IIB1 and IIB2 metabolized the fatty acid at rates lower than IA1 (Table IV), these heme proteins were highly selective epoxygenases, generating EET mixtures as their only reaction products (Table IV and Fig. 3). P-450s IA2 and IIC11 displayed lesser regioselectivity generating mixtures of C15-C20 alcohols, HETEs, and EETs (Table IV). Cytochromes IIA1 and IIC12 did not catalyze arachidonic acid metabolism to a significant extent.

The epoxygenase metabolites generated by P-450s IA2, IIA1, IIC11, IIB1, and IIB2 (21, 6, 62, 100, and 100% of the total reaction products, respectively) were resolved into the corresponding EET regioisomers by normal phase HPLC and then
Cytochrome P-450 Epoxide Properties

EET formation as nearly equimolar mixtures (Table V). Different experiments with SE. 115% of the mean for all cases. Reaction rates are given in nanomoles of EETs formed per min/nmol of purified P-450 (at 30 °C) with S.E. <15% of the mean values. Averaged (n = 3) turnover rates are in nanomoles of product formed per min/nmol of P-450 (at 30 °C) with S.E. <10% of the mean values. Total rate

### Table IV

| Cytochrome P-450 form | Turnover rate | \( C_{V} \) \(-\, C_{alcohol} | HETEs | EETs |
|-----------------------|---------------|-----------------|------|------|
| IA2                   | 0.04          | 44              | 35   | 21   |
| IA1                   | 0.70          | 87              | 7    | 6    |
| IIC11                 | 0.67          | 19              | 19   | 62   |
| IIB1                  | 0.30          | <1              | <1   | 100  |
| IIB2                  | 0.11          | <1              | <1   | 100  |

### Table V

Regiosiomer composition of the EETs generated by reconstituted cytochrome P-450 forms

| Regiosiomer | IA2 | IA1 | IIC11 | IIB1 | IIB2 |
|-------------|-----|-----|-------|------|------|
| 5,6-EET     | ≤4  | <=4 | ≤8    | ≤5   |      |
| 8,9-EET     | 15  | 17  | 23    | 27   | 27   |
| 11,12-EET   | 58  | 22  | 29    | 37   | 26   |
| 14,15-EET   | 23  | 61  | 44    | 30   | 42   |
| Total rate  | 0.07| 0.04| 0.41  | 0.30 | 0.11 |

Quantified by liquid scintillation spectrometry. A comparative analysis of the relative epoxygenase activities showed that none of the five reconstituted enzymes displayed selectivity for the epoxidation of a single olefin with exclusion of the others. Thus, in addition to a general low reactivity at the 5,6-olefin, all forms catalyzed epoxidation at the 8,9-, 11,12-, and 14,15-olefins (Table V). P-450 IA2 and IA1 displayed the highest degree of regiospecificity with 11,12- and 14,15-EET accounting for 58% (form IA2) and 81% (form IA1) of their total epoxygenase activity (Table V). On the other hand, those P-450 forms with the highest epoxygenase turnover numbers, i.e. IIC11, IIB1, and IIB2, catalyzed 8,9-, 11,12-, and 14,15-EET formation as nearly equimolar mixtures (Table V).

The contribution of individual P-450 forms to the overall stereochemical properties of the microsomal arachidonic acid epoxygenase was studied by characterizing the chirality of the EETs generated by reconstituted systems containing P-450s IA2, IA1, IIC11, IIB1, and IIB2. To facilitate comparisons, the data in Tables VI-VIII illustrate the stereochemistry of the metabolites resulting from epoxidation at the fatty acid 8,9-, 11,12-, and 14,15-olefins. P-450s IA2, IA1, IIB1, and IIB2 epoxidized the 8,9-olefin stereospecifically, generating (8S,9R)-EET with 93, 87, 86, and 90% purity, respectively (Table VI). Significantly, with these four heme proteins, substrate binding results in oxygen insertion at the same si, re-face of the 8,9 double bond. On the other hand, catalysis by the rat liver constitutive P-450 IIC11 produced nearly racemic 8,9-EET (Table VI). With the exception of P-450 IIC11, which formed 11,12-EET in a roughly 1:1 ratio of antipodes, epoxidation of the 11,12 double bond also proceeded with high enantiofacial selectivity. The (11S,12R)-EET enantiomer was generated by P-450s IA1, IIB1, and IIB2 with 84% purity (Table VII). Importantly, the P-450 IA2 arachidonic acid 11,12-epoxygenase was unique. This enzyme catalyzed highly asymmetric oxygenation at the opposite enantiotopic face of the 11,12-olefin (re,si-face) with (11R,12S)-EET formed in 95% purity (Table VII). Finally, a similar analysis performed with the 14,15-EET metabolite is shown in Table VIII. Approximately 60% of the total P-450 IA1 epoxygenase activity leads to the stereoselective formation of 14,15-EET in a nearly
Cytochrome P-450 Epoxygenase, Stereochemical Properties

6:1 ratio of antipodes (Table VIII). On the other hand, forms IA2, IIB1, and IIB2 showed a generalized lower enantiofacial selectivity toward the 14,15-olefin when compared with the 8,9- and 11,12-double bonds (Table VIII). With the exclusion of P-450 IIC11, which produced nearly racemic mixtures of 14,15-EET, all four forms catalyzed preferential oxygen addition to the \( \text{r,si}-\)face of the 14,15-double bond.  

To date, no crystallographic information is available for any microsomal P-450. Information about the topology of the heme environment and of the active site has been approached by indirect observations of substrate selectivity and region- and stereochemical product analysis. A role for the porphyrin propionic acid side chains in substrate anchoring via H-bonding or cation chelate has been proposed (33). Importantly, methyl arachidonate is not a substrate for P-450 epoxidation nor does the addition of the ester to a microsomal suspension produce any spectral manifestation of active site binding (30). Jerina et al. (34) have proposed the presence of a hydrophobic depression in the active site of P-450. The absolute orientation of the prosthetic heme group for the major PB-inducible form of rat liver P-450 has been reported (35). Additionally, it has been suggested that the active site has a lipophilic binding area over pyrrole ring C and that ring B is sterically encumbered (36). With the exception of P-450 IIC11, the stereochemical properties of the EETs formed by either microsomal or purified P-450s revealed an unprecedented high stereoselectivity for the oxidation of such an unbiased acrylic molecule. The above indicates that the active site molecular coordinates responsible for heme-fatty acid spatial orientation are remarkably rigid and structured. As discussed for the microsomal enzymes, purified P-450s IIB1, IIB2, and IA1 epoxidized the arachidonate 8,9- and 11,12-olefins with the same enantiotopic sidedness and opposite to that of the 14,15-olefin (Fig. 4). Since in reconstituted systems the asymmetry of the epoxygenase reaction is under the control of a single protein catalyst, we propose, therefore, a very similar or common geometry for the substrate binding sites of cytochromes P-450 IIB1, IIB2, and IA1 and significantly different from that of form IA2 (Tables VI–VIII).

The experimental data presented provide a coherent explanation for the effects of PB induction on the enantiofacial selectivity of the microsomal epoxygenase(s), i.e. the EET stereochemical inversion correlates with net increases in the specific contents of P-450s IIB1 and IIB2. On the other hand, with the exception of the 11,12-epoxygenase activity of P-450 IA2, none of the purified enzymes studied appeared to contribute significantly to the chirality of the EETs generated by NF-induced or non-induced microsomal fractions. These results suggest the presence in the microsomal membrane of yet unidentified P-450 epoxygenases with unique enantiofacial selectivities.

The biological relevance of the epoxygenase reaction has been established by the demonstration of the in vivo biosynthetic origin of its reaction products (6). Thus, epoxidation of arachidonic acid represents a novel route for the oxidation of the fatty acid and, more importantly, for the generation of novel pools of membrane phospholipids containing esterified EETs moieties (9). Efforts to demonstrate enzymatic, NADPH-dependent, or NADPH-independent epoxidation of arachidonyl phosphatidylcholine or arachidonyl phosphatidylcholine by rat liver homogenate or microsomal or cytosolic fractions proved unsuccessful (37). The available evidence supports a sequential mechanism in which P-450 epoxidation of free arachidonic acid is followed by subsequent EET acylation. The in vitro stereochemical properties of the P-450 epoxygenases reported here suggest a role for P-450 forms IIB1 and IIB2 in the enzymology of rat liver in vivo EET formation (6). The demonstration of an enzymatic route for in vivo formation of oxidized phospholipids has significant implications for cell membrane physiology and for a potentially decisive role for P-450 in its control. Alterations in the fatty acid composition of membrane phospholipids have important consequences for the structural integrity and the functional properties of cellular membranes (38). The oxidation of phospholipid-bound fatty acids has documented effects on membrane properties including changes in membrane ion permeability (39), alterations in the enzymatic activity of several membrane-bound enzymes (40, 41), as well as changes in membrane fluidity and fusogenic properties (42, 43).

From the foregoing evidence as well as published data (6, 25), we conclude that the regio- and enantioselectivity of the arachidonic acid epoxygenase is cytochrome P-450 protein-dependent. Additionally, the data show that, in contrast to the lipoxigenase and cyclooxygenase members of the arachidonate cascade, the enantioselectivity of the epoxygenase is under in vivo regulatory control. These observations as well as the documented presence in rat liver of unique phospholipids containing an EET-esterified moiety suggest important functional roles for these prominent heme proteins in controlling membrane physicochemical properties and thus function.

REFERENCES

1. Needleman, P., Turk, J., Jaschik, B. A., Morrison, A. R., and Leftowith, J. B. (1986) Annu. Rev. Biochem. 55, 69–102, and references therein.
2. Fitzpatrick, F. A., and Murphy, R. C. (1989) PharmacoL Rev. 40, 229–241, and references therein.
3. Capdevila, J., Chacos, N., Werringloer, J., Prough, R. A., and Estabrook, R. W. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5302–5306.
4. Morrison, A. R., and Pascoe, N. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7375–7378.
5. Oliw, E., Lawson, J. A., Brauch, A. R., and Oates, J. A. (1981) J. Biol. Chem. 256, 9924–9931.
6. Karara, A., Dishman, E. A., Blair, I., Falck, J. R., and Capdevila, J. (1989) J. Biol. Chem. 264, 19822–19827.
7. Chacos, N., Falck, J. R., Wixtrom, C., and Capdevila, J. (1982) Biochem. Biophys. Res. Commun. 104, 916–922.
8. Capdevila, J., Pramanik, B., Napol, J. L., Manna, S., and Falck, J. R. (1984) Arch. Biochem. Biophys. 231, 511–517.
9. Capdevila, J. H., Kishore, V., Dishman, E. A., Blair, I., and Falck, J. R. (1987) Biochem. Biophys. Res. Commun. 146, 639–644.
10. Falck, J. R., Schueler, V., Jacobson, H., Siddhanta, A., Pramanik, B., and Capdevila, J. H. (1987) J. Lipid Res. 28, 840–846.
11. Toto, R. J., Siddhanta, A., Manna, S., Pramanik, B., Falck, J. R., and Capdevila, J. H. (1987) Biochem. Biophys. Acta 919, 132–139.
12. Capdevila, J. H., Falck, J. R., Dishman, E., and Karara, A. (1990) Methods Enzymol. 187, 385–394.
13. Chacos, N., Capdevila, J., Falck, J. R., Mann, S., Wixtrom, C., Gill, S. S., Hammock, B. D., and Estabrook, R. W. (1983) Arch. Biochem. Biophys. 223, 639–648.
14. Omura, T., and Sato, R. (1964) J. Biol. Chem. 239, 2370–2378.
15. Wxman, D. J. (1984) J. Biol. Chem. 259, 15481–15490.
16. Guengerich, F. P., Danner, G. A., Wright, S. T., Martin, M. V., and Kaminsky, L. S. (1982) Biochemistry 21, 6019–6030.
17. Guengerich, F. P. (1978) Biochemistry 17, 3633–3639.
18. Guengerich, F. P., Martin, M. V., Beaune, P. H., Kremers, P., Wolff, T., and Wxman, D. J. (1986) J. Biol. Chem. 261, 5051–5060.
Cytochrome P-450 Epoxigenase, Stereochemical Properties

19. Yasukochi, Y., and Masters, B. S. S. (1976) J. Biol. Chem. 251, 5337-5344
20. Hammonds, T. D., Blair, I. A., Falck, J. R., and Capdevila, J. H. (1989) Anal. Biochem. 182, 300-303
21. Moustakis, C. A., Viala, J., Capdevila, J., and Falck, J. R. (1985) J. Am. Chem. Soc. 107, 5283-5286
22. Mosset, P., Yagadiri, P., Sun-Lumin, Capdevila, J. H., and Falck, J. R. (1996) Tetrahedron Lett. 27, 6055-6058
23. Foureman, G. L., Harris, C., Guengerich, F. P., and Bend, J. R. (1989) J. Pharmaco Exp. Ther. 248, 492-497
24. Wistuba, D., Nowotny, H. P., Trager, O., and Shcurig, V. (1989) Chirality 1, 127-136
25. Falck, J. R., Manha, S., Jacobson, H. R., Estabrook, R. W., Chacos, N., and Capdevila J. (1984) J. Am. Chem. Soc. 106, 3334-3336
26. Capdevila, J. H., Kim, Y. R., Wixtrom, C., Falck, J. R., Manha, S., and Estabrook, R. W. (1985) Arch. Biochem. Biophys. 243, 8-19
27. Nebert, D. W., and Gonzalez, F. J. (1987) Annu. Rev. Biochem. 56, 945-993, and references therein
28. Gonzalez, F. J. (1989) Pharmacol. Rev. 40, 243-288, and references therein
29. Thakker, D. R., Yagi, H., Koreeda, M., Lu, A. Y. H., Levin, W., Wood, A. W., Conney, A. H., and Jerina, D. M. (1977) Chem. Biol. Interactions 16, 281-300
30. Falck, J. R., Lumin, S., Blair, I., Dishman, E., Martin, M. V., Waxman, D. J., Guengerich, F. P., and Capdevila, J. H. (1990) J. Biol. Chem. 265, 11244-11249
31. Corey, E. J., Iguchi, S., Albriqth, J. O., and De, B. (1983) Tetrahedron Lett. 24, 37-40
32. Sterk, H., Konrat, R., and Honig, H. (1989) J. Mol. Liq. 40, 101-115
33. Estabrook, R. W., Martinez-Zadillo, G., Young, S., Peterson, J. A., and McCarthy, J. J. (1975) J. Steroid Biochem. 6, 419-425
34. Jerina, D. M., Michaud, D. P., Feldman, R. J., Armstrong, R. N., Vyas, K. P., Thakker, D. R., Yagi, H., Thomas, P. E., Ryan, D. E., and Levin, W. (1982) in Microsomes, Drug Oxidations and Drug Toxicity (Sato, R., and Kato, R., eds) pp. 185-201, Wiley-Interscience, New York
35. Ortiz de Montellano, P. R., Kunze, K. L., and Beilan, H. S. (1983) J. Biol. Chem. 258, 45-47
36. Kunze, K. L., Mangold, B. L. K., Wheeler, C., Beilan, H. S., and Ortiz de Montellano, P. R. (1983) J. Biol. Chem. 258, 4202-4207
37. Ballou, L. R., Lam, B. K., Wong, P. Y. K., and Cheung, W. Y. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6990-6994
38. Sevanian, A. (1988) in Cellular Antioxidant Defense Mechanisms (Chow, C. K., ed) Vol. II, pp. 78-95, CRC Press, Inc., Boca Raton, FL
39. Frei, B., Winterhalter, K. H., and Richter, C. (1985) Eur. J. Biochem. 149, 633-639
40. Van Kuijk, F. J. G., Sevanian, A., Handelman, G. J., and Dratz, E. A. (1987) Trends Biochem. Sci. 12, 31-34
41. Sevanian, A., Wratten, M. L., McLeod, L. L., and Kim, E. (1988) Biochim. Biophys. Acta 961, 316-327
42. Gamache, D. A., Fawzy, A. A., and Franson, R. C. (1988) Biochim. Biophys. Acta 958, 116-124
43. Gast, K., Zirmer, D., Ladhoff, A. M., Schreiber, J., Koelsch, R., Kretschmer, K., and Lasch, J. (1982) Biochim. Biophys. Acta 686, 99-109
44. Guengerich, F. P., Wang, P., and Davidson, N. K. (1982) Biochemistry 21, 1998-1976
45. Nebert, D. W. (1989) DNA 8, 1-13
46. Rampersaud, A., Waxman, D. J., Ryan, D. E., Levin, W., and Waltz, F. G. (1985) Arch. Biochem. Biophys. 243, 174-183
47. Hayashi, S., Morohashi, K., Yoshioka, H., Okuda, K., and Omura, T. (1988) J. Biochem. (Tokyo) 103, 858-862
Cytochrome P-450 enzyme-specific control of the regio- and enantiofacial selectivity of the microsomal arachidonic acid epoxygenase.
J H Capdevila, A Karara, D J Waxman, M V Martin, J R Falck and F P Guenguerich

J. Biol. Chem. 1990, 265:10865-10871.

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