Liver Microsomal Epoxide Hydrase

SOLUBILIZATION, PURIFICATION, AND CHARACTERIZATION

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Epoxide hydrase was solubilized from liver microsomes of phenobarbital-treated rats by treatment with cholate and purified to apparent homogeneity by ammonium sulfate fractionation and column chromatography in the presence of the nonionic detergent Emulgen 911 on DEAE-cellulose and hydroxyapatite. The purified enzyme preparation had a single major band with a molecular weight of 53,000 to 54,000 on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Other studies indicated that in the absence of sodium dodecyl sulfate, purified epoxide hydrase exists as high molecular weight aggregates. The preparation was essentially free of heme and flavin, but still contained small amounts of lipids and Emulgen 911.

Many aromatic and olefinic compounds, such as fatty acids, steroids, drugs, as well as environmental contaminants, are metabolized in mammals by enzymes localized in liver microsomes (1-5). Metabolic conversion of these compounds by the mixed function oxidase system has long been considered a means for the detoxification and excretion of a variety of foreign substances. Recent investigations into the mechanisms by which the hepatic monooxygenase system catalyzes the hydroxylation of aromatic and olefinic compounds have shown that intermediate arene and alkene oxides are formed (6-8). Arene oxides, in addition to isomerizing to phenols and conjugating with glutathione via glutathione-S-oxide transferase, react readily with a variety of nucleophiles including DNA, RNA, and protein (8-11). These highly reactive arene oxides are also converted to dihydrodiols via the microsomal enzyme epoxide hydrase (6-8). Since arene oxides of polycyclic aromatic hydrocarbons have been implicated as ultimate carcinogens, the conversion of epoxides to dihydrodiols by epoxide hydrase may be an important step in the detoxification of these compounds (7-11).

In order to elucidate the role of metabolism in the cytotoxicity and carcinogenicity of polycyclic aromatic hydrocarbons, a basic understanding of the properties and mechanism of action of the enzymes involved in these activation and inactivation processes is essential. In the past few years, several laboratories have purified and characterized the cytochrome P-450 system responsible for the initial oxidation reaction (12-16). Procedures for the partial purification of epoxide hydrase from guinea pig liver (17), human liver (18), and rat liver (19) have also been reported. The present paper describes the solubilization and purification of rat liver microsomal epoxide hydrase in a nearly homogeneous state.

EXPERIMENTAL PROCEDURES

Epoxide Hydrase Assay—Epoxide hydrase activity was measured using a radiometric assay based on the conversion of [3H]styrene oxide to [3H]styrene glycol (20). The standard incubation mixture contained 100 μl of 0.5 mM Tris buffer containing 0.1% Tween 80 (pH was 9 at room temperature: 8.7 at 37°C), 1600 nmoles of [3H]styrene oxide (0.3 μCi/μmol) in 20 μl of acetonitrile, enzyme, and water in a final volume of 320 μl. The rate of product formation was linear with respect to time of incubation (Fig. 1A) and protein concentration (Fig. 1B) of the purified epoxide hydrase to be described. The pH optimum of the reaction was 8.7 at 37°C (Fig. 1C). One unit of epoxide hydrase is defined as the amount catalyzing the formation of 1.0 nmol of product/min under the described assay conditions.

Preparation of Microsomes—Male, Long-Evans rats (from Blue Spruce Farms, Altamont, N. Y.) weighing 50 to 60 g were administered phenobarbital intraperitoneally at a dose of 100 mg/kg body weight once daily for 3 days. Induced animals were used since the microsomal epoxide hydrase activity, like cytochrome P-450, is also enhanced by phenobarbital pretreatment (20). Liver microsomes were prepared as previously described (12).

Solubilization and Purification of Epoxide Hydrase—In a typical preparation, 2.7 g of microsomal protein were suspended in 183 ml of a mixture containing 45 ml of glycerol, 21 ml of 1 mM potassium phosphate buffer (pH 7.7), 2.1 ml of 0.1 mM dithiothreitol, 2.1 ml of 0.1 mM EDTA, and 0.25 mM sucrose. The mixture was sonicated with a Biosoanik III (Browning Scientific Inc., Rochester, N. Y.) equipped with a 3/8-inch standard probe tip at full output for four 30-s intervals, while the temperature was maintained below 5°C with an ice-salt bath. Gener- ally, four to five batches (i.e. up to a total of 15.2 g of microsomal protein) were prepared, and these batches were combined after sonication. Sodium cholate (10% solution) was then added so that the final concentration was 1 mg of cholate/mg of protein, and the mixture was stirred on ice for 20 min.

Solid ammonium sulfate was added to 40% saturation (24.3 g/100 ml). The mixture was stirred for 20 min, centrifuged at 25,000 × g for 20 min, and the precipitate was discarded. Additional solid ammonium sulfate was added to the supernatant fraction to 50% saturation (6.3g/100 ml). The resulting precipitate was dissolved in 0.05 M potassium
phosphate buffer, pH 7.7, to a final volume of 36 ml for each 2.7 g of starting microsomal protein, and the mixture was centrifuged at 160,000 x g for 1 hour to remove insoluble material. The redish supernatant fraction was dialyzed overnight against 12 liters of 0.02 M potassium phosphate buffer, pH 7.7, containing 20% glycerol, 10^{-4} M dithiothreitol, 10^{-4} M EDTA, and 0.1% cholate and centrifuged at 35,000 x g for 20 min. This preparation was designated as "ammonium sulfate fraction" and was stable for several weeks when stored at -20° under N₂.

Prior to column chromatography on DEAE-cellulose, the ammonium sulfate fraction was dialyzed for 3 to 4 hours against 6 liters of 0.005 M Tris buffer, pH 8.5 (at 4°), containing 0.1% cholate. The dialyzed sample was applied slowly (40 to 50 ml/hour) to a Whatman DE52 column (2.5 x 55 cm) previously equilibrated with 0.005 M Tris, pH 8.5, containing 0.5% Emulgen 911. As shown in Fig. 2, epoxide hydrase was eluted with 0 to 0.5% linear gradient of [3H]Emulgen 911 (185,000 cpm/mg) in 0.005 M Tris, pH 8.5, as described under "Experimental Procedures." Because of the continuous changes in detergent concentration, fraction sizes were variable: Fractions 1 to 80, 18 ml each; 81 to 120, 10 ml each; 121 to 136, 14 to 16 ml each. Solid line represents epoxide hydrase activity; dotted line represents Emulgen 911 concentration determined by \( ^{3}H \) counts, and broken line represents the theoretical linear Emulgen gradient. Since the labeled \( ^{3}H \)Emulgen in the column fractions interfered with the hydrase assay using \( ^{3}H \)styrene oxide as substrate, aliquots of the column fractions minus substrate were assayed as controls, and the resulting radioactivity in the ethyl acetate extracts was subtracted from that obtained with the same sample in the presence of substrate.

Several additional comments should be made about this DE52 column. Fraction size (collected by drop counting) was quite variable because of the continuous increase in detergent concentration in the eluting fractions. Fraction size markedly decreased while epoxide hydrase was eluting from the column. These changes in volume are useful as a marker in localizing epoxide hydrase activity. Fractions containing epoxide hydrase are usually light yellow, whereas most of the red material was retained on the column. High Emulgen 911 concentration in the DE52 fraction (2 mg of detergent/mg of protein) inhibits epoxide hydrase activity, and thus reaction rates were not linear when more than 50 µg of protein were used in the assay at this stage.

Before the DE52 fraction was subjected to column chromatography on hydroxylapatite, the sample was dialyzed for 3 hours against 2 liters of 0.01 M potassium phosphate buffer, pH 7.4, to remove glycerol. The dialyzed sample was applied slowly (10 to 15 ml/hour) to a hydroxylapatite column (3 x 4.1 cm) previously equilibrated with 0.01 M potassium phosphate buffer, pH 7.4. The column was first washed with between 400 and 600 ml of 0.01 M potassium phosphate buffer, pH 7.4, to remove Emulgen 911. The column was washed until the absorbance of the eluent at 275 nm (due to Emulgen 911) was less than 0.02. Less than 10% of the epoxide hydrase activity was eluted from the column by this procedure. Epoxide hydrase was eluted from the column with a linear gradient of potassium phosphate buffer consisting of 200 ml of 0.1 M potassium phosphate, pH 7.4, and 290 ml of 0.5 M potassium phosphate, pH 7.4. Protein content was determined by monitoring the fractions at 280 nm. Since the ratio of epoxide hydrase activity to \( A_{280} \) was generally greater in the first part of the elution curve (Fig. 3, Fractions 78 to 85), only those fractions with a high ratio were combined. The pooled fraction was concentrated through an XM50 membrane, dialyzed against 0.01 M potassium phosphate buffer, pH 7.4, and centrifuged at 35,000 x g for 20 min. This final preparation, designated as the "hydroxylapatite fraction or HA fraction," generally had a specific activity of 400 to 470 units/mg of protein and was stable for several months at -20°.

Pooled fractions from the latter part of the elution curve were yellow and had a specific activity of 260 to 380 units/mg of protein. When subjected to a second hydroxylapatite column, the specific activity of this fraction could be increased above 400 units/mg only if the fractions from the first part of the elution curve were also pooled with it. A hydroxylapatite fraction with a specific activity of 460 units/mg was used for most of the studies reported in this paper.

Disc Gel Electrophoresis—Polyacrylamide gel electrophoresis was performed in the presence of sodium dodecyl sulfate in the discontinuous
ous buffer system by the method of Neville (21). Protein bands were detected by staining with Coomassie blue according to the method of Fairbanks et al. (22).

**Amino Acid Analysis**—The amino acid composition of purified epoxide hydrase was determined after acid hydrolysis according to the method of Spackman et al. (23) with a Joel model 6AH automatic analyzer equipped with an automatic sample storage/injection system and a programmer. Tryptophan was determined by hydrolysis in 4 M methane sulfonic acid containing 1% tryptamine according to the method of Liu and Chang (24). Half-cystine was determined as cysteic acid as described by Hirs (25).

**Other Methods**—Protein was determined by the method of Lowry et al. (26) using bovine serum albumin as a standard. Microsomal lipids and lipids in the purified epoxide hydrase were extracted from 2 ml of protein by the method of Bligh and Dyer (27).

**RESULTS**

**Purification of Epoxide Hydrase**—Typical results for each step in the purification of epoxide hydrase from liver microsomes of phenobarbital-treated rats are shown in Table I. The final preparation was purified 40-fold, and the recovery varied between 5 and 12%.

The steps for the solubilization of epoxide hydrase from rat liver microsomes with sodium cholate, as well as the subsequent ammonium sulfate fractionation, were originally designed for the purification of rat liver microsomal cytochrome P-450 (28). Although the yield and purification of epoxide hydrase after these steps were not high, it was possible to remove many other contaminating microsomal components such as phospholipids, cytochrome $b_5$, and NADPH-cytochrome $c$ reductase by using solubilization with cholate and fractionation with ammonium sulfate. In fact, these steps were found to be a necessary prerequisite for success in the subsequent steps of the purification.

Column chromatography of the ammonium sulfate fraction on DEAE-cellulose at low ionic strength (0.005 M Tris) and high pH (8.5) with a linear gradient of Emulgen 911 (0 to 0.5%) was very effective for removal of large amounts of contaminating proteins such as cytochromes P-450 and $b_5$ without any significant loss of epoxide hydrase. The recovery of protein from the column was generally 10% or less, whereas recovery of epoxide hydrase activity ranged from 60% to greater than 90%.

Although elution of various components from an ion exchange resin like DE52 is usually accomplished with a salt gradient, elution of epoxide hydrase from the column was mainly affected by increasing detergent concentration. Comparison of the theoretical linear Emulgen gradient to the experimentally determined gradient (Fig. 2) established that a large amount of Emulgen 911 remained bound to the protein on the DE52 column and that elution of Emulgen coincided with the elution of epoxide hydrase. The concentrated DE52 fraction contained approximately 2 mg of Emulgen 911/mg of protein.

Most of the detergent in the DE52 fraction did not bind to the hydroxylapatite column or could be easily removed by washing the column with low ionic strength potassium phos-

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**Table I**

<p>| Purification of liver microsomal epoxide hydrase |
|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein</th>
<th>Activity</th>
<th>Specific activity</th>
<th>Yield</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes</td>
<td>234</td>
<td>13,100</td>
<td>13,987</td>
<td>10.5 (10-17)*</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Ammonium sulfate fraction</td>
<td>168</td>
<td>3,518</td>
<td>66,517</td>
<td>18.9 (18-26)</td>
<td>48 (40-50)*</td>
<td>1.8</td>
</tr>
<tr>
<td>DE52 column fraction</td>
<td>23</td>
<td>230</td>
<td>64,799*</td>
<td>282 (207-290)</td>
<td>47 (30-47)</td>
<td>26.8</td>
</tr>
<tr>
<td>Hydroxylapatite column fraction</td>
<td>8.6</td>
<td>19.4</td>
<td>8,034</td>
<td>414 (400-470)</td>
<td>5.8 (5-12)</td>
<td>39.4</td>
</tr>
</tbody>
</table>

*The values in parentheses are the range of specific activity and yield obtained in a series of purification experiments ($n = 5$).

*This fraction contained a high concentration of Emulgen 911 which interfered with the hydrase assay as well as the protein determination. It was thus not possible to accurately calculate total hydrase activity, specific activity, and yield so that the values obtained for this step should be considered an approximation.
phate buffer (Fig. 3). Although a small portion of the epoxide hydrase activity was eluted from the column along with the detergent by 0.01 M potassium phosphate buffer (pH 7.4), the bulk of the activity was eluted by a linear gradient of potassium phosphate (0.1 to 0.5 M, pH 7.4) of higher ionic strength. As will be described, the concentrated HA fraction (pooled from Fractions 78 to 88) was highly purified. A higher yield was not obtained because Fractions 89 to 110 had much lower specific activities and were discarded. Despite the removal of more than 98% of the [3H]Emulgen by washing with low ionic strength phosphate buffer, the fractions containing the bulk of the epoxide hydrase activity still contained a small amount of [3H]Emulgen. The final HA fraction contained approximately 0.08 to 0.1 mg of Emulgen 911/mg of protein. This low concentration of detergent did not affect epoxide hydrase activity even at high protein concentrations (see Fig. 1B).

**Purity and Molecular Weight**—The purity and the minimal molecular weight of the HA fraction were examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Fig. 4 shows that the purified epoxide hydrase preparation contained a single major band in contrast to the numerous bands observed with liver microsomes obtained from phenobarbital-treated rats. These results indicate that this epoxide hydrase preparation is near homogeneity. When proteins of known molecular weight were included as standards, the molecular weight of the polypeptide chain of epoxide hydrase was estimated to be 53,000 to 54,000 (not shown).

Several lines of evidence indicate that purified epoxide hydrase exists as high molecular weight aggregates in the absence of sodium dodecyl sulfate. Sedimentation velocity studies in the absence of detergent or sodium dodecyl sulfate revealed that the purified epoxide hydrase was polydisperse. On the other hand, if centrifuged in the presence of 0.1% deoxycholate, the epoxide hydrase preparation had a sharp boundary with an $s_{20,w}$ value of 14.7. Equilibrium centrifugation of epoxide hydrase in the presence of 0.1% deoxycholate gave an apparent molecular weight of 650,000, assuming a partial specific volume of 0.72 ml/g. Gel permeation chromatography with a calibrated Sepharose 4B column in the presence of 0.1% deoxycholate yielded an apparent molecular weight of 600,000 for the purified epoxide hydrase.

**Absorption Spectrum**—The absolute absorption spectrum of the purified epoxide hydrase had a single major band around 280 nm (Fig. 5). A shoulder around 290 nm is indicative of a high tryptophan content. Even though the purified epoxide hydrase still contained Emulgen 911, the contribution by the detergent (absorption maximum, 275 nm) in the ultraviolet region appeared to be small. Based on the detergent content of the hydrase determined by [3H]Emulgen and the extinction of Emulgen 911 at 275 nm, the contribution of Emulgen 911 at 275 to 280 nm was approximately 10% of the observed absorbance in this region. Absence of an absorption peak or shoulder in the 320 to 560 nm region indicated the absence of heme or flavin. The epoxide hydrase solution used for the spectral analysis was colorless; but at high protein concentrations (e.g., 2 mg/ml), the preparation was a very faint yellow, indicating the presence of a trace amount of heme-containing or flavin-containing components.

**Amino Acid Composition**—Amino acid analysis revealed that the purified epoxide hydrase had a relatively high content of tryptophan, tyrosine, methionine, and phenylalanine (Table Wcrosomes Purified Epoxide Hydrase 5OirU IflPrx 02 240 260 320 360 400 440 460 520 560 WAVE LENGTH (nm)

**Fig. 4.** Polyacrylamide gel electrophoresis of microsomes and purified epoxide hydrase in the presence of sodium dodecyl sulfate. Electrophoresis was carried out as described under "Experimental Procedures." Migration was from top to bottom.
II). It also had a large number of nonpolar residues which may explain the tendency of the protein to aggregate in aqueous buffer during isolation. The high tryptophan and tyrosine content was responsible for the high extinction of hydrase at 290 nm (Fig. 5). A molecular weight of 54,000 determined by amino acid composition is in very good agreement with the value determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and thus indicates the presence of a single polypeptide chain in the purified epoxide hydrase.

Lipid Content—Based on the results of chromatography of lipid extracted from purified epoxide hydrase and lipid extracted from microsomes, both the phospholipid and neutral lipid content of the purified hydrase preparation was decreased by at least 300- to 500-fold/unit of epoxide hydrase activity as compared to microsomes. Addition of microsomal lipid to the purified epoxide hydrase has no effect on enzymatic activity. Lipid does not appear to be required for epoxide hydrase activity as evidence by the following findings. Repeated extraction of lyophilized microsomes with butyl alcohol and acetone, which removed all the neutral lipids and greater than 90% of the phospholipids (29), did not affect epoxide hydrase activity; and addition of microsomal lipid to the lipid-depleted microsomes did not enhance epoxide hydrase activity.

Activators and Inhibitors—Metal-chelating agents such as EDTA and α,α′-dipyridyl at 10⁻⁴ M had no effect on the activity of purified epoxide hydrase, while sulphydryl-binding agents (iodoacetamide and ρ-hydroxymercuribenzoate) at 10⁻⁴ M, only inhibited the reaction by 15%. As reported previously by others (30, 31), metyrapone (1.5 × 10⁻³ M) and 1-(2-isopropylphenyl)-imidazole (1.5 × 10⁻³ M) enhanced the rate of reaction by 220%. The mechanism by which these compounds stimulate hydrase activity is not clear and will require further investigation.

In contrast to the stimulatory effect on a partially purified epoxide hydrase, while sulfhydryl-binding agents (iodoacetamide and ρ-hydroxymercuribenzoate) at 10⁻⁴ M, only inhibited the reaction by 15%. As reported previously by others (30, 31), metyrapone (1.5 × 10⁻³ M) and 1-(2-isopropylphenyl)-imidazole (1.5 × 10⁻³ M) enhanced the rate of reaction by 220%. The mechanism by which these compounds stimulate hydrase activity is not clear and will require further investigation.

Since epoxide hydrase plays an important role in the metabolism of polycyclic aromatic hydrocarbons, purified hydrase could be a valuable tool for mechanism studies. For example, purified hydrase could be coupled with a hydrase-free cytochrome P-450 or cytochrome P-448 system to establish the enzymatic conversion of various polycyclic hydrocarbons to dihydrodiols via arene oxides. As reported in a recent paper (33), we have used such a coupled system and demonstrated that several benzo[a]pyrene oxides are obligatory intermediates in the metabolism of benzo[a]pyrene to a variety of metabolites. In the absence of epoxide hydrase, a purified cytochrome P-448-containing system metabolized benzo[a]pyrene to phenols and quinones. Upon addition of purified epoxide hydrase, the rate of total metabolism of benzo[a]pyrene was unchanged; but significant amounts of dihydrodiols were produced at the expense of phenols. With this coupled system, arene oxides of benzo[a]pyrene were shown to rearrange primarily to phenols in the absence of epoxide hydrase. Such a coupled system could also be used in a tissue culture system to study the mechanism of chemical carcinogenesis and mutagenesis caused by many polycyclic hydrocarbons since the relative amounts of arene oxides, phenols, and dihydrodiols could be manipulated by controlling the level of epoxide hydrase in the system. Many of these studies are now in progress in our laboratory.

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**Note Added in Proof**—We have recently found that the solvent used to dissolve [³H]styrene oxide affects the reaction rate of epoxide hydrase. For example, if tetrahydrofuran is used to replace acetonitrile, the rate of hydration of styrene oxide is increased. Thus, the specific activity of the purified enzyme using [³H]styrene oxide as substrate and tetrahydrofuran as the solvent would be 1200 instead of 400 to 470.

**DISCUSSION**

The present paper describes the solubilization and purification of liver microsomal epoxide hydrase from phenobarbital-treated rats. The purified enzyme appears to be nearly homogeneous, with an apparent molecular weight of 600,000 to 650,000. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed that the purified epoxide hydrase preparation contains a single polypeptide with a molecular weight of 53,000 to 54,000. It contains essentially no heme or flavin, and does not appear to require metal ions since EDTA or α,α′-dipyridyl did not affect the activity. Metal analysis of the purified epoxide hydrase indicates the presence of insignificant amounts of iron (0.03 mol/mol), zinc (0.01 mol/mol), and copper (0.02 mol/mol). Manganese and molybdenum are not detectable.

Although epoxide hydrase activity was assayed at each step in the purification procedure using styrene oxide as a representative substrate, several K-region arene oxides have also been shown to serve as substrates for the purified enzyme. These include phenanthrene 9,10-oxide, benzo[a]anthracene 5,6-oxide, and benzo[a]pyrene 4,5-oxide (32).

**Table II**

<table>
<thead>
<tr>
<th>Residue</th>
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<tr>
<td>Lys</td>
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<tr>
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<tr>
<td>Arg</td>
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</tr>
<tr>
<td>Try</td>
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</tr>
<tr>
<td>Cys</td>
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</tr>
<tr>
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<tr>
<td>Leu</td>
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<td>Tyr</td>
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<tr>
<td>Phe</td>
<td>29</td>
</tr>
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</table>

*Total residues: 468; molecular weight, 54,123.*
REFERENCES

3. Manering, G. J. (1971) Metabolism 20, 228-245
Liver microsomal epoxide hydrase. Solubilization, purification, and characterization.
A Y Lu, D Ryan, D M Jerina, J W Daly and W Levin


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