Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1663–1667, March 1985
Biochemistry

Rapid purification of cytosolic epoxide hydrolase from normal and clofibrate-treated animals by affinity chromatography

(chalcone oxide/terpenoid epoxide/peroxisome proliferator/stilbene oxide/clofibrate)

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Communicated by Robert L. Metcalf, November 15, 1984

ABSTRACT **Epoxide hydrolase from liver cytosol (cEH) of both normal and clofibrate-treated mice can be bioselectively adsorbed onto an affinity column prepared from epoxy-activated Sepharose and 7-methoxycitronellyl thiol. The free ligand is a modest inhibitor of cEH (I_{50}, $\approx 3 \times 10^{-4}$ M) and lacks the epoxide function necessary for it to be turned over as a substrate. This study demonstrates that a methoxy group can be used to mimic an oxirane in a vertebrate system. Bioselective elution of cEH can be accomplished with several chalcone oxides, which are selective potent inhibitors (I_{50}, 1–50 $\times 10^{-7}$ M), and activity can be recovered by dialysis. This procedure thus enhances the purification by offering independent opportunities for selective binding and selective elution. Conservatively, a 40%–80% recovery of partially inhibited enzyme activity can be achieved in 4–48 hr with a 30- to 90-fold purification. The purified cEH from clofibrate-induced animals was essentially homogeneous by NaDodSO$_4$/PAGE and had an apparent subunit molecular weight of 58,000. The cEHs from normal and clofibrate-induced animals appeared identical by NaDodSO$_4$/PAGE. Since the cEH activity in normal and clofibrate-treated animals is due to the same enzyme, the increase in cEH activity caused by selected hypolipidemic agents appears to be true induction.**

Epoxide hydrolases (EC 3.3.2.3) are essential for the removal of endogenous and exogenous oxirane-containing molecules (1–4). In mammalian systems, these ubiquitous enzymes serve an important protective function in the detoxification of potential mutagenic or otherwise deleterious metabolic byproducts. Considerable effort has been expended in isolating and characterizing the enzymes involved, and three main forms can be distinguished: a cytosolic form (cEH) and a microsomal form (mEH), possessing different substrate specificities, pH optima, molecular weights, and immunoreactivities (5, 6), and a second microsomal form apparently selective for cholesterol 5,6-epoxide and related compounds (7, 8). The microsomal form hydrating arene oxides has been most studied and has been purified from rat, mouse, rabbit, and human livers (1). In contrast, the more recently discovered cytosolic form is more poorly understood in terms of its molecular properties. A convenient source of homogeneous cEH was required to proceed with the protein chemistry of this enzyme.

cEH has been purified from rabbit (9), human (10), and mouse liver (11). These laborious purifications require multiple steps, and low yields of 0.5%–10% are reported for these procedures, with 180- to 550-fold purification. We now report the preparation of a bioselective adsorbent for cEH, and we describe its use for the rapid and high yield purification of cEH from mouse liver. Clofibrate and other hypolipidemic agents have been shown recently to be unique in their ability to increase cEH activity in cytosol from mouse liver (12). The affinity purification used here demonstrates the identity of the cEH from control and clofibrate-induced animals, thus providing evidence that clofibrate is a true inducer of cEH. Finally, we propose a hypothesis to account for the enzyme induction and the binding efficacy of the affinity column.

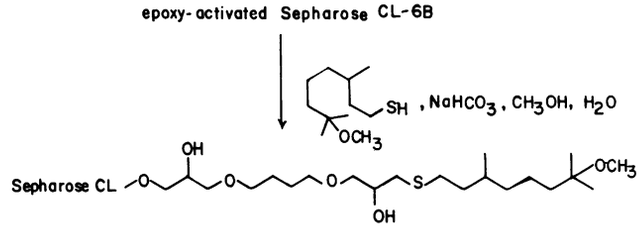

FIG. 1. Synthesis of the affinity resin MCT on epoxy-activated Sepharose CL-6B (MCT-Sepharose).

MATERIALS AND METHODS

Enzyme Source and Assays. Livers from control and clofibrate-fed mice were homogenized in 76 mM sodium phosphate buffer (pH 7.4) and then centrifuged for 20 min at 11,500 × *g* followed by 60 min at 105,000 × *g* to give a 10% (wt/vol) cytosol fraction (12).

cEH activity was determined in 76 mM sodium phosphate buffer (pH 7.4) on 1:10 dilutions of column fractions (0.2% effective cytosol concentration) at 37°C by monitoring the conversion of *trans*-[^{3}H]stilbene oxide (50 μM; 68 mCi/mmol; 1 Ci = 37 GBq) to the diol using a water–dodecane partition assay (12, 13). mEH was measured at pH 7.4 and pH 9.0 using *cis*-[^{3}H]stilbene oxide, and glutathione transferase was assayed with *cis*-[^{3}H]stilbene oxide as described (13). Activities are expressed in units of nmol of substrate conjugated or hydrolyzed per min and specific activities are given as units/mg. Protein concentrations were determined by the Bradford method (14) with standardization using bovine serum albumin.

Preparation of 7-Methoxycitronellylthiol-Sepharose and cEH Inhibitors. Sepharose CL-6B was epoxy-activated according to described procedures (15, 16), and an aliquot (0.3 g) was assayed for the free pendant epoxy functionality by addition of 1.5 ml of 1.3 M $Na_2S_2O_3$ (pH 7.0) and then back-titration of the resultant base using 0.01 M HCl. Epoxy-activated resin (6.4 g, ≈85 microequivalents) was added to 10 ml of methanol/0.1 M $NaHCO_3$ (1:1), pH 8.8, and then a methanol solution (10 ml) of 66 mg (320 μmol) of 7-methoxycitronellylthiol was added (Fig. 1) (MCT; prepared in four steps from citronellol; see ref. 17). The slurry was swirled for 24 hr

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Abbreviations: MCT, 7-methoxycitronellylthiol; cEH, cytosolic epoxide hydrolase; mEH, microsomal epoxide hydrolase.

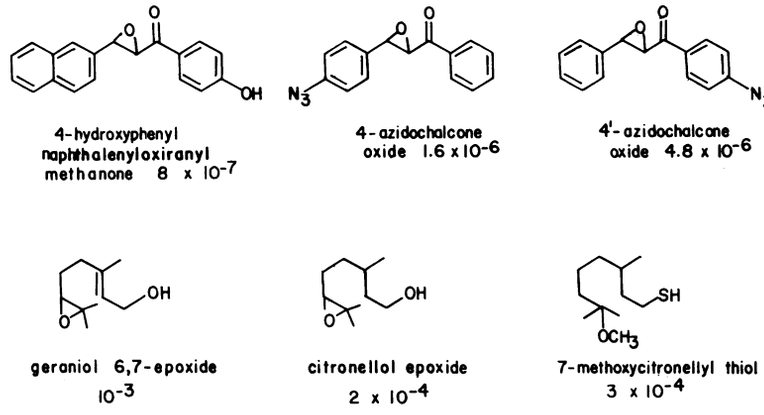

FIG. 2. Inhibitors of cEH and their molar I_{50} values for *trans*-[^{3}H]stilbene oxide hydrolysis.

at room temperature, washed in a sintered glass funnel with 20 vol each of methanol/water (1:1), methanol, methanol/water (1:1), water, 0.5 M NaCl, 1% Lubrol-PX (Sigma), water, ethanol/water, and then stored at 4°C in absolute ethanol. The chalcone oxides (Fig. 2) were prepared by epoxidation of the corresponding chalcones in basic methanolic hydrogen peroxide. Geraniol 6,7-epoxide and citronellol epoxide (Fig. 2) were prepared by oxidation of geranyl acetate or citronellyl acetate with *m*-chloroperbenzoic acid in chloroform followed by ester hydrolysis. Stock solutions of all inhibitors were prepared at 50 mM in ethanol.

Affinity Chromatography on MCT-Sepharose. *Standard procedures.* Affinity purifications were performed at 2°C using coolant-jacketed minicolumns (internal diameter, 8 mm). Routine separations were conducted using a 1.5- to 2.5-ml bed (8 × 25 mm) of MCT-Sepharose preequilibrated in a 76 mM sodium phosphate buffer (pH 7.4) containing 0.1 mM sodium EDTA/0.1 mM dithiothreitol. Cytosol was diluted to 2% (wt/vol) in the above buffer, pumped onto the MCT-Sepharose column at 0.2–0.3 ml/min, and 2- to 3-ml fractions were collected automatically (Fig. 3).

Elution with buffer equal to the quantity of cytosol applied reduced cEH and protein levels to background. Lubrol-PX (0.3%–1.0%) eluted cEH activity, but the net purification was low. A phosphate buffer pH gradient down to pH 5.0 failed to elute cEH, although it has been determined that the enzyme is stable at that pH. Elution of cEH was accomplished with several inhibitors of the enzyme: citronellol epoxide, geraniol 6,7-epoxide, 4-azidochalcone oxide, 4'-azidochalcone oxide, and 4-hydroxyphenyl naphthalenyloxiranyl methanone (see Fig. 2).

Column loading was assessed using a 100-μl bed of MCT-Sepharose in a 1-ml tuberculin syringe. Enzyme-rich clofibrate-induced cytosol [2.0% (wt/vol); 20 ml; 430 units] was passed through this microcolumn at room temperature. Negligible cEH activity was eluted despite discoloration and discontinuities in the resin bed. The theoretical capacity of this 100-μl bed of resin (≈0.6 microequivalents of ligand) is >5 liters of 2% cytosol. However, the binding of other hydrophobic material in cytosol to MCT-Sepharose is expected to reduce this capacity, because the resin bed and flow characteristics deteriorated considerably in this experiment. The resin was washed with buffer (6 ml) and then with 4 ml of 1 mM 4-azidochalcone oxide. A dramatic sharp peak of cEH containing at least 280 units, based on inhibitor removal by dialysis, was eluted.

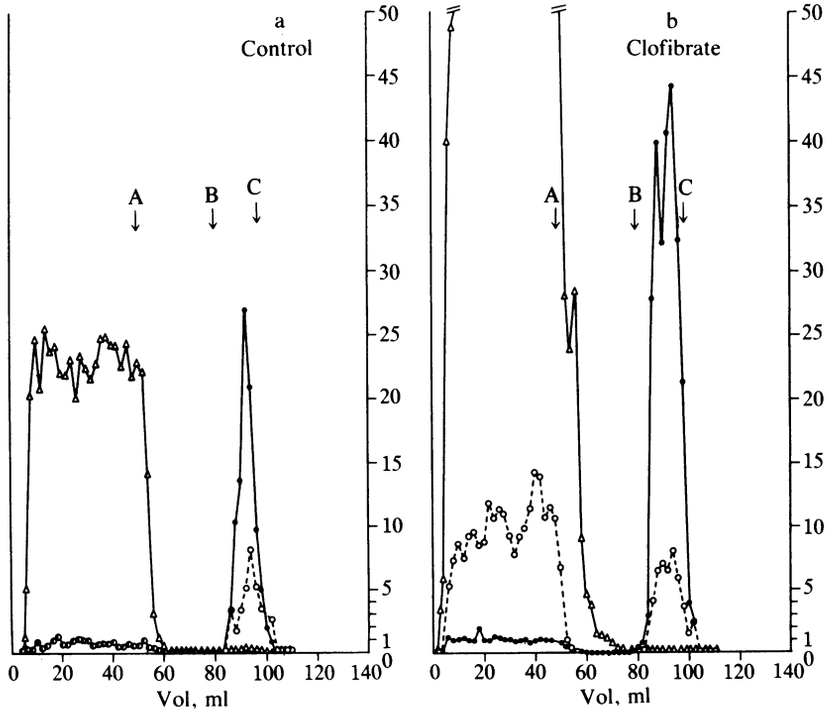

FIG. 3. Comparison of affinity purification of cEH from (*a*) control and (*b*) clofibrate-induced mouse liver by elution with 4-azidochalcone oxide. Elution of EH activities and glutathione transferase activity: △, glutathione transferase measured with 50 μM *cis*-[^{3}H]stilbene oxide at pH 7.4; ●, cEH measured by *trans*-[^{3}H]stilbene oxide assay at pH 7.4; ○, mEH measured by *cis*-[^{3}H]stilbene oxide at pH 9.0. Glutathione transferase and *trans*-[^{3}H]stilbene oxide are expressed as units/ml; *cis*-[^{3}H]stilbene oxide is expressed as units/ml × 10^{-1}. Elution code: A, 76 mM sodium phosphate buffer, pH 7.4; B, pH step gradient, 6 ml each of pH 7.0, 6.5, 6.0, 5.5, 5.0; C, 4-azidochalcone oxide, 5 ml each of 1 × 10^{-5} M, 1 × 10^{-4} M, 1 × 10^{-3} M.

Preparative separation. A large scale purification of cEH from the cytosol from 12 clofibrate-induced mouse livers was performed using a 10-ml (8 × 80 mm) bed of MCT-Sepharose. Cytosol (2%; 750 ml containing 960 mg of protein) was pumped through this column at 0.5 ml/min with occasional monitoring of cEH activity in the eluate. Minimal cEH activity was detected in the loading fractions or the wash. A total of 2.3×10^4 units of *trans*-[^{3}H]stilbene oxide activity was thus applied in 36 hr. After washing the resin with buffer (500 ml at 1.0 ml/min), cEH was eluted with 50 ml of 0.5 mM 4-azidochalcone oxide followed by 20 ml of buffer and 50 ml of 0.5 mM geraniol 6,7-epoxide. The active enzyme fraction contained 9.7 mg of purified cEH (in 62 ml) with at least 5600 units of enzyme activity based on partial removal of the inhibitor by dialysis.

Storage and regeneration. MCT-Sepharose was stored in ethanol containing ≈0.1% butylated hydroxyanisole as a preservative (i.e., to prevent oxidation of sulfur and subsequent loss of binding activity). MCT-Sepharose was regenerated after use by washing with detergent, 0.5 M NaCl, ethanol, and then phosphate buffer to remove proteins and ligands; it can be reused six or more times with little loss of binding activity.

Polyacrylamide Gel Electrophoresis. Molecular weight determinations and homogeneity of cEH eluted with various detergents and inhibitors were assessed by discontinuous PAGE using $NaDodSO_4$ according to Laemmli (18) on 0.75-mm gels (12% acrylamide in separating gel, 5% acrylamide in stacking gel) (Figs. 4 and 5). After electrophoresis at 4°C at 15 mA per gel, $NaDodSO_4$/PAGE gels were stained with 0.25% Coomassie brilliant blue R-250. Gels were cast on Gelbond-PAG (FMC, Rockland, ME) to facilitate handling and storage.

Dialysis. Dialysis was performed using Spectrapor (Spectrum Medical Industries, Los Angeles) membrane tubing (M_r cutoff, 12,000–14,000). For measurement of cEH activity, the enzyme was stabilized by addition of a phosphate buffer (pH 7.4) containing 1 mM 2-mercaptoethanol/1 mM dithiothreitol/5% (vol/vol) sucrose/1 mg of bovine serum albumin per ml, and dialyzed against 20% (vol/vol) sucrose in the same buffer. Absence of stabilizing agents resulted in complete loss of enzyme activity during 12-hr dialysis at 4°C.

Hydrophobic Chromatography on Phenylsepharose. Earlier work in these laboratories (unpublished results) indicated that Phenylsepharose (Pharmacia) would bind cEH and then release it upon elution with 0.1%–0.3% Lubrol-PX. Thus, 40 ml of 2.0% (wt/vol) cytosol was pumped onto a 2-ml bed of prewashed Phenylsepharose at 4°C, followed by 30 ml of buffer. A frontal analysis, detected by the *trans*-[^{3}H]stilbene oxide assay, occurred after <30 ml of cytosol had been pumped through. After washing with buffer, a modest peak of activity was eluted with 1 mM 4-azidochalcone oxide.

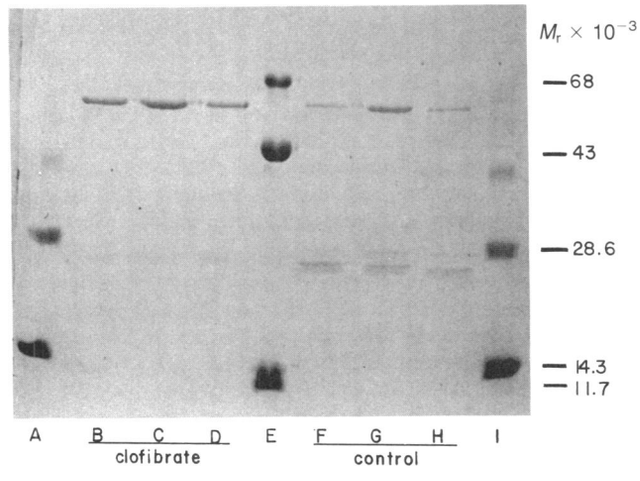

FIG. 4. $NaDodSO_4$/PAGE showing differences in cEH eluted with 4-azidochalcone oxide from clofibrate-induced cytosol (lanes B–D) and control cytosol (lanes F–H). In each set of three, the bands from left to right represent the front, middle, and tail of the eluted peak of cEH activity. Lanes A, E, and I contain molecular weight standards as indicated.

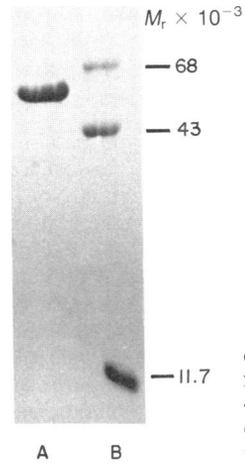

FIG. 5. $NaDodSO_4$/PAGE showing overloaded band (70 μg of protein) of clofibrate-induced cytosolic EH eluted with 4-azidochalcone oxide from a 100-μl bed of MCT-Sepharose (lane A) and protein standards (lane B).

RESULTS AND DISCUSSION

Bioselective Adsorption and Elution of cEH. The selection of a suitable bioselective adsorbent and an appropriate bioselective eluant were both of crucial importance. Indeed, the results below provide a clear demonstration of the synergistic effect on affinity purification that can be obtained by using a bioselective eluant of a structural class that is chemically different from the bioadsorbent ligand.

The chalcone oxides were chosen as eluting agents on the basis of their low I_{50} values for *trans*-[^{3}H]stilbene oxide hydrolysis for cEH but their relatively poor performance as inhibitors for mEH and glutathione transferases (19). Moreover, a large variety of substituted chalcone oxides were available such that electronic and solubility properties could be varied to optimize elution with the inhibitor as well as removal of the inhibitor after elution (19).

The choice of the affinity ligand was less straightforward. Attempts to couple 4- or 4′-hydroxychalcone to epoxy-activated Sepharose or to couple the corresponding glycidyl ethers to Sepharose CL-6B produced affinity gels that either irreversibly bound enzyme activity or showed essentially no binding of cEH (unpublished results). Attempts to prepare covalently linked epoxychalcone oxides were also unsuccessful in producing a bioselective adsorbent. This problem was resolved by noting the high affinity of the cytosolic mouse liver enzyme for hydration of terpene epoxides, in particular the 6,7-epoxygeranyl phenyl ethers, a class of potent insect juvenile hormone mimics (20, 21).

We chose the MCT prepared previously in these laboratories (17) as a ligand that combined four key features. First, the thiol function would allow attachment to epoxy-activated Sepharose under mildly basic (pH 9) conditions. Second, the 7-methoxy function should serve as an effective mimic of the epoxide without being a substrate for EH. Third, the bioselective ligand and eluant were very different in structure, allowing two independent opportunities for selective binding and selective elution. Fourth, the affinity gel should be stable to proteases, esterases, and hydrolases because only ether and thioether linkages were present.

MCT-Sepharose effectively retains cEH from mouse liver cytosol. Approximately 50% of the protein elutes at the solvent front during application of the crude cytosol to the gel,

with <1% of the *trans*-[^{3}H]stilbene oxide hydrolytic activity present. The capacity of the MCT-Sepharose is ≈6 microequivalents per ml of swollen gel. Typical runs were done with a conservative loading of 5–75 mg of total protein per 1.5 ml of gel, and cytosol (2% wt/vol) was pumped through the resin at 0.25–0.50 ml/min at 4°C for optimum binding. More concentrated cytosol or more rapid flow rates caused greater leakage of cEH activity with the solvent front. Nonetheless, a 100-μl minicolumn used at ambient temperature easily retained cEH from 25 mg of total protein, and a 10-ml column effectively held cEH from ≈1 g of cytosol protein. We estimate that the real capacity is at least one order of magnitude higher.

Elution of cEH from MCT-Sepharose can be accomplished with a variety of cEH inhibitors (Fig. 2 and Table 1). Although a pH gradient did not elute cEH, the nonionic detergent Lubrol-PX did elute cEH at 0.3%–1.0% detergent. The purification factor from the detergent elution was very low, but this experiment confirmed that cEH could be both retained and eluted from MCT-Sepharose. The use of citronellol epoxide or 4-azidochalcone oxide resulted in the elution of a sharp peak of cEH activity. The purification factor from the use of citronellol epoxide is considerably lower, but the concentration of this inhibitor/substrate required to achieve elution is nearly 1/10th (≈0.1 mM) that required for 4-azidochalcone oxide (≈1 mM). Also, it is important to note that citronellol epoxide removes all the cEH from the column so that there is none remaining when a chalcone oxide "chaser" is used. When 4'-azidochalcone oxide or 4-hydroxyphenylnapthalenyloxiranyl methanone was used to elute cEH, lower recoveries of somewhat less homogeneous enzyme were obtained.

The homogeneity and molecular size of the eluted cEH were assessed by NaDodSO$_4$/PAGE. Citronellol epoxide elutes major impurities at M_r 24,000 and 30,000, in addition to the clear minor band of cEH at M_r 58,000; azidochalcone oxide elutes cEH more selectively. The value of M_r 58,000 for cEH under these conditions agrees with the values of 57,000–59,000 for rabbit, human, and mouse liver cEHs (9–11). Gel filtration suggests a molecular weight of ≈130,000 for native cEH (22, 23), so it is proposed (10, 11, 23) that mouse liver cEH consists of two identical monomeric units.

In comparing our purification factors with published values, it appears that our 35- to 90-fold purification gives an enzyme of greater homogeneity than reportedly 180-fold purified material. This is due to the residual chalcone oxide present in the eluted protein. Dialysis with 1% bovine serum albumin to stabilize the purified enzyme gave a 2- to 4-fold increase in activity after 2 hr; however, with longer times, enzyme activity declined significantly. All of our reported purifications are thus highly conservative in terms of the homogeneity of the protein and the overall yield of enzyme activity.

The possibility existed that MCT-Sepharose functioned only as a hydrophobic column with the apparent selectivity due only to the choice of good cEH inhibitors as eluants. However, a frontal analysis rapidly occurred with Phenylsepharose, indicating low capacity of Phenylsepharose relative to MCT-Sepharose. Elution with 4-azidochalcone oxide gave a peak of modest cEH activity, but examination of this material by NaDodSO$_4$/PAGE showed very little cEH at M_r 58,000; instead, two intense bands at M_r 24,000 and 28,000 were present. Thus, MCT-Sepharose exhibits bioselective adsorption of cEH rather than only nonspecific hydrophobic interactions with cEH.

Comparison of Control and Clofibrate-Induced Enzymes. Chronic administration of the hypolipidemic drug clofibrate (ethyl α-[4-chlorophenoxy]-α-methyl propionate) to mice leads to a 2.5-fold increase in *trans*-[^{3}H]stilbene oxide hydrolytic activity of the liver cytosol (12). Clofibrate is a peroxisome-proliferating drug (24), which, among other actions, inhibits sterol biosynthesis (25, 26), and it is believed that cEH is associated with mitochondria and peroxisomes *in vivo* (27, 28). We wished to determine whether the induced enzyme was the same as that in control animals. To this end, two identical affinity columns were prepared and run with freshly obtained cytosol from livers of control and clofibrate-fed mice. Sixty milliliters of 2% cytosol was applied in both cases, and identical washing and elution regimes were followed. Eluted fractions were assayed for glutathione transferase activity (using *cis*-[^{3}H]stilbene oxide at pH 7.4), mEH activity (using *cis*-[^{3}H]stilbene oxide at pH 9) and cEH activity (using *trans*-[^{3}H]stilbene oxide at pH 7.4).

The cEH peak was eluted as expected with 4-azidochalcone oxide, and both enzymes eluted under identical conditions (Fig. 3). Glutathione transferase activity was not retained by MCT-Sepharose and eluted with the solvent front in both preparations. *cis*-[^{3}H]stilbene oxide hydrolytic activity eluted only under the *trans*-[^{3}H]stilbene oxide peak in control cytosol, as expected for the ≈1/50th lower activity of cEH for *cis*-[^{3}H]stilbene oxide as substrate relative to *trans*-[^{3}H]stilbene oxide. In the clofibrate-induced cytosol, some *cis*-[^{3}H]stilbene oxide activity was detected in the solvent front in addition to that under the *trans*-[^{3}H]stilbene oxide peak. We believe this activity reflects the presence of a mEH-like enzyme in the cytosol.

Table 1. Purification of cEH on MCT-Sepharose with different elution conditions

Eluant	Control or clofibrate, nmol/min*	Protein applied, mg	Total activity,† units	Specific activity, units/mg	Protein eluted, mg	Total activity,‡ units	Specific activity, units/mg	% activity recovered	Purification factor‡
Lubrol	Control (frozen)	5.6	24.8	4.50	2.3	17.1	7.4	60	X2
4-AZCO	Control (frozen)	31.3	121	3.86	0.194	69.0	356	57	X92
Citronellol epoxide	Control (frozen)	49.5	157	3.20	1.32	129	97	82	X31
4-AZCO	Control (fresh)	58.9	454	7.71	0.765	177	231	39	X30
4-AZCO	Clofibrate (fresh)	76.8	1095	14.3	0.798	556	697	51	X49
4-AZCO	Clofibrate (frozen) (100-μl column)	25.6	430	16.8	0.300	278§	927	65	X55
4-AZCO	Clofibrate (frozen) (10-ml column)	960	22,700	23.6	9.7	5568	574	24	X24

4-AZCO, 4-azidochalcone oxide.
*Unless otherwise indicated, columns were 8 mm (internal diameter) with 1.5–2.5 ml of MCT-Sepharose.
†EH was assayed with *trans*-[^{3}H]stilbene oxide (50 μM; 69 mCi/mmol) by a standard partition assay.
‡Without dialysis to remove excess inhibitor. This then results in an underestimate of the cEH activity actually present.
§Dialyzed 12 hr vs. sucrose/phosphate buffer.

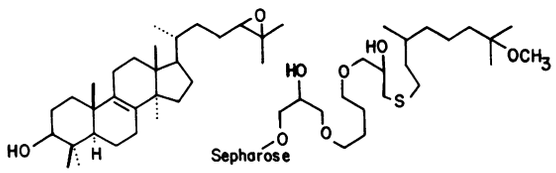

FIG. 6. Proposed analogy of side chain of lanosterol 24,25-epoxide with MCT-Sepharose.

Analysis of the control and clofibrate cEHs by NaDodSO$_4$/PAGE demonstrated the apparent identity of molecular sizes of the two enzymes (Fig. 4). The clofibrate-induced sample is essentially homogeneous (M_r, 58,000), while the control cytosol contains three contaminants (M_r, 23,500, 24,500, and 26,500). Even when >70 μg of protein is loaded in a single well, the clofibrate-induced enzyme from the MCT-Sepharose appears as only a single band by NaDodSO$_4$/PAGE under conditions with which <1 μg would be easily detectable (Fig. 5). Moreover, isoelectric focusing also shows a single band for clofibrate-induced cEH from the affinity column (unpublished results). Using HPLC on a DEAE-type anion exchange column (Synchropak AX-300) with 0–0.5 M sodium acetate in 25 mM Tris acetate (pH 7.9) at 0.5 ml/min, >98% of all *trans*-[^{3}H]stilbene oxide activity and 95% of protein (Coomassie and A_{280} assays) eluted under a single peak at 12 ml (void volume, 4 ml) corresponding to 0.45 M NaOAc.

Role of cEH and Basis of Bioselective Adsorption to MCT-Sepharose. We hypothesize that cEH has a major role in the hydration of endogenous lipid epoxides, in particular, those involved as by-products of steroid biosynthesis (4). The hypolipidemic drug clofibrate selectively induces cEH levels in mouse liver and simultaneously causes proliferation of peroxisomes (12, 24). It is possible that the cEH induction is a secondary response to peroxisome proliferation—i.e., to remove the additional epoxides formed, because probucol (a nonperoxisome proliferating hypolipidemic drug) does not induce higher cEH levels (12). In support of this hypothesis, we cite several observations from this study. First, the control and induced forms of cEH are identical by NaDodSO$_4$/PAGE and two other methods, indicating that the induction process leads to an increased production of an existing enzyme. Second, MCT-Sepharose possesses a ligand that can effectively mimic the side chain of 24,25-epoxylanosterol or of an epoxidized squalene (Fig. 6). Thus, the selective binding of cEH (in contrast to mEH or glutathione transferase) to the affinity resin suggests that terpene epoxides may be among the natural endogenous substrates for this enzyme. It was also possible to elute the cEH activity with citronellol epoxide, a terpene epoxide and a structural mimic of an epoxidized sterol side chain. Third, the connection between clofibrate induction of cEH, peroxisome proliferation, and the known inhibition of cholesterol biosynthesis by clofibrate (26) suggests that the induced cEH may be produced to detoxify squalene 2,3,24,25-bisepoxide, lanosterol 24,25-epoxide, or other potential angiotoxic oxygenated sterols (4, 29). These epoxides could arise as a result of increased peroxisome activity in generating the oxygenated sterols that are believed to be the proximal effectors of the inhibition of cholesterol biosynthesis (26, 30).

We believe this hypothesis forms the basis for further study of the role of mammalian hepatic cEH with endogenous substrates. Moreover, the availability of a rapid and highly selective affinity chromatography method now makes the study of the enzymology, physiology, and protein chemistry of the soluble epoxide hydrolases an easily accessible undertaking. The MCT-Sepharose system also provides a clear demonstration of the power of bioselective adsorption onto a substrate analog followed by bioselective elution with an inhibitor possessing a structure very different from that of the bound ligand.

We acknowledge the essential contributions of our co-workers Mr. S.-K. Park and Mr. W.-S. Eng (Stony Brook) for synthesis of ligands and inhibitors; Dr. D. E. Moody and Ms. D. N. Loury (University of California, Davis) for providing mouse liver cytosol and for performing *cis*-[^{3}H]stilbene oxide assays; and Drs. P. Cheung, R. M. Roe, and M. Silva (University of California, Davis) for helpful discussions and assistance in the laboratory. G.D.P. was supported by National Institutes of Health Grant GM 30899, the Alfred P. Sloan Foundation, the Camille and Henry Dreyfus Foundation, and the Herman Frasch Foundation. This work was performed during the sabbatical of G.D.P. with B.D.H. B.D.H. was supported by National Institutes of Health Grant ES-02710 and a National Institute of Environmental Health Sciences RCDA (ES-00107). G.D.P. is a Fellow of the Alfred P. Sloan Foundation (1981–1985) and a Camille and Henry Dreyfus Teacher Scholar (1981–1986).

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