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Affinity Purification of Cytosolic Epoxide Hydrolase Using Derivatized Epoxy-Activated Sepharose Gels[1]

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Improved affinity chromatography procedures for the purification of cytosolic epoxide hydrolase are described. An earlier affinity purification method using immobilized 7-methoxycitronellyl thiol (MCT) sporadically produced final enzyme preparations containing major impurities. To eliminate these impurities, we tested alternate ligands, spacer arms, and ligand concentrations. A series of alkyl and aryl thiols coupled to epoxy-activated Sepharose were found to exhibit markedly different binding characteristics as compared with commercially available alkyl- and aryl-Sepharose gels. Using one of these new matrices, benzylthio-Sepharose, cytosolic epoxide hydrolase from mouse liver was purified over 100-fold, appeared homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and was obtained with 60–90% recovery of enzyme activity. The impurities previously observed with the MCT–Sepharose procedure were reduced or eliminated by using an MCT ligand concentration of 5 microequivalents per gram or less. MCT–Sepharose and benzylthio-Sepharose provide rapid and convenient one-step procedures for obtaining purified cytosolic epoxide hydrolase from numerous species and tissues. © 1988 Academic Press, Inc.

KEY WORDS: affinity chromatography; epoxide hydrolase; enzyme purification; epoxy-activated Sepharose.

Epoxide hydrolases (EC 3.3.2.3) catalyze the addition of water to epoxide-containing molecules of endogenous and exogenous origin (1,2). Several different forms of epoxide hydrolases have been identified in mammalian tissues, including a "microsomal epoxide hydrolase" which hydrates numerous epoxidized xenobiotics including arene oxides (1,3,4), a "cholesterol epoxide hydrolase" (also microsomal) which catalyzes the hydrolysis of cholesterol oxides (5,6), a recently identified "leukotriene A_4 hydrolase" (7), and a "cytosolic epoxide hydrolase" that hydrolyzes a variety of epoxides not on cyclic systems (2,8–10).

Classical purification techniques have been published for the purification of cytosolic epoxide hydrolase from human (11), rabbit (12), and mouse (13,14) liver. These multiple-step procedures gave low yields of 0.5–35% with 120- to 550-fold purification. Recently, an affinity chromatography procedure was developed, using 7-methoxycitronellyl thiol (a weak inhibitor) coupled to epoxy-activated Sepharose CL-6B to selectively bind the enzyme and 4-azidochalcone oxide (a stronger inhibitor) for its selective elution (15). This method offered a rapid, one-step procedure for obtaining high yields of essentially homogeneous enzyme. However, while using this new purification procedure in our studies of the protein chemis-

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try of the enzyme, we often encountered major impurities in our final preparations. In the process of attempting to understand and remedy this problem, a new, commercially available column ligand was found which consistently provided an enzyme preparation of equal or greater purity than the previous method. This improved single-step affinity chromatography procedure using benzylthio-Sepharose is presented, along with the results of a number of modifications to the original method—including the essential importance of using low MCT[3] ligand concentrations. The benzylthio-Sepharose and MCT–Sepharose procedures are compared for a number of mammalian species and tissues. In addition, the differences in binding characteristics between a newly prepared series of alkylthio- and arylthio-Sepharose gels and the corresponding commercially available gels are discussed.

MATERIALS AND METHODS

Chemicals. Citronellol, 3,7-dimethyl-1-octanol, *trans*-stilbene oxide (TSO), 1,4-butanediol diglycidyl ether (95%), ethylene glycol diglycidyl ether, sodium dodecyl sulfate (SDS), and the various alkyl and aryl thiols were obtained from Aldrich Chemical Co. (Milwaukee, WI). Clofibrate was a gift from Ayerst Laboratories. 4-Fluorochalcone oxide (16), citronellol epoxide, and [^{3}H]TSO (68 mCi/mmol) (17) were prepared previously in this laboratory. Sepharose CL-6B and phenyl-Sepharose CL-4B were purchased from Pharmacia. Bio-Rad protein dye reagent was purchased from Bio-Rad Laboratories, (Richmond, CA). Dithiothreitol (DTT) and bovine serum albumin, fraction V, were obtained from Sigma (St. Louis, MO).

[3] Abbreviations used: MCT, 7-methoxycitronellyl thiol; TSO, *trans*-stilbene oxide; SDS, sodium dodecyl sulfate; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; DMOT, 3,7-dimethyl-1-octanyl thiol.

Synthesis of 7-methoxycitronellyl thiol. Citronellol was converted to 7-methoxycitronellol by alkoxymercuration–basic borohydride reduction (18) and purified to greater than 99% purity (GLC) by flash chromatography on silica gel using hexanes/ethyl acetate (3:2). The corresponding thiol was then prepared via an *S*-acetyl derivative using a Mitsunobu procedure as described by Volante (19) and detailed by Prestwich *et al.* for citronellyl thiol (20). The thiol ester was purified by flash chromatography (silica gel, hexanes/ethyl acetate 9:1) to greater than 99% purity (GLC) prior to reduction to the thiol. An essential modification in the original method for the thiol ester reduction (Prestwich, personal communication) involved the dropwise addition of the thiol ester (1 mmol in 2 ml ether) to a stirred 1-ml solution of 1.0 M lithium aluminum hydride in ether under nitrogen at 0°C. After stirring for 15 min at room temperature, any excess $LiAlH_4$ was destroyed by the addition of 1 ml of a saturated aqueous solution of Na^+/K^+ tartrate:tartaric acid (1:1). The ether layer was then separated, dried over sodium sulfate, and concentrated to give the product which was used without further purification. Maintenance of free thiols under an inert atmosphere is essential to prevent disulfide formation.

Synthesis of affinity matrices. Sepharose CL-6B was epoxy-activated and coupled to the various thiol ligands as described previously (20,21) with some modifications. Sepharose CL-6B was washed with 10 vol each of H_2O, MeOH/H_2O (1:1), and 0.1 M NaOH. To 10 g of moist gel was added a 20-ml solution containing 3 mg/ml $NaBH_4$, 0.3 M (final concentration) NaOH, and 0.5–4 ml (optimum approximately 2 ml for benzylthio-Sepharose) of 1,4-butanediol diglycidyl ether. The slurry was swirled at room temperature for 5 h using an orbital shaker. The epoxy-activated gel was then washed in a sintered glass funnel with 20 vol each of H_2O, MeOH/H_2O (1:1), MeOH, MeOH/

may be possible to change the affinity of the column by using a different ligand concentration ... a range is available
. lower concentration if elution is a problem
. higher concentration to increase binding

H_2O (1:1), and H_2O—taking care to ensure that the pH of the water was above 6. The gel was then suction-filtered to near dryness and an aliquot (0.3 g) assayed for free epoxy functionality by addition of 1.5 ml of 1.3 M $Na_2S_2O_3$ (pH 7.5) followed by back titration of the resultant base with 0.010 M HCl (15). An approximately fivefold excess of thiol in 5 ml of MeOH was added to 5 g of epoxy-activated Sepharose in 10 ml of 0.1 M $NaHCO_3$/MeOH (1:1). After swirling for 24 h, the derivatized Sepharose was washed with 40 vol each of MeOH/H_2O (1:1), MeOH, MeOH/H_2O (1:1), H_2O, 0.5 M NaCl, H_2O, 0.001 M HCl (to hydrolyze unreacted epoxy groups), H_2O, and EtOH/H_2O (1:1) and then stored at 4°C in absolute EtOH containing ca. 0.1% butylated hydroxyanisole.

Enzyme sources. Male Swiss-Webster mice 20–25 g (Bantin–Kingman, Fremont, CA) were fed *ad libitum* for 14 days a diet of ground Purina laboratory chow containing a final concentration of 0.5% (w/w) clofibrate dissolved in 10 vol of corn oil. This regimen has been previously shown to increase cytosolic epoxide hydrolase activity in mice 2.5-fold (22–24). Livers from adult male and female Rhesus monkeys were obtained through the Northern California Primate Research Center (Davis, CA). Two frozen human liver samples were obtained from Dr. Charles Tyson, SRI Int. (Menlo Park, CA). Both were from caucasian adults with normal livers. Cytosols were prepared from these livers as previously described (22) and were used immediately or stored at −70°C until required.

Affinity chromatography. All purification procedures were performed at 4°C using 76 mM sodium phosphate buffer at pH 7.4, containing 0.1 mM EDTA (Buffer A). After the affinity gel was washed with 100 vol of Buffer A, a 0.5-ml bed was poured in a 5-mm i.d. Econo-Column (Bio-Rad Laboratories). In a typical experiment, 15 ml of 8% (w/v) cytosol was pumped through the column at 0.1–0.2 ml/min and the eluant collected as the "nonbinding fraction." The column was washed with an equal volume of Buffer A ("buffer wash fraction") to remove loosely bound, contaminating proteins. The enzyme was then eluted with 4 ml of 0.5 mM 4-fluorochalcone oxide using gravity flow ("elution fraction"). 4-Fluorochalcone oxide has been shown to give results identical to those obtained with the previously used, but potentially reactive, 4-azidochalcone oxide (25). A 1.0 mM solution of citronellol epoxide used in place of the 4-fluorochalcone oxide produced results identical to those with the benzylthio-Sepharose gel.

Dialysis. To remove the 4-fluorochalcone oxide or citronellol epoxide inhibitors, the eluted enzyme solution was dialyzed in Spectrapor 2 membrane tubing (M_r cutoff 12,000–14,000, Spectrum Medical Industries, Los Angeles) for 30 min to 2 h against Buffer A. The inclusion of DTT (1 mM) in the dialysis buffer, or the addition of bovine serum albumin (100 μg/ml) to the eluted enzyme solution, had no apparent effect on stability of the enzyme during dialysis.

Assays. Cytosolic epoxide hydrolase activity was monitored with [^{3}H]TSO by a partition assay as described previously (2,17), except that bovine serum albumin (100 μg/ml) was added to the assay buffer to stabilize the purified enzyme. Protein concentrations were determined by the Bradford method (26), using bovine serum albumin as the standard.

Gel electrophoresis. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemmli (27) on 0.75-mm gels (12% acrylamide in separating gel, 4% acrylamide in stacking gel). Molecular-weight standards included phosphorylase B (94 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20 and 21 kDa) and lysozyme (14.3 kDa). Protein bands were visualized by staining with Coomassie brilliant blue R-250.

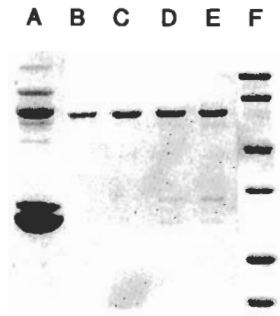


FIG. 1. SDS–PAGE of mouse liver cytosolic epoxide hydrolase purified using MCT–Sepharose and benzylthio-Sepharose gels. Lane A, MCT–Sepharose from original study; lane B, benzylthio-Sepharose; lane C, 5 μeq/g MCT–Sepharose; lane D, 7.5 μeq/g MCT–Sepharose; lane E, 14 μeq/g MCT–Sepharose; lane F, molecular-weight standards.

RESULTS AND DISCUSSION

Problems with previous method. The novel affinity purification procedure of Prestwich and Hammock (15) offered a rapid and convenient method for obtaining highly purified cytosolic epoxide hydrolase. The technique used 7-methoxycitronellyl thiol linked to epoxy-activated Sepharose CL-6B to bind the enzyme and a chalcone oxide inhibitor for its elution. As compared with previously published classical procedures for purifying the enzyme from mammalian liver (11–14), the new method offered much higher yields in a fraction of the time the others required. However, with continued use of the new purification technique in our laboratory, we often encountered major impurities in the final enzyme preparations from mouse liver, ranging from below 5% to above 50% as visualized on SDS–polyacrylamide gels. The molecular weight of the major protein impurity ranged from 22 to 26 kDa. This impurity was even observed when using the same batch of MCT–Sepharose as that used in the initial study (see Fig. 1, lane A). The appearance of this 22- to 26-kDa band was also a sporadic problem with other species. In an effort to reduce or eliminate the impurities in the purified enzyme preparations, we explored a number of modifications to the original procedure, using cytosol from mouse liver.

Possible degradation of cEH. One possibility was that the 22- to 26-kDa protein originated as a breakdown product of the purified enzyme. The addition of a protease inhibitor, phenylmethylsulfonyl fluoride, at a final concentration of 0.1 mM, to the buffers used for tissue preparation and purification had no effect on the level of impurities. Furthermore, repeated freeze–thaw cycles, or prolonged storage of the purified enzyme under conditions which resulted in complete loss of enzyme activity, did not alter its appearance on electrophoresis. Incubation with a variety of proteases also failed to generate peptide fragments identical to the impurities (25). Thus, it appeared that degradation was not an important factor.

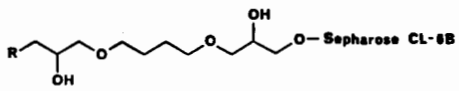
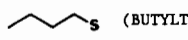
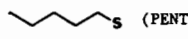
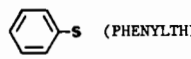
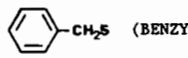
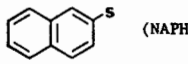
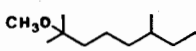
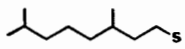
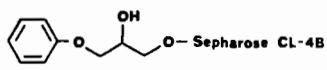
Spacer arm effects. In an attempt to determine if the impurities observed in our purified enzyme preparations resulted from the length of the spacer arm (28), we synthesized and tested an MCT–Sepharose gel in which ethylene glycol diglycidyl ether was used in place of 1,4-butanediol diglycidyl ether to generate the spacer arm. This shortening of the spacer arm by two carbon atoms had no significant effect.

In the case of phenyl-Sepharose CL-4B and phenylthio-Sepharose, though, which differ primarily in the length of the spacer arm (4 atoms vs 13 atoms), a dramatic difference in results was observed. Phenylthio-Sepharose provided a high yield of very purified enzyme, whereas phenyl-Sepharose CL-4B did not retain the enzyme from the cytosol passed through the column under identical conditions (see Table 1 and Fig. 2, lanes E and F). It would appear that the series of alkylthio- and arylthio-Sepharose matrices synthesized in this study (which includes phenylthio-Sepharose) possess binding characteristics markedly different from the analagous alkyl- and aryl-Sepharose columns available commercially—which con-

tain a shorter spacer arm. Phenyl-Sepharose and other hydrophobic interaction matrices with short spacer arms are likely to emphasize interactions with lipophilic regions on the surface of proteins, whereas lipophilic ligands connected to Sepharose with long spacer arms may emphasize high-affinity binding to hydrophobic pockets. However, at high ligand concentrations, one might anticipate that even those affinity gels with long spacer arms will emphasize interactions with hydrophobic regions on the surfaces of many proteins. These alkylthio- and arylthio-Sepharose gels, discussed in greater detail below, can be conveniently prepared from epoxy-activated Sepharose and commercially avail-

TABLE 1

AFFINITY CHROMATOGRAPHY OF CYTOSOLIC EPOXIDE HYDROLASE FROM MOUSE LIVER ON DERIVATIZED EPOXY-ACTIVATED SEPHAROSE GELS

AFFINITY MATRIX	PERCENT RECOVERY OF TOTAL ACTIVITY		
	NONBINDING	BUFFER WASH	ELUTION
			
R =			
 (BUTYLTHIO-)	90	0.8	0
 (PENTYLTHIO-)	86	1.9	0
 (HEXYLTHIO-)	14	5.6	38
 (OCTYLTHIO-)	4.0	0.3	48
 (PHENYLTHIO-)	16	14	66
 (BENZYLTHIO-)	12	4.6	75
 (NAPHTHYLTHIO-)	3.6	1.7	70
 (7-METHOXYCITRONELLYL THIO-) [MCT]	4.6	1.4	92
 (3,7-DIMETHYL-1-OCTANYL THIO-) [DMOT]	3.2	0.2	2.3
 PHENYL-SEPHAROSE	56	15	0.8

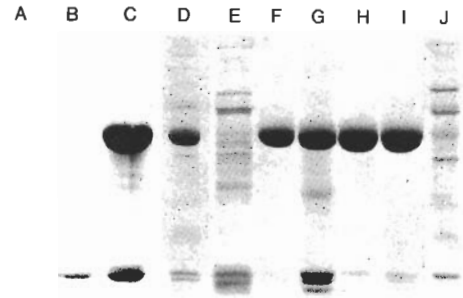


FIG. 2. SDS–PAGE of epoxide hydrolase purified from 2% (w/v) mouse liver cytosol using derivatized Sepharose gels. Lane A, butylthio-Sepharose; lane B, pentylthio-Sepharose; lane C, hexylthio-Sepharose; lane D, octylthio-Sepharose; lane E, phenyl-Sepharose CL-4B; lane F, phenylthio-Sepharose; lane G, naphthylthio-Sepharose; lane H, benzylthio-Sepharose; lane I, MCT–Sepharose; lane J, DMOT–Sepharose.

able thiols and may provide a useful new series of affinity matrices for hydrophobic interaction chromatography.

Potential precolumns to remove impurities. The aforementioned alkylthio- and arylthio-Sepharose gels were originally synthesized and tested for the purpose of serving as precolumns that might bind the apparently hydrophobic lower molecular-weight impurity while allowing the enzyme to pass through. This approach was based on preliminary results obtained with phenyl-Sepharose CL-4B as a precolumn to MCT–Sepharose; the cytosol was passed through a 1-ml bed of phenyl-Sepharose and then directly onto the MCT–Sepharose gel. The purified enzyme preparation that resulted appeared to be essentially free of impurities by SDS–PAGE, although the yield was extremely low. In an attempt to achieve the same results, but with higher yields, we tested a series of alkyl and aryl ligands coupled to epoxy-activated Sepharose CL-6B (Table 1). To reduce the number of variables being examined in the experiments, we first tested the affinity gels as individual columns rather than as precolumns to MCT–Sepharose. The ability of the derivatized Sepharose gels to retain the major impurity, while allowing passage of the enzyme, was assessed, in addition to examination of which proteins were eluted with 4-fluorochalcone oxide. In the C_4–C_8 alkyl series, the butyl and pentyl ligands did not retain much activity, although they did bind the lower molecular-weight impurity which eluted with 4-fluorochalcone oxide (Fig. 2, lanes A and B). This protein exhibited no measurable enzyme activity with TSO. The hexyl and octyl ligands retained >80 and >95%, respectively, of the enzyme activity applied in the cytosol, and to our surprise, highly purified cEH was eluted from both columns (Fig. 2, lanes C and D). Similarly, in the aryl series, the phenyl and benzyl ligands both provided essentially homogeneous enzyme (Fig. 2, lanes F and H), although the yield was significantly higher for the benzyl column. These unexpected findings led to the separate development of benzylthio-Sepharose as an alternative (and more easily synthesized) gel matrix for the purification (see below). The naphthyl ligand produced results comparable to those of the phenyl and benzyl ligands, with the exception that the lower molecular-weight impurities were present in significant amounts (Fig. 2, lane G).

Nature of the binding. The above data provided strong evidence that the binding of the enzyme to the affinity matrix results from hydrophobic interactions and not from bioselective adsorption. This hypothesis was supported by the results from the MCT–Sepharose and DMOT-Sepharose gels (Table 1 and Fig. 2, lanes I and J). The only difference between these ligands is the absence in DMOT of the methoxy group (which was thought to mimic the epoxide moiety). Although both gels retained greater than 90% of activity during loading of the cytosol, they differed strikingly in the amount of purified enzyme eluted (Table 1). The results suggest that the enzyme is bound more tightly by the more lipophilic DMOT ligand. Binding of enzyme to the affinity matrix may occur by

TABLE 2

PURIFICATION OF MOUSE LIVER cEH ON BENZYLTHIO-SEPHAROSE

	Specific activity (nmol/min/mg)	Total activity (nmol/min)	Protein (mg)
Crude cytosol	13.0	1200	92
Nonbinding fraction	0.2	15	87
Buffer wash	1.3	7	5
Elution (dialyzed)	1460	1060	0.7

interactions between the lipophilic ligand and hydrophobic regions postulated by Mullin and Hammock (16) to exist on either side of the enzyme's catalytic center.

Since the binding process does not appear to be sufficiently specific to account for the high purification factors observed in the affinity chromatography of the enzyme, the very high selectivity probably results from specific (bioselective) elution. This was illustrated previously (25) by the much lower purification factors obtained by elution of the enzyme with Lubrol-PX, a nonionic detergent, as compared with elution using chalcone oxide inhibitors.

Novel benzylthio-Sepharose affinity matrix. Results for the single-step affinity purification from mouse liver using benzylthio-Sepharose are shown in Table 2. The data represent a 110-fold purification with 89% recovery of enzyme. This protocol, which has been repeated at least 60 times, routinely provides milligram quantities of purified enzyme in 60–90% overall yield with a specific activity that ranges from 900 to 1500 (mean = 1200) nmol/min/mg protein. This compares with values of 1490 (13), 1330 (14), and 930 (15) nmol/min/mg for mouse liver enzyme obtained by other procedures. The purified enzyme appears homogeneous by SDS–PAGE (Fig. 1, lane B) with a M_r of approximately 60,000, which is in agreement with previous findings (14,15). An important advantage of the benzylthio-Sepharose matrix is the commercial availability of the ligand, which avoids the four-step synthesis required for MCT.

Effect of ligand concentration. The recovery (and purity) of enzyme was found to be very dependent on the concentration of benzylthiol ligand bound to the Sepharose gel (Fig. 3). At the optimum ligand concentration, nearly complete binding of enzyme to the affinity matrix occurs, followed by essentially complete elution with 4-fluorochalcone oxide. As the ligand concentration falls below the optimum, increasing amounts of enzyme pass through the column without binding (as evidenced by high recovery of

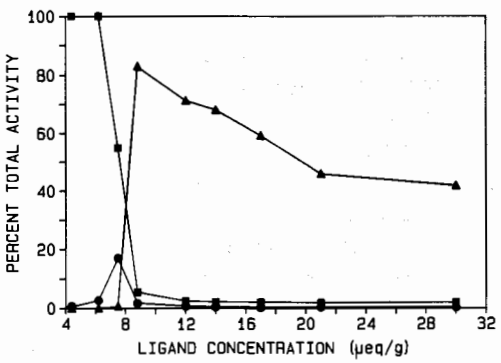


FIG. 3. Optimization of ligand concentration. Affinity chromatography of 8% (w/v) mouse liver cytosol was performed using benzylthio-Sepharose containing varying concentrations of benzyl mercaptan ligand. The ligand concentration was determined by assay of free epoxy functionality present in the epoxy-activated Sepharose. Fractions were collected and assayed as described under Materials and Methods: ■, non-binding fraction; ●, buffer wash fraction; ▲, dialyzed elution fraction.

activity in the nonbinding fraction), and the recovery of purified enzyme is decreased. At high ligand concentrations, essentially complete binding of the enzyme occurs, but only partial elution is attained—even with the use of higher inhibitor concentrations. Furthermore, the eluted enzyme from columns with high ligand concentration contains significant amounts of impurities. An apparent "equivalency point" for benzylthio-Sepharose is observed near a ligand concentration of 7.5 μeq/g, at which a significant portion of activity is found in the buffer wash fraction, while almost no activity is present in the dialyzed elution fraction.

The pronounced effect of ligand concentration on enzyme purity found with benzylthio-Sepharose provided a clue for eliminating the impurities in the MCT–Sepharose enzyme preparations. We prepared and tested a series of MCT–Sepharose gels of varying ligand concentration from 5 to 14 μeq/g gel. The mouse liver enzyme from the 5 μeq/g column appeared homogeneous by SDS–PAGE, while the 7.5 and 14 μeq/g columns showed increasing amounts of the lower molecular-weight impurity (Fig. 1, lanes C–E). The 5 μeq/g MCT–Sepharose gel provides results with purified mouse liver enzyme essentially identical to those listed above for benzylthio-Sepharose. The key factor in obtaining a consistently homogeneous enzyme preparation from mouse liver with MCT–Sepharose appears, therefore, to be the use of a ligand concentration of 5 μeq/g or less, rather than the 5–15 μeq/g originally recommended (15).

Stability of purified enzyme: Effect of buffer modifiers and enzyme concentration. The stability at 4°C of undialyzed mouse liver enzyme eluted at approximately 150 μg/ml is shown in Fig. 4. The increase in specific activity with time is likely due to the slow hydrolysis of the 4-fluorochalcone oxide inhibitor by the purified enzyme. The absence of any marked stabilizing effect of argon purging of buffers or inclusion of DTT is in contrast to the results of Meijer and DePierre (14,29), who reported such procedures essential for maintaining activity of the purified enzyme.

As we scaled up the purification procedure, applying 40–50 ml of mouse liver cytosol onto the same volume of affinity gel, more concentrated preparations of purified enzyme were obtained. At concentrations above 300 μg/ml, we observed no loss of enzyme activity in either dialyzed or undialyzed elution fractions after 6 months at 4°C.

Stability of the purified enzyme under assay conditions. At the 1 μg/ml concentration of purified mouse liver enzyme used routinely in assays, a rapid and dramatic fall in activity was observed at 37°C, with over 50% of the enzyme activity lost within 10 min, and greater than 90% within 25 min. We have found, however, that the presence of bovine serum albumin (0.1 mg/ml) significantly stabilizes the activity of the dialyzed

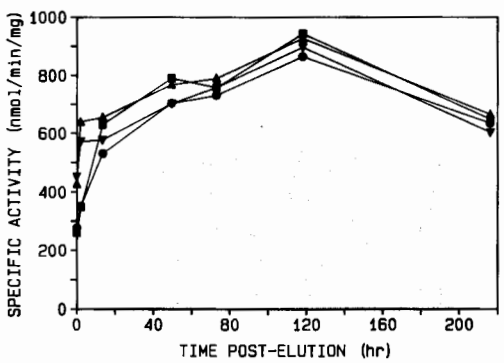


FIG. 4. Stability of purified mouse liver cytosolic epoxide hydrolase. The specific activity of undialyzed, purified enzyme maintained at 4°C was monitored (using *trans*-stilbene oxide) over a 10-day period. The enzyme was purified with benzylthio-Sepharose from a single preparation of cytosol using: ■, sodium phosphate buffer (76 mM, pH 7.4); ●, phosphate buffer + 1 mM dithiothreitol; ▼, argon-purged phosphate buffer; or ▲, argon-purged phosphate buffer + 1 mM dithiothreitol. Values represent the means of triplicate determinations with the standard deviation for all points less than 10%. Similar trends were observed when the experiment was repeated.

enzyme, with more than 85% of original activity being retained after 1 h at 37°C.

Application of techniques to other species and tissues. The benzylthio-Sepharose purification procedure has been successfully applied to mouse liver from three other strains of mice (Balb/c, C57B1, and SEC/R1) as well as to rabbit liver, bovine liver, and human placenta (unpublished data). Preliminary results from rat and Rhesus monkey, although, indicate that this method is not always directly transferable to other species.

The MCT–Sepharose purification procedure appears to be more universally applicable to the purification of cytosolic epoxide hydrolase from other species and tissues. It has previously been used to purify enzyme from human, Rhesus monkey, baboon, rabbit, and rat liver (30). However, when the lower ligand concentration MCT–Sepharose gels synthesized in this study were used, significantly improved results were obtained. Rhesus monkey liver, for example, exhibited the same trend as mouse liver, with a homogenous preparation obtained with the 5 μeq/g MCT gel and increasing amounts of impurities resulting from the higher ligand concentrations (Fig. 5). Human liver enzyme was purified more than 500-fold using the 5 μeq/g MCT–Sepharose gel. The purified enzyme was obtained in greater than 60% yield with a specific activity (TSO) of 250–280 nmol/min/mg. Similarly, a 4 μeq/g MCT column has been successfully applied to the purification from human placenta as well as from mammary glands of mice and Rhesus monkeys (unpublished results).

For those tissues in which the benzylthio-Sepharose procedure can be successfully applied, it is the method of choice due to its ease of preparation. For both benzylthio-Sepharose and MCT–Sepharose, our experience to date indicates that for the best results one needs to optimize the ligand concentration for each tissue and species examined.

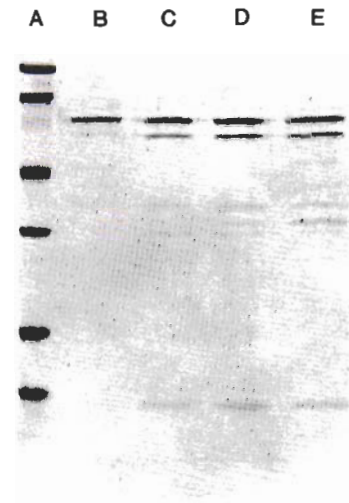


FIG. 5. SDS–PAGE of cytosolic epoxide hydrolase purified from livers of rhesus monkeys using MCT–Sepharose gels of varied ligand concentration. Lane A, molecular-weight standards; lane B, 5 μeq/g MCT–Sepharose; lane C, 7.5 μq/g MCT–Sepharose; lane D, 10 μeq/g MCT–Sepharose; lane E, 14 μeq/g MCT–Sepharose.

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