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EPOXIDE HYDRASE IN HUMAN LIVER BIOPSY SPECIMENS: ASSAY AND PROPERTIES

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Abstract—A sensitive assay for the determination of epoxide hydrase activity in needle biopsy specimens of human liver has been developed with [^{3}H]styrene oxide as substrate and was used for the study of some properties of human epoxide hydrase. Levels of epoxide hydrase in liver of man are 4·71 ± 0·41 nmoles styrene glycol/mg protein per min, comparable to guinea-pig (5·00 ± 0·38) rather than to Rhesus monkey (13·16 ± 0·88). Human hepatic epoxide hydrase which was found exclusively in the microsomal fraction, was solubilized with Cutscum, a neutral detergent, and purified. The enzyme was remarkably stable as long as it was particle-bound. Neither dialysable cofactors nor endogenous activators or inhibitors appeared to exist in the homogenate. The optimum pH for the purified enzyme was 9. The apparent K_m was 0·38 mM and the apparent V_{max} was 62·1 nmoles product/mg protein per min with respect to styrene oxide as the substrate. The product, styrene glycol, had no inhibitory effects. High (5–17 mM) concentrations of substrate, styrene oxide, markedly inhibited epoxide hydrase activity at low (0·2 mg/ml) but not at high (2 mg/ml) concentrations of protein, indicating that this inhibition may be due to the alkylating properties of the substrate, styrene oxide. Sulfhydryl reagents slightly but significantly inhibited the enzyme suggesting that no sulfhydryl group is essentially involved in the catalytic mechanism at the active site, but that sulfhydryl group(s) may be of importance for holding the enzyme molecule in the optimal conformation for maximal activity. Chelating agents, a carbonyl reagent and β-diethylaminoethyl diphenylpropylacetate (SKF 525-A) had no effect.

ARENE oxides† and alkene oxides† are now recognized as intermediates in the oxidative metabolism of many aromatic[1–6] and olefinic[7,8] compounds. These intermediates are reactive electrophiles and for several of them it has been established that they bind to tissue constituents such as protein, RNA and DNA.[9,10] Alkylation of such important molecules would be expected to produce disturbance of cell functions and, indeed, it has been reported that arene oxides are more potent carcinogens[11,12] and mutagens[13,14] than the parent hydrocarbons. Several alkene oxides have also been reported to be effective carcinogens[15,16] and mutagens.[17]

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† Compounds which formalistically result from the epoxidation of one of the double bonds of an aromatic nucleus are called arene oxides. Those resulting from the epoxidation of an olefinic double bond are called alkene oxides.[19]

Epoxide hydrases transform such epoxides to much less reactive vicinal diols (Ref. 18 and references therein). Levels and activity of these enzymes may therefore be of critical importance with respect to accumulation of such epoxides and thus with respect to mutagenicity and carcinogenicity of aromatic and olefinic compounds. Since previous studies on these enzymes have been performed with rodents (for a complete review see Ref. 19), it appeared of great interest to see whether this enzymic activity was also present in human and, if so, to investigate the properties of human epoxide hydrase(s). An assay was therefore developed which allows the determination of epoxide hydrase activity in 10–20 mg needle biopsy specimens of human liver. Using this assay, some properties of human epoxide hydrase have been investigated.

MATERIALS AND METHODS

Enzyme preparations. Needle biopsy specimens (10–20 mg) of human liver were immediately placed in 100 μl chilled isotonic (1·19 per cent) KCl containing 10 mM phosphate buffer (pH 7·4). The samples were maintained at 0–5° for as short a time as possible (<4 hr). The medium was then removed and replaced by 100 μl 10 mM phosphate buffer (pH 7·4) containing, except where stated, 1% w/w Cutscum, a neutral detergent (Fisher Scientific Company, Pittsburg, Pa.). The samples were then immediately frozen in Dry Ice/acetone and stored at −70° until used. After thawing the tissue was disintegrated in this medium by ultrasonication (MSE ultrasonic disintegrator) at 20 kHz for 5 sec. The homogenate was then centrifuged at 27,000 *g* for 15 min at 0–5° and the resulting clear supernatant was used for the assay of epoxide hydrase activity and protein content.

The biopsy specimens used in this study were from individuals who had not been treated with drugs known or suspected to induce microsomal drug metabolizing enzyme(s) for at least 2 weeks before the biopsy specimen was taken. Pathological examinations showed no gross lesions in any of the biopsy specimens used.

In order to establish the optimal conditions for the assay of epoxide hydrase activity in needle biopsy specimens of human liver and in order to compare this epoxide hydrase activity with other species, 10-mg samples were removed from the livers of animals and treated as described above for human biopsy specimens. Samples from Swiss white Webster mice (25–40 g), Sprague–Dawley rats (150–250 g), guinea-pigs (strain not determined) (300–500 g), Blau Wiener rabbits (2·5–3·0 kg) and Rhesus monkeys (6·3–6·5 kg) were used. All animals were male with the exception of one Rhesus monkey.

Microassay of epoxide hydrase activity. The substrate, [^{3}H]styrene oxide, was synthesized as described[20] but with a higher specific radioactivity (5·46 mCi/m-mole) in order to increase the sensitivity. Incubation was at non-saturating concentrations (0·2 mM) of the substrate which was dissolved in acetonitrile (10 μl per assay) and added to 70 μl of chilled 0·2 M Tris–HCl (pH 9·0) containing 0·04% w/w Tween 80. Ten or 20 μl of the clear liver extract were then added and the volume was made up to 100 μl with 10 mM phosphate buffer (pH 7·4) containing 1% Cutscum. The incubation was for 5 min at 37° and was terminated by two extractions of the substrate with 4 ml petroleum ether (b.p. 40–60°). Three hundred μl of distilled water were added prior to removal of the organic phase by aspiration. The product, styrene glycol was then extracted into 2 ml ethyl acetate. Three hundred μg of carrier styrene

glycol in 0·1 ml ethyl acetate were added to a 1·5 ml aliquot of the extract. The solution was concentrated under a stream of argon and the styrene glycol purified by thin-layer chromatography (silica gel 60 F254, Merck) with benzene–chloroform–ethyl acetate (1:1:1, v/v). The band corresponding to styrene glycol (R_f: 0·2) was visualized under u.v. light, removed, extracted with 1 ml methanol and assayed by liquid scintillatation spectrometry in 10 ml toluene containing 60 mg butyl PBD (Ciba-Geigy, Basel). Recovery of enzymatically prepared product, [^{3}H]styrene glycol, through the entire procedure was 78 ± 4% (n = 3). Values given in this paper have been corrected for recovery. Complete incubation mixtures with no enzyme served as blanks. These blanks were not significantly different (0·35 > P > 0·30) from boiled (100°, 10 min) enzyme blanks for all tissues studied. The protein concentrations were determined as described.[21] Bovine serum albumin was used as a standard in presence of the same medium which contained the enzyme preparation.

Subcellular fractionation, solubilization and purification of a human epoxide hydrase. All procedures were performed at 0–5°. Six needle biopsy specimens of human liver were pooled in 600 μl of isotonic (0·25 M) sucrose containing 10 mM phosphate buffer (pH 7·4) and homogenized in 1 ml glass–glass Potter–Elvejem tissue grinders. Homogenates were centrifuged at 600 g for 15 min to remove cell debris and nuclei. The resulting supernatant was then centrifuged at 8500 g for 15 min to obtain a mitochondrial pellet. The postmitochondrial supernatant was centrifuged at 100,000 g for 1 hr to obtain a microsomal pellet and a final supernatant of non-particulate cell constituents. Mitochondrial and microsomal pellets were resuspended in 400 μl 10 mM phosphate buffer (pH 7·4). Ten and 20 μl aliquots of these preparations were assayed for epoxide hydrase and glucose-6-phosphatase activity and for protein concentration. Cutscum (final concentration 1 per cent) was added prior to epoxide hydrase assay. The remainder of the microsomal suspension was diluted to 1 ml with 10 mM phosphate buffer (pH 7·4) containing Cutscum (final concentration 1 per cent), gently stirred for 15 min and then centrifuged at 100,000 g for 1 hr. The supernatant was passed through glasswool, thereby removing a whitish material from the preparation, and brought to a saturation of 25 per cent with respect to $(NH_4)_2SO_4$. This caused precipitation of the active protein which upon centrifugation at 27,000 g for 15 min rose to the surface. This latter portion was resuspended in 1 ml 10 mM phosphate buffer (pH 7·4) and passed through a small Sephadex G-200 column which had been equilibrated and was eluted with the same buffer. Active fractions were combined and concentrated by negative pressure dialysis to a final volume of approximately 0·8 ml (Table 3).

Statistics. The method of least squares was used to calculate regression lines and student's t-test for determination of the significance of differences between means.[22] P-values of 0·001 or less were considered to be significant. The measure of variation in this study is the standard error of the mean (S.E.M.).

RESULTS AND DISCUSSION

Assay

A simple radioassay for determination of epoxide hydrase activity has been previously developed.[20] This procedure is very rapid and the sensitivity is satisfactory as long as enough tissue with relatively high epoxide hydrase activity is available.

A slight modification[23] allowed detection of epoxide hydrase activity in organs where levels of this enzyme(s) are low compared to liver, providing that the amount of tissue available was not limited. Even the modified assay proved, however, to be too insensitive for the determination of epoxide hydrase activity in 10–20 mg needle biopsy specimens of human liver. A procedure has now been developed which easily allows with one such specimen duplicate epoxide hydrase assays at two protein concentrations and determination of the protein content.

Homogenization of a single 10–20 mg biopsy specimen in 100 μl of medium using either small glass–glass, Teflon–glass or plexiglass–glass Potter–Elvejem tissue grinders proved unsatisfactory in terms of reproducibility and recovery of enzyme activity probably due to incomplete and too foamy homogenization of such small volumes. Tissue disintegration by ultrasonication, however, gave quite reproducible results. Thus, the assay of eight 10-mg samples of guinea pig liver, each sonicated in 100 μl of 10 mM phosphate buffer (pH 7·4) gave a S.E.M. of <8 per cent.

The sensitivity of the reported assay[23] is limited by the blank. Using routine conditions (10 g guinea-pig liver homogenized in 30 ml medium), the blank can very easily be kept below 3 per cent as compared to the active sample (activity defined as counts/min above blank). However, the blank is more than 200 per cent (again compared to the active sample) if epoxide hydrase activity is determined by this method with a 10-mg liver biopsy specimen. Since the reported assay[23] already purifies the product extensively, the radioactivity in the blank appears to result mainly from nonenzymatic formation of styrene glycol. Effort was therefore directed to finding conditions leading to a more favorable sample/blank ratio.

Table 1 shows that the medium used during the disintegration procedure with sonication is of great importance in terms of sensitivity of the assay. Thus, a medium consisting of 1% Cutscum and 10 mM phosphate buffer resulted in a sample/blank ratio which was about three times higher than with phosphate buffer alone. Relatively large standard errors (Table 1 and 2) are due to the fact that they refer to individual preparations of 10-mg liver samples. Table 2 shows that the sensitivity of the assay increases at non-saturating concentrations of the substrate (the apparent

TABLE 1. EFFECT OF VARIOUS HOMOGENIZATION MEDIA ON THE SENSITIVITY OF AN EPOXIDE HYDRASE MICROASSAY WITH [^{3}H]STYRENE OXIDE AS SUBSTRATE*

Medium		Activity over blank (%)
Dilute buffer:	10 mM Tris–HCl pH 7·4	42 ± 7
	10 mM phosphate pH 7·4	45 ± 6
Isotonic media:	0·15 M KCl†	67 ± 7
	0·25 M sucrose†	69 ± 6
Detergents:	1% Nonidet P40†	85 ± 8
	1% Triton X-100†	94 ± 8
	1% Cutscum†	131 ± 11

* Ten mg fresh guinea-pig liver were disintegrated in 100 μl medium by ultrasonication. Twenty μl of this preparation were incubated at 37° for 5 min with 2·2 mM [^{3}H]styrene oxide as substrate. Assay as described in Methods. Values are means ± S.E.M. from three preparations. Deviation from ideal linearity with respect to protein concentration was <10 per cent in all media.

† Containing 10 mM phosphate buffer (pH 7·4).

TABLE 2. EFFECT OF SUBSTRATE ([^{3}H]STYRENE OXIDE) CONCENTRATION ON THE SENSITIVITY OF AN EPOXIDE HYDRASE MICROASSAY*

Substrate concentration (mM)	Activity over blank (%)
2·2	137 ± 12
0·8	181 ± 16
0·4	358 ± 41
0·2	561 ± 48
0·1	422 ± 51
0·05	348 ± 52

* Ten mg fresh guinea-pig liver in 100 μl 10 mM phosphate buffer (pH 7·4) containing 1% Cutscum were disintegrated by ultrasonication. Twenty μl of this preparation were incubated at 37° for 5 min. Assay as described in Methods. Values are means ± S.E.M. from three preparations. Deviation from ideal linearity with respect to the protein concentration was <10 per cent at substrate concentrations between 0·2 and 2·2 mM.

K_M for styrene oxide was, in a purified epoxide hydrase preparation from guinea-pig liver approx. 0·5 mM[24]). Thus, the sensitivity at a substrate concentration of 0·2 mM was increased by more than 300 per cent as compared to that at a substrate concentration of 2·2 mM, although enzymatic product formation was considerably lower under these non-saturating conditions (Fig. 4). However, the decrease in nonenzymatic product formation was even more pronounced under the latter conditions leading to a higher sample/blank ratio. The data in Table 2 refer to preparations from guinea-pig liver in a medium containing Cutscum. Very similar increases in sensitivity were obtained with rat liver and with isotonic KCl containing no Cutscum (data not stated). Variation of the incubation temperature influenced enzymatic product formation and blank in an almost parallel fashion. Thus, a broad optimum for the sample/blank ratio was observed from 27 to 37° with rat and from 32 to 47° with guinea-pig liver preparations which were disintegrated by ultrasonication in the presence of a detergent, cutscum (data not stated). Extending the incubation time from 5 to 30 min resulted in very little increase of the sample/blank ratio (21 ± 2%, $n = 3$), whereas the linearity with respect to protein concentration was more satisfactory (deviation from ideal linearity <10 per cent) if the incubation time was 5 min only.

Thus, at conditions where conversion to product glycol was linear with respect to protein concentration, a total increase in sensitivity by a factor of about 13 was achieved. This sensitivity is of great importance where the biological material is limited, such as in studies with human needle biopsy specimens and should also prove useful for the study of epoxide hydrases in tissues or organisms where levels of these enzymes are very low. Values (product formation/mg protein/min) obtained with this more sensitive assay are slightly different, namely 28 ± 4 per cent higher, than values obtained with the same enzyme preparation using the previous more rapid but less sensitive assay.[20] This is due to the fact that the presence of the detergent, Cutscum, in the incubation medium increases the specific activity by 91 ± 4

per cent, while the lower substrate concentration decreases the specific activity by 33 ± 2 per cent.

Properties of human hepatic epoxide hydrase(s)

Stability. For technical reasons, human liver biopsy specimens were stored at 0–5 in isotonic KCl before they were frozen and kept at −70°. Storage at 0–5 never exceeded 4 hr. No loss of activity was observed with 10-mg pieces of guinea-pig and rat liver ($0{\cdot}40 > P > 0{\cdot}35$ and $0{\cdot}20 > P > 0{\cdot}15$ respectively; $n = 3$ animals for each species) which had been stored under the above conditions for 24 hr. One human specimen which was obtained 10 min after biopsy was divided into two portions, one of which was assayed immediately and the other after 18 hr at 0–5°. Activity was not significantly ($0{\cdot}25 > P > 0{\cdot}20$) different in the two portions. Another human biopsy specimen was divided into two portions, one for immediate assay, the other for assay after 6 weeks at −70°. The activity of the latter was only slightly (7 per cent) decreased as compared to the former. After heating at 60° for 5 min 61 ± 7 per cent ($n = 2$ biopsies) of the initial activity were still present, whereas boiling (10 min) fully destroyed the enzymatic activity.

Epoxide hydrase activity in human liver as compared to other species. Figure 1 shows that levels of human hepatic epoxide hydrases are nearer to those of rodents, in particular guinea-pig, than to those of Rhesus monkey. For some of the species presented here the results are slightly different from those reported previously[20] due to differences in the assay procedure as discussed above. No significant ($0{\cdot}2 > P > 0{\cdot}1$) differences in epoxide hydrase activity were observed between sexes in human. In the case of Rhesus monkeys, the levels obtained with one female were not different from those of males. In the other species only males were used.

Subcellular distribution. Epoxide hydrase activity was found predominantly in the microsomal fraction if homogenization was performed in isotonic non-ionic (sucrose) medium using Potter–Elvejem tissue grinders. Moreover, the portion of the total epoxide hydrase activity associated with the mitochondrial and with the non-particulate fraction (Fig. 2) was even smaller than that of glucose-6-phosphatase, a microsomal marker enzyme.[25] Human hepatic epoxide hydrase, therefore, appears to be localized exclusively in the endoplasmic reticulum.

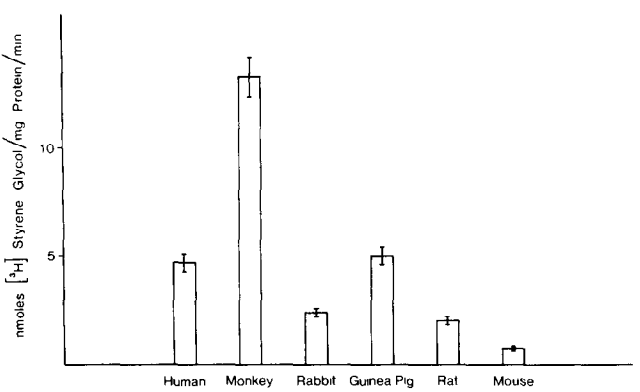

FIG. 1. Epoxide hydrase activity in human liver as compared to other species. Samples were from four individuals for each species except for Rhesus monkey (only three individuals but six preparations). Values represent means ± S.E.M.

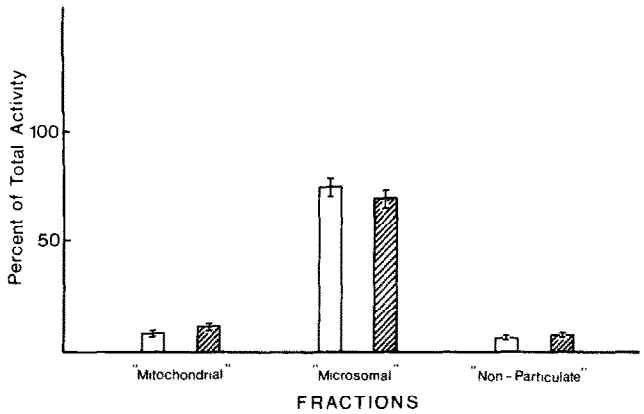

FIG. 2. Epoxide hydrase (□) and glucose-6-phosphatase (▨) activity in subcellular fractions of homogenates from human liver biopsy specimens. Glucose-6-phosphatase activity was assayed as described[30] and the liberated phosphate determined by the method of Lowry.[31] Epoxide hydrase activity was assayed as described in Methods. Values represent means ± S.E.M. ($n = 3$). The activities in the 600 g supernatant are referred to as total activities, which were for epoxide hydrase 0·620 ± 0·047 nmoles styrene glycol/mg liver per min and for glucose-6-phosphatase 0·125 ± 0·019 nmoles phosphate/mg liver per min. Recoveries were 89 ± 6·8 per cent for epoxide hydrase and 88 ± 5·9 per cent for glucose-6-phosphatase.

pH Optimum. In the crude homogenate, the enzyme displays a very broad pH profile with its optimum between 7 and 9. After solubilizing the enzyme from the microsomal membranes and further purification mainly by $(NH_4)_2SO_4$ precipitation and Sephadex G-200 chromatography (Table 3), a sharp pH profile with a clear optimum at 9 was observed (Fig. 3). Nonenzymatic hydration was <5 per cent at pH values between 6 and 11.

Independence of cofactors and absence of endogenous inhibitors. Table 4 shows for homogenate (see footnote*) and solubilized preparations (see footnote † and ‡) that metal ions were not required for maximal activity. Thus, repeated dialysis, Sephadex G-25 chromatography or chelating agents had no significant effects ($P > 0·1$ in all cases) on epoxide hydrase activity. The fact that dialysis and Sephadex G-25 chromatography did not alter enzyme activity also means that the presence of endogenous low molecular weight activators or inhibitors is unlikely. Moreover, if a

TABLE 3. PURIFICATION OF AN EPOXIDE HYDRASE FROM HUMAN LIVER BIOPSY SPECIMENS

Fraction	Protein recovery (%)	Activity recovery (%)	Sp. act.*	Purification factor
1 Homogenate	100	100	1·83	1
2 Microsomal fraction after detergent (Cutscum) treatment	19·3	137	13·0	7·1
3 High speed supernatant after removal of top layer	8·0	125	28·6	15·6
4 Precipitation by $(NH_4)_2SO_4$ and Sephadex G-200 chromatography	0·43	14·3	60·8	33·2

* Assay of epoxide hydrase activity as described in Methods, except that the activity in the homogenate was determined in the absence of Cutscum. Specific activity expressed as nmoles [^{3}H]styrene glycol/mg protein/min.

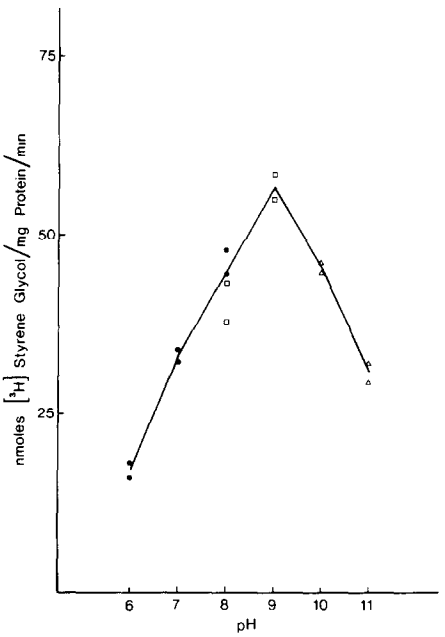

FIG. 3. Activity of a purified human hepatic epoxide hydrase as a function of pH in 0·35 M phosphate (●), Tris (□) or glycine (△) buffer. Values represent individual determinations.

"complete" preparation of a biopsy specimen which had been disintegrated by ultrasonication in 10 mM phosphate buffer containing 1% Cutscum was mixed in different proportions with the purified enzyme (preparation 4 of Table 3) the resulting activity was always additive. Thus, the increase in specific activity during purification (Table 3) does not appear to be due to the removal of an inhibitor present in the original homogenate.

TABLE 4. EFFECT OF VARIOUS TREATMENTS AND POTENTIAL INHIBITORS ON HUMAN EPOXIDE HYDRASE

Treatment	Activity (% of controls)
Dialysis*	98 ± 4·7
Sephadex G-25†	94 ± 7·1
EDTA (5 mM)‡	90 ± 7·0
α,α′-Dipyridyl (5 mM)‡	101 ± 4·1
N-Ethylmaleimide (1 mM)‡	79 ± 3·6§
p-Chloromercuribenzoate (1 mM)‡	76 ± 2·0§
Iodoacetamide (1 mM)‡	87 ± 3·5§
Hydroxylamine (0·01–1 mM)‡	101 ± 2·7
β-Diethylaminoethyl diphenylpropylacetate (SKF 525-A) (0·2–2 mM)‡	96 ± 7·2
Styrene glycol (2 mM)‡	95 ± 3·1

* Whole homogenate was dialyzed (2 × 12 hr) against 1000 vol of 10 mM phosphate buffer (pH 7·4).

† Solubilized epoxide hydrase (fraction 3 of Table 3) was used. A portion of this fraction which was kept for the same length of time at the same temperature served as a control.

‡ Purified epoxide hydrase (fraction 4 of Table 3) was used. Agents, except styrene glycol and SKF 525-A, were preincubated with enzyme for 5 min before addition of substrate.

§ Significantly ($P < 0.001$) different from controls.

K_m *and* V_{max}. A Lineweaver–Burk analysis[26] of [^{3}H]styrene glycol formation with the purified human hepatic epoxide hydrase was performed using 0·1–0·5 mM [^{3}H]styrene oxide as substrate at a protein concentration of 0·24 mg/ml. An apparent K_m of 0·38 mM and an apparent V_{max} of 62·1 nmoles product/min/mg protein was obtained (correlation coefficient 0·977). The K_m (0·42 mM, correlation coefficient 0·969), determined with an epoxide hydrase preparation which had been purified from guinea-pig liver using the same procedure as described in Methods for the human enzyme, was not significantly different from the K_m of the preparation from human liver ($0·2 > P > 0·1$).

Effect of high concentrations of substrate, of product diol and of carbonyl and sulfhydryl reagents. High (5–17 mM) concentrations of substrate (styrene oxide) inhibited the activity of the purified epoxide hydrase preparation by 20–50 per cent at a protein concentration of 0·24 mg/ml. Increasing the protein concentration by addition of bovine serum albumin gradually alleviated and finally prevented this inhibition (Fig. 4). This may indicate that the inhibition is due to the reaction of the electrophilically reactive epoxide substrate with nucleophilic moieties of the enzyme, since this effect would be expected to be less pronounced at higher protein concentrations. Styrene oxide is, in fact, an active alkylating agent.[27] Styrene glycol, the hydration product of styrene oxide, did not inhibit the enzymatic hydration of the latter. Sulfhydryl reagents and a non-specific alkylating reagent, iodoacetamide, slightly but significantly inhibited the enzymatic activity (Table 4), indicating that sulfhydryl groups may have some importance in holding the enzyme protein in a conformation which is more favorable for its catalytic activity but that either no sulfhydryl group or a relatively inaccessible group is of importance in the active site of the enzyme. A carbonyl reagent, hydroxylamine did not exert any influence on the activity of the enzyme β-diethylaminoethyl diphenylpropylacetate (SKF 525-A), which potently inhibits several reactions catalyzed by microsomal drug metabolizing enzymes,[28] had no effect on epoxide hydrase activity (Table 4). Epoxide hydrase preparations, which

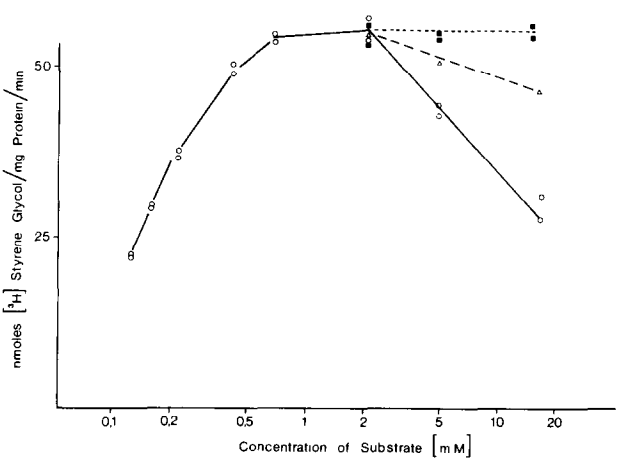

FIG. 4. Enzymatic hydration of styrene oxide with a purified human hepatic epoxide hydrase as a function of substrate concentration at protein concentrations of 0·24 mg/ml (○) 0·50 mg/ml (△) or 2 mg/ml (■). Epoxide hydrase concentration was the same in all experiments. Higher protein concentrations were achieved by addition of bovine serum albumin. Values represent individual determinations.

had been purified from rat and guinea-pig liver under identical conditions as described in Methods for the human enzyme, behaved similarly with respect to carbonyl and sulfhydryl reagents, product diol and high concentrations of substrate.*

CONCLUSION

A microassay has been developed which allows determination of epoxide hydrase activity in 10–20 mg needle biopsy specimens of human liver. The procedure is based on an existing radioassay of this enzyme using [^{3}H]styrene oxide as substrate but the sensitivity is improved by a factor of at least 13 when compared to the most sensitive modification of the reported assay.[23] In previous studies it had been shown with a purified epoxide hydrase from guinea-pig liver that the enzyme which is responsible for the hydration of this substrate, styrene oxide, also hydrates a large variety of other alkene and arene oxides.[18,29]

The microassay now allowed the demonstration of the existence of a human hepatic epoxide hydrase and the investigation of some properties of this enzyme(s) in needle biopsy specimens. The enzyme(s) is very stable as long as it is particle-bound. No low molecular weight cofactor is required for maximal activity and no endogenous activator or inhibitor appears to be present in the homogenate. The levels of human hepatic epoxide hydrase are similar to or greater than that found in rodents but markedly lower than the levels in Rhesus monkeys. With the aid of this sensitive assay it was possible to follow subcellular fractionation, solubilization and purification of this enzyme from as little as six pooled needle biopsy specimens. The enzyme appears to be exclusively localized in the endoplasmic reticulum. Epoxide hydrase, in the purified preparation, has a pH optimum of 9 and a K_m of 0·38 mM and a V'_{max} of 62·1 nmoles product/mg protein per min with styrene oxide as substrate. The product, styrene glycol, does not inhibit the enzyme, nor does β-diethylaminoethyl diphenylpropylacetate (SKF 525-A), an inhibitor of several biotransformations of foreign compounds mediated by microsomal enzymes. Sulfhydryl reagents, on the other hand, slightly but significantly inhibit the enzyme. These properties are quite similar to the properties of an epoxide hydrase which had been purified from guinea-pig liver.[24] Establishment of similarities between human and rodent epoxide hydrase appears to be especially important in view of current attempts to develop *in vivo* activators or inducers of this enzyme in rodents. Several properties of the enzyme which had not previously been studied in animals, such as additivity of enzyme activity after combining crude homogenates with the purified preparation, influence of alkylating or carbonyl reagents on enzyme activity, inhibition of the enzyme by high concentrations of the substrate, styrene oxide, and protection from this inhibition by addition of bovine serum albumin, were also found to be similar in human and rodent (rat and guinea-pig) epoxide hydrase. Thus, studies of epoxide hydrase from the guinea-pig or the rat appear to have relevance also with respect to the human enzyme.

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* F. Oesch, unpublished results.

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