Biochemical Pharmacology, Vol. 35, No. 2, pp. 231–235, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

SUBCELLULAR LOCALIZATION OF EPOXIDE HYDROLASE IN MOUSE LIVER AND KIDNEY

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(*Received* 11 *April* 1985; *accepted* 18 *July* 1985)

Abstract—The subcellular distribution of epoxide hydrolase activity towards TSO and HEOM in mouse liver and kidney was investigated using zonal rotor centrifugation. Epoxide hydrolase activity towards TSO was found predominantly in the soluble fraction with peroxisomes accounting for activity in the particulate fractions. Renal particulate activity towards HEOM was found predominantly in the microsomes.

Epoxide hydrolases (EC 3.2.2) catalyse the hydration, to *trans* dihydrodiols, of a wide range of arene and alkene oxides, many of which can be generated within the cell by the action of microsomal monooxygenases [1]. Many carcinogens and mutagens (e.g. benzo(*a*)pyrene and aflatoxin B) are activated by conversion to epoxides.

In vertebrates epoxide hydrolase activity has been found in a variety of tissues [2] and has been associated with both microsomal [1] and cytosolic [3] subcellular fractions. The microsomal and cytosolic fractions appear to contain different forms of the enzyme, on the grounds that they contrast markedly in their activities towards certain substrates [4–7] and appear to be immunologically different [7]. *Trans*-stilbene oxide (TSO)‖ was found to be a very good substrate for the cytosolic enzyme but a poor substrate for the microsomal enzyme [8]. Waechter *et al.* [9] found activity towards TSO in mouse hepatic peroxisomes. In the present study we confirm this and report the subcellular distribution of epoxide hydrolase activity in mouse kidney using the substrates TSO and HEOM (1,2,3,4,9,9-hexachloro-6,7-epoxy,1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4-methanonaphthalene).

MATERIALS AND METHODS

Chemicals. (^{14}C)-*trans*-stilbene oxide was a gift from C. Timms (Institut für Toxikologie, University of Mainz). HEOM was prepared as previously described [10]. *n*-Dodecane, 1-naphthylacetate, 1-naphthylphosphate, sodium pyrophosphate and palmitoyl-CoA were purchased from Sigma (London) Chemical Company (Poole, U.K.), hydrogen peroxide from BDH (Poole, U.K.), pyridine, hexamethyldisilazane, trimethylchlorosilane from Koch-Light Ltd. (Colbrook, Bucks, U.K.) and fluorochemical FC-43 from 3M Chemical Company (Bracknell, U.K.). All other reagents were of analytical grade.

Animals. Adult male albino mice, MF1 strain, were used in this study.

Sub-cellular fractionation. Preparation of post-nuclear supernatants (PNS) from kidneys and from livers of albino mice, treatment of PNS with pyrophosphate, and rate and density dependent banding in sucrose gradients in a BXIV zonal rotor were performed as previously described [11]. Homogenization was in 3 mm imidazole buffer pH 7.2 containing 10% (w/w) sucrose. Following the preparation of PNS, the supernatants were treated with 100 mm pyrophosphate pH 8.2 to give a final concentration of 6–7 mm pyrophosphate in the PNS [12]. Treatment with pyrophosphate specifically lowers the density of endoplasmic reticulum by removing loosely bound proteins and ribosomes.

Pyrophosphate treated PNS was loaded onto a 500 ml linear sucrose gradient of 15–32% (w/w) resting on a cushion of 60% (w/w) sucrose in a BXIV zonal rotor. An overlay of 7% (w/w) sucrose was added so that sample and overlay measured 40 ml. Centrifugation was for 8 min at 16,000 rpm, 4°, and the gradient was unloaded with 60% (w/w) sucrose. Peroxisome enriched fractions prepared from PNS by rate dependent banding were loaded onto a stepped gradient comprising 5 ml each 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56 and 59% (w/w) sucrose resting on a bed of 60% (w/w) sucrose in a BXIV zonal rotor. Centrifugation was for 4 hr at 23,000 rpm, 4°, and the gradient unloaded with fluorochemical FC-43 (density 1.9 g/ml). The high density portion of the gradient was divided into 4 ml fractions; the lower density region into larger fractions.

Assays. The following marker enzymes were assayed according to the procedures described in the accompanying references: non-specific esterase, acid and alkaline phosphatase, using 1-naphthyl-phosphate as substrate, catalase, palmitoyl-CoA oxidase and succinate dehydrogenase [13], protein [14] and glucose-6-phosphate dehydrogenase [15].

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‖ Abbreviations used: TSO, *trans*-stilbene oxide; HEOM, (1,2,3,4,9,9-hexachloro-6,7,-epoxy-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4-methanonaphthalene); PNS, post nuclear supernatant.

Epoxide hydrolase activity towards TSO was measured according to the method of Gill *et al.* [8] using ^{14}C labelled TSO as substrate. Epoxide hydrolase activity towards HEOM was assayed using the method of Craven *et al.* [10], the *trans*-diol product being determined by GLC analysis of the trimethyl-silane derivative.

Presentation of fractionation results. The distribution of enzymes between subcellular fractions is given in the form of frequency histograms following the practice of de Duve [16].

RESULTS

The subcellular markers used in this study were: catalase and palmitoyl-CoA oxidase for peroxisomes, non-specific esterase for microsomes, succinate dehydrogenase for mitochrondria, acid phosphatase for lysosomes, alkaline phosphatase for plasma membrane (liver) or brush border (kidney) and glucose-6-phosphate dehydrogenase (NADP dependent) for soluble fraction.

Rate-dependent banding was used to prepare a peroxisome enriched fraction from kidney PNS, the results of which are presented in Fig. 1. This peroxisome fraction (labelled P in Fig. 1) had respectively 63% and 21% of the catalase and esterase in the PNS but less than 3% of the glucose-6-phosphate dehydrogenase and was associated with 10% of PNS protein and 13% of TSO-directed epoxide hydrolase. Figure 2 shows the distribution of markers obtained by density dependent banding of this peroxisome

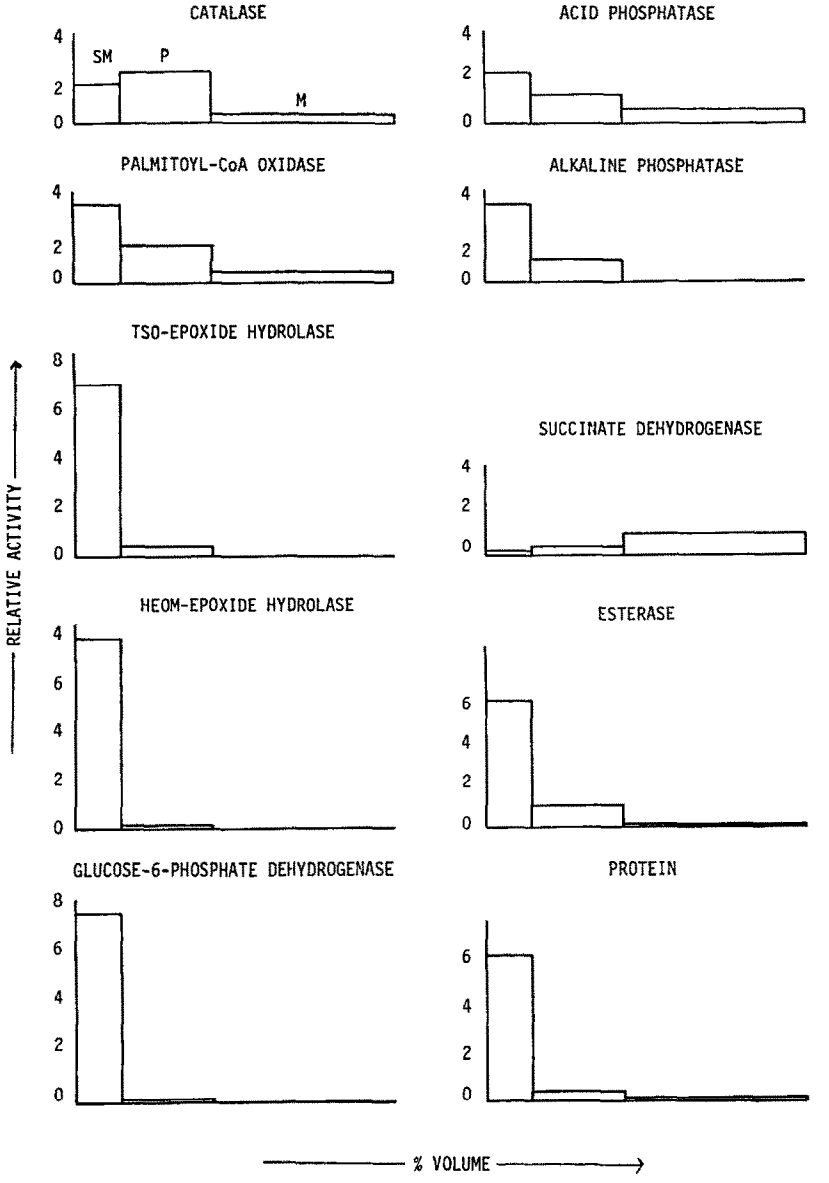

Fig. 1. Rate-dependent banding in sucrose of kidney PNS. Experimental conditions are described in Materials and Methods: SM, soluble and microsomal fraction; P, peroxisome-enriched fraction; M, mitochondrial fraction.

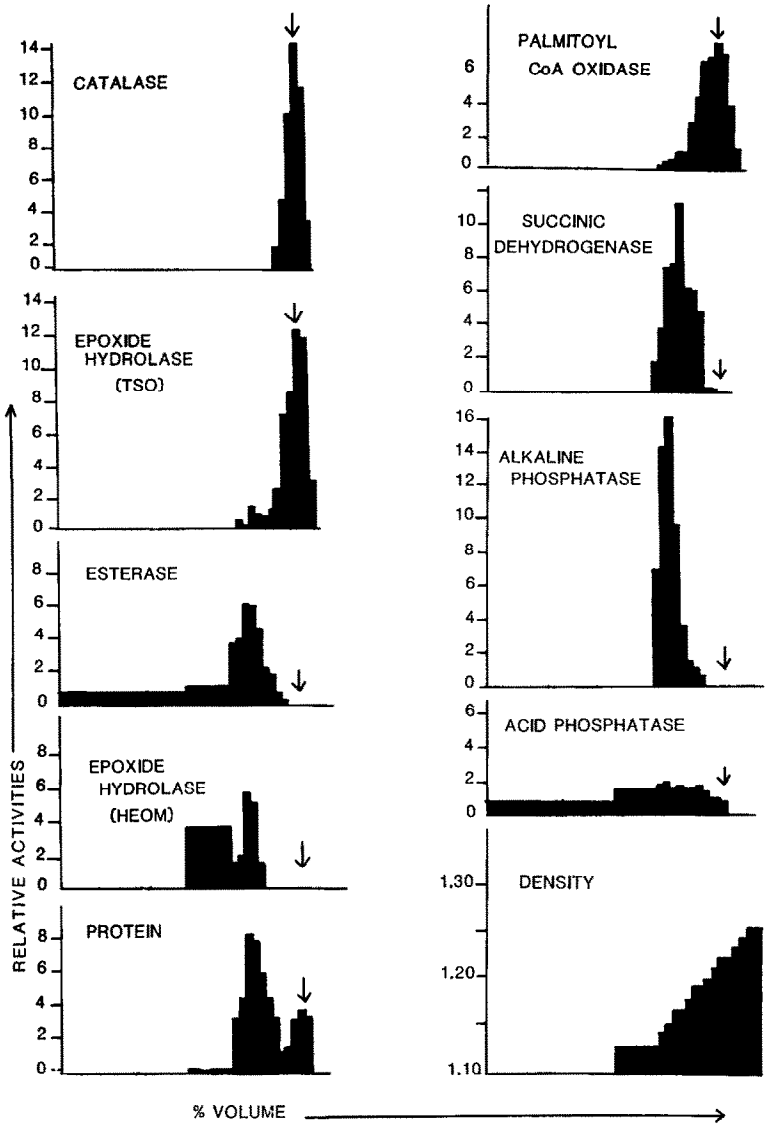

Fig. 2. Density-dependent banding in sucrose of a peroxisome enriched fraction prepared by rate-dependent banding of kidney PNS. Experimental conditions are described in Materials and Methods. Enzyme recoveries from this gradient were as follows: catalase 92%, esterase 90%, protein 70%, palmitoyl CoA oxidase 105%, succinic dehydrogenase 87%, alkaline phosphatase 93%, acid phosphatase 62%, epoxide hydrolase TSO substrate 113%, epoxide hydrolase HEOM substrate 104%. Arrow indicates peak peroxisomal (catalase) fraction.

enriched fraction. Using catalase and palmitoyl-CoA oxidase as markers, peroxisomes are well separated from all other organelles. The particulate TSO-directed epoxide hydrolase activity appears to be exclusively localized in the peroxisomes with none associated with microsomes or mitochondria. In contrast the epoxide hydrolase which metabolizes HEOM is not associated with peroxisomes and most appears to be microsomal as is the case with rat liver homogenates [10].

Leakage of catalase from organelle preparations in sucrose solutions has been reported [17]; it is, however, a moot point as to how much of the non-sedimentable catalase of homogenates arises from leakage from damaged peroxisomes and how much is simply cytosolic catalase. Some workers favour the idea that in rodents catalase is exclusively peroxisomal and any found in the soluble fraction is due to leakage during the purification procedure [18]. Recently it has been reported from electron micrograph studies of guinea-pig liver that some catalase is cytosolic [19] and it has been postulated that catalase is transported from peroxisomes to the cytosol where it is destroyed. The situation in mouse kidney is at present unknown. If it is assumed that all catalase in mouse kidney is peroxisomal, and that peroxisomes damaged during homogenization lose catalase and epoxide hydrolase activities to the same extent, then our results indicate that approximately 80% of the TSO-directed epoxide hydrolase is in the soluble

fraction (and remains with glucose-6-phosphate dehyrogenase in the sample band of the rate-dependent gradient—Fig. 1) and the remaining 20% activity is associated with the peroxisomes. If the same assumptions are made for palmitoyl-CoA oxidase activity as for catalase then 70% of the TSO-directed epoxide hydrolase is in the soluble fraction and 30% is associated with peroxisomes.

Mouse liver was examined by the same density gradient techniques as those described for kidney. Again most of the TSO-directed epoxide hydrolase was in the soluble fraction with particulate activity localised exclusively in the peroxisomes (Fig. 3). The specific activity of TSO-directed epoxide hydrolase in the peak peroxisomal fraction from liver (30 ± 2.7 nmol/mg protein per min) was nearly twice that found for kidney (19.6 ± 1.8 nmol/mg protein per min).

DISCUSSION

TSO has been used to differentiate between microsomal and "cytosolic" forms of epoxide hydrolase, the epoxide being a substrate predominantly for the cytosolic forms of the enzyme [8]. Previous workers have reported that in the liver the epoxide hydrolase activity toward TSO is located in peroxisomes as well as cytosol [9]. We have confirmed this observation and found that this is also the case with mouse kidney. Virtually no activity towards this substrate was found in microsomes of either mouse liver or kidney. In studies upon microsomal epoxide hydrolases of mouse liver, Guenthner and Oesch [20] found a form of microsomal epoxide hydrolase which was capable of hydrating TSO. This enzyme was catalytically and immunologically distinct from both cytosolic epoxide hydrolase and a previously known

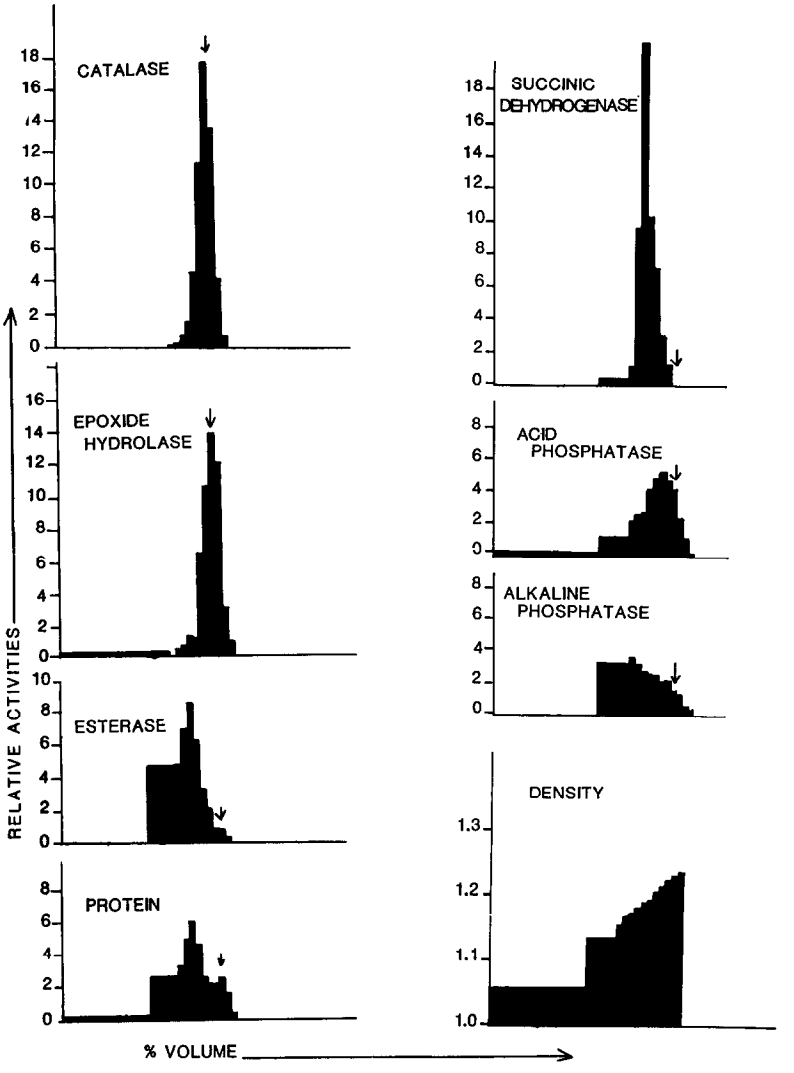

Fig. 3. Density-dependent banding in sucrose of a peroxisome-enriched fraction prepared by rate-dependent banding of liver PNS. Experimental conditions are described in Materials and Methods. Enzyme recoveries from this gradient were as follows: catalase 111%, esterase 84%, protein 90%, succinic dehydrogenase 85%, acid phosphatase 87%, alkaline phosphatase 79%, epoxide hydrolase (substrate–TSO) 65%. Arrow indicates peak peroxisomal (catalase) fraction.

microsomal form which only poorly hydrates TSO. The reason why no microsomal activity toward TSO was found in this study is unclear; however this could be due to a strain difference between the mice used in the two studies or the loss of this enzyme activity on storage of the microsomal samples used in this study.

The renal peroxisomal epoxide hydrolase does not act on the epoxide HEOM to any significant degree. Particulate HEOM epoxide hydrolase appears to be associated predominantly with microsomes.

Our work indicates the presence of multiple forms of epoxide hydrolase in mouse kidney, therefore mirroring the situation in liver where a multiplicity of forms have been found [7, 21]. From the studies reported here it would appear that a large proportion of the epoxide hydrolase activity towards TSO (~80%) is in the cytosol.

The toxicological significance of peroxisomal epoxide hydrolase is at present unknown. Microsomal epoxide hydrolases are located in the membrane where the monooxygenase system is also located and therefore is in an ideal position to detoxify epoxides produced by the monooxygenase system. However, no monooxygenase system has yet been identified in peroxisomes. We have observed that mouse kidney peroxisomal epoxide hydrolase activity is not inhibited by crotonyl-CoA, the substrate for peroxisomal enoyl-CoA hydratase, and therefore the two enzymes are presumably different. One possible role for peroxisomal epoxide hydrolase has been suggested; the hydration of cholesterol epoxide [22] in a possible peroxisomal involvement in cholesterol metabolism.

Acknowledgements—The authors wish to thank Mr C. H. Walker for helpful discussion during the preparation of this manuscript and Mr S. D. Hallam for technical assistance. The work was supported by a grant from the National Kidney Research Fund.

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