Epoxide hydrolase activity in isolated peroxisomes of mouse liver

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Using *trans*-stilbene oxide as substrate, the subcellular distribution of epoxide hydrolase was investigated in livers from DBA/2 mice. The highest specific activities were found in cytosolic and light mitochondrial fractions. Isopycnic subfractionation of the light mitochondrial fraction showed that the organelle-bound *trans*-stilbene oxide hydrolase is localized in peroxisomes.

Epoxide hydrolase *Peroxisome* *Subcellular distribution*

1. INTRODUCTION

Epoxide hydrolases (EC 3.3.2.3) catalyze the hydration of arene and alkene oxides to *trans*-dihydrodiols. Activity has been found in different subcellular fractions including the cytosol [1–4] and seems to vary with the substrate used. However, it is apparent that two different enzymes with different structural and immunological properties are found in liver cytosolic and microsomal fractions [1,5,6]. Using *trans*-β-ethylstyrene oxide or *trans*-stilbene oxide (TSO) as substrates, authors in [4] recently demonstrated significant epoxide hydrolase activity in mouse liver heavy and light mitochondrial fractions. This enzyme was reported to have properties similar to those of the cytosolic enzyme [4]. From additional experiments, the authors concluded that the observed hydrolase activity is localized in mitochondria. Using a recently developed method for the isolation of peroxisomes by means of metrizamide gradient centrifugation (in preparation), we present evidence that the organelle-bound TSO-hydrolase activity is localized in peroxisomes.

Abbreviation: TSO, *trans*-stilbene oxide

2. MATERIALS AND METHODS

2.1. *Materials*

Metrizamide (Nyegaard, Oslo) was obtained from Dr Molter GmbH (Heidelberg). Cytochrome *c* (grade III), *o*-nitrophenyl acetate, *p*-nitrophenyl-β-glucuronide and bovine serum albumin (fraction V) were purchased from Sigma GmbH (München). Titanium oxysulfate was obtained from Riedel-de Haen (Seelze) and glycolic acid from Merck AG (Darmstadt). All other chemicals were also of the purest analytical grade available.

2.2. *Preparative procedures*

Male DBA/2 mice (15–21 g) were obtained from GR Bomholdgård (Ry, Denmark). They were fasted overnight and livers were perfused with physiological saline via the portal vein, prior to killing the animals. The livers were pooled, weighed, minced with scissors and homogenized with 0.25 M buffered sucrose. Homogenization was carried out in a Potter-Elvehjem homogenizer, cooled in an ice-water bath.

The filtered homogenate was fractionated as in [7] into a heavy (B) and light (D) mitochondrial fraction, a microsomal fraction (G) and a final

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supernatant (F) using a Beckman-rotor Typ JA-20.

The light mitochondrial fraction D was further subfractionated by isopycnic centrifugation in the Beckman VTi50 vertical rotor using a continuous metrizamide gradient.

2.3. *Biochemical analyses*

Epoxide hydrolase activity was measured with [^{14}C]TSO as substrate (provided by Dr W. Küng, CIBA-GEIGY), as in [8]. Thin-layer radiochromatography of ethyl acetate extracts obtained following the partition assay were performed to identify [^{14}C]erythro-1,2,-dihydroxy-1,2-diphenylethane as the reaction product [8]. Cytochrome *c* oxidase [9], esterase [10], β-glucuronidase [11], catalase [12] and α-hydroxy acid oxidase [12] were assayed according to the published procedures. With the exception of catalase and cytochrome *c* oxidase, which are defined in [12] and [9], respectively, enzyme activities are given in units: 1 U $\simeq$ 1 μmol product formed/min. Protein concentrations were estimated as in [13] using bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

The distribution of the enzyme activities in the initial fractions B, D, G and F is shown in fig.1. The activity of cytochrome *c* oxidase, a mitochondrial marker enzyme, was predominantly found in fraction B (75%), although the highest specific activity of this enzyme was detected in fraction D which contained only 14% of the total activity. Esterase, an endoplasmic reticulum marker enzyme, was mainly recovered in the microsomal fraction G (75%). Only low levels of these two enzymes were found in the cytosolic fraction F. The peroxisomal marker enzymes catalase and α-hydroxy acid oxidase, as well as the lysosomal marker enzyme β-glucuronidase, were more or less evenly distributed over the 4 fractions B, D, G and F, whereby these enzymes exhibited the highest specific activity in fraction D. These findings correspond well with the observations in [7], in which

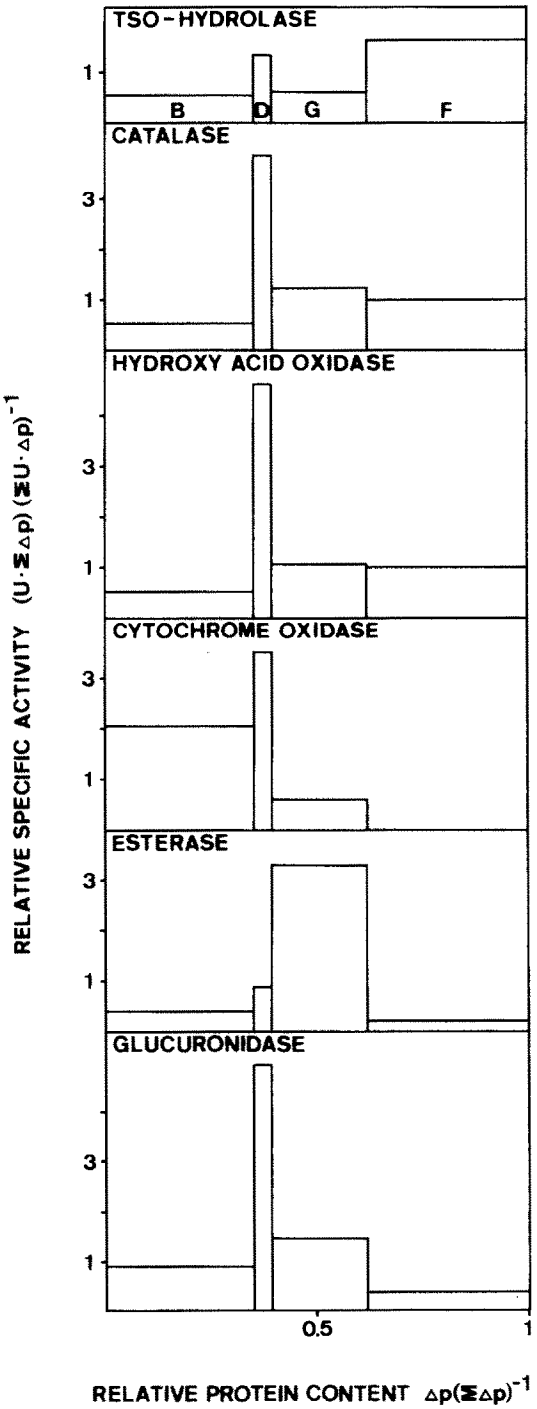

Fig.1. Distribution of TSO-hydrolase and marker enzyme activities in the initial fractions B, D, G and F. Abbreviations: U, total units of an enzyme found in a fraction; ΣU, total units found in all fractions: Δp, total protein content of a fraction; $\Sigma\Delta$p, total protein content of all fractions. ΣU/g liver were: TSO-hydrolase, 0.123; catalase, 77; α-hydroxy acid oxidase, 709; cytochrome *c* oxidase, 27; esterase, 334; β-glucuronidase, 1.5. $\Sigma\Delta$p/g liver was 110 mg.

Triton WR-1339-treated rats and a similar fractionation procedure were used, albeit without routinely separating microsomal and cytosolic fractions (G and F).

Epoxide hydrolase activity was measured with TSO which is known to be a substrate for the cytosolic but not for the microsomal hydrolase [8]. As expected, more than half of the total TSO-hydrolase activity (62%) was recovered in the cytosolic fraction F (spec. act. 1.83 units/mg protein); 38% of the TSO-hydrolase activity was distributed among the organelle fractions B, D and G, whereby the highest specific activity was observed in fraction D (1.49 units/mg protein). Authors in [4] also found 60% of the total TSO-hydrolase activity in the cytosolic fraction and the remaining 40% distributed within their organelle fractions, although these authors used a somewhat different fractionation procedure. The distribution of epoxide hydrolase within the organelle fractions B, D and G differed from that of the respective marker enzymes investigated (fig.1). Striking differences were observed if the TSO-hydrolase activity was compared with that of esterase or cytochrome *c* oxidase; suggesting that the hydrolase activity may not be linked to either the endoplasmic reticulum nor the mitochondria. The differences in comparison with catalase, α-hydroxy acid oxidase and β-glucuronidase appeared to be less marked. Nevertheless, only 15% of the organelle-bound hydrolase activity (B, D, G) was recovered in fraction D, whereas the recovery of the peroxisomal and lysosomal markers in this fraction was between 25 and 30%.

The subcellular localization of organelle-bound TSO-hydrolase was further investigated by means of isopycnic subfractionation of fraction D on linear metrizamide gradients in a vertical rotor. The results of a representative experiment are shown in fig.2. Between 85–125% of the various marker enzyme activities were recovered from the gradient. The highest protein concentrations were found at low density fractions 9–13. Cytochrome

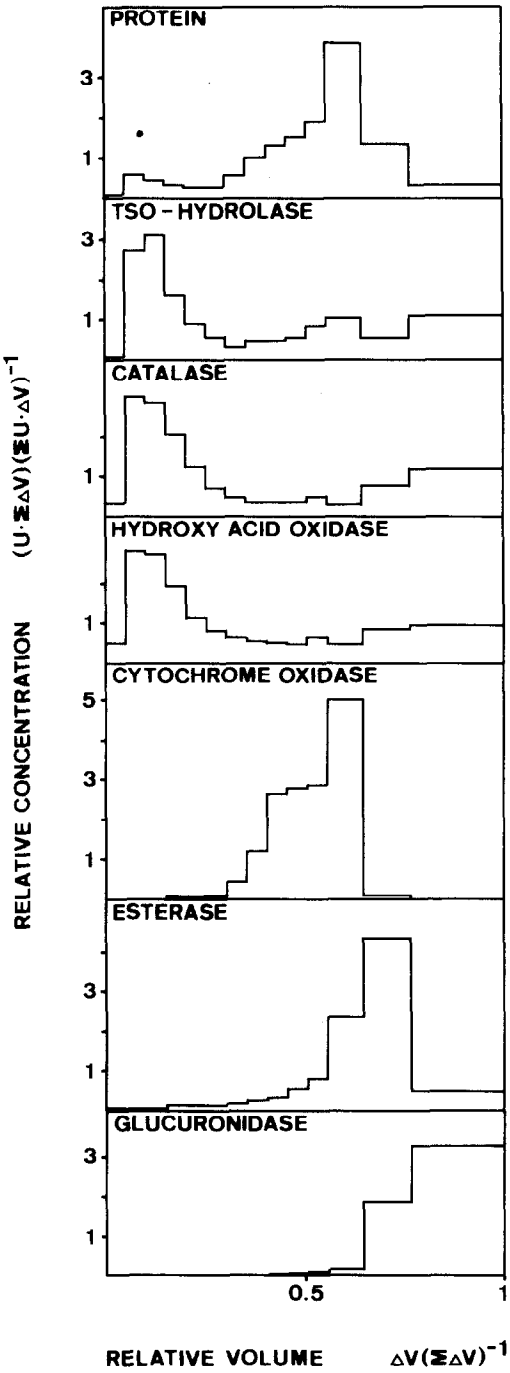

Fig.2. Distribution of protein and enzyme activities following isopycnic density gradient centrifugation of fraction D. ΔV represents the volume of a fraction and $\Sigma\Delta V$ the total gradient volume (39.4 ml). The following total amounts were recovered from the gradient: protein, 48.5 mg; TSO-hydrolase, 0.039 U; catalase, 94 U; α-hydroxy acid oxidase, 1404 U; cytochrome *c* oxidase, 27 U; esterase, 112 U; and β-glucuronidase, 2.7 U.

c oxidase and esterase activities, the marker enzymes for mitochondria and endoplasmic reticulum, respectively, were mainly localized in this part of the gradient. Lysosomal β-glucuronidase activity remained mainly at the top of the gradient. Visual inspection of the gradients always revealed a diffuse band at high densities near the bottom of the gradient. The material of this band was collected in fractions 2 and 3. Biochemical analysis (fig.2) as well as electron microscopic examination (not shown) of these two fractions emphasized that they consisted of highly purified peroxisomes.

About 70% of the TSO-hydrolase activity applied to the gradient was recovered. This activity was distributed in a manner similar to that of the peroxisomal marker enzymes. The specific epoxide hydrolase activity in fractions 2 and 3 was about 4-times higher than that of fraction D (i.e., the fraction applied to the gradients). The two peroxisomal marker enzymes catalase and α-hydroxy acid oxidase were enriched 5-fold and 6-fold, respectively. The slightly lower enrichment factor of the TSO-hydrolase and its lower recovery in comparison with catalase (70% *vs* 94%) may be accounted for by the instability of the enzyme in the metrizamide-gradient fractions. In this respect it should be mentioned that the hydrolase activity in metrizamide-containing gradient fractions was lost within 2 days, whereas it was quite stable in fraction D which was devoid of metrizamide. Nevertheless, the specific activity of the hydrolase within the peroxisomal fractions was more than 3-times higher than that in cytosolic fractions.

These findings provide strong evidence that the organelle-bound TSO-hydrolase activity is localized in peroxisomes rather than in mitochondria, as suggested in [4]. The exact localization of the enzyme within peroxisomes (membrane, matrix or nucleoid) remains to be established. Recently, it was found [14] that in rat liver fractions the microsomal hydration of cholesterol 5,6 α-oxide is catalysed by an epoxide hydrolase which differs markedly from the well-characterized microsomal enzyme. Other studies [15] have shown that cholesterol epoxide is also hydrolysed by rat liver cytosolic fractions; thus, it is tempting to speculate that microsomal cholesterol 5,6 α-oxide hydrolase [14] is actually located in peroxisomes present in the microsomal fractions. In addition, an involvement of peroxisomes in the cholesterol metabolism has been proposed in [16].

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