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Epoxide hydrolase activity in the mitochondrial fraction of mouse liver

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The intense interest in the metabolic fate of epoxidized xenobiotics is due to several factors. For instance, epoxides are often intermediates in the lipophile to hydrophile conversions necessary for the excretion of olefinic and aromatic compounds by living systems[1], and are widely encountered in man's diet from both natural and man-made sources. Some of these epoxidized compounds may alkylate proteins and nucleic acids and thus include some of the most potent cytotoxins, mutagens and carcinogens known[2]. In mammals, epoxides may rearrange, deoxygenate to olefins, react with glutathione to form conjugates, or be hydrolysed by water to yield 1,2-diols with or without enzymatic catalysis[1,3,4]. The enzymes which catalyse the formation of diols are known as epoxide hydrolases (EC 3.3.2.3), and their subcellular distribution is the subject of this report. Early data showed that styrene oxide hydrolase activity was associated with the microsomal subcellular fraction[5]. Epoxide hydrolase activity was subsequently demonstrated on the nuclear[6], Golgi apparatus and plasma membranes[7], and in the cytosol of the cell[8,9], leaving the mitochondria as the last major cellular organelle assumed to be devoid of epoxide hydrolase activity. We now report strong evidence for the occurrence of substantial epoxide hydrolase activity in the mitochondria.

While studying the subcellular distribution of the microsomal epoxide hydrolase, Oesch *et al.*[5] reported substantial styrene oxide hydrolase activity in a crude mitochondrial fraction prepared in isotonic KCl. However, the same study showed that mitochondria prepared in isotonic sucrose had low styrene oxide hydrolase activity which was not significantly different from that of the microsomal marker enzyme glucose-6-phosphatase. Analogous subcellular distribution studies in this laboratory using an epoxidized terpene or fatty acid as substrate showed high epoxide hydrolase activity in the mitochondrial fraction[10,11]. However, in the case of these two substrates, the specific activity of the hydrolase was increased rather than decreased by repeated washing of the mitochondria. As no marker enzymes were used in these studies it was not possible to show definitively the presence of epoxide hydrolase activity in the mitochondria. Thus, the following experiments designed to yield 'pure mitochondria' were performed.

Using the procedure described in Fig. 1 legend, the purity of the mitochondrial and submitochondrial fractions was monitored with several enzymes. Subcellular distribution of epoxide hydrolase activity was monitored with benzo(*a*)pyrene 4,5-oxide (BPO)[12] and *trans*-β-ethylstyrene oxide (TESO)[13] as

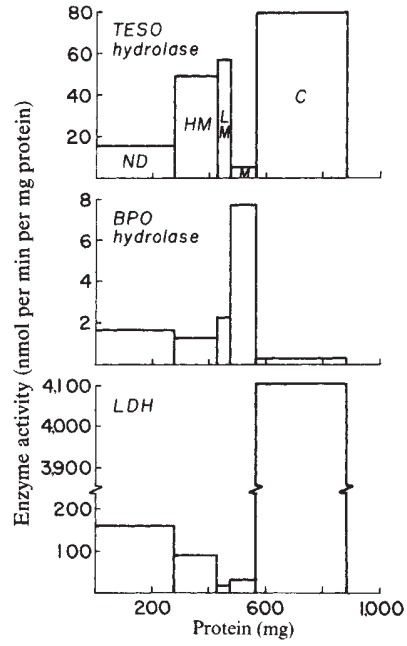

Fig. 1 Histograms of the subcellular distribution of epoxide hydrolase activity using TESO, *trans*-β-ethylstyrene oxide (upper), or BPO, benzo(*a*)pyrene 4,5-oxide (middle), as substrates and LDH, lactate dehydrogenase, with pyruvate as substrate (lower). Livers from 10-week old, male Swiss-Webster mice were rinsed in ice-cold 0.25 M sucrose and homogenized in 0.25 M sucrose/10 mM Tris-HCl, *p*H 7.4 buffer in a loose-fitting Potter-Elvejhem homogenizer to give a 10% w/v homogenate. The homogenate was centrifuged at 600*g* for 10 min, to give the nuclei and debris fraction (ND). The heavy mitochondria (HM) were pelleted at 6,500*g* for 10 min, the light mitochondria (LM) at 12,000*g* for 10 min, and the microsomes (M) at 100,000*g* for 60 min, leaving the cytosolic fraction (C) as the supernatant. Pelleted fractions were washed in the same conditions and resuspended in buffer (sodium phosphate, I = 0.2 M, *p*H 7.4 or Tris-HCl, 0.1 M, *p*H 9.0) for analysis. The ND, HM, LM, M and C fractions had 270, 160, 41, 87 and 320 mg of protein, respectively, from a preparation using three mouse livers. Separation of lysosomes and peroxisomes from mitochondria was achieved from livers, treated with Triton WR-1339 at 850 mg per kg 3.5 days before they were killed, by differential centrifugation as above followed by sucrose density gradient centrifugation[15]. The lysosomal and peroxisomal fractions had less than 2 and 28%, respectively, of the epoxide hydrolase activity in the crude mitochondrial fraction. The relatively high epoxide hydrolase activity in the peroxisomal fraction is probably due to mitochondrial contamination as the separation of these two fractions was not clear cut as determined by enzyme markers. Mitochondria were separated into inner membrane, outer membrane and the intermembrane and matrix fractions by swelling, shrinking and sonication, followed by sucrose density gradient centrifugation[16]. The values reported here are averages of two or three determinations from a preparation using three mouse livers. Of the total epoxide hydrolase activity present in the crude cell homogenate, 70 and 78% of the TESO and BPO hydrolysing activity is recovered in the various subcellular fractions.

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substrates. Significant TESO hydrolase activity, ~25% of the total recovered in the subcellular fractions, was observed in the mitochondrial fraction (Fig. 1), although the highest level was found in the cytosol. The subcellular distribution of epoxide hydrolase activity is very similar when *trans*-stilbene oxide (TSO) is used as substrate. Both TESO and TSO are negligibly hydrated by the microsomal epoxide hydrolase and the immunologically similar nuclear epoxide hydrolase. In contrast, the mitochondrial fraction poorly hydrates BPO, a substrate widely used to monitor epoxide hydrolase activity. Further, the low specific activity of BPO hydrolase in the mitochondrial fraction indicates minimal contamination of this fraction by nuclear and/or microsomal membranes as BPO hydrolase is a good marker enzyme for the microsomal and nuclear membranes[1,6]. Repeated washing of the mitochondrial fractions leads to a substantial decrease in BPO hydrolase specific activity and most of this activity is associated with the outer mitochondrial membrane. As styrene oxide and BPO hydrolase activity in the microsomes are immunologically identical[14], our data support the hypothesis of Oesch[1] that styrene oxide hydrolase activity in the mitochondrial fraction is probably due to microsomes adhering to the outer mitochondrial membrane. In contrast, the TESO hydrolase specific activity in the mitochondrial fractions increases following repeated washings.

Using lactate dehydrogenase, a widely accepted marker for the cytosol, we demonstrated that epoxide hydrolase activity in the mitochondrial and nuclear fractions does not result from cytosolic contamination (Fig. 1). Results using additional marker enzymes including cytochrome oxidase[15], NADPH cytochrome *c* reductase[16], cytochrome P_{450} (ref. 17), citrate synthase[18] and adenylate kinase[19] also support the relative purity of the subcellular fractions. Use of citrate synthase and adenylate kinase also showed that the cytosolic epoxide hydrolase activity does not arise from mitochondrial lysis during homogenization, as only 0.6 and 2% of the mitochondrial activity of these enzymes, respectively, was found in the cytosol.

Subsequent experiments using livers from mice treated with Triton WR-1339 (ref. 15) indicated that lysosomes and peroxisomes, monitored by acid phosphatase[19] and urate oxidase[20], respectively, have minimal TESO hydrolase activity. As such, epoxide hydrolase activity in the mitochondrial fraction does not arise from contamination by these organelles. When mitochondria are disrupted, a preponderance of the epoxide hydrolase activity is associated with the matrix and intermembrane space fraction with TESO as substrate and less activity is associated with the inner and outer mitochondrial membranes. Most of the activity associated with either of these membranes can be removed with a single wash, indicating contamination by the matrix and intermembrane space fraction. The mitochondrial fraction is able to hydrate a wide variety of epoxidized compounds including epoxidized fatty acids and glycidal ethers, with a spectrum of activity similar to that of the cytosolic epoxide hydrolase, but distinct from that of the microsomal enzyme.

As the nuclei and mitochondria are excluded from the activation/deactivation systems used in many short-term mutagenicity assays[21], these systems may not provide a valid picture of the ability of cells to degrade potentially mutagenic epoxides. However, this effect may be more quantitative than qualitative, because the mitochondrial epoxide hydrolase demonstrates a spectrum of activity similar to that of the cytosolic epoxide hydrolase.

The present study and published literature[1,6,7,22] illustrate the apparently ubiquitous nature of epoxide hydrolases in cellular components. Although the information needed to understand fully the biological role of epoxide hydrolases is lacking, their ability to metabolize some xenobiotics is clear. The association of epoxide hydrolases with the mitochondria and nuclei of the cell may serve to protect the structural integrity of these organelles as well as their nucleic acid content. It was recently shown[23,24] that mitochondrial DNA is more easily alkylated by polyaromatic hydrocarbons and their diol-epoxides than nuclear DNA. Such results are not altogether surprising because of the nature of mitochondrial DNA and the oxidases occurring in the mitochondria[25,26] which can activate the polyaromatic hydrocarbons to reactive electrophiles. Although the mitochondrial epoxide hydrolase activity reported here does not appreciably metabolize BPO, these epoxide hydrolases can potentially protect the mitochondria from those reactive epoxides which are substrates. Therefore, our study certainly illustrates the need for increased research on these important enzymes using a variety of potential substrates in all subcellular components.

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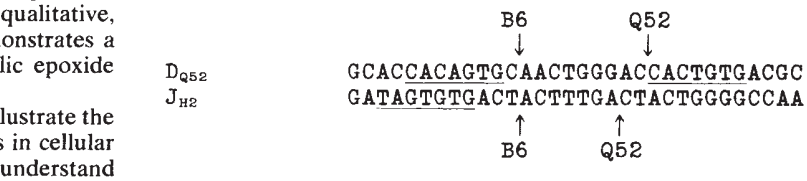
Errata

In the letter 'Human immunoglobulin variable region genes—DNA sequences of two V_κ genes and a pseudogene' by D. L. Bentley and T. H. Rabbitts, *Nature* **288,** 730–733 (1980), Fig. 3 shows the DNA sequence of a human V_κ pseudogene (not V_λ).

In the article 'Sequence and organization of the human mitochondrial genome' by S. Anderson *et al.*, *Nature* **290,** 457–465, line 56 on page 462 should read '... of the anticodon of mammalian mt RNAs is *not* known, it is ...'

In the article 'Identification of D segments of immunoglobulin heavy-chain genes and their rearrangement in T lymphocytes' by Y. Kurosawa *et al.*, *Nature* **290,** 565–570, the arrows indicating recombination sequences in the text figure on page 570 were positioned incorrectly. The correct version is shown below:



Corrigendum

In the letter 'A nonrandom component in cosmic rays of energy $\geq 10^{14}$ eV' by C. L. Bhat *et al.*, *Nature* **288,** 147–149 (1980), the RA values shown in Figs 2 and 3 have been found to be incorrect. The correct values are those obtained by subtracting the values given from 24 hours. This excludes the pulsar PSR 0525+21 as a possible origin. The results are consistent with a point-source origin in the RA range 20 ± 3 hours.