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Short Communication: Cytosolic Epoxide Hydrolase in Human Placenta

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INTRODUCTION

Epoxide hydrolases (EC 3.3.2.3) catalyse the hydrolysis of often mutagenic, carcinogenic and cytotoxic epoxides to generally less reactive and more easily excreted 1,2-diols (Oesch, 1973; Wixtrom and Hammock, 1985). These epoxides may arise from cytochrome P450-catalysed oxidation of olefinic and aromatic xenobiotics including many drugs used clinically, such as the antiepileptic drug carbamazepine—whose epoxide metabolite may account for the drug's teratogenic activity (Lindhout, Hoppener and Meinardi, 1984). Several forms of epoxide hydrolase have been identified in mammalian tissues. These forms differ in molecular weight, substrate specificity, pH optima and immunoreactivities. They include a 'microsomal epoxide hydrolase' that hydrolyses a wide range of epoxides including those on cyclic systems, a 'cholesterol epoxide hydrolase' (also microsomal) that catalyses the hydrolysis of cholesterol epoxides and related compounds, a recently reported 'leukotriene A_4 hydrolase' (McGee and Fitzpatrick, 1985) and a 'cytosolic epoxide hydrolase' that hydrolyses a number of epoxides not on cyclic systems. The cytosolic epoxide hydrolase (cEH) is involved in the hydrolysis of a variety of epoxidized lipids (Gill and Hammock, 1979; Sevanian, Stein and Mead, 1981; Chacos et al, 1983) including some eicosanoid intermediates of lipoxygenase metabolic pathways. It may also be responsible for conversion of the insulin secretagogue hepoxilins to trioxilins (Pace-Asciak, Klein and Speilberg, 1986). We have partially purified cEH from human term placenta, as it is a readily available source of human tissue, and have measured the cEH activity in both cytosol and purified preparations using *trans*-stilbene oxide. To our knowledge, this is the first report of cEH in human placenta.

MATERIALS AND METHODS

Enzyme sources

Term placentae were obtained within minutes of delivery from normal pregnancies in which none of the mothers had regular drug intake. Cytosolic fractions were prepared essentially as previously described (Hammock and Ota, 1983) and were used immediately or stored at −70°C until required.

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Assays

Cytosolic epoxide hydrolase activity was monitored with [^{3}H]-*trans*-stilbene oxide (TSO) by a partition assay as described previously (Gill, Ota and Hammock, 1983) except that bovine serum albumin (0.1 mg/ml) was added to the assay buffer to stabilize activity. Protein was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Purification

Affinity purifications of cEH were performed at 4°C using benzylthio-Sepharose or 7-methoxycitronellylthio-Sepharose matrices (Wixtrom, Silva and Hammock, 1987). In a typical experiment, 50 ml of 15 per cent (w/v) placenta cytosol was pumped through a 0.5 ml column of affinity resin pre-equilibrated with 76 mM, pH 7.4 phosphate buffer. The eluent was collected as the 'non-binding fraction'. The column was then washed with 20 ml of phosphate buffer ('buffer wash'), and the enzyme eluted with 4 ml of 0.5 mM 4-fluorochalcone oxide (a cEH inhibitor). The eluted enzyme solution was dialysed for two hours against phosphate buffer to remove the inhibitor ('dialysed fraction').

Electrophoresis and Immunoblotting

The partially purified cEH was electrophoresed on a sodium dodecyl sulphate-12 per cent polyacrylamide gel (SDS-PAGE) according to Laemmli (1970). Protein bands on one portion of the gel were visualized by silver staining. Proteins from the remaining two portions of the gel were transferred electrophoretically onto nitrocellulose paper. Immunoblotting was performed as described by Towbin, Staehelin and Gordon (1979) using a 1:500 dilution of mouse or human liver cEH antiserum and a 1:2000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad Immuno-Blot Assay Kit).

RESULTS AND DISCUSSION

The placenta is believed to serve a protective role for the fetus through its metabolism of a variety of xenobiotics. Epoxide hydrolases are capable of detoxifying epoxidized xenobiotics by catalysing their hydrolysis to 1,2-diols. Previous studies have examined in detail the microsomal form of epoxide hydrolase in human placenta (Pacifici and Rane, 1982, 1983). This report provides the first information on the presence of a cytosolic epoxide hydrolase in human placenta.

The levels of cEH activity present in the cytosol from three human placentae are shown in Table 1. These rates of hydrolysis of TSO are quite low when compared with the values of 0.2 to 6.6 nmol/min/mg reported for cytosol from human liver (Wang, Meijer and Guengerich, 1982; Silva and Hammock, 1987). The higher level of cEH activity (4 nmol/min/mg for styrene oxide) observed by Pacifici et al (1983) in human fetal liver may indicate that it is a more important site for detoxification of these epoxides than is the placenta.

Table 1. Epoxide hydrolase in human placenta cytosol

Mother	Age (years)	Placental cytosolic epoxide hydrolase activity (nmol TSO diol formed/min/mg ± s.e.m.)
LK	33	0.067 ± 0.003
KD	29	0.042 ± 0.003
SG	35	0.039 ± 0.002

Table 2. Affinity purification of cytosolic epoxide hydrolase from human placenta

	Specific activity (nmol/min/mg)	Total activity (nmol/min)
Crude cytosol	0.054	11.0
Non-binding fraction	0.009	2.0
Buffer wash	ND	0.01
Elution (dialysed)	38	1.3

ND = not done.

To facilitate a comparison (by SDS-PAGE and immunoblotting) of the placental enzyme with cEH from mouse and human liver, an affinity purification procedure previously developed for mouse liver was used to obtain a 700-fold enrichment of placental cEH. Table 2 shows the results for the affinity purification of cEH from human term placenta using 7-methoxycitronellylthio-Sepharose. Identical results were obtained using benzylthio-Sepharose. The specific activity is low when compared with values of 150 to 250 nmol/min/mg observed for the human liver enzyme (Wang, Meijer and Guengerich, 1982; Wixtrom and Silva, unpublished results). This is probably a result of both the instability of purified cEH at very low protein concentrations (Wixtrom, Silva and Hammock, 1987) and the high levels of impurities present.

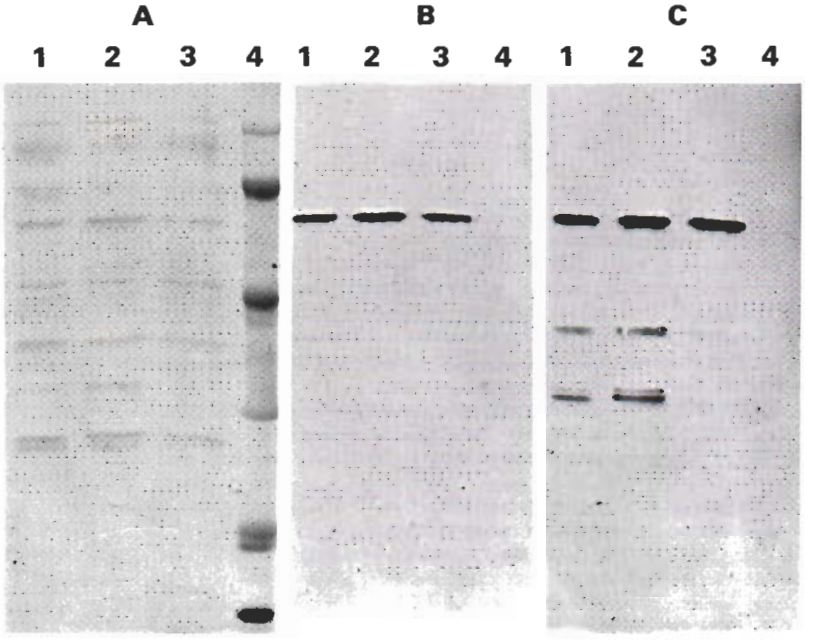

Figure 1. SDS–PAGE and immunoblotting of placental cEH. Samples of 700-fold purified cEH (2.5 μg/lane) were electrophoresed on an SDS-polyacrylamide gel: (A) silver-stained SDS-PAGE; (B) immunoblot with mouse liver cEH antiserum; (C) immunoblot with human liver cEH antiserum. Lane 1: SG placenta; Lane 2: LK placenta; Lane 3: KD placenta; Lane 4: MW standards; these standards were phosphorylase B (M_r 94 000), bovine serum albumin (M_r 68 000), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 29 000), soybean trypsin inhibitor (M_r 20 000, 21 000) and lysozyme (M_r 14 300). SDS-polyacrylamide gels and immunoblots of purified human and mouse liver cEH showed the same M_r 59 000) and intensity of reaction with antibodies as did the placental enzyme (data not shown). Further details about the electrophoresis and blots are given in 'Materials and methods'.

The electrophoretic patterns and immunoblots of the partially purified cEH preparations are shown in Figure 1. The antibodies raised against purified mouse and human liver cEH both reacted strongly with a protein of M_r 50 000 present in all three of the placenta cytosols, which corresponds directly with the M_r of cEH observed in all other species examined to date. The reaction of human liver cEH antiserum with the lower M_r cytosolic proteins (Figure 1 C) has been observed previously in immunoblots of partially purified human liver cEH and is probably attributable to impurities present in the enzyme preparation used to raise the rabbit antibodies. The above results provide further evidence for the similarity of cEH among liver and extrahepatic tissues of mice and humans, both in terms of immunological identity and molecular mass.

SUMMARY

It has been shown that cytosol from human term placenta contains cytosolic epoxide hydrolase activity. This cytosolic epoxide hydrolase was enriched more than 700-fold by affinity chromatography and appears similar to the enzyme from mouse and human liver in terms of molecular mass (M_r 59 000) and antigenic reactivity.

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