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EXTRAHEPATIC SULFATION OF PHENOLS

BOVINE LUNG AND INTESTINAL PHENOL SULFOTRANSFERASES

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Abstract—Phenol sulfotransferases (PST) from bovine lung and small intestine were purified about 1000-fold. PST from bovine small intestine, similarly as the bovine lung enzyme, catalyzes sulfation of only exogenous phenols. A single thermostable form of PST, active with high concentrations of phenol (K_m = 1.43 mM) was found in the small intestine. The effect of divalent cations on the activity of the two phenol sulfotransferases was determined. The molecular weight of the native enzymes was estimated as about 69,000 and subunit molecular weight determined by sodium dodecyl sulfate gel electrophoresis as 35,000. In double immunodiffusion tests the bovine lung PST showed antigenic identity with the bovine small intestine enzyme but complete immunological incompatibility with rat liver sulfotransferase.

Conjugation with sulfate is an important route of metabolism of various phenolic compounds and is known to involve the action of phenol sulfotransferase (PST, 3′-phosphoadenylsulfate:phenol sulfotransferase, EC 2.8.2.1). It has been demonstrated that organs other than liver are able to metabolize phenolic molecules [1–3]. The lungs represent the first line of defence with respect to compounds entering the body via the respiratory tract, and gut with respect to orally administered substances [4, 5].

The activity of phenol sulfotransferase has been demonstrated in human small intestine [6] and human and bovine lungs [7, 8]. In our previous study at least two molecular forms of PST differing in thermal stability have been found in both human and bovine lung [8]. The differences in substrate specificity between human and bovine lung enzymes were related to the presence of different molecular forms of PST demonstrated in both tissues [8].

The differences observed between the human and bovine lung phenol sulfotransferases in substrate specificity and thermal stability led us to further characterization of the bovine lung enzyme as well as to isolation and characterization of PST from bovine small intestine.

MATERIALS AND METHODS

Materials

Reagents. [^{35}S]PAPS (sp. act. 1.1–2.8 Ci/mmole) was obtained from New England Nuclear and stored at −20°. DEAE-cellulose DE-11 was from Whatman Biochemicals (Maidstone, Kent, U.K.), Sepharese 4B, Sephacryl S-200, Sephadex G-100 from Pharmacia (Uppsala, Sweden). Bacto Adjuvant, Complete Freund was from DIFCO Laboratories (Detroit, MI), 2-mercaptoethanol from Calbiochem (Los Angeles, CA), 2-naphthol was a product of Merck; other acceptor substrates were purchased from Sigma.

Marker proteins. Bovine serum albumin, chicken ovalbumin, aldolase, horse myoglobin, carbonic anhydrase (Sigma Chemical Co.) were used.

All other chemicals were of analytical grade.

Tissues

Bovine lung and jejunum part of small intestine were obtained from a slaughterhouse just after the animals were killed. The fresh mucosa was shaved and used for PST purification.

Purification procedure

The purification of bovine lung and small intestine mucosa phenol sulfotransferases was carried out using techniques described previously [7]. PST was found to be practically confined to cytosole and almost absent from other subcellular fractions. In the salt fractionation procedure a maximum increase in specific activity was obtained using 0–40% and 35–55% ammonium sulfate for the lung and small intestine enzyme, respectively.

On DEAE-cellulose both enzymes emerged as a single, symmetrical peak. Subsequently the purified enzymes were retained on the *p*-hydroxyphenylacetic acid agarose affinity column and were eluted with a linear gradient of sodium phosphate buffer, pH 7.4. The specific activity of the enzymes after the last step of purification, Sephadex G-100 chromatography, was about 1000-fold that of the crude extract. The results of purification are summarized in Table 1.

PST from rat liver was isolated and purified using the procedure described by Borchardt and Schasteen [9]. The purification was carried through the Sephacryl S-200 chromatography step.

PST activity

PST activity was measured using [^{35}S]3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the sulfate donor. The assay mixture consisted of 10 μM sodium phosphate buffer, pH 6.5, variable amounts of phe-

Table 1. Purification of bovine lung and small intestine phenol sulfotransferases

Step	Procedure	Lung PST			Intestine PST		
		Specific activity (nmole/mg/min)	Purification factor	Yield (%)	Specific activity (nmole/mg/min)	Purification factor	Yield (%)
	9000 *g* supernatant	3.5×10^{-3}	1	100	2.6×10^{-3}	1	100
1	100,000 *g* supernatant	5.2×10^{-3}	1.5	95	3.8×10^{-3}	1.5	96
2	$(NH_4)_2 SO_4$ fractionation	9.8×10^{-3}	2.8	73	1.1×10^{-2}	4.2	76
3	DEAE-cellulose chromatography	5.1×10^{-2}	14.6	56	4.8×10^{-2}	18.5	52
4	Affinity chromatography	8.2×10^{-1}	234.3	34	6.9×10^{-1}	265.4	40
5	Sephadex G-100 chromatography	3.1	885.7	26	2.45	942.3	31

Approximately 30 g of bovine lung and 20 g of mucosa from small intestine was used for the purification of phenol sulfotransferases.

nolic sulfate acceptor, 0.45 μCi [^{35}S]PAPS (0.29 μM), and the enzyme preparation in a total volume of 1 ml. The reaction mixtures were incubated for 15 min at 37°. Blanks were assayed as described above, except that the phenolic substrate was omitted.

The amount of ^{35}S-labeled products was determined by the method of Foldes and Meek [10] as described previously [8].

Protein determination

Protein was determined according to Lowry *et al.* [11] or spectrophotometrically by the method of Warburg and Christian [12], with crystalline bovine serum albumin as a standard.

Thermal stability study

Phenol sulfotransferase after the last step of purification was incubated in a shaker bath at various temperatures and for various periods of time. Then the samples were cooled to 4° and assayed for activity after the addition of buffer and substrates. Blanks without an accepting substrate were determined in parallel at each temperature and time point studied.

Molecular weight determination

Molecular weight estimation was performed using a Sephadex G-100 column (1 × 30 cm) previously equilibrated with 10 mM sodium phosphate buffer, pH 6.5, as described by Andrews [13].

Horse myoglobin (17,000), ovalbumin (45,000), bovine serum albumin (68,000), and aldolase (158,000) were used as standards (5 mg each). Fractions of 2 ml were checked for enzyme activity and protein content.

Polyacrylamide gel electrophoresis

Electrophoresis was performed in sodium acetate buffer, pH 5.5 [14] at 4° and 2 mA for 15 min, followed by 6 mA per gel for about 60 min. Protein was stained with 0.5% Amido Black in 7% (v/v) acetic acid. PST activity was detected in 2-mm gel slices eluted with sodium phosphate buffer, pH 6.5.

SDS-polyacrylamide gel electrophoresis was carried out as described by Weber and Osborn [15] in 0.1 M sodium phosphate buffer, pH 7.0, containing 0.1% (w/v) SDS at room temperature and 8 mA per gel. Proteins were detected by staining the gels in the Coomassie blue/methanol/acetic acid solution for 2 hr at room temperature. The gels were extensively destained in methanol/acetic acid solution and stored in 7.5% acetic acid solution. The subunit molecular weight was estimated from a calibration curve obtained from a simultaneous electrophoresis of the following protein markers: bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (29,000), and myoglobin (17,200).

Immunochemical studies

Guinea pigs were immunized with high-purity PST from bovine lung and rat liver. In the course of 20 days guinea pigs received three injections of 200–300 μg of purified phenol sulfotransferase, in 0.2 ml Freund's adjuvant. After approximately 30 days the animals were bled through cardiac puncture. The antisera obtained were stored at −20°.

The double immunodiffusion test was carried out according to Ouchterlony [16]. Experiments were performed on 1% agarose in 0.85% saline. Immunodiffusion plates were kept at room temperature for 48 hr, whereupon they were washed successively with saline and water, dried and stained with 0.5% (w/v) Amido Black in glacial acetic acid–methanol (1:9).

RESULTS

Enzyme activity

The specific activity assayed in the presence of 50 μM 2-naphthol was 3.5×10^{-3} nmole/mg protein/min and 2.6×10^{-3} nmole/mg protein/min or 2.05×10^{-1} nmole/g wet tissue/min and 3.1×10^{-2} nmole/g/min in the crude extracts from bovine lung and small intestine, respectively. It was similar to the activity of PST from human lung [7] and human small intestine (not shown).

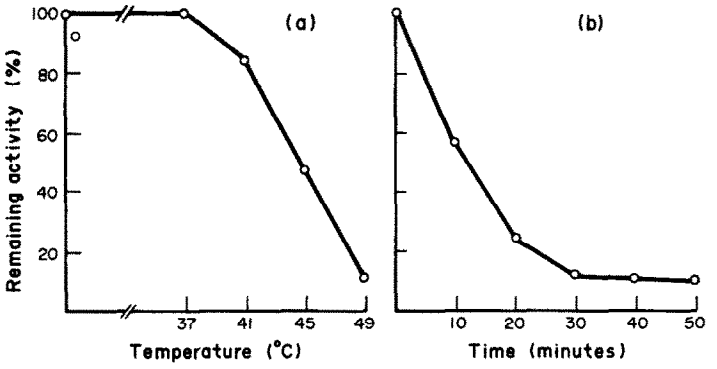

Fig. 1. Thermostability of bovine small intestine phenol sulfotransferase. The activity was measured after 15 min preincubation at the indicated temperature (a), and after preincubation at 45° for the indicated time (b), with 5 mM phenol as substrate. Each point represents the mean of three determinations.

Substrate specificity and affinity

The bovine small intestine phenol sulfotransferase, similarly as the bovine lung PST [8], acts on various exogenous phenols but does not catalyse the sulfation of endogenous catecholamines such as adrenaline, noradrenaline, dopamine. It does not use either L-tyrosine or dopa as substrates.

With phenol as an accepting substrate the K_m value of the small intestine sulfotransferase was 1.43 mM, whereas for the bovine lung enzyme two K_m values of 40 and 909 μM were reported [8].

Thermal stability

The bovine small intestine PST, like lung sulfotransferase [8], was very stable at high temperature. After 15 min preincubation at 45°, the enzyme assayed with 5 mM phenol retained about 50% of its initial activity (Fig. 1a), and about 10% of the activity after 50 min preincubation (Fig. 1b).

Effect of divalent cations

Bovine lung and small intestine sulfotransferases were activated by Mg^{2+} and inhibited by Zn^{2+}. At the ion concentration of 5 mM, after 15 min incubation at 37° with $MgCl_2$ the activity of the lung and small intestine PST increased by about 20 and 45%, respectively, whereas in the presence of $ZnCl_2$ only 3 to 5% of the initial activity was retained.

Mn^{2+} inactivated the lung enzyme and, like Mg^{2+} at low concentrations it activated the small intestine PST (Fig. 2).

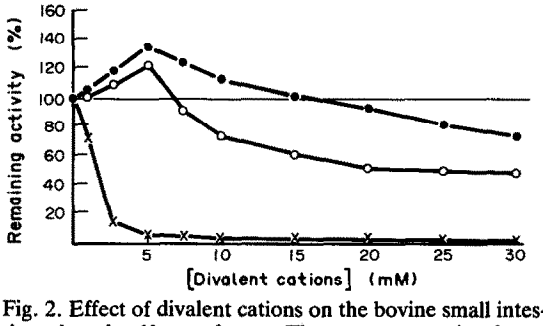

Fig. 2. Effect of divalent cations on the bovine small intestine phenol sulfotransferase. The enzyme was incubated for 15 min in the absence or presence of different concentrations of $MgCl_2$ (●), $MnCl_2$ (○), and $ZnCl_2$ (×). The activity was measured with 50 μM 2-naphthol as substrate. Each value represents the mean of three determinations.

Molecular weight

The molecular weight of both the purified bovine lung and small intestine PST was estimated by gel filtration on Sephadex G-100 to be about 69,000. The subunit molecular weight determined by SDS electrophoresis was 35,000 (Fig. 3).

Electrophoretic properties

The purified lung and small intestine PST was subjected to polyacrylamide gel electrophoresis at pH 5.5. The lung PST showed a slightly lower mobility towards the cathode than the intestinal enzyme (Fig. 4).

Immunochemical properties

In the double immunodiffusion test, the purified sulfotransferase from bovine lung showed antigenic identity with the bovine small intestine enzyme (Fig. 5I), but did not react with PST from rat liver (Fig. 5II, III). In the same test in the presence of two mixed antisera, the rat liver and bovine lung phenol sulfotransferases gave two crossing over precipitation lines evidencing complete immunological incompatibility of the two enzymes (Fig. 5IV).

DISCUSSION

Phenol sulfotransferase is a cytosolic enzyme which plays an important role in the conjugative mechanisms. The presence of different molecular forms of the enzyme in various tissues has been reported [17–19]. Both thermostable and thermolabile forms of PST were found in human brain, platelet and lung [19, 20, 8]. The thermostable forms show high affinity to exogenous phenols, and the thermolabile forms to catecholamines and millimolar concentrations of phenols [19].

During the purification procedure of bovine lung

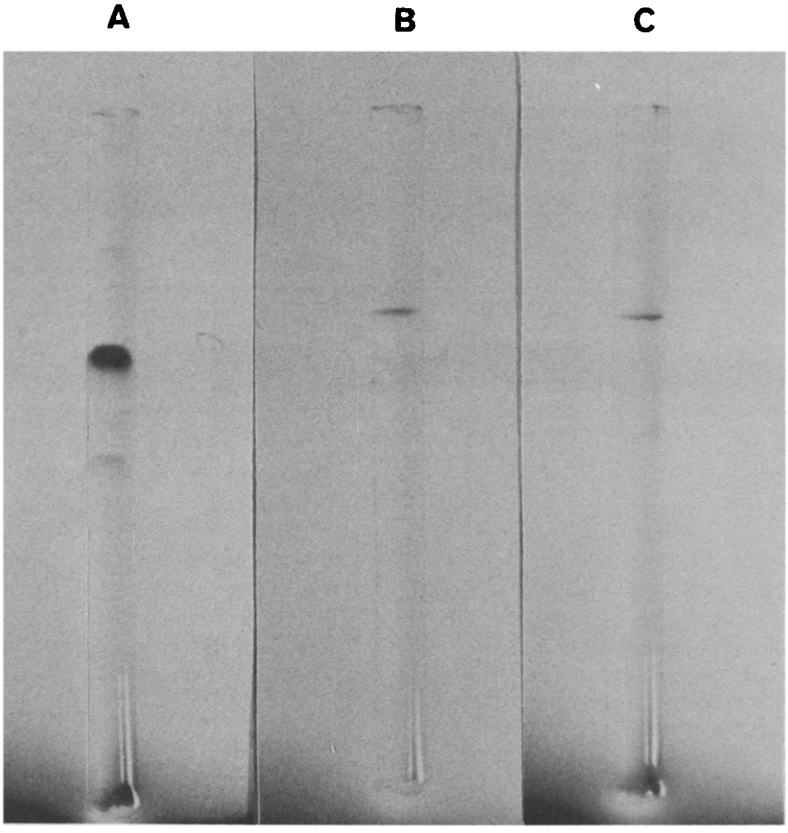

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of bovine small intestine and lung phenol sulfotransferases. The electrophoresis was carried out as described under Materials and Methods. (A) Marker proteins: egg albumin (45,000), carbonic anhydrase (29,000), myoglobin (17,200); (B) lung PST; (C) small intestine PST. About 10 μg of protein was applied to the gel.

and small intestine phenol sulfotransferase, a symmetrical peak of activity was observed without any indications of isozyme forms similar to those reported earlier for the rat liver enzyme [17, 21].

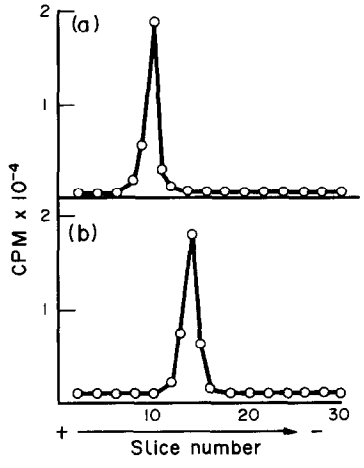

Fig. 4. Polyacrylamide gel electrophoresis of bovine lung (a) and small intestine (b) phenol sulfotransferases. The electrophoresis was carried out at pH 5.5 as described under Materials and Methods. About 100 μg of protein was applied to the gel.

Both investigated in current study enzymes, similar to other non-hepatic sulfotransferases, e.g. from rat kidney, stomach [22] and rat small intestine (data not shown) as well as from bovine cerebral gray matter and brain microvessel endothelial cell monolayers [23], are active only with exogenous phenols. As others active with exogenous phenols sulfotransferases [24, 25, 7, 23] they are very stable at high temperature. They both are activated by Mg^{2+} and strongly inactivated by Zn^{2+}. Their molecular weight of 69,000 as well as subunit molecular weight of 35,000 show that both bovine lung and small intestine sulfotransferases are dimers, unlike the human lung PST [7] but similar to rat liver enzymes, isolated by Sekura and Jakoby [21].

The adsorbance on DEAE-cellulose, substrate specificity and affinity, thermostability, molecular weight of native and denaturated forms, as well as immunological identity of bovine lung and small intestine PST show that those two enzymes might be the same form of phenol sulfotransferase. The absence of the thermolabile sulfotransferase, active with catecholamines in bovine lung and jejunum as well as in bovine brain [23], shows that those compounds are metabolised there by pathways other than sulfation.

Our present results support the earlier observations that the metabolism of various exo- and

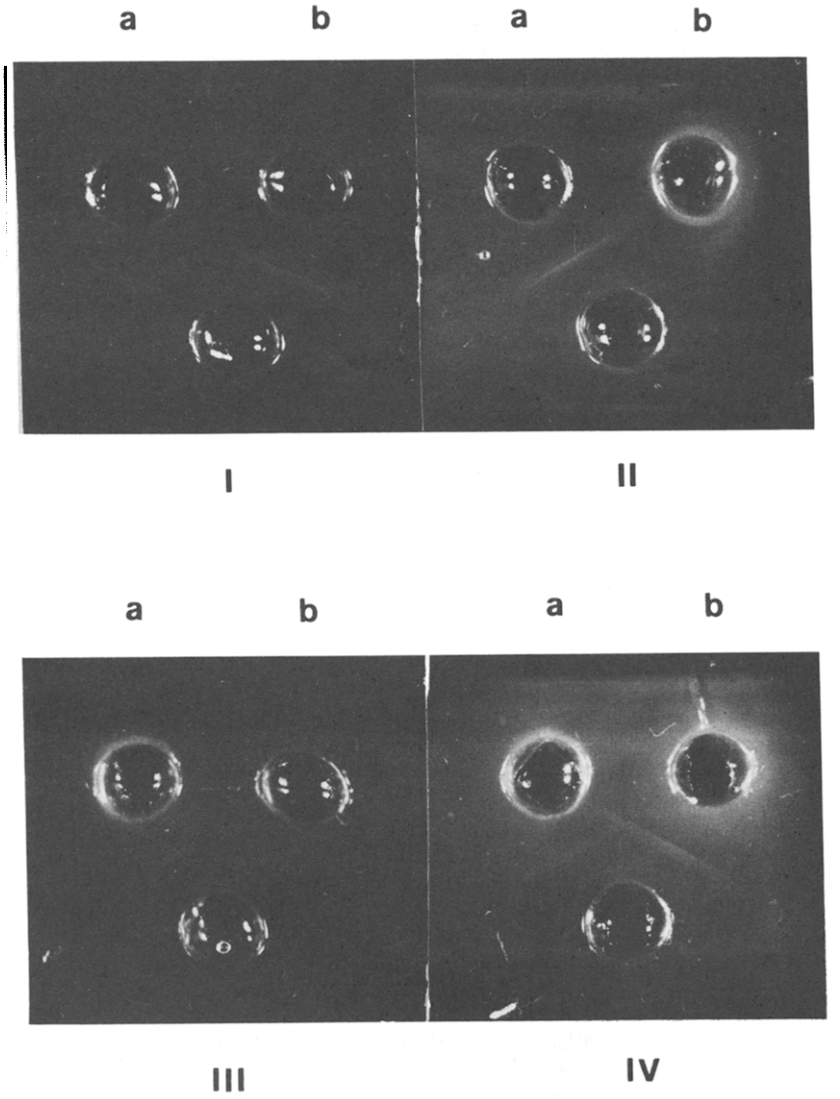

Fig. 5. Double immunodiffusion of bovine lung and rat liver antiserum against PST from bovine lung, bovine small intestine and rat liver. Center well, antibody against: I, II, bovine lung PST; III, rat liver PST; IV, both bovine lung and rat liver PSTs. Ia, IIa, IIIb, IVb, PST from bovine lung; Ib, PST from bovine small intestine; IIb, IIIa, IVa, PST from rat liver.

endogenous compounds is not only tissue- but also species-dependent.

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