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Human ileum phenol sulfotransferase

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Sulfation is an important pathway for the biotransformation of phenolic drugs and amine neurotransmitters in humans and other animal species. The gastrointestinal tract represents the first line of defence against noxious substances present in the diet or administered orally. A normal human diet may contain up to 600 mg of phenolic compounds daily [1]. Powell *et al.* [1] demonstrated that only conjugated phenol is present in portal blood, 72% as phenyl sulfate.

Phenol sulfotransferase (PST) which catalyses sulfation is a cytosolic enzyme. At least two different molecular forms of the enzyme were found in human blood platelet, brain, lung and liver [2–6]. These forms differ in their substrate specificity, thermal stability and sensitivity to inhibitors. The thermostable form (TS) catalyses the sulfation of micromolar concentrations of exogenous phenols, the thermolabile form (TL) catalyses the sulfation of catecholamines and millimolar concentrations of phenols [2–4]. However, the TS form present in human liver can also sulfate dopamine at high concentrations [6]. On the other hand, bovine small intestine PST [7], as well as rat stomach [8] and small intestine enzymes (Barańczyk-Kuźma *et al.*, unpublished), do not use endogenous catecholamines as substrates.

Experiments presented in this communication concern the human ileum PST and were performed to determine whether the properties of human small intestine phenol sulfotransferase are similar to those of human lung [4, 9] and other human tissues enzymes [2, 3, 6], or to PSTs from the gastrointestinal tract of other species [7, 8].

Methods and results

Materials. Reagents: [^{35}S]PAPS (sp. act. 1.1–2.8 Ci/mmole) was obtained from New England Nuclear (Boston, MA) and stored at −20°. Phenol and 2-naphthol were products of Merck (Darmstadt, F.R.G.); other acceptor substrates were purchased from Sigma Chemical Co. (Poole, U.K.). Sephadex G-100 and Dextran 2000 were from Pharmacia (Uppsala, Sweden), and acrylamide and methylenebisacrylamide were from Fluka (Buchs, Switzerland). Marker proteins: bovine serum albumin, chicken ovalbumin, aldolase, horse myoglobin (Sigma) were used. All other chemicals were of analytical grade.

Tissues. Human small intestine (ileum part) was obtained at the time of surgery of 56–68 year old patients (four female and five male) undergoing clinically indicated partial ileum resection for the removal of the colon carcinoma. The fresh tissues with no pathological changes observed by histological methods were used for PST extraction.

PST extraction. PST was extracted from the mucosa or from enterocytes isolated according to Weiser [10]. The fresh mucosa or enterocytes were homogenized in 3 vol. of

10 mM sodium phosphate buffer, pH 6.5, containing 0.25 M sucrose and 3 mM 2-mercaptoethanol. The homogenate was centrifuged for 15 min at 9000 *g*. The supernatant was decanted and centrifuged at 100,000 *g* for 90 min yielding the cytosolic fraction. All steps were carried out at 4°. The fresh cytosol was used for the assay of PST activity and stored at −30° for other studies.

PST activity and affinity. The activity of human small intestine PST was assayed with different phenolic substrates using the barium hydroxine method [11] as described previously [4]. The activity assayed in the cytosolic fraction obtained from isolated enterocytes was almost the same as that in shaved mucosa (Table 1). The enzyme, similarly to dog small intestine PST (not shown), but different from bovine [9] and rat (not shown) intestinal sulfotransferases, catalyses the sulfation of both exogenous phenols and endogenous catacholamines, showing the highest activity with dopamine (Table 1).

Human small intestine PST shows a high affinity to dopamine with a K_m value of 5.0 ± 0.8 μM calculated by the method of Eisenthal and Cornish-Bowden [12]. With phenol the Lineweaver–Burk double reciprocal plot had a biphasic course (Fig. 1). The K_m value at low phenol concentrations (1–200 μM) was 28.6 ± 2.4 μM, and at higher concentrations (200–5000 μM) it was 170 ± 5.7 μM.

Thermal stability. Human ileum PST assayed with 50 μM dopamine or 2.5 mM phenol was more thermolabile at high temperature than that measured with 50 or 250 μM phenol (Fig. 2A). After preincubation at 41° from 10 to 50 min the enzyme assayed with 50 μM dopamine and at all concentrations of phenol used showed very similar thermal stability (Fig. 2B). After 20 min preincubation at 43° less than 10% of the initial PST activity was retained when assayed with 50 μM dopamine or 2.5 mM phenol, but more than 30% when assayed with 50 μM phenol (Fig. 2C).

K_m values for human small intestine PST were assayed after 15 min preincubation at 41° or 43° with phenol as the variable substrate. After preincubation at 41° the biphasic curve in the Lineweaver–Burk double reciprocal plot was still present and two K_m values were similar to those observed without preincubation were obtained: 18.0 ± 4.5 and 151 ± 12 μM. Human ileum PST after 15 min preincubation at 43° was inhibited by phenol at the concentrations exceeding 100 μM and only one K_m value of 18.0 ± 4.5 μM could be calculated.

Table 1. Activity of human ileum phenol sulfotransferase with different substrates

	Enterocytes	Mucosa
Substrate	Specific activity, pmol/mg/min	
Phenol	2.8 ± 0.18	3.5 ± 0.13
2-Naphthol	7.5 ± 0.35	8.3 ± 0.16
p-Nitrophenol	1.6 ± 0.21	1.9 ± 0.09
Dopamine	11.0 ± 0.18	14.6 ± 0.31

Each value is the mean ± SE (N = 9).

Electrophoretic properties and molecular weight. Polyacrylamide gel electrophoresis was performed in sodium acetate buffer, pH 5.5, according to Sakai and Gross [13] as described previously [4]. The human ileum PST showed a higher mobility towards the cathode than the bovine small intestine enzyme (Fig. 3).

The molecular weight of human small intestine phenol sulfotransferase was estimated, by gel filtration on the calibrated Sephadex G-100 column, to be about 69,000. Thus it was the same as that of bovine small intestine PST [7], but about twice as high as human lung enzyme [4].

Discussion

Phenol sulfotransferases isolated from various tissues show different substrate specificity [14, 8, 7]. The human ileum PST as other human extrahepatic phenol sulfotransferases [2–4] as well as the human liver enzyme [6]

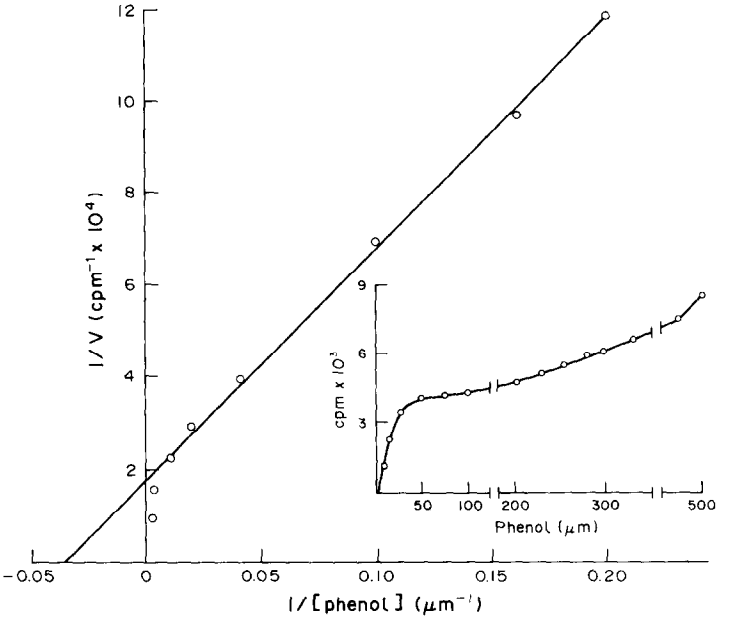

Fig. 1. Lineweaver–Burk plot of human enterocyte PST with phenol as the variable substrate. Each value represents the mean of three determinations.

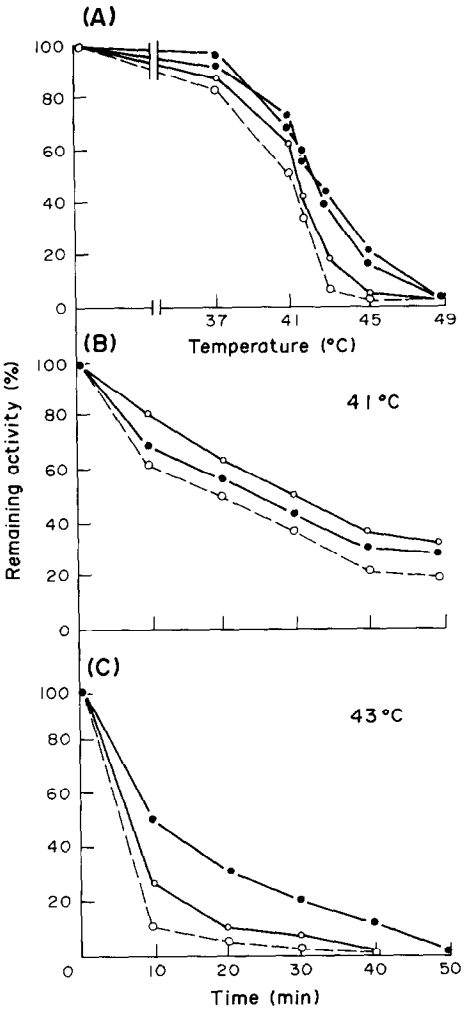

Fig. 2. Thermal stability of human small intestine PST. The enzyme was preincubated for 15 min at various temperatures (A) and for various time intervals (B, C). Activity was measured with 50 μM dopamine (○); 2.5 mM phenol (◦); 250 μM phenol (●); 50 μM phenol (•). Each value represents the mean of three determinations on a single sample of intestine.

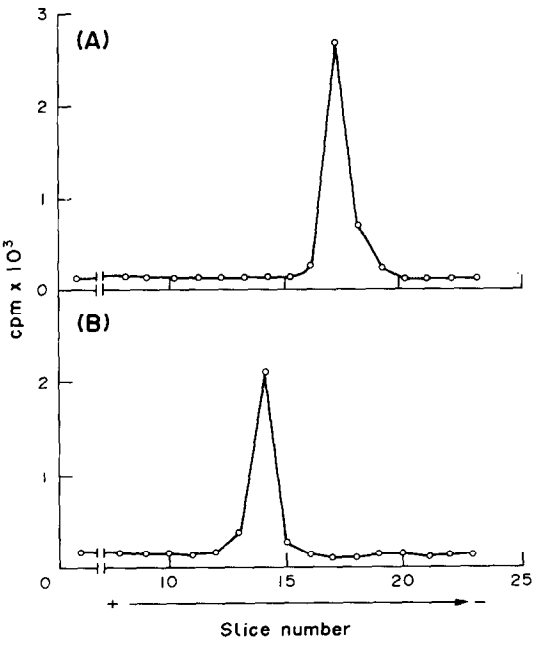

Fig. 3. Polyacrylamide gel electrophoresis of human (A) and bovine (B) small intestinal phenol sulfotransferases. The electrophoresis was carried out at pH 5.5. About 100 μg of protein was applied to the gel.

is active with both exogenous phenols and dopamine. The affinity of the ileum enterocyte enzyme towards dopamine is very close to that of human lung PST [4] and the thermolabile form isolated by Sundaram and Weinshilboum [15] from human jejunum, but much higher than the affinity towards phenol. Similarly, as with human lung [4] and platelet PSTs [16], two K_m values were calculated for the enterocyte enzyme with phenol as a variable substrate. The ileum phenol sulfotransferase which is active with dopamine and shows low affinity towards phenol (K_m = 170 ± 5.7 μM) is less thermostable than the enzyme which shows higher affinity to phenol (K_m = 28.6 ± 2.4 μM). The affinity of both TS and TL forms of human enterocyte PST towards phenol is much higher than that of TS form of the bovine small intestine enzyme [7]. It is also higher than that of jejunum and ileum PST from rat (Barańczyk-Kuźma *et al.*, unpublished).

This preliminary study of human ileum enterocyte phenol sulfotransferase suggests that the enzyme, like other phenol sulfotransferases from human tissues contains at least two molecular forms which can sulfate both exo- and endogenous phenolic compounds. Metabolism of those compounds by the sulfation pathway seems to be more species- than tissue-dependent.

In summary, phenol sulfotransferase (PST) was isolated from human ileum enterocytes. Phenol, 2-naphthol, *p*-nitrophenol and dopamine were tested as substrates. K_m values for phenol and dopamine were determined before and after preincubation at 41° and 43°. Ileum PST was found to be thermostable with low concentration of phenol and thermolabile with dopamine and higher concentrations of phenol. The molecular weight of native enterocyte PST was estimated as 69,000. The differences between electrophoretical mobility of human ileum and bovine small intestine phenol sulfotransferases were shown.

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Characterization and post-natal development of rat cerebellum tyrosylprotein sulfotransferase

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Tyrosine sulfation has been demonstrated to be a widespread post-translational modification of a variety of functionally diverse, biologically active peptides and proteins [1, 2]. For several of the identified tyrosine sulfated proteins and peptides, such as cholecystokinin (CCK*) [3], gastrin [4], phyllokinin [5], leukosulfakinin [6] and C4 of complement [7], sulfation has been shown to result in the optimization of biological activity.

Sulfoconjugation of the tyrosyl residues of proteins and peptides has been demonstrated to be catalyzed primarily within the Golgi apparatus by the enzyme, tyrosylprotein sulfotransferase (TPST) [8, 9]. This enzyme utilizes 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the sulfate donor and requires that the acceptor tyrosine residue be surrounded by a highly acidic amino acid domain within the protein [8, 10]. Using the acidic, synthetic polymer, poly Glu_6,Ala_3,Tyr_1 (EAY), we have demonstrated recently that TPST displays a fairly wide tissue distribution in the rat [9]. Of the tissues examined, rat liver possesses the highest specific activity, more than 2.5-fold higher than that in the other tissues examined. TPST activity also displays a variable distribution in rat brain, with the cerebellum and pituitary containing the highest specific activity.

Little is known about the factors that influence or promote tyrosine sulfation of peptides and proteins within the brain and other tissues. For example, one such factor that has not been addressed is the post-natal development of TPST, as well as whether the formation of this enzyme during development correlates with the synthesis of biologically active tyrosine sulfated proteins and peptides. In this study, we begin to address these latter questions by observing post-natal developmental changes of TPST activity in both rat cerebellum and liver.

EAY (average mol. wt 47,000), MES buffer, Lubrol-PX and unlabeled PAPS were purchased from the Sigma Chemical Co. (St Louis, MO), and bicinchoninic acid protein assay solution was obtained from Pierce (Rockford, IL). [^{35}S]-5′-PAPS (0.6 to 1.0 Ci/mmol) was purchased from New England Nuclear (Boston, MA). All other chemicals used were the purest available from commercial sources.

Timed pregnant rats were obtained from Holtzman Laboratory Animals (Madison, WI) and housed indi-

* Abbreviations: CCK, cholecystokinin; TPST, tyrosylprotein sulfotransferase; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; EAY, polymer of Glu_6,Ala_3,Tyr_1; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; and MES, a-(*N*-morpholine)ethanesulfonic acid.