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LUNG PHENOL SULFOTRANSFERASES

THERMAL STABILITY OF HUMAN AND BOVINE ENZYMES

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Abstract—Phenol sulfotransferase (PST) from human and bovine lung was purified about 1000-fold and the activity was measured with different acceptor substrates. Human lung PST catalyzes sulfation of both exo- and endogenous phenols, but the bovine lung enzyme only exogenous phenols. This difference in substrate specificity seems to be related to the presence of at least two different molecular forms of the enzyme: a thermostable and a thermolabile form in human lung, and two thermostable forms in bovine lung. Like the human platelet PST, the thermostable form from human lung is active with low concentrations of phenol (K_m = 45 μM) and the thermolabile form with dopamine and high concentrations of phenol (K_m = 909 μM). Bovine lung, which shows no catecholamine sulfating activity, contains two thermostable phenol sulfotransferases, both active with phenol but differing in their affinity to this substrate (K_m = 40 μM and 335 μM, respectively).

3′-Phosphoadenylsulfate : phenol sulfotransferase (PST, EC 2.8.2.1) is a cytoplasmic enzyme which transfers the sulfate group from a physiological donor, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to various phenolic acceptor compounds. The enzyme catalyzes the sulfation of many phenolic drugs and their metabolites [1–3]. Formation of sulfate monoesters is an important route for inactivation of catecholamine neurotransmitters in such tissues as brain [4, 5], blood platelets [6, 7], liver [8, 9] and human lung [10]. However, some organs, e.g. rat kidney [11] and bovine small intestine (Barańczyk-Kuźma and Szymczyk, unpublished), do not show PST activity towards biogenic amines.

On the basis of substrate specificity, Rein *et al.* [12] suggested the presence of at least two forms of PST in human platelets: "P", or phenol, and "M", or monoamine, forms.

Two independently regulated forms of phenol sulfotransferase in human platelets were also described by Reither and Weinshilboum [6] and Reither *et al.* [13]. One form was thermostable and the other thermolabile. The thermostable PST shows high affinity towards exogenous compounds such as phenol. *p*-nitrophenol, 6-OH melatonin. The thermolabile form catalyzes the sulfate conjugation of catecholamines and millimolar amounts of phenols.

The fact that phenol sulfotransferase occurs in multiple molecular forms, as well as the role of sulfation in drug and catecholamine metabolism in non-hepatic tissues, led us to attempt characterization of PST from human and bovine lung.

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°. 2-Naphthol was a product of Merck; other acceptor substrates were purchased from Sigma, Aldrich and Eastman, 2-mercaptoethanol was from Calbiochem (Los Angeles, CA), DEAE-cellulose DE-11 from Whatman Biochemicals (Maidstone, Kent, U.K.), sodium dodecylsulfate agarose from Sigma Chemical Company and Sephadex G-100 from Pharmacia (Uppsala, Sweden).

All other chemicals were of analytical grade.

Tissues

Human lungs were obtained at autopsy from persons 40–65 years old; the organs with no pathological changes observed by histological methods were used. Fresh bovine lungs were taken from a slaughterhouse immediately after killing of the animal.

PST assay

PST activity was measured using [^{35}S]3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the sul-

Table 1. Activity of human and bovine lung phenol sulfotransferases with different substrates

Substrate (30 μM)	Human lung PST	Bovine lung PST
	% activity	
2-Naphthol	100	100
p-Nitrophenol	100	96
Phenol	70	48
Salicylamide	56	45
p-Methylphenol	72	90
o-Methoxyphenol	340	105
Adrenaline	250	0
Noradrenaline	28	0
Dopamine	500	0
DOPA	0	0
Tyrosine	0	0

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fate donor. The assay mixture consisted of 10 mM sodium phosphate buffer, pH 6.5, variable amounts of phenolic 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-labelled products was determined by the method of Foldes and Meek [14]. The reaction was stopped by addition of 0.1 M barium acetate, 0.1 M barium hydroxide, and 0.1 M zinc sulfate (200 μl each). The resulting precipitate was removed by centrifugation and the barium hydroxide–zinc sulfate precipitation step repeated. A 1 ml aliquot of the resulting supernatant was subjected to liquid scintillation spectrometry.

Protein determination

Protein was determined according to Lowry *et al.* [15] or spectrophotometrically by the method of Warburg and Christian [16], with crystalline bovine serum albumin as a standard.

Thermal stability experiments

Phenol sulfotransferases after the last step of purification were 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.

RESULTS

Purification procedure for human and bovine lung phenol sulfotransferase

The purification was carried out using techniques similar to those described earlier [10]. The specific activity of the enzyme after the last step of purification assayed with 30 μM 2-naphthol as an acceptore substrate was 2.5 and 3.1 nmole/mg/min for human and bovine lung PST, respectively which represented about 1000-fold purification over the activity in the crude homogenates.

Enzyme activity

Activity of the purified human and bovine lung PST's was assayed using different exo- and endogenous phenolic substrates at the concentration of 30 μM (Table 1). It is of interest that neither adrenaline nor dopamine was sulfated by bovine lung PST, but the human lung enzyme showed a very high activity with either catecholamine. Noradrenaline was also sulfated only by the human lung enzyme. Tyrosine and DOPA at the concentrations from 1 to 500 μM were not sulfated by the enzymes studied.

Effect of dopamine and phenol on PST activity

Human lung PST. Human lung enzyme shows a high affinity to dopamine with K_m value of 1.5 μM. A marked substrate inhibition was observed at dopamine concentrations exceeding 50 μM (Fig. 1).

No inhibition was observed with phenol at the concentration up to 5 mM. Lineweaver–Burke double reciprocal plot of the results gave a biphasic curve (Fig. 2). The K_m value at low phenol concentrations (10–500 μM) was 45 μM and with higher concentrations (0.5–5 mM) it was 909 μM.

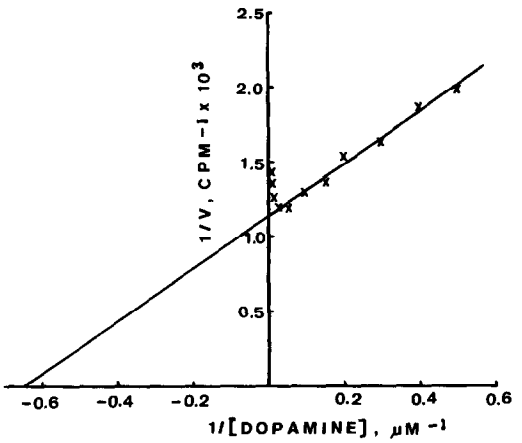

Fig. 1. Lineweaver–Burke plot of human lung PST with dopamine as the variable substrate. Assay was performed as described in Materials and Methods using the purified enzyme and the constant concentration (0.29 μM) of [^{35}S] PAPS (0.45 μCi). The reaction mixture was incubated for 15 min at 37°. Each value represents the mean of three determinations.

Bovine lung PST. The enzyme did not sulfate adrenaline, noradrenaline, dopamine and DOPA at the concentrations of 1–500 μM either at pH 6.5 or 7.9.

In the presence of phenol as an accepting substrate, the Lineweaver–Burke double reciprocal plot gave a biphasic curve (Fig. 3). No substrate inhibition was observed up to phenol concentration of 10 mM. The K_m values calculated by the method of Eisenthal and Cornish-Bowden [17] were 40 and

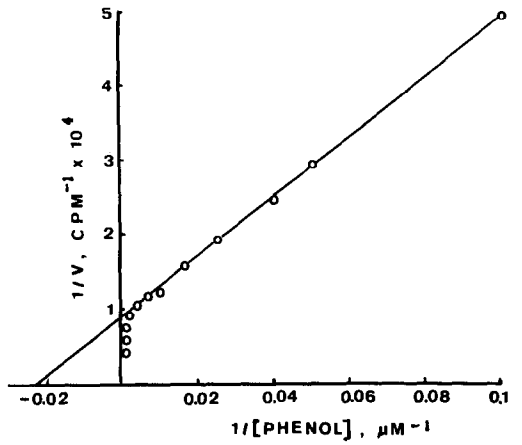

Fig. 2. Lineweaver–Burke plot of human lung PST with phenol as the variable substrate. Activity was assayed as described in Materials and Methods using the purified enzyme and the constant concentration (0.29 μM) of [^{35}S] PAPS (0.45 μCi). The reaction mixture was incubated for 30 min at 37°. Each value represents the mean of three determinations.

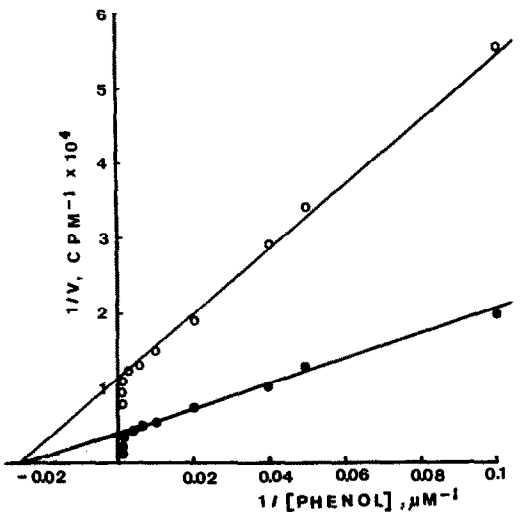

Fig. 3. Lineweaver–Burke plots of bovine lung PST. Activity was assayed as described in Materials and Methods using the purified enzyme, the constant concentration (0.29 μM) of [^{35}S]PAPS (0.45 μCi) and different concentrations of phenol. The reaction mixture was incubated for 30 min at 37°. Each value represents the mean of three determinations. ●, enzyme without preincubation; ○, enzyme after 15 min preincubation at 45°.

335 μM for the two forms of the enzyme, respectively.

Thermal stability

Human and bovine lung PSTs were preincubated for 15 min at 37–49°. Human lung PST activity measured with 30 μM dopamine and 2.5 mM phenol was more thermolabile than that measured with lower concentrations (50 and 500 μM) of phenol (Fig. 4). After 15 min preincubation at 41° the enzyme assayed with 50 μM phenol retained about 70% of its activity, but in the presence of 30 μM dopamine only 30% (Figs. 4 and 5). After preincubation at 43° with 50 or 500 μM phenol about 40% of the activity was retained, but not more than 10% with dopamine or 2.5 mM phenol (Figs. 4 and 5).

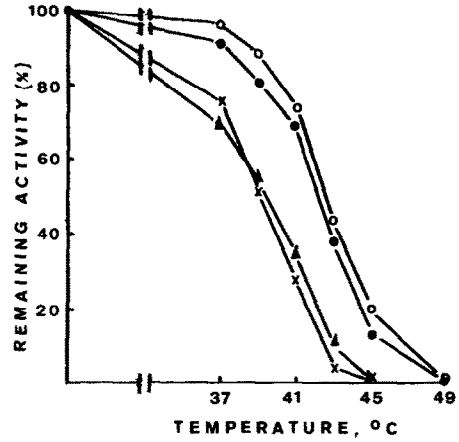

Fig. 4. Thermal stability of human lung PST with dopamine and different concentrations of phenol. The enzyme was preincubated for 15 min at the temperature indicated. Each value represents the mean of three determinations. ×, 30 μM dopamine; ●, 50 μM phenol; ○, 500 μM phenol; △, 2.5 mM phenol

Bovine lung PST was equally stable with all the phenol concentrations used (50, 500, 2000 μM) (Fig. 6a). The enzyme retained about 50% of its initial activity after 15 min preincubation at 45° (Fig. 6b) and was still active after 50 min preincubation at that temperature. It also showed very high thermal stability with different exogenous phenolic compounds such as 2-naphthol, *p*-nitrophenol, *p*-methylphenol, *o*-methoxyphenol.

K_m values for both human and bovine lung enzymes were assayed after 15 min preincubation at 43° and 45°, respectively, using phenol as a variable substrate.

Human lung PST after 15 min preincubation at 43° was inhibited by phenol at the concentrations

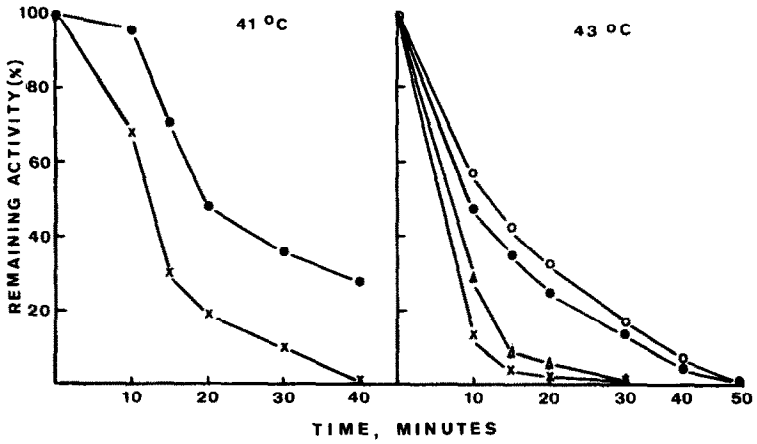

Fig. 5. Thermal stability of human lung PST. The enzyme was preincubated at 41° and 43° for the time indicated. PST activity was measured with dopamine and phenol. Each value represents the mean of three determinations. ×, 30 μM dopamine; ●, 50 μM phenol; ○, 500 μM phenol; △, 2.5 mM phenol

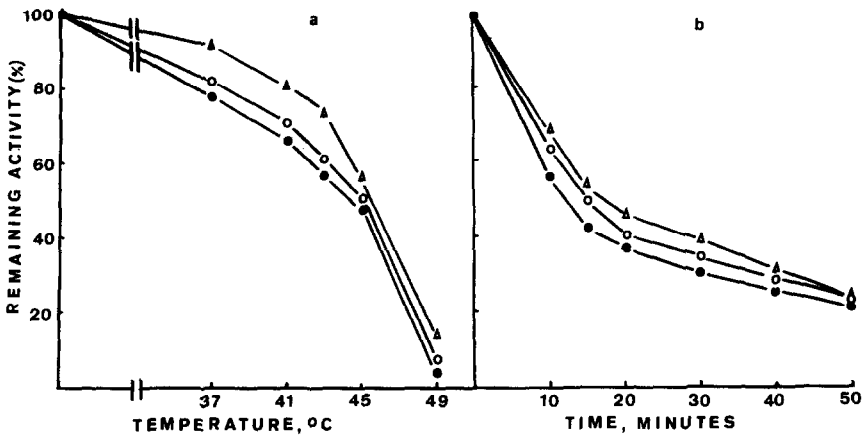

Fig. 6. Thermal stability of bovine lung PST. The enzyme was preincubated for 15 min at various temperatures (a) and at 45° for various time intervals (b). Activity was measured with different concentrations of phenol. Each value represents the mean of three determinations. ●, 50 μM phenol; ○, 500 μM phenol; △, 2.5 mM phenol

exceeding 1 mM and only one K_m value of 45 μM could be calculated (Fig. 7).

No substrate inhibition up to 8 mM phenol was observed in the case of the bovine lung enzyme after 15 min preincubation at 45°. The biphasic curve in the Lineweaver–Burke double reciprocal plot was still present and two K_m values, very similar to those observed without preincubation, were obtained: 38.5 and 339 μM respectively (Fig. 3).

DISCUSSION

Phenol sulfotransferase activity has been detected in the liver [8, 9] and brain [4, 18] of various mammals. It was also found in rat kidney and stomach [11] as well as in human blood [6, 19], lung [10] and cultured skin fibroblasts [20]. Sulfate esters of 1-naphthol are present in human bronchus and colon tissue [21, 22]. Four homogeneous forms of phenol sulfotransferase were found in rat liver [8, 23]. These forms, differing in electric charge, amino-acid composition, optimal pH and substrate specificity were designed I–IV. PST I and II were specific mainly for exogenous phenols, whereas forms III and IV were active with phenols and catecholamines.

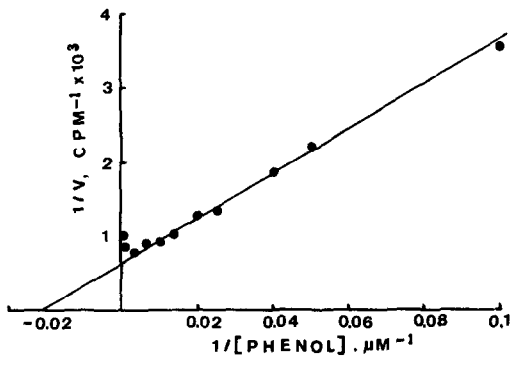

Fig. 7. Lineweaver–Burke plot of human lung PST after 15 min preincubation at 43°. Activity was assayed as described in Materials and Methods using the purified enzyme, the constant concentration (0.29 μM) of [^{35}S]PAPS (0.45 μCi) and different concentrations of phenol. The reaction mixture was incubated for 30 min at 37°. Each value represents the mean of three determinations.

Phenol sulfotransferases isolated from different tissues show different substrate specificity. The brain enzyme is active mainly with a large group of catecholamines and their metabolites [4, 18], rat stomach PST sulfates exogenous phenols and some catecholamine metabolites [11], rat kidney enzyme-only exogenous phenols [11]. Human lung PST is active with both, exo- and endogenous substrates, whereas bovine lung enzyme only with exogenous phenols (Table 1).

It seems that the differences in substrate specificity between human and bovine lung enzymes are related to the presence of at least two forms of the enzyme in this tissue: a thermostable (TS) and a thermolabile (TL) in human lung and two thermostable (TS) forms in bovine lung.

All thermostable forms are specific toward phenol, but the bovine lung enzymes show different affinity toward this compound, with K_m = 40 and 335 μM, respectively. The thermolabile enzyme, present only in human lung, similarly to the TL form from platelets, is active with dopamine and high concentrations of phenol (K_m = 909 μM).

It appears that specificity of PST and the pattern of distribution of its molecular forms is not only tissue- but also species-dependent.

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REFERENCES

1. A. J. Cummings, M. L. King and B. K. Martin, *Br. J. clin. Pharmac.* **29**, 150 (1967).
2. R. T. Williams, in *Detoxication Mechanisms*, p. 297. Wiley, New York (1979).

3. C. Reither and R. M. Weinshilboum, *Clin. pharmac. Ther.* **32**, 612 (1982).
4. K. J. Renskers, D. F. Kevin and J. A. Roth, *J. Neurochem.* **34**, 1362 (1980).
5. E. J. M. Pennings, R. Vrielink and G. M. J. Van Kempen, *Biochem. J.* **173**, 299 (1978).
6. C. Reither and R. M. Weinshilboum, *J. Pharmac. exp. Ther.* **221**, 43 (1982).
7. G. Rein, V. Glover and M. Sandler, *Clin. chim. Acta* **111**, 247 (1981).
8. W. B. Jakoby, R. D. Sekura, E. S. Lyon, C. J. Marcus and J. L. Wang, in *Enzymatic Basis of Detoxication* (Ed. W. B. Jakoby), p. 199. Academic Press, New York (1980).
9. R. T. Borchardt and C. S. Schasteen, *Biochim. biophys. Acta* **708**, 272 (1982).
10. A. Barańczyk-Kuźma, *Biochem. Med.* **000** (1985).
11. A. Barańczyk-Kuźma, R. T. Borchardt and C. L. Pinnick, *Acta biochim. Polon.* **32**, 35 (1985).
12. G. Rein, V. Glower and M. Sandler, in *Phenolsulfotransferase in Mental Health Research* (Eds. M. Sandler and E. Usdin), p. 98. Macmillan, New York (1981).
13. C. Reither, G. Mwaluke, J. Dunnete, J. Van Loon and R. Weinshilboum, *Archs. Pharmac.* **324**, 140 (1983).
14. A. Foldes and J. L. Meek, *Biochim. biophys. Acta* **327**, 365 (1973).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. O. Warburg and W. Christian, *Biochem. Z.* **310**, 384 (1941).
17. R. Eisenthal and A. Cornish-Bowden, *Biochem. J.* **139**, 715 (1974).
18. A. Barańczyk-Kuźma, R. T. Borchardt, C. S. Schasteen and C. L. Pinnick, in *Phenolsulfotransferase in Mental Health Research* (Eds. M. Sandler and E. Usdin), p. 55. Macmillan, New York (1981).
19. E. J. M. Pennings and G. M. J. Van Kempen, *Clin. chim. Acta* **134**, 199 (1983).
20. A. Crooks, X. O. Breakefield, C. H. Sulens, C. M. Castiglione and J. K. Coward, *Biochem. J.* **176**, 187 (1978).
21. E. M. Gibby and G. M. Cohen, *Biochem. Pharmac.* **33**, 739 (1984).
22. E. M. Gibby and G. M. Cohen, *Br. J. Cancer.* **49**, 645 (1984).
23. R. D. Sekura and W. B. Jakoby, *J. biol. Chem.* **254**, 5658 (1979).