

Properties of phenol sulfotransferase from bovine brain

Dorota Drobisz and Anna Barańczyk-Kuźma

Chair and Department of Biochemistry, Institute of Biopharmacy, Warsaw Medical School, S. Banacha 1, 02-097 Warsaw, Poland

Phenol sulfotransferase (PST¹, EC 2.8.2.1) catalyses sulfate conjugation of exo- and endogenous compounds bearing phenolic functional groups [1]. The PST-catalysed reaction represents a major metabolic pathway for detoxication of catecholamine neurotransmitters of some mammals in the brain [2, 3]. The presence of different molecular forms of PST in brain of several mammalian species has been reported [4 - 7].

In earlier studies it has been demonstrated that a thermostable PST form is present in the bovine brain microvessel endothelial cells, forming the blood-brain barrier [8, 9]. This form is active with many deaminated and/or methylated catecholamine metabolites which suggests that sulfation plays a role in regulating

catecholamine movement between the blood and the central nervous system [10].

In the present work we have studied the properties of phenol sulfotransferase from bovine brain cortex.

The bovine brain was obtained from a local slaughterhouse immediately after the animal had been killed. PST was extracted from fresh, superficial gray matter scraped off from the entire cerebral cortices. The homogenate was prepared in 10 mM sodium phosphate buffer, pH 7.6, containing 1 mM dithiothreitol and 0.25 M sucrose. Then it was centrifuged at $15000 \times g$ for 15 min, and recentrifuged at $100000 \times g$ for 60 min yielding the cytosolic fraction. The PST activity was determined according to Foldes & Meek [11] with $0.45 \mu\text{Ci } [^{35}\text{S}]3'$ -phosphoadeno-

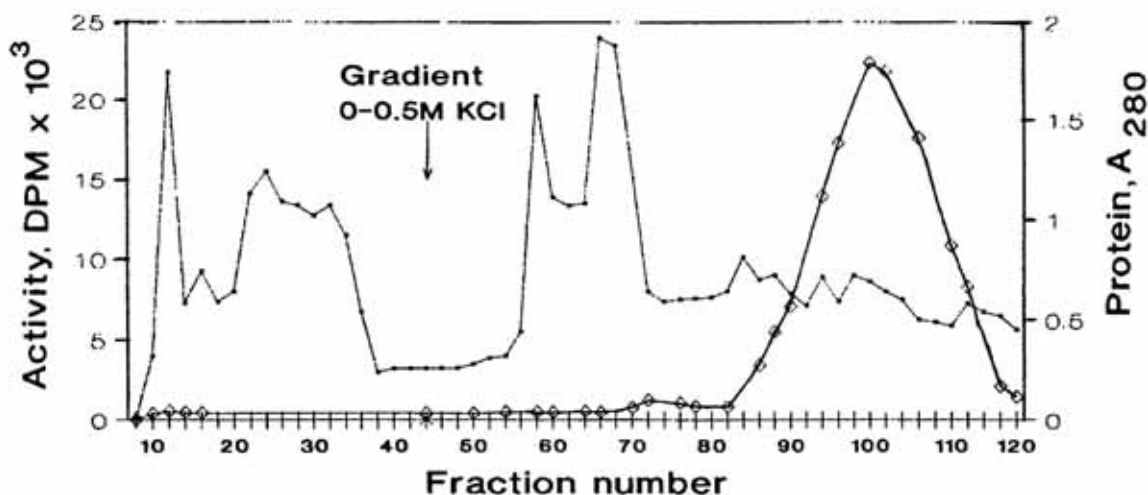


Fig. 1. DEAE-cellulose chromatography of phenol sulfotransferase from bovine brain cortex. The cytosol (about 200 mg protein) was applied on the column (20 x 2 cm) equilibrated with 10 mM sodium phosphate buffer, pH 7.6, containing 1 mM dithiothreitol. Fractions of 5 ml were collected, PST activity with 1 mM *p*-nitrophenol (□), and protein (●) were determined

¹Abbreviations: PST, phenol sulfotransferase; for other abbreviations see legend to Fig. 2

sine-5'-phosphosulfate (0.29 mM) as a sulfate donor. In the presence of 1 mM *p*-nitrophenol the specific activity of the cytosolic fraction was 0.74 ± 0.07 pmol/mg protein per minute. Like in bovine brain microvessel endothelial cells [10] no PST activity was observed with catecholamines at the concentration up to 1 mM.

A single PST form was eluted from DEAE-cellulose with a linear KCl gradient (Fig. 1). This form was active with catecholamine metabolites but not with catecholamines themselves (Fig. 2 A). Among catecholamine metabolites the highest PST activity was observed with 500 μ M MOPEG and HVA, the deaminated and methylated derivatives of norepinephrine and dopamine, respectively. The enzyme sulfated also some drugs having a phenolic or catechol ring, such as: analgetics, salicylamide and paracetamol, antihypertensive α -methyl dopa, adrenergics, phenylephrine, propranolol, but not octopamine or metaraminol (Fig. 2 B). 6-Hydroxydopamine, a neurotoxin which de-

stroys catecholaminergic nerve terminals was not metabolized by bovine cortex PST either.

The optimum pH for bovine brain PST in the presence of *p*-nitrophenol was 8.0; the enzyme affinity towards this substrate was quite low with K_m 0.2 ± 0.02 mM, but higher than for MOPEG (K_m 0.67 ± 0.03 mM).

The brain cortex enzyme showed a similar thermostability on preincubation at the 37°C to 49°C as that from brain microvessel endothelial cells. Its relative molecular mass determined on Sephadex G-100 calibrated column was about 64000, and isoelectric point estimated by agarose gel electrophoresis, with ampholine in the pH range of 3.5 - 10.0, was 7.6 (not shown).

The activity of bovine gray matter PST was inhibited by Mn^{2+} and Zn^{2+} . At the ion concentration of 5 mM only 23% and 13% of the initial activity was observed after 10 min preincubation with $MnCl_2$ and $ZnCl_2$, respectively (Fig. 3). No inhibition was observed with Mg^{2+} at the concentrations up to 10 mM. Catecho-

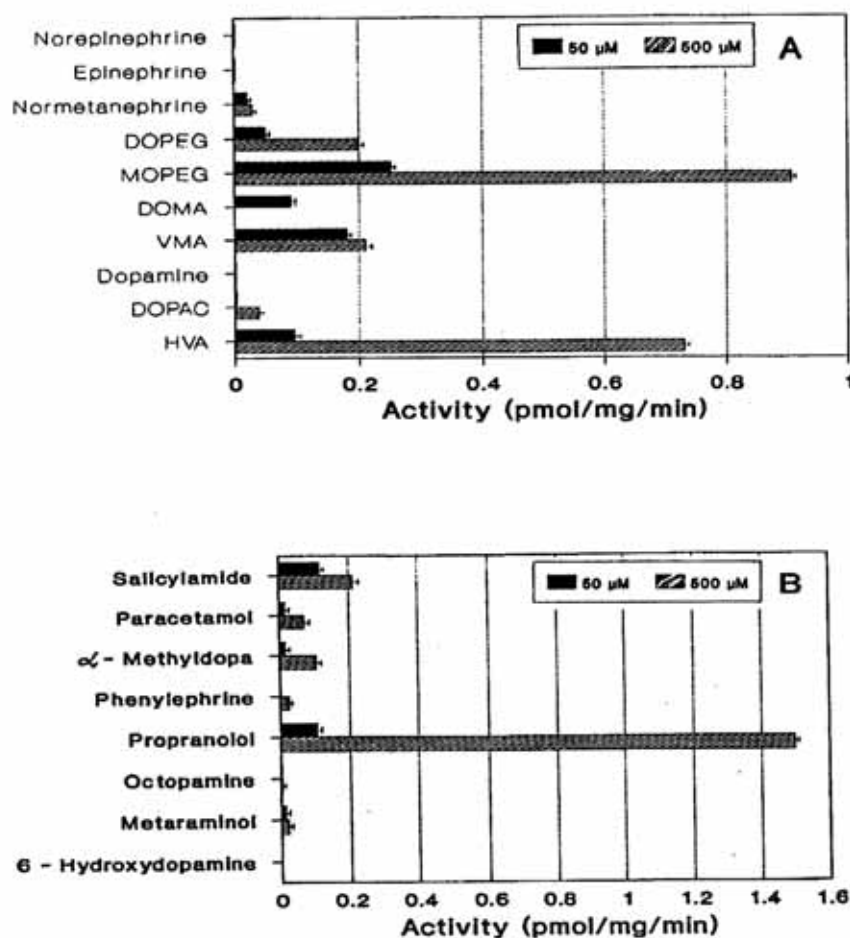


Fig. 2. Substrate specificity of phenol sulfotransferase from bovine brain cortex.

Activity of PST after DEAE-cellulose chromatography was assayed using 50 and 500 μ M catecholamines and their derivatives (A), and drugs (B). Each value is the mean of three determinations. DOPEG, 3,4-dihydroxyphenylethylene glycol; MOPEG, 3-methoxy-4-hydroxy-phenylethylene glycol; DOMA, 3,4-dihydroxymandelic acid; VMA, 3-methoxy-4-hydroxymandelic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid

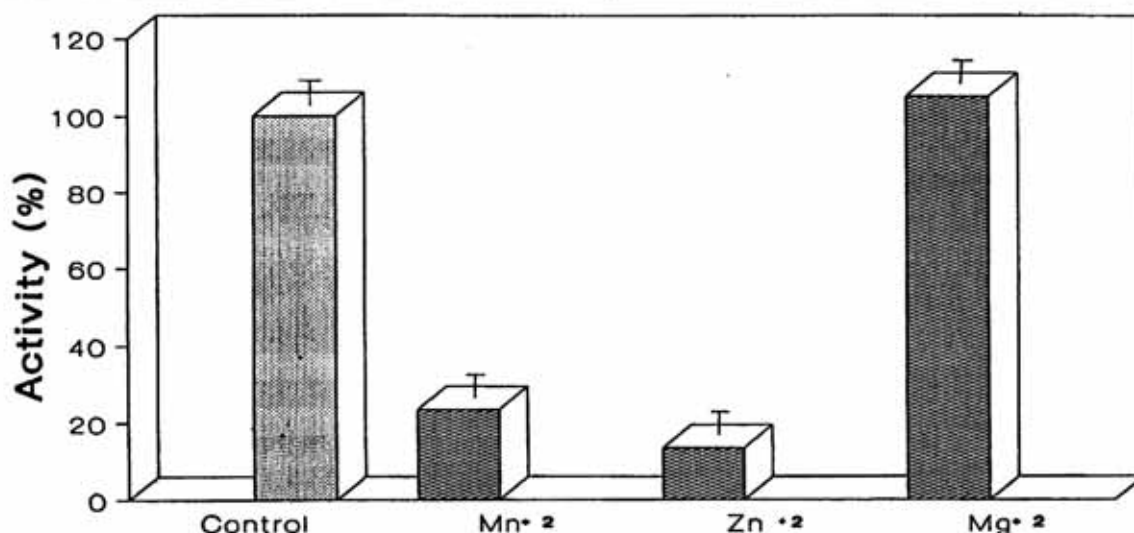


Fig. 3. The effect of ions on the activity of phenol sulfotransferase.

The activity was determined after 10 min preincubation of the enzyme with 5 mM ion solution in 10 mM sodium phosphate buffer, pH 8.0, using 1 mM *p*-nitrophenol as an acceptor substrate. The activity after preincubation with buffer alone (2.75 pmol/mg per min) was taken as 100. Each value is the mean of three determinations

lamines (dopamine, norepinephrine, epinephrine) at the concentrations up to 1 mM did not inhibit bovine brain PST. The enzyme was very sensitive on 1,2-dichloro-4-nitrophenol, the specific inhibitor of sulfation [12], with I_{50} 0.2 mM. It was also inactivated by -SH groups reagents: *N*-ethylmaleimide and reduced glutathione, with I_{50} 0.1 mM and 2.5 mM, respectively, as well as by phenylglyoxal, a compound specific for arginine residues (I_{50} 0.3 mM).

Thus, it can be concluded that only one PST form is present in the bovine brain cortex. The properties of this form appear to be similar to those of the monkey brain cortex PST II [7]. Both enzymes, as well as that isolated by us from the bovine gray matter microvessel endothelial cells [10] do not sulfate catecholamines, but they do sulfate catecholamine metabolites and different exogenous phenolic compounds. The results of this study show that in bovine brain cortex, sulfation plays a role in inactivation of various drugs, and support our earlier suggestion that it can regulate movement of catecholamines between the blood and the central nervous system.

REFERENCES

- Jakoby, W.B., Sekura, R.D., Lyon, E.S., Markus, C.J. & Wang, J.L. (1980) in *Enzymatic basis of detoxification* (Jakoby, W.B., ed.) p. 199, Academic Press, New York.
- Richter, D. (1940) *J. Physiol.* **98**, 361 - 374.
- Renskers, K.J., Foer, K.D. & Roth, J.A. (1980) *J. Neurochem.* **34**, 1362 - 1368.
- Schanberg, S.M., Breese, G.R., Schldkraut, J.J., Gordon, E.K. & Kopin, I.J. (1968) *Biochem. Pharmacol.* **17**, 2006 - 2008.
- Barańczyk-Kuźma, A., Borchardt, R.T., Schasteen, C.S. & Pinnick, C.L. (1981) *Psychopharmacol. Bull.* **17**, 50 - 51.
- Young, W.F., Jr., Okazaki, H., Laws, E.R., Jr. & Weinshilboum, R.M. (1984) *J. Neurochem.* **43**, 706 - 715.
- Barańczyk-Kuźma, A., Audus, K.L. & Borchardt, R.T. (1992) *Acta Biochim. Polon.* **39**, 153 - 158.
- Barańczyk-Kuźma, A., Audus, K.L. & Borchardt, R.T. (1986) *J. Neurochem.* **46**, 1956 - 1960.
- Audus, K.L. & Borchardt, R.T. (1987) *Ann. N. Y. Acad. Sci. U.S.A.* **507**, 9 - 18.
- Barańczyk-Kuźma, A., Audus, K.L. & Borchardt, R.T. (1989) *Neurochem. Res.* **14**, 689 - 691.
- Foldes, A. & Meek, J.L. (1973) *Biochim. Biophys. Acta* **327**, 365 - 374.
- Mulder, G.J. & Scholtens, E. (1977) *Biochem. J.* **165**, 553 - 559.