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PROPERTIES OF PHENOL SULPHOTRANSFERASE FROM BRAIN OF THE MONKEY *RHESUS MACACA*

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Conjugation with sulphate catalysed by phenol sulphotransferase (PST, EC 2.8.2.1) plays a very important role in inactivation and removal of catecholamines as well as in inactivation of various exogenous phenolic compounds. Due to the activity of this enzyme more than 70% of catecholamines present in human blood serum occurs in the form of sulphate esters [1] and more than 90% of catecholamines is excreted in the same form in urine [2, 3]. In our earlier studies we have demonstrated a significant involvement of PST in inactivation of catecholamines and their metabolites in the monkey *Rhesus macaca* brain cortex microvessel endothelial cells, forming the blood-brain barrier [4, 5]. In the present work we have studied the properties of phenol sulphotransferase from brain cortex of the same monkey species.

The cortex of *Rh. macaca* brain was obtained frozen in solid carbon dioxide. The homogenate was prepared in 10 mM sodium phosphate buffer, pH 7.4 containing 1 mM dithiothreitol and 0.25 M sucrose. Then it was centrifuged at 15 000 × *g* for 15 min, and recentrifuged at 100 000 × *g* for 60 min. The supernatant was used for the studies. The PST activity was determined according to Foldes & Meek [6] with $[^{35}S]$3′-phosphoadenosine-5′-phosphosulfate (PAPS) as a sulphate residue donor. The effect of

inhibitors was assayed after 10 min preincubation of the enzyme at 37°C in the presence of the compound studied and 10 mM sodium phosphate buffer, pH 6.5. Thermostability of the enzyme was determined after 15 min preincubation at the temperature indicated.

On DEAE-cellulose ion-exchange chromatography PST from brain cortex of *Rh. macaca* separated into two forms: non-adsorbed (PST I) and adsorbed (PST II) (Fig. 1). Cationic PST I was active with *p*-nitrophenol (pNP) as a substrate, whereas it was inactive towards catecholamine. The

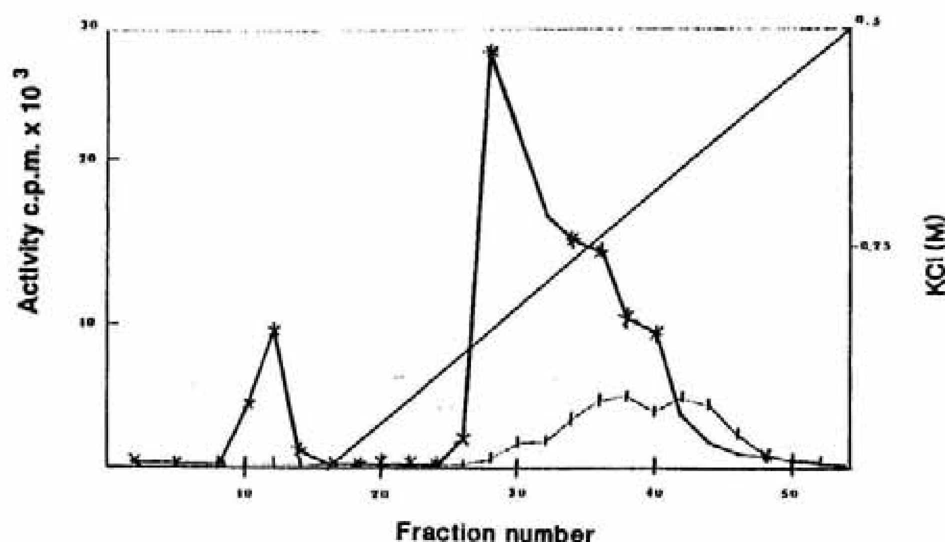

Fig. 1. DEAE-cellulose chromatography of phenol sulphotransferase from monkey brain cortex. The 100 000 × *g* supernatant (about 100 mg of protein) was applied on the DEAE-cellulose column (20 × 1 cm) equilibrated with 10 mM sodium phosphate buffer, pH 7.4, containing 1 mM dithiothreitol. The enzyme was eluted with the same buffer and then with a linear KCl concentration gradient. Fractions of 5 ml were collected and the PST activity towards 50 μM pNP (*) or 50 μM dopamine (+) was determined

main, anionic form, PST II, was active with both pNP and catecholamines, as well as with their metabolites (Table 1). The affinity of PST I to pNP was lower than that of PST II, the K_m values being 200 and 100 μM, respectively. The affinity of PST II to catecholamines was much higher than that to exogenous pNP, the K_m values being: 2.4 μM for dopamine, 14.3 μM for epinephrine, and 100 μM for norepinephrine. The two forms of the enzyme

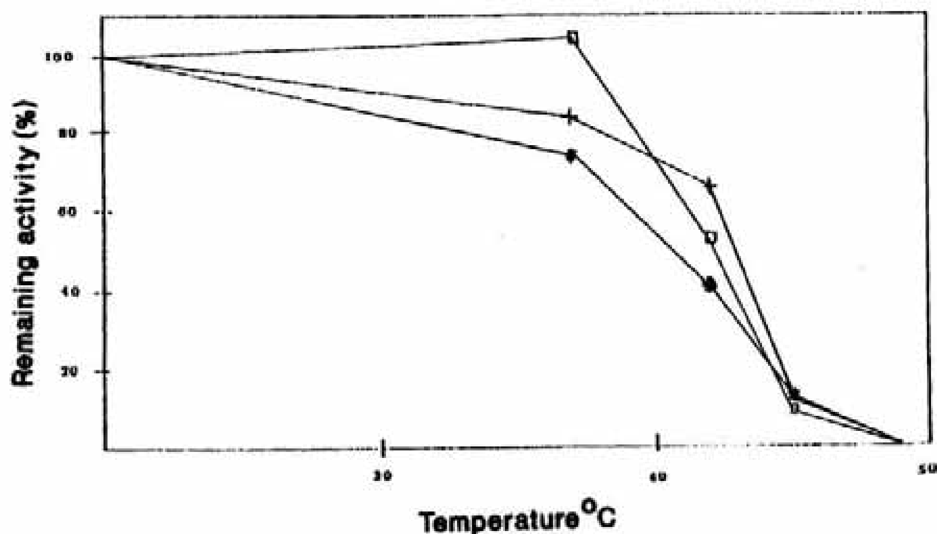

Fig. 2. Thermostability of phenol sulphotransferase from monkey brain cortex. The enzyme forms obtained after DEAE-cellulose chromatography were preincubated at the temperature indicated. The activity of PST I was determined with 1 mM pNP (+) and that of PST II with 1 mM pNP (✱) or 50 µM dopamine (□) as substrates. Each point is the mean values of 4 determinations

Table 1

Substrate specificity of phenol sulphotransferase (PST II) from monkey brain cortex

Substrate (50 µM)	Activity (%)
Dopamine	100
Epinephrine	97
Norepinephrine	38
Methyldopamine	100
Homovanillic acid	13
3,4-Dihydroxyphenylacetic acid	14
DOPA	0
Methyl-DOPA	50

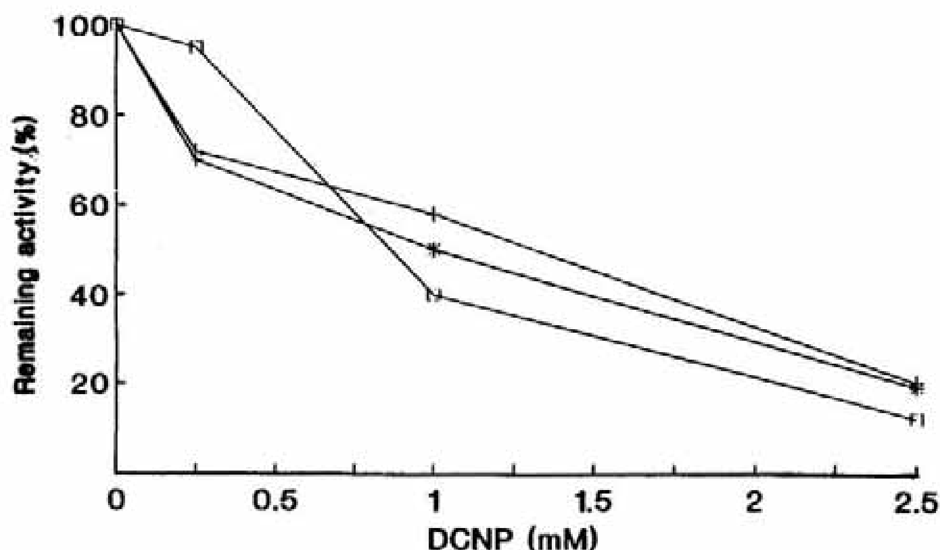

Fig. 3. The effect of 1-chloro-2,4-dinitrophenol (DCNP) on the activity of phenol sulphotransferase from monkey brain cortex. PST I and PST II were preincubated with the inhibitor at the concentrations indicated. The activity of PST I was determined with 1 mM pNP (+) and that of PST II with 1 mM pNP (*) or 50 mM (□) dopamine as substrates. Each point is the mean values of 4 determinations

showed a similar, substrate-independent heat stability (Fig. 2) and susceptibility to 1-chloro-2,4-dinitrophenol (CDNP), a specific inhibitor of sulphatation (Fig. 3). The two forms differed in molecular weight, which estimated by gel filtration on Sephadex G-100 was about 35 000 for PST I and 66 000 for PST II. Both enzyme forms were inactivated by *N*-ethylmaleimide (NEM). Addition of a substrate (pNP or PAPS) to the NEM-inactivated PST I did not reactivate this enzyme (Fig. 4) indicating that –SH groups are not directly involved in binding of these substrates. However, the activity of PST II inactivated by NEM was restored in the presence of pNP or PAPS (Fig. 5). A similar effect was observed also after addition of dopamine. It seems that, in the case of PST II, active –SH groups are located at the binding sites of either of these substrates.

It can be concluded that brain cortex of the monkey *Rh. macaca* contains two different forms of phenol sulphotransferase; both are able to inactivate exogenous phenols, but only the main, anionic form shows an affinity towards catecholamines. Substrate specificity of the enzyme forms is not

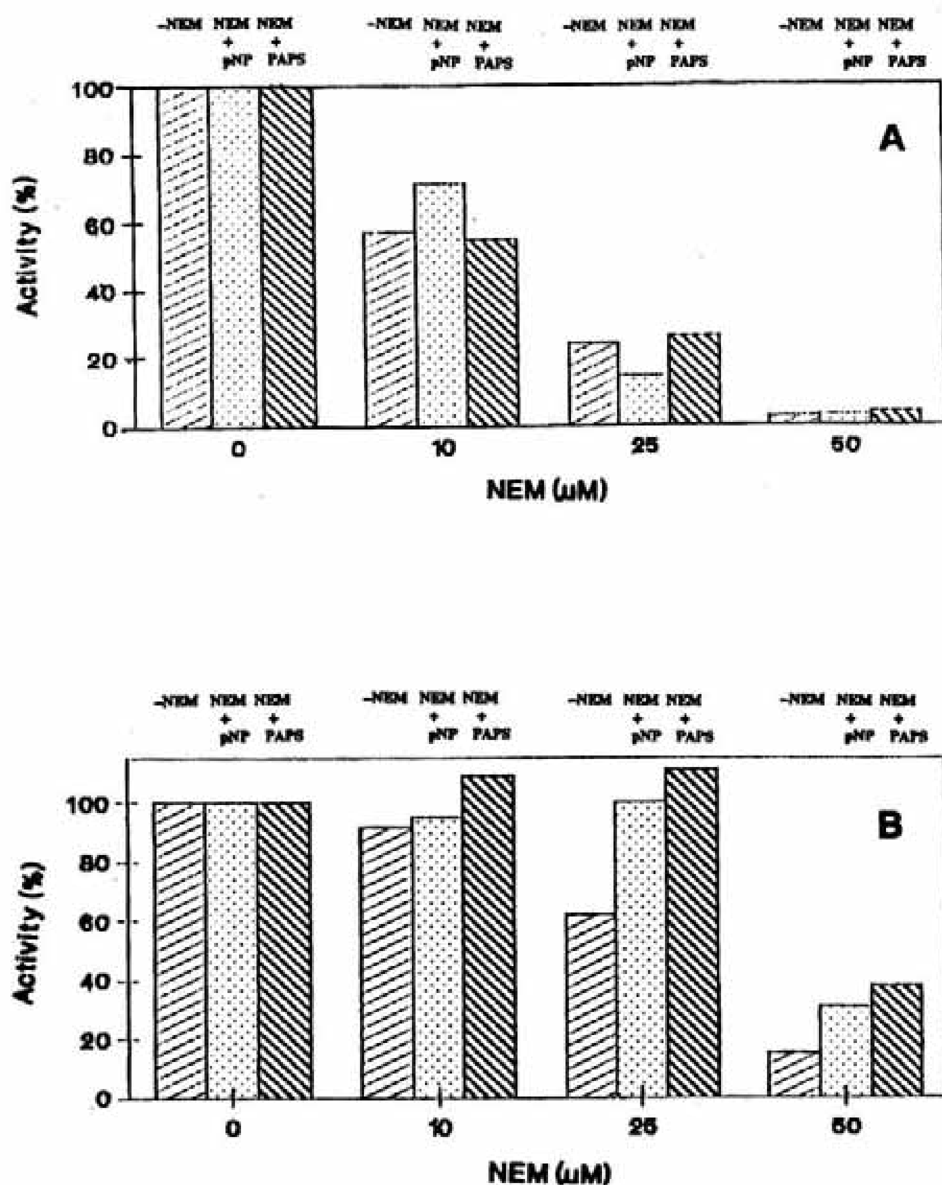

Fig. 4. Inhibition by *N*-ethylmaleimide of PST I (A) and PST II (B) and protective effect of substrates. The enzyme was preincubated with NEM at the concentrations indicated

related to their thermostability, as it has been observed for sulphotransferases isolated from human tissues [7, 8, 9].

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