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# Correlation of phenol sulphotransferase activities in the liver and platelets of rat

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**Abstract**—Phenol sulphotransferase (PST) activity in rat platelet cytosol for *p*-nitrophenol (PNP) sulphation was found to have a similar dependence on PNP concentration and thermostability to that in rat liver cytosol. The activities of PST isoenzyme for the sulphation in the μM range of PNP in rat platelets and rat liver were significantly correlated. Thus, measurement of PST activity in platelets could be a useful and practical method for predicting this activity in liver.

Phenol sulphotransferase (EC 2.8.2.1) (PST) catalyses sulphate conjugation of exogeneous phenolic compounds such as acetaminophen (paracetamol) and salicylamide and endogeneous monoamines such as dopamine and tyramine. PST exists mainly in the liver but is present in other tissues such as kidney and intestine (Anderson & Weinshilboum 1979). Since Hart et al (1979) observed the existence of PST in human platelets, with dopamine and phenol as substrates, several studies on PST in human platelets have been reported. Reiter et al (1983) partially separated human platelet PST by ion exchange chromatography into two forms, one thermostable and active with *p*-nitrophenol (PNP) in the μM range and the other, thermolabile and active with PNP in the mM range. Correlations of PST activities in human platelets and psychiatric disease (Sandler et al 1981) or of the thermostable PST activities in human platelets and urinary excretion of acetaminophen sulphate after oral dosing (Reiter & Weinshilboum 1982) have been noted; however, the real PST activities are not necessarily obtained from these studies because of the variation in absorption of orally dosed acetaminophen. Although a significant correlation of PST activities in human platelets and kidney or gut has also been reported (Anderson et al 1981), the relationship of the activities in platelets and liver has yet to be adequately explained.

We reported that the PST activity-substrate concentration profile for PNP sulphation is biphasic in rat liver cytosol (Mizuma et al 1983a) and in rat isolated hepatocytes (Mizuma et al 1982), indicating the existence of several forms of PST in rat liver (Mizuma et al 1983b). Partial purification of PST in rat liver cytosol by chromatography on DEAE-cellulose, or chromatofocusing, gave three fractions I, II and IV (Nakamura et al 1987) corresponding to the isoenzymes defined by Sekura & Jakoby (1979, 1981). When these purified fractions were used, sulphation in the μM range of PNP was found to be catalysed only by fraction IV, but in the mM range of PNP, the three fractions were active (Nakamura et al 1987). IV corresponded to the M enzyme of human platelets that Rein et al (1982) reported active with the monoamines. We also reported the sulphation of acetaminophen was catalysed primarily by fraction IV (Mizuma et al 1984).

The PNP concentration-dependent forms of PST observed in both rat liver and human platelets warranted attention because, if PST activities in the liver and platelets can be correlated, the activity in platelets, which are easily isolated, should provide some indication of that in the liver. Such a correlation would be of value clinically. In the present study, the relation of PST activities in rat liver cytosol and rat platelet cytosol have been examined.

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## Materials and methods

*Materials.* 3′-[$^{35}$S]Phosphoadenosine 5′-phosphosulphate ([$^{35}$S]PAPS, 1·0–1·5 Ci mmol$^{-1}$) was obtained from New England Nuclear, Boston, MA, USA. Bovine serum albumin (BSA, fraction V) and dithiothreitol were purchased from Sigma Chemical Co., St Louis, MO, USA. All other chemicals and reagents were of analytical grade or better.

*Preparation of rat liver cytosol and platelet cytosol.* Wistar male rats (ca 300 g) were used. The preparation of liver cytosol was as described by Nakamura et al (1987). The final supernatant from centrifugation at 100000 *g* was diluted with 0·062% BSA in 5 mM potassium phosphate buffer (pH 7·5) to give the cytosol fraction for assay of PST activity. Rat platelet cytosol was prepared by a slightly modified method of Anderson & Weinshilboum (1980). Blood was collected in a polyethylene tube containing 2% sodium ethylenediamine tetraacetate (EDTA) from rats (1:3 blood v/v), before isolation of the liver, centrifuged at 200 *g* for 10 min at 20°C and the supernatant transferred to another tube; the procedure was repeated twice. The collected supernatant was then centrifuged at 16300 *g* for 10 min at 4°C; the platelet pellet obtained rinsed twice with 2 mL of 0·9% NaCl. This was discarded and the platelet pellet resuspended in 2 mL of 5 mM potassium phosphate buffer (pH 7·5). The suspension was sonicated at 80 Watts twelve times, 15 s each time (Ohtake Works, Tokyo). The final suspension was centrifuged at 100000 *g* for 60 min at 4°C. The supernatant was mixed with 0·062% BSA in 5 mM potassium phosphate buffer (pH 7·5) to give the cytosol fraction for measuring PST activity.

*Assay of PST activity.* The PST activity in both rat liver and platelet cytosol was measured by the method of Anderson & Weinshilboum (1980) and described by Nakamura et al (1987). The protein content in the liver and platelet cytosol was determined by the method of Lowry et al (1951) using BSA as standard. The sensitivity limit of PST activity in the assay was 0·001 pmol (mg protein)$^{-1}$ min$^{-1}$.

## Results and discussion

The PST activity-PNP concentration profile in rat platelets was similar to that in rat liver cytosol (Mizuma et al 1983b; Nakamura et al 1987), but weaker, with a peak at around 1 μM of PNP and a second peak at a concentration exceeding 1 mM. The thermostability of PST in rat platelet cytosol was also similar to that of the PST in rat liver cytosol (Mizuma et al 1983b) (Fig. 1). The activity was more thermolabile in the μM than in the mM concentration range of PNP. One fraction of PST, which was obtained from rat liver cytosol by chromatography on DEAE-cellulose or by chromatofocusing, was active with PNP at 1 μM (Nakamura et al 1987). This fraction corresponded to PST fraction IV, the isoenzyme defined by Sekura & Jakoby (1979,

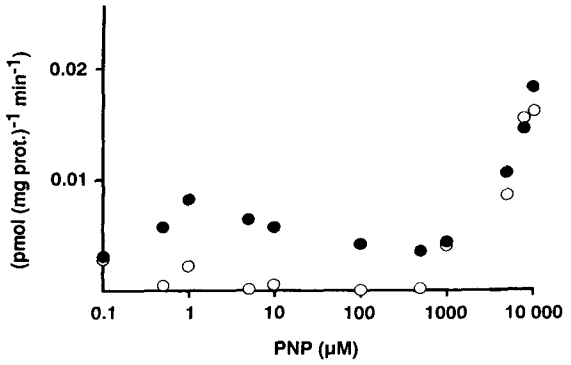


FIG. 1. Effect of substrate concentration on phenol sulfotransferase (PST) activity for *p*-nitrophenol (PNP) sulphation in rat platelet cytosol. Untreated platelets (●); heated platelets (41°C, 15 min) (○).

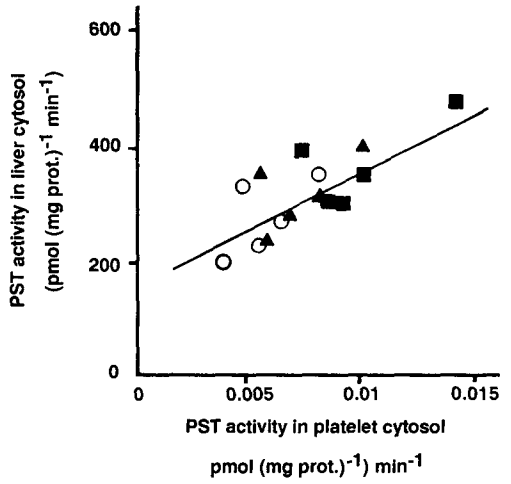


FIG. 2. Correlation of PST activities for PNP sulphation in rat liver cytosol and rat platelet cytosol. A regression line, $y = 21005x + 155$, and correlation coefficient, 0·755 ($P < 0·01$), were obtained by linear regression analysis. A significant difference of the intercept on the ordinate from zero was not found ($P > 0·05$). PNP concentrations: 0·7 μM (■); 0·35 μM (▲); 0·2 μM (○).

1981) or the M enzyme of Rein et al (1982), since this fraction was active not only with β-naphthol and PNP but also with monoamine. Thus, PST activity for PNP in the μM concentration range in rat platelets and rat liver may be considered due to fraction IV. This PST activity corresponded to that in human platelets although PNP concentration-dependent thermostability differed (Reiter et al 1983).

We have found the sulphation of acetaminophen in the μM range (human therapeutic range) to be catalysed primarily by fraction IV (Mizuma et al 1984). Thus, the activities of fraction IV in rat platelet cytosol and rat liver cytosol were examined using PNP in the same concentration range (0·2, 0·35 and 0·7 μM) where substrate inhibition does not occur. In Fig. 2, each symbol represents one set of data for both activities obtained from the same rat. Although some individual variation was observed, the PST activities were found to be statistically significantly correlated.

Estimation of drug metabolism rates in man is often valuable for establishing a dosage regimen, since the metabolism of a drug such as acetaminophen or salicylamide is a predominant factor in determining the elimination rate in-vivo. The correlation of PST activities in platelets and liver is therefore meaningful pharmacokinetically. Thus, measurement of PST activity in platelets could be a useful and practical method for predicting this activity in liver.

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