SOLUBILIZATION AND PURIFICATION OF GLUCURONYL TRANSFERASE FROM RABBIT LIVER MICROSOMES *

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Mammalian liver microsomes contain an enzyme system (glucuronyl transferase)[1] which catalyzes the transfer of the glucuronyl moiety from UDP-glucuronic acid to various phenolic, carboxylic acid and amine acceptors (Axelrod et al., 1958; Dutton and Storey, 1954; Isselbacher, 1956). Previous attempts (Isselbacher, 1956) to solubilize this enzyme system by techniques such as sonic oscillation, organic solvents, or bile salts have been unsuccessful. These procedures have not as yet yielded significant or stable enzyme activity in the supernatant fraction following centrifugation at 105,000 x g. Pogell and Leloir (1961) recently reported the "solubilization" of glucuronyl transferase from guinea pig microsomes following digitonin treatment; however, they indicated that centrifugation was only at 10,000 x g for 10 minutes.

Imai and Sato (1960) have described the solubilization of an aromatic hydroxylase from liver microsomes with the aid of a heat-treated venom of Trimeresurus flavoviridis. Using a modification of their technique, we have obtained a soluble preparation from rabbit liver microsomes which is active in the synthesis of glucuronic acid conjugates of various phenolic (p-nitrophenol, o-aminophenol), and carboxylic acid (anthranilic acid, bilirubin) acceptors. It is of interest that heated preparations of Crotalus adamanteus were ineffective in solubilizing the enzyme activity.

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1 This enzyme system has also been referred to as UDP-transglucuronylase.

In the routine experiments p-nitrophenol was used as an acceptor and the transferase activity was measured spectrophotometrically at 400 mμ as described previously (Isselbacher, 1956). The incubation mixture contained in addition to the enzyme, 0.25 μmoles p-nitrophenol, 0.1 μmoles UDP-glucuronic acid (Sigma Chemical Co.) and 25 μmoles Tris buffer, pH 7.4 in a total volume of 0.3 ml. The incubation was at 37° for 20 minutes and the reaction was stopped with ethanol. An aliquot was then added to 0.1 N NaOH for the spectrophotometric assay.

Rabbit liver was homogenized with four volumes of 0.1 M KCl in a Waring blendor for one minute at 4200 r.p.m. After centrifugation at 8500 x g, microsomes were sedimented at 105,000 x g in a Spinco ultracentrifuge and resuspended in 0.1 M KCl. The microsomal suspension was adjusted to pH 9.0 with 0.5 M Tris buffer and to this was added 0.1 volumes of a heated 2% solution of Trimeresurus flavoviridis[2] prepared as described by Imai and Sato (1960). After standing for 16 hours at 4°, the pH was adjusted to 7.4 with 1 N HCl and the preparation recentrifuged at 105,000 x g for 2 hours. The enzyme activity in this venom-treated supernatant fraction can be lyophylized and stored at -15° for 3 to 4 weeks with only minimal loss of activity. The venom-treated supernatant usually showed a 3- to 5-fold increase in specific activity (Table I) and the total units of transferase activity always exceeded the activity in the microsomal fraction. The apparent increase in transferase activity is probably related to the presence of β-glucuronidase which serves to hydrolyze the product (p-nitrophenyl glucuronic acid) formed by the transferase reaction. At pH 7.4, the venom-treated supernatant fraction contained only 15 to 20 per cent of the β-glucuronidase activity of the microsomal fraction.

A further purification of the enzyme catalyzing the synthesis of p-nitrophenyl glucuronic acid has been achieved. Saturated $(NH_4)_2SO_4$

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[2] The venom was a gift of Dr. K. Nakamura, National Institutes of Health, Tokyo, Japan.

(pH 6.5) was added to the venom supernatant fraction and the protein precipitating between 30 and 40 per cent saturation was redissolved in 0.02 M Tris buffer (pH 6.5). After dialysis against the same buffer, the preparation was added to $Ca_3(PO_4)_2$ gel (1 mg gel per mg protein). The supernatant fraction thus obtained usually showed a 12-fold increase in specific activity over the activity in the microsomes (Table I) but the enzyme was much less stable than the venom-treated fraction. Some protection against loss of activity was afforded by the addition of sulfhydryl compounds such as mercaptoethanol or glutathione. Consistent with this effect was the finding that p-hydroxymercuribenzoate and p-chloromercuriphenyl-sulfonic acid are potent inhibitors of the enzyme system.

TABLE I

PARTIAL PURIFICATION OF GLUCURONYL TRANSFERASE FROM RABBIT LIVER

Fraction	Protein	Specific activity	Units*
	mg	units/mg. protein	
Microsomes	1210	0.78	945
Venom treatment	745	3.05	2270
$(NH_4)_2SO_4$	280	5.60	1570
$Ca_3(PO_4)_2$	152	9.10	1380

* A unit is defined as that amount of enzyme required to produce an optical density change of 1.0 per 20 minutes at 400 mμ.

It has also been noted that venom-treated supernatant fraction of rabbit liver microsomes contained only negligible activity for the synthesis of N-glucuronic acid conjugates, using aniline as an acceptor

(Axelrod et al., 1958). This suggests that this transferase may be distinct from the system involved in the synthesis of the ether- and ester-type glucuronic acid conjugates. To date, with the purification steps described above, no definite evidence has been obtained as to whether the ether- and ester-type conjugates are synthesized by a single enzyme. Studies aimed at resolving this question are in progress.

In view of the success obtained with certain venom preparations in the solubilization of glucuronyl transferase and aromatic hydroxylase (Imai and Sato, 1960) from liver microsomes, it seems quite likely that the venom technique may find wider application and prove to be of value in the solubilization of other microsomal or particulate enzymes.

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