Short Communications

SC 11038

Solubilization and activation of liver UDP glucuronyltransferase by EDTA*

Liver homogenates are capable of transferring the glucuronyl moiety from UDP-glucuronic acid to a series of compounds having phenolic, carboxylic or amino groups. The enzyme which catalyzes this transfer reaction, UDP-glucuronyltransferase (UDP-glucuronate glucuronyltransferase, EC 2.4.1.17), has been localized in the microsomal fraction by ISSELBACHER[1]. He obtained the enzyme in solution by sonication, but it proved to be very unstable. It was later reported that a soluble and stable preparation of the enzyme could be obtained by treating the liver microsomal fraction with 0.01 M EDTA at pH 9.0 (see ref. 2). A "soluble" preparation has also been described by POGELL AND LELOIR[3]. This preparation, obtained by treatment of liver microsomes with digitonin, was activated by albumin. ISSELBACHER has recently reported that snake venom from *Trimesurus flavoridis* solubilizes and activates UDP-glucuronyltransferase from rabbit liver. The activation was attributed in part to inhibition or removal of glucuronidase activity[4].

The present report is concerned with studies on the properties of UDP-glucuronyltransferase activity obtained by the use of EDTA. The acceptor used for the transfer reaction was phenolphthalein[5]. Protein determinations were done with the Folin–Ciocalteu reagent[6]. It has been found that treatment with EDTA results in

TABLE I

PURIFICATION OF UDP-GLUCURONYLTRANSFERASE

The reaction mixture (0.05 ml) contained 0.3 M Tris buffer (pH 8.0), 1.5 mg bovine serum albumin, 0.04 M $MgCl_2$, 3 mM mercaptoethanol, and 1 mM phenolphthalein. Enzyme and water were added to a final volume of 0.1 ml. The reaction was started by the addition of 0.1 μmole of UDP-glucuronic acid (0.005 ml). A blank tube with no UDP-glucuronic acid was run simultaneously. Incubations were done at 37° for 5–10 min. The reaction was stopped by the addition of 3.0 ml of 0.4 M glycine buffer (pH 10.5). The absorbancy at 555 mμ of the blank was matched against the absorbancy of the tube with UDP-glucuronic acid. The decrease in absorbancy (1 cm light path) of the latter was taken as UDP-glucuronyltransferase activity. One unit represents a decrease in absorbancy of 1.0 in 10 min. At 555 mμ and pH 10.5, phenolphthalein has an extinction coefficient of 12.6 $cm^2/\mu mole$.

	Enzyme fraction	*Total volume (ml)*	*Total units*	*Total protein (mg)*	*Specific activity (units/mg protein)*	*Recovery (%)*
I	Liver homogenate	480	4416	13 610	0.32	
II	Ammonium sulfate, 0–42% fraction	312	4417	5 491	0.81	100
III	Microsomal fraction treated with EDTA	190	4454	3 344	1.34	100
IV	Soluble enzyme	573	2093	2 184	0.94	47
V	Ammonium sulfate, 20–45% fraction	350	1580	840	1.88	36

* A preliminary report was submitted to the meeting of the Society for Pediatric Research at Atlantic City, New Jersey, May 1962.

10- to 20-fold activation of transferase activity but that this activation is largely undetectable unless albumin and Mg^{2+} are included in the assay mixture.

A rabbit liver was homogenized in 4 parts of 0.15 M NaCl and centrifuged at 5000 × g × 15 min. The supernatant was brought to 42% $(NH_4)_2SO_4$ saturation with ammonium sulfate and the precipitate suspended in 0.1 M NaCl. This suspension was centrifuged at 80 000 × *g* for 40 min. After another wash with 0.1 M NaCl, the microsomal pellets were suspended in 0.01 M EDTA–0.15 mM mercaptoethanol (pH 9.0) and dialyzed overnight against 3.0 l of the same buffer. The dialyzed solution was adjusted to pH 9.0 and centrifuged at 80 000 × *g* for 1 h. The precipitate was washed with the EDTA buffer 4 to 6 times. The supernatants were combined and after making them 1 mM with $MgCl_2$ they were fractionated with ammonium sulfate. The precipitate obtained between 20 and 45% saturation was collected and dialyzed against 0.05 M Tris, 1 mM EDTA, 1 mM $MgCl_2$ (pH 8.0).

A flow sheet of this procedure is shown in Table I. Samples from the first two steps were dialyzed against the EDTA–mercaptoethanol buffer in order to obtain comparable activation in all fractions before assay. The original homogenate had 336 units before dialysis, so that the activation produced by EDTA in this case amounts to 13-fold.

The activation accompanying dialysis against EDTA is not fully demonstrated unless albumin and $MgCl_2$ are added to the assay mixture (Table II). Neither of these

TABLE II

ACTIVATION OF UDP GLUCURONYLTRANSFERASE BY ALBUMIN AND $MgCl_2$ AFTER TREATMENT WITH EDTA

The assay mixture (0.05 ml) contained, before additions indicated in the table, 0.15 μmole Tris buffer (pH 8.0), 150 mμmoles mercaptoethanol, and 50 mμmoles phenolphthalein. The final volume was 0.1 ml. The protein concentration in both fractions was 4.9 mg/ml.

Enzyme fraction	*Additions*	*Activity (units/ml)*	*Activation factor*
II	None	1.08	
(Microsomal fraction	$MgCl_2$ (2 μmoles)	0.86	0.79
before EDTA treatment)	albumin (1.5 mg)	0.82	0.76
	$MgCl_2$ (2 μmoles) and albumin (1.5 mg)	0.88	0.80
	$MgCl_2$ (2 μmoles), albumin (1.5 mg) and EDTA (0.5 μmoles)	0.74	0.68
III	None	1.65	1.53
(Microsomal fraction	$MgCl_2$ (2 μmoles)	1.75	1.62
after EDTA treatment)	albumin (1.5 mg)	7.50	6.94
	$MgCl_2$ (2 μmoles) and albumin (1.5 mg)	9.50	8.79

two agents produce an increase of the enzyme activity in homogenates that have not been treated with EDTA (Fraction II). Activation with both agents was larger than with either alone. Activation with albumin was greater and more reproducible than that obtained with $MgCl_2$ alone. The optimum concentration of albumin required to obtain the increase in enzyme activity was 1.5 mg/ml; higher concentrations inhibited the reaction.

The addition of EDTA to the reaction mixture did not substitute for dialysis; it is clear that the effect of EDTA is not immediate (Table II). Activation similar to that obtained with EDTA was achieved by dialysis against 0.05 M glycine buffer (pH 9.0), but the enzyme did not become soluble.

The activation of glucuronide conjugase can not be attributed to inhibition of contaminating glucuronidase which could not be detected in the undialyzed preparations under the conditions used for the assay.

It was found that the enzyme activity could not be tested in the presence of SO_4^{2-} ion. A SO_4^{2-} concentration of 0.1 M inhibited about 43%. The addition of $MgCl_2$ was necessary during ammonium sulfate fractionation. It was found that the precipitate obtained in the absence of $MgCl_2$ tended to pack poorly, was brown in color and quite insoluble; Mg^{2+} had also to be present in the buffer employed to dissolve the precipitate obtained with ammonium sulfate. $MnCl_2$ activated the reaction, the increase in rate being 75% of that produced by $MgCl_2$.

The optimum pH for stability was found to be about 7.9. After 21 days at 2° at pH 8.0, 60% of the activity was recovered, and in a sample stored in the freezer (−15°) for 1 year 50% was recovered. These studies were carried out with samples from Fraction V.

Attempts to purify the enzyme further were hampered because manipulation by the usual procedures rendered it extremely unstable. A series of compounds were tested for their ability to protect Fraction V against denaturation at 37° for 1 h and at 45° for 0.5 h. Protection was afforded by 2 mM glutathione, 0.5 mM mercaptoethanol and 5 mM D-glucosamine. The protection was 30, 50 and 100%, respectively, the last value being the activity of the sample before incubation. The protection by glucosamine was investigated in the range 0.5–50 mM. Maximum protection was obtained at 5 mM. Glucosamine added to the assay mixture in this range of concentration had no effect on the rate of the reaction.

The rate *versus* substrate concentration was measured with different concentrations of UDP-glucuronic acid. The K_m, as obtained by the double-reciprocal-plot method of Lineweaver–Burk was 0.14 mM.

This work was supported by a grant from the Association for the Aid of Crippled Children.

Department of Microbiology,
New York University School of Medicine,
New York 16, New York (U.S.A.)

ELIAS HALAC
ELENA BONEVARDI

1 K. J. ISSELBACHER, *Recent Progr. Hormone Res.*, 12 (1956) 134.
2 E. HALAC, JR., *Am. J. Diseases Children*, 100 (1960) 594.
3 B. M. POGELL AND L. F. LELOIR, *J. Biol. Chem.*, 236 (1961) 293.
4 K. J. ISSELBACHER, *Biochem. Biophys. Res. Commun.*, 5 (1961) 243.
5 A. K. BROWN AND W. W. ZUELZER, *J. Clin Invest.*, 37 (1957) 334.
6 O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.

Received October 30th, 1962