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STUDIES OF THE ACTIVATION OF UDP-GLUCURONYLTRANSFERASE

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SUMMARY

Low concentrations of the detergents Triton X-100 and deoxycholate enhanced the activity of rat liver microsomal *p*-nitrophenol glucuronyltransferase 3-fold without radically altering the activities of pyrophosphatase or β-glucuronidase. Similar concentrations of the detergents, however, had no significant activating effect on the glucuronyltransferase activity of guinea pig liver microsomal fractions prepared in identical manner. Triton X-100 still activated the rat liver enzyme when competition between glucuronyltransferase and pyrophosphatase for UDP-glucuronate was reduced at a higher concentration of the sugar nucleotide and when β-glucuronidase had been substantially inhibited with saccharo-1,4-lactone. It is concluded that the closely associated pyrophosphatase and β-glucuronidase activities are not involved in the activation of the rat liver enzyme by detergents. The activation of rat liver glucuronyltransferase and the differences between that enzyme and guinea pig liver glucuronyltransferase are discussed.

INTRODUCTION

When liver microsomal fractions from a number of species (mainly rat, but also rabbit and ox) are treated with detergents such as Triton X-100 and deoxycholate, the apparent activity of glucuronyltransferase (UDP-glucuronate glucuronyltransferase, EC 2.4.1.17) is increased several fold[1–4]. Adlard and Lathe[5] have attributed these effects in the rat to the inhibition of a microsomal pyrophosphatase (nucleoside diphosphate sugar pyrophosphatase) which, in the absence of detergent, competes with glucuronyltransferase for the substrate UDP-glucuronate, (UDP-GlcUA). It has been suggested[6] that inhibition by detergent of β-glucuronidase (EC 3.2.1.31) present in the microsomal preparations might also contribute to the apparent detergent-stimulation of glucuronyltransferase. Microsomal fractions from guinea pig liver appear to respond differently to detergents. We have found[6], in agreement with Mowat

Abbreviation: UDP-GlcUA, UDP-glucuronate.

and Arias[7], that glucuronyltransferase in such preparations is not activated by detergents. Since it is known that the activities of both pyrophosphatase and β-glucuronidase are very much lower in guinea pig liver microsomal preparations than in similar preparations from the rat[8], these findings lend support to the idea that inhibition of these hydrolases is involved in the stimulation of glucuronyltransferase in rat liver microsomal fraction. However, the strength of this evidence is diminished by the observation of Leuders and Kuff[1] that Triton X-100 did in fact stimulate the activity of glucuronyltransferase in a guinea pig liver microsomal fraction under certain experimental conditions. It may well be, as we have suggested[6], that the apparent discrepancy between our results and those of Mowat and Arias[7] on one hand and those of Leuders and Kuff[1] on the other, is the result of differences in such factors as time and conditions of storage of microsomal suspensions or to minor differences in fractionating techniques or ratio of microsomal protein to detergent.

Therefore, to establish whether or not there is a real difference in the response to detergents of glucuronyltransferase activity of rat and guinea pig liver, we have compared the effects of Triton X-100 and deoxycholate on microsomal fractions prepared from the two sources in identical manner. We also have measured the effects of the detergents on the pyrophosphatase and β-glucuronidase activities of the same preparations in order to investigate the role of these enzymes in the activation of glucuronyltransferase.

MATERIALS AND METHODS

Male rats (Wistar, 200–250 g) and guinea pigs (Hartley, 600–700 g) were obtained from Fisons Pharmaceuticals, Loughborough, Leicestershire, U.K. The animals were starved overnight and liver microsomal fractions prepared as described previously[9]. Within 1 h of preparation the microsomal suspensions (5 mg of protein/ml, final concentration) were preincubated at room temperature for 10 min with 12.5 mM Tris–HCl buffer (pH 8.0) and various concentrations of detergents in the range 0–0.5%. Portions of the mixtures were then taken as follows for the assay of enzyme activities; for glucuronyltransferase, 0.2 ml; for pyrophosphatase, 0.4 ml; for β-glucuronidase, 0.3 ml. In all assays the incubation tubes were shaken at 37 °C for 20 min.

Glucuronyltransferase activity was measured with *p*-nitrophenol as acceptor essentially according to Hollmann and Touster[10]. The incubation medium contained UDP-GlcUA (0.167 or 0.501 mM), 50 mM KH_2PO_4–KOH buffer (pH 7.28) and enzyme in a total volume of 0.6 ml.

Pyrophosphatase activity was estimated using an incubation medium of final volume 1.2 ml containing UDP-GlcUA (0.167 or 0.501 mM), 50 mM Tris–HCl buffer (pH 7.28 or 8.9) and enzyme. The reaction was terminated by 0.8 ml of 12.5% trichloroacetic acid and after centrifugation 1 ml of the supernatant solution was assayed for the inorganic phosphate released[11]. In control incubations trichloroacetic acid was added before enzyme. Using 0.167 mM UDP-GlcUA the product appeared linearly with time until 20% of the substrate had been hydrolysed.

β-Glucuronidase activity was estimated using a medium of 1 ml volume containing 0.2 mM *p*-nitrophenylglucuronide, 50 mM KH_2PO_4–KOH buffer (pH 7.28) or 50 mM acetate buffer (pH 5.0) and enzyme. The reaction was terminated by 2 ml of

0.1 M NaOH and 1 ml of water. After centrifuging, the absorbances of control and assay mixtures were measured at 400 nm (ref. 12). The *p*-nitrophenol was released linearly with time until 45% of the glucuronide had been hydrolysed.

Microsomal protein was measured using a biuret method[13] standardised with bovine serum albumin and saccharo-1,4-lactone was prepared according to Levvy[14].

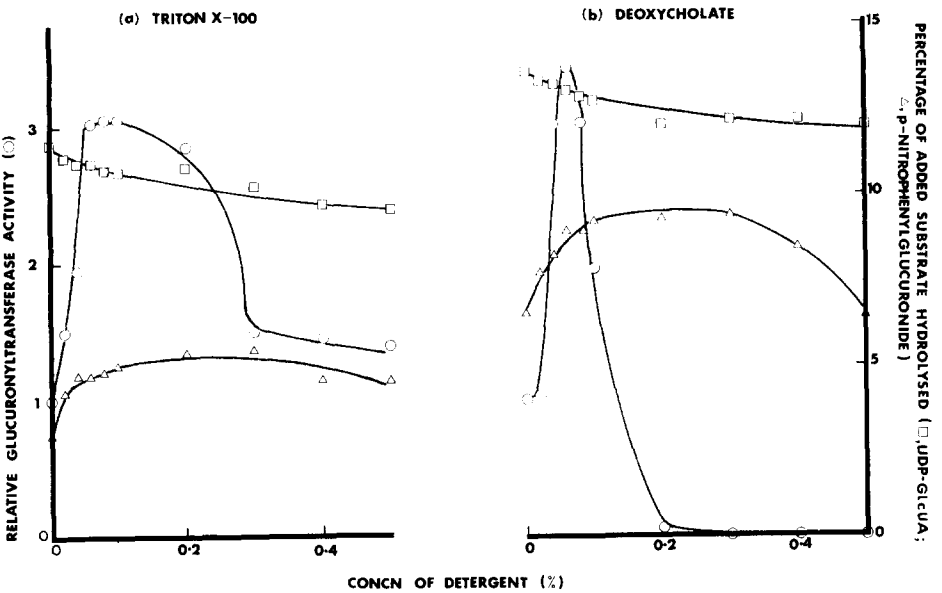

Fig. 1. The effects of detergents on rat liver microsomal glucuronyltransferase, pyrophosphatase and β-glucuronidase activities. Glucuronyltransferase activities are expressed relative to those obtained in the absence of detergent. UDP-GlcUA concentration in the assays of glucuronyltransferase and pyrophosphatase was 0.167 mM. All assays were performed at pH 7.28.

RESULTS AND DISCUSSION

In agreement with previous work[1-4], Triton X-100 and deoxycholate caused substantial stimulation of the glucuronyltransferase activity of freshly prepared rat liver microsomal suspensions (Fig. 1 shows typical results). Both detergents, at their optimal concentrations, increased 3-fold the enzyme activity of untreated preparations, which in these experiments ranged from 188 to 208 pmoles of glucuronide formed/min per mg of protein. Activation depended on the concentration of surfactant, maximal stimulation occurring near 0.1% Triton X-100 or 0.06% deoxycholate. The effect was critically concentration-dependent with deoxycholate, which, at concentrations greater than 0.1% was strongly inhibitory. In a similar series of experiments, however, in which rat liver glucuronyltransferase activity had been activated to 446–475 pmoles/min per mg of protein by storing the microsomal suspensions for 2 days at 0 °C, the detergents failed to produce further stimulation.

In contrast to these findings Fig. 2 shows that, under the same experimental conditions, Triton X-100 at concentrations near 0.06% produced only a slight activation of the glucuronyltransferase activity of freshly prepared guinea pig liver micro-

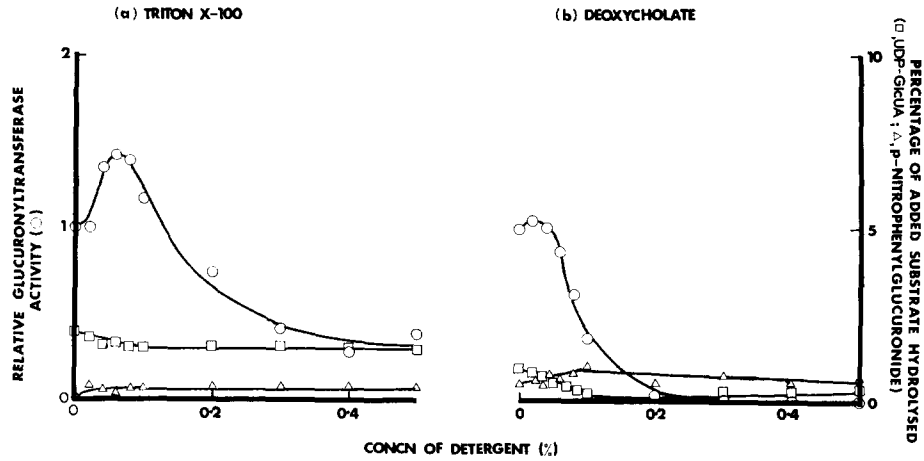

Fig. 2. The effect of detergents on guinea pig liver microsomal glucuronyltransferase, pyrophosphatase and β-glucuronidase activities. Glucuronyltransferase activities are expressed relative to those obtained in the absence of detergent. UDP-GlcUA concentration in the assays of glucuronyltransferase and pyrophosphatase was 0.167 mM. All assays were performed at pH 7.28.

somal fractions while deoxycholate strongly inactivated the enzyme. These preparations possessed high glucuronyltransferase activity in the absence of detergent (912–1496 pmoles/min per mg of protein).

These results show that the enzymes of rat and guinea pig liver differ markedly in their response to detergents under these experimental conditions. The effect of Triton X-100 on the guinea pig enzyme is not exactly the same as reported in our previous work[6] when we found that the detergent inhibited enzyme activity over the whole concentration range studied. However, in the earlier experiments the microsomal suspensions had been stored frozen at −18 °C which may account for the apparent discrepancy. Deoxycholate, on the other hand, inhibited the activities of both fresh and frozen preparations. These results do not support the finding that guinea pig liver glucuronyltransferase is strongly activated by Triton X-100 (ref. 1).

The data of Table I confirm the conclusion[8] that the activities of pyrophosphatase and β-glucuronidase are higher in liver microsomal fractions of rat than in those of guinea pig. These results also show that, at the pH of the glucuronyltransferase assay used here (pH 7.28), the activities of β-glucuronidase and pyrophosphatase were considerably lower than at their respective pH optima. β-Glucuronidase had a particularly low activity at pH 7.28.

The large activations of glucuronyltransferase in the rat caused by Triton X-100 and deoxycholate were accompanied by only very modest inhibition of pyrophosphatase activity (Fig. 1). Since the amounts of UDP-GlcUA removed by the hydrolase were reasonably low even in the absence of detergent, it is considered that abolition by detergent of the competition between pyrophosphatase and glucuronyltransferase for UDP-GlcUA is unlikely to be responsible for the activation of glucuronyltransferase. The detergent-activation was accompanied by an increase in β-glucuronidase activity (Fig. 1). This increase, however, was so small as to militate against the enzyme's involvement in the stimulation of glucuronyltransferase activity. However, since the

TABLE I

THE PYROPHOSPHATASE AND β-GLUCURONIDASE ACTIVITIES OF RAT AND GUINEA PIG LIVER MICROSOMAL FRACTIONS

pH 8.9 is optimal for pyrophosphatase (ref. 15), pH 5.0 for β-glucuronidase (ref. 16). The activity of a purified bovine liver preparation (Sigma Type B-3, 0.1 mg) is included for comparison.

Enzyme	*Source*	*Concn of added substrate (mM)*	*pH of assay*	*Percentage of added substrate hydrolysed*
Pyrophosphatase	Rat liver microsomal	0.167	7.28	11.5
		0.167	8.9	14.1
		0.501	7.28	5.3
		0.501	8.9	11.2
	Guinea pig liver microsomal	0.167	7.28	1.4
		0.167	8.9	5.7
		0.501	7.28	0.6
		0.501	8.9	3.2
β-Glucuronidase	Rat liver microsomal	0.2	7.28	1.8
		0.2	5.0	37.4
	Guinea pig liver microsomal	0.2	7.28	0.2
		0.2	5.0	15.8
	Bovine liver, purified	0.2	7.28	0.4
		0.2	5.0	41.3

extremely low levels of pyrophosphatase and β-glucuronidase activities present in guinea pig liver microsomal fractions were virtually unaffected by all the concentrations of both detergents, and since no significant activation of glucuronyltransferase occurred in these preparations (Fig. 2), it could be argued that these microsomal hydrolases might nevertheless play a part in the glucuronyltransferase activation in the rat.

When the concentration of UDP-GlcUA was increased 3-fold (to 0.501 mM) the activity of rat liver microsomal pyrophosphatase was increased but the percentage of substrate hydrolysed was decreased (Table I). The experiment of Fig. 1a was repeated using this higher concentration of UDP-GlcUA in the assays of glucuronyltransferase and pyrophosphatase. As might be expected, glucuronyltransferase activity in the absence of detergent was higher at the elevated UDP-GlcUA concentration (446 pmoles/min per mg of protein) than at the lower substrate concentration. Nevertheless, 0.1% Triton X-100 still produced a 3-fold activation (Fig. 3a). Using this higher concentration of UDP-GlcUA the percentage of substrate removed by pyrophosphatase was not only decreased but also was unaltered by all concentrations of the detergent between 0.02 and 0.5% (Fig. 3a). These data provide no support for the theory of Adlard and Lathe[5] that the simultaneous inhibition of pyrophosphatase causes the activation of glucuronyltransferase by detergent. However, it is noteworthy that these authors measured glucuronyltransferase activity with 0.25 mM UDP-GlcUA and pyrophosphatase activity with 0.5 M sugar nucleotide. Since the present work utilises identical concentrations of UDP-GlcUA in both assays it is considered that our results are more pertinent to the question of glucuronyltransferase activation.

To investigate further and possible involvement of β-glucuronidase in the activation of rat liver enzyme a microsomal suspension was treated with Triton X-100 in presence of saccharo-1,4-lactone, a specific inhibitor of the hydrolyase[14]. Fig. 3b

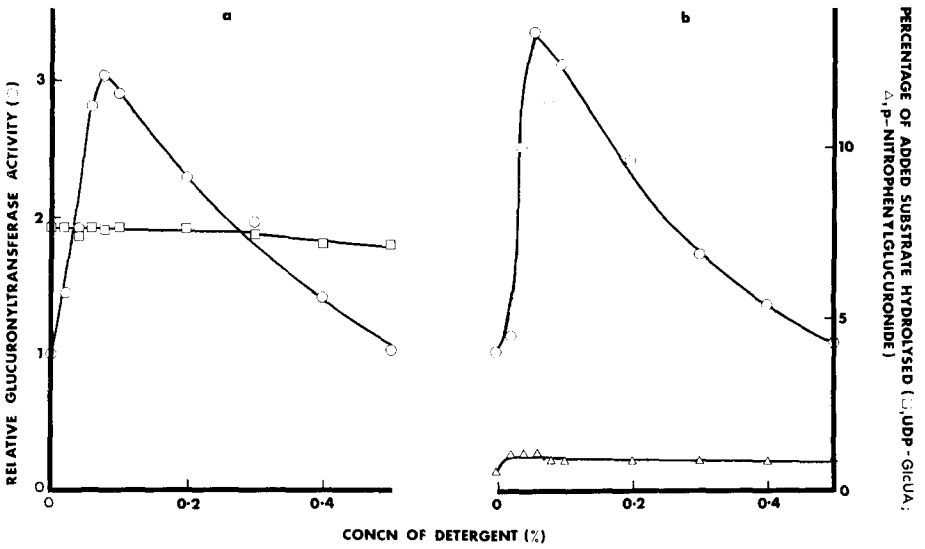

Fig. 3. The involvement of pyrophosphatase and β-glucuronidase activities in the activation of rat liver glucuronyltransferase by Triton X-100. Glucuronyltransferase activities are expressed relative to those obtained in the absence of detergent. All assays were performed at pH 7.28. (a) UDP-GlcUA concentration in the assays of glucuronyltransferase and pyrophosphatase was 0.501 mM. (b) Microsomal fraction was preincubated with the various concentrations of detergent in presence of 1.2 mM saccharo-1,4-lactone and glucuronyltransferase activities measured with 0.167 mM UDP-GlcUA.

shows that β-glucuronidase was indeed strongly inhibited by the lactone and that the residual activity was essentially unaffected by all the concentrations of Triton X-100 used. Nevertheless, the detergent still produced 3-fold activation of glucuronyltransferase. From these data we conclude that β-glucuronidase activity in rat liver microsomal fraction is not involved in glucuronyltransferase activation.

The present results provide direct evidence against a role for the closely associated pyrophosphatase and β-glucuronidase activities in the activation of rat liver microsomal glucuronyltransferase by detergents. Previous authors have put forward alternative theories to account for activation. It has been proposed[2,3], that, by altering the structure of the microsomal membrane, detergents unmask active sites on the enzyme which are normally inaccessible to substrates due to the location of this protein in the membrane. Vessey and Zakim[4] postulate, on the other hand, that the phospholipid membrane normally constrains glucuronyltransferase in a conformation of reduced activity and that detergent treatment removes these restraining forces permitting the enzyme to assume a fully active conformation. If either of these theories holds for the rat our observation that guinea pig glucuronyltransferase is not substantially activated by detergents suggests that this enzyme in intact microsomal fractions may already be in a maximally active form, *i.e.* fully accessible to substrates or in a fully active conformation. However, our results clearly show that high concentrations of detergent inactivate both rat and guinea pig liver enzymes and suggest that the overall effect observed is the net result of two processes, *viz.* activation at low concentration and inactivation at higher concentrations. Failure of detergents to activate

guinea pig liver glucuronyltransferase may indicate that this enzyme is more readily inactivated than that of the rat.

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