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SEPARATION AND PARTIAL PURIFICATION OF TWO DIFFERENTIALLY INDUCIBLE UDP-GLUCURONYLTRANSFERASES FROM RAT LIVER

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Conjugation with glucuronic acid by microsomal UDP-glucuronyltransferase (GT, EC 2.4.1.17) is a major pathway by which the body inactivates and eliminates a wide variety of lipid-soluble xenobiotics and endogenous compounds (1). It was shown previously that the microsomal enzyme inducing agents phenobarbital (PB) and 3-methylcholanthrene (MC) differentially stimulate GT reactions(2). This was taken as evidence for the existence of multiple GT's. To clarify the mechanism of the effect of PB and MC on GT and to characterize the inducible GT's enzyme purification is required. In this communication we report a substantial purification and separation of 1-naphthol-GT and morphine-GT which are preferentially induced by MC and PB, respectively.

Materials and Methods. Liver microsomes were prepared, and the solubilization and ammonium sulfate precipitation were carried out as previously described(2). The 50-70% ammonium sulfate precipitate was dissolved in buffer I (0.02 M Tris-HCl,pH 7.7, containing 10% glycerol, 0.25% Brij 58 (Atlas,Essen) and 0.1 mM dithioerythritol), and applied to a Bio-Gel A-1.5m column (2 x 60 cm) equilibrated with the same buffer. GT (1-naphthol as substrate), which eluted shortly after the void volume, was then applied to a DEAE-cellulose column (2 x 30 cm) equilibrated with buffer I and eluted with

400 ml of a linear KCl-gradient (0-0.5 M) in buffer I. Fractions containing enzyme activity were dialyzed against buffer II, in which Tris-HCl, pH 7.7 of buffer I was replaced by potassium phosphate buffer, pH 6.5. After dialysis the enzyme was applied to a CM-cellulose column (2 x 20 cm) equilibrated with buffer II. After extensive washing with this buffer the enzyme was eluted with 0.2 M buffer II. The enzyme was then concentrated by dialysis against polyethylene glycol 20000 (Serva, Heidelberg) and further purified by thin-layer isoelectric focussing (3) in a 'TLC-Double Chamber' (Desaga, Heidelberg) at 4°C. Gels on glass plates (20 x 20 cm) consisted of Sephadex G-75 superfine containing 1.6% ampholytes pH 5-8 (LKB, Bromma, Sweden), 0.06% Brij 58 and 2.5% glycerol. Isoelectric focussing was performed at 300 V for 8 h and then at 800 V for 2 h. 1 cm portions of the gel were scraped off the plates and eluted with buffer I. The fractions were assayed for enzyme activity and dialyzed for 48 h against buffer I to remove the ampholytes. After protein determination (4) GT preparations were lyophilized and dissolved in 2% SDS, 0.05 M Na_2CO_3 and 10% ß-mercaptoethanol to give a protein concentration of 1 mg/ml. SDS-gel electrophoresis was performed on slab-gels (5). Phospholipids were determined as described (2). GT (1-naphthol as substrate) was assayed according to Bock et al. (6) and GT (morphine as substrate) according to Del Villar et al. (7).

Results and Discussion. GT (1-naphthol as substrate) was partially purified from livers of rats treated with PB or MC (Table 1). During purification the phospholipid/protein ratio progressively decreased. The specific enzyme activity in the MC-group was higher than in the PB-group at all purification steps. We believe that the lack of convergence of the specific activity is due to an increased stability of the enzyme in the MC-group. Substrate specific forms of GT could be clearly separated at the DEAE-cellulose step (Fig.1). When the purification was started from

Table 1. Partial purification of rat liver microsomal GT (1-naphthol as substrate)

Purification step	GT (nmol/min/mg protein)		Phospholipid (mg/mg protein)
	PB-treatment	MC-treatment	MC-treatment
Homogenate	11 (0.8)[a]	38 (2.5)[a]	-
Microsomes	79 (4.0)[a]	218 (7.8)[a]	0.34
Ammonium sulfate	173	495	0.22
Bio-Gel A-1.5m	358	606	0.12
DEAE-cellulose	370	1448	0.01
CM-cellulose	941	2230	-
Isoelectric focussing	(1800)[b]	(4000)[b]	-

[a] Activity in native homogenates or microsomes, [b] Calculated from the distribution of protein and GT activity after thin-layer isoelectric focussing. For purification experiments 8 male Sprague-Dawley rats (200-250 g) were treated with PB or MC. PB was injected i.p. at a dosage of 100 mg/kg/day, and MC was given once i.p. at a dosage of 40 mg/kg, dissolved in olive oil. Treated rats were sacrificed on the 4th day. The mean specific activities of peak fractions of several purification experiments is shown (n=4). The phospholipid/protein ratio in the PB-group was not significantly different from the MC-group

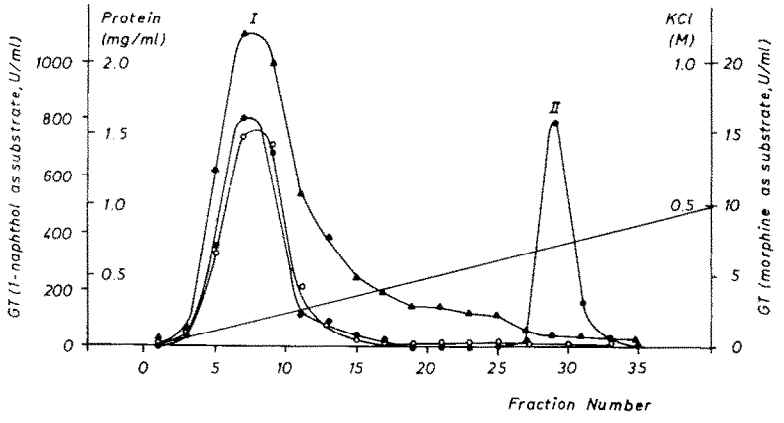

Fig.1. DEAE-cellulose chromatography of rat liver GT's. PB-treated rats were used. Partially purified GT's (Bio-Gel A-1.5m step, Table 1) were applied to a DEAE-cellulose column equilibrated with buffer I and eluted with a linear KCl-gradient (0-0.5 M) containing the same buffer. o, GT (1-naphthol as substrate); •, GT (morphine as substrate); ▲, protein. Enzyme units, nmol/min. Each fraction contained approximately 8 ml.

PB-treated rats, GT (morphine as substrate) could be separated into two peaks. The specific activity of peak II was 20-times higher than that of peak I. Quite different results were obtained in puri-

fication experiments from MC-treated rats. The specific activity of morphine-GT in peak II was only 1/6 compared with the PB-group whereas the specific activity of 1-naphthol-GT was 3.9-fold higher than in the PB-group (Table 1). The purification experiments suggest that PB and MC differentially stimulate separate GT's. A separation of GT's (morphine and 4-nitrophenol as substrates) was also reported by Del Villar et al. (7). 1-Naphthol-GT was further purified by CM-cellulose chromatography and thin-layer isoelectric focussing. Two isoelectric points were observed at pH 6.5 and 7.0, the specufic activity being higher at pH 7.0. Enzyme preparations eluted at pH 7.0 were analyzed by SDS-gel electrophoresis. Two protein bands were observed in enzyme preparations from both PB- and MC-treated rats with molecular weights of about 48000 and 52000. Morphine-GT (Fig.1,peak II) contained several bands with molecular weights >56000 but did not show the protein bands present in the 1-naphthol-GT preparation. The SDS-gel protein pattern of 1-naphthol-GT and morphine-GT suggests that both enzymes contain different polypeptides.

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