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PRELIMINARY COMMUNICATIONS

FUNCTIONAL HETEROGENEITY OF UDP-GLUCURONOSYLTRANSFERASES IN DIFFERENT MEMBRANES OF RAT LIVER

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Glucuronidation of molecules of very different shape suggests the existence of several enzymes with different acceptor specificities. In the endoplasmic reticulum, two kinds of activities have already been characterized. The late fetal activity (1), which is enhanced by 3-methylcholanthrene (2) is able to conjugate planar molecules or group I substrates (3). The neonatal activity (1), inducible by phenobarbital (2), shows specificity towards bulky aglycones or group II substrates (3). Although the endoplasmic reticulum of hepatocytes is known as the major site of xenobiotic glucuronoconjugation, we have recently detected the presence of UDP-glucuronosyltransferase (EC 2.4.1.17) with 4-nitrophenol as the substrate, in the plasma membrane fraction of rabbit liver, which can be induced by phenobarbital (4). Such an activity has also been discovered in Golgi apparatus and in nuclear membranes (5, 6). In order to establish if the enzyme heterogeneity according to the type of substrate used also exists in the different subcellular organelles, we have investigated UDP-glucuronosyltransferase activity towards 10 aglycones in plasma membranes (PM), Golgi apparatus, nuclear envelope and rough or smooth endoplasmic reticulum (RER, SER) of rat liver.

Materials and Methods. Male Sprague-Dawley rats (160–180 g) were purchased from Iffa-Credo (St Germain sur l'Arbresle/France). After 15 hour fasting, the animals, in groups of three to four, were sacrified, the livers perfused with 0.15 M NaCl, cut into small pieces and homogenized in two parts in the following buffer systems: a) 1 mM $NaHCO_3$, pH 7.4 for preparation of PM according to Neville's technique (7). The corresponding pellet obtained after centrifugation on the sucrose gradient was used as the nuclear envelope fraction. b) 0.5 M sucrose, 50 mM Tris, 5 mM $MgCl_2$, pH 7.0 for both preparations of SER and RER (8) and Golgi membranes (9). Protein content was measured by the method of Lowry et al (10) with bovine serum albumin as standard. RNA concentration and NADPH cytochrome c reductase activity were determined by the methods of Fleck and Begg (11) and William and Kamin (12) respectively. Alkaline phosphatase activity was estimated on a fast centrifugal analyzer (Cobas, Roche Bioelectronique, France) with a Bio Mérieux kit (ref. 61 371). Glutamate dehydrogenase was measured using a Boehringer kit (ref. 124 320), and gamma-glutamyltransferase according to Szasz's method (13). The activity of galactosyltransferase was determined as described by Morré (9). Glucuronidation rates were estimated by Mulder and Van Doorn's procedure (14) after the detergent requirement for optimal activation had been determined for each subcellular fraction with the following substrates concentrations: 4.5 mM UDP-glucuronate, 0.25 mM aglycone. The reaction was followed at 37°C on a Cobas-Bio-centrifugal analyzer. Bilirubin UPD-glucuronosyltransferase was measured according to Heirwegh et al (15).

Results and discussion.

Evaluation of fraction purity. Table 1 indicates the enzyme activity or amount of known markers of subcellular fractions in rat liver. RNA was preferentially associated with the RER fraction and was found to be present in similar amounts to those obtained by Stasiecki et al (6) and Craft et al (16) except in SER where its concentration is somewhat higher. There is currently no good marker for the ER fraction (17). However, we have measured the NADPH cytochrome c reductase activity which is present to a greater extent in the SER fraction (5, 6). In the other endomembranes, its amount is negligible except in the Golgi apparatus. As proposed by Ito and Palade (18), this activity could be due mainly to an endogeneous reductase which represents about 30% of the amount found in the microsomal fraction (17). Whatever the fraction studied, we found there was little contamination by mitochondria as estimated by measurement of glutamate dehydrogenase activity. UDP-galactose N acetylglucosamine galactosyltransferase is a preferentially dictyosomal marker enzyme (19) with which our Golgi frac-

tion was greatly enriched (35 fold the homogenate activity). Alkaline phosphatase is particularly a marker of liver bile canalicular PM (20). The enrichment factor of the PM fraction over the whole homogenate was 43. The Golgi apparatus contained 31 % of the activity of PM but the intrinsic presence of alkaline phosphatase in the Golgi membranes cannot be excluded, as explained by Bergeron et al (21) for another PM component, AMPase. Gamma-glutamyltransferase activity was detectable in all subcellular fractions. This glycoprotein which can be measured in microsomes, is preferentially located in PM especially after drug induction (22, 23). Finally the quality of the subcellular fractions was also checked by electron microscopy.

Table 1. Evaluation of fraction purity

	Rough Endoplasmic Reticulum	Smooth Endoplasmic Reticulum	Golgi Apparatus	Plasma Membrane	Nuclear Envelope	Mitochondria
RNA	0.17 ± 0.03 (100)	0.09 ± 0.02 (53)	0.04 ± 0.01 (24)	0.02 ± 0.01 (12)	0.03 ± 0.01 (18)	N.D.
NADPH cytochrome c reductase	114.9 ± 9.3 (81)	142.7 ± 22.3 (100)	48.9 ± 7.8 (34)	9.6 ± 0.8 (7)	0.7 ± 0.2 (1)	N.D.
Galactosyltransferase	0.020 ± 0.001 (1)	0.33 ± 0.10 (9)	3.75 ± 0.80 (100)	0.23 ± 0.13 (6)	0.003 ± 0.002 (0.1)	N.D.
Alkaline phosphatase	3.0 ± 2.9 (3)	4.1 ± 1.6 (5)	28.2 ± 22.1 (31)	90.7 ± 57.9 (100)	2.6 ± 0.3 (3)	N.D.
Gamma-glutamyl transferase	1.96 ± 0.45 (42)	2.12 ± 0.84 (45)	2.12 ± 1.10 (45)	4.70 ± 1.74 (100)	1.20 ± 0.42 (26)	N.D.
Glutamate dehydrogenase	18.1 ± 10.1 (4)	18.9 ± 7.5 (4)	N.D.	43.3 ± 6.1 (9.6)	0.10 (0)	449 ± 118 (100)

. Activities are listed in nmol/min/mg protein and RNA content in mg/mg protein.
. Values are expressed as the mean ± SD of at least five different preparations.
. Number in brackets represent the percentage of specific marker activity or content, when compared with the value found in the specific subcellular fraction.
. N.D.: not determined

Glucuronidation of various substrates in the different subcellular fractions. We determined the activity of UDP-glucuronosyltransferase in different endomembranes of rat liver towards 10 substrates, after maximal activation with Triton X-100: three of the late fetal group or group I, 4-methylumbelliferone, 4-nitrophenol, eugenol, and five of the neonatal group or group II, 4-hydroxybiphenyl, 2-aminophenol, borneol, morphine, chloramphenicol, and two endogeneous substrates : testosterone and bilirubin. Whatever the aglycone used, the rate of glucuronidation was quite the same in the RER and SER fractions; only the activity level was different (fig. 1). For example, activity of the group I substrates was at least two times higher than that measured with the group II aglycones. Identical results have been obtained by Boutin et al in rat liver microsomal fractions (24). The nuclear envelope glucuronidated essentially the group I substrates, since the activities represented 18 to 22 % of that found in the ER. These results were in good agreement with those of Stasiecky et al (6) and Elmamlouk et al (25). In contrast, the group II substrates were poorly transformed by the nuclear envelope. Interestingly, it appeared that carcinogenic compounds such as 3-methylcholanthrene enhance only the glucuronidation rate of group I substrates (25). In nuclear membranes UDP-glucuronosyltransferase activities may provide a specific protective role against damage to genetic material. On the other hand, the Golgi and PM fractions had approximately the same glucuronidation activity whatever the substrate. They represented 18 to 31 % of the specific activity measured in the ER for group I substrates, as found by von Bahr et al (5) and Stasiecky et al (6). For the other aglycones, however, the extent of conjugation by these fractions reached that found with the ER and it was even identical for the bulkiest substrates used such as morphine, chloramphenicol and testosterone with the PM fraction and bilirubin with the Golgi fraction. The nuclear envelope, RER and SER thus seemed to be more specialized for the conjugation of group I substrates, whilst in the Golgi apparatus and PM, all the substrates appeared to have a glucuronoconjugation capacity at a similar level. Some workers have attempted to describe the molecular basis by which the two groups may be distinguished; Wishart et al (26) have rejected molecular weight and lipophilicity parameters but the thickness or bulkiness of the molecules may play an important role as shown by Okulicz-Kozarin et al (3). Hence it seems that the Golgi apparatus and PM possess the same potential capacity to conjugate the bulkiest substrates than the ER. Conjugation of bilirubin could be catalysed by a different UDP-glucuronosyltransferase since it is mainly enhanced by pregnenolone 16 alpha-carbonitrile (27) and clofibrate (28). Our results show that bilirubin glucuronoconjugation, measured on the basis of specific activity, occurs not only in the ER but also to the same extent in the Golgi apparatus and half as much as in the PM.

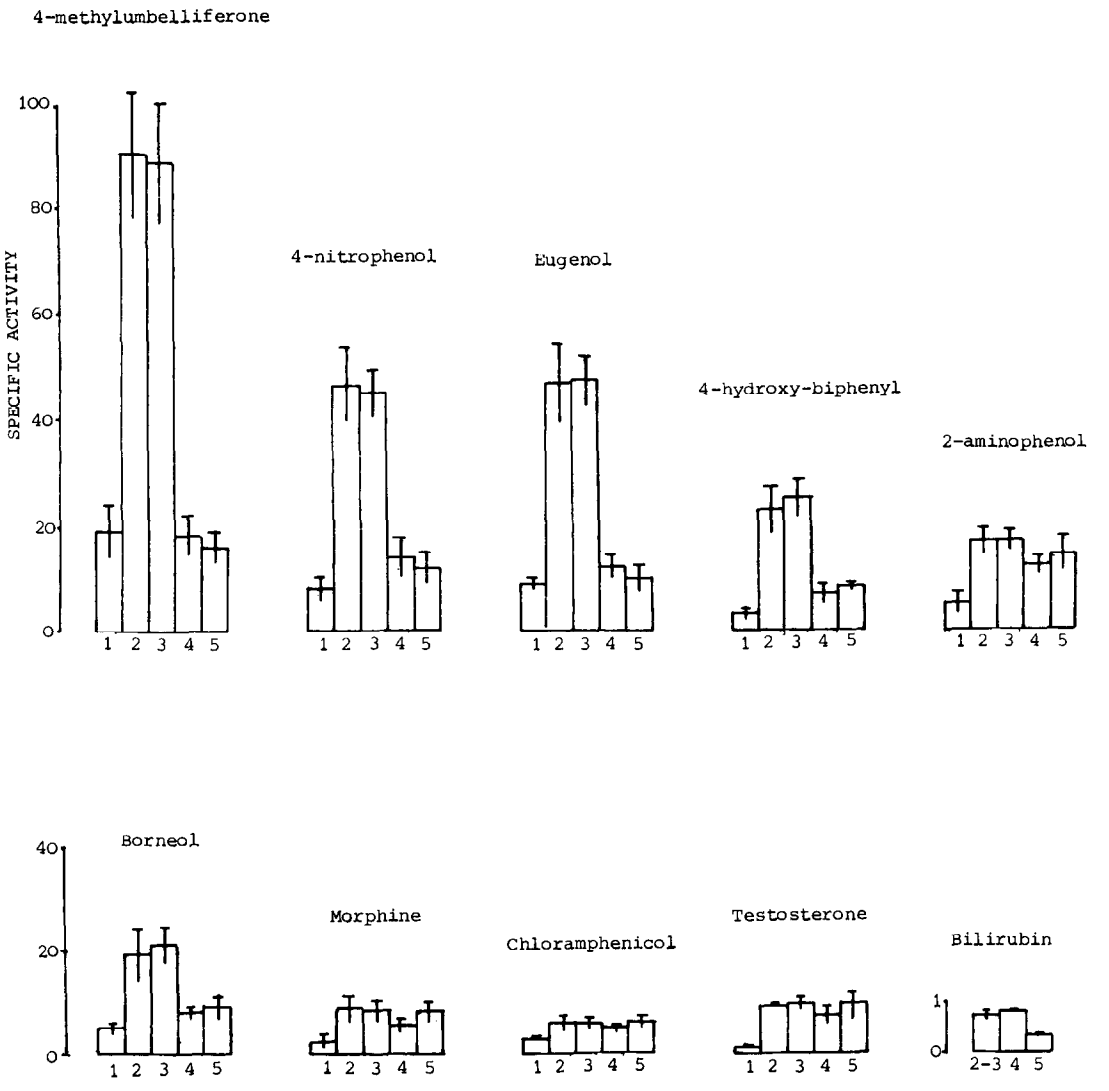

Figure 1: UDPGT activities towards various substrates.

Specific activities in fractions are expressed as nmoles aglycone conjugated per minute per mg protein. All data are mean values ± SD of at least six separate experiments except for bilirubin conjugation (mean ± SEM). Fractions are: 1: nuclear envelope, 2: RER, 3: SER, 4: Golgi apparatus, 5: plasma membrane, 2-3: ER.

In conclusion, the results provide evidence that more than one type of UDP-glucuronosyltransferase activities exist in the different endomembranes. The question now is to determine on what the apparent specialization of the different organelles in the glucuronidation of certain substrates is based. The functional heterogeneity could be due to either a different lipid microenvironment which could modulate both substrate accessibility and enzyme conformation (29), or a non random distribution of different isoenzymes among the organelles in rat liver.

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