UDP-Glucosyltransferase Activity of Housefly Microsomal Fraction

By ANTONIO MORELLO* and YOLANDA REPETTO*

Department of Biochemistry, University of Chile, Faculty of Medicine, P.O. Box 6671, Santiago, Chile

(Received 7 August 1978)

Housefly UDP-glucosyltransferase activity towards *p*-nitrophenol was demonstrated in a system *in vitro*. The activity is localized in the microsomal fraction, requires UDP-glucose, is slightly stimulated by Mg^{2+} and is activated optimally over a wide range of detergent concentration. Phenobarbital increases the enzyme(s) activity about 3-fold, with *p*-nitrophenol as substrate, which differs from the corresponding mammalian glucuronyltransferase.

Housefly microsomal fraction has been shown to catalyse actively the NADPH-linked cytochrome P-450 reactions which lead to the conversion of numerous xenobiotics into more polar compounds (Morello *et al.*, 1971). Types of attack include aromatic hydroxylation, *O*- and *N*-dealkylation and aliphatic hydroxylation. The resulting compounds or their intermediates are further metabolized either by conjugation with glutathione through the action of soluble glutathione transferases (Fukami & Shishido, 1966; Morello *et al.*, 1968) or, as has been shown *in vivo*, by conjugation with glucose (Smith, 1977).

UDP-glucuronyltransferases (EC 2.4.1.17) occurs in mammals (Dutton & Burchell, 1977); however, in plants and invertebrates, conjugation does not involve glucuronidation, but rather glucosidation (Leakey & Dutton, 1975; Dutton & Burchell, 1977; see Smith, 1977). In vertebrates glucosidation does not occur to any significant extent, because UDP-glucosyltransferases (EC unclassified) appear to be limiting (see Dutton & Burchell, 1977).

Essentially no information is available, however, on the intracellular localization of UDP-glucosyltransferases in insects, their latency and their response to inducers.

In the present work, we report on the housefly UDP-glucosyltransferase activity towards *p*-nitrophenol. The enzyme(s) is microsomal, is activated by detergents, requires UDP-glucose and is induced by phenobarbital.

Materials and Methods

Chemicals

UDP-glucose, UDP-glucuronic acid, β -glucosidase, β -glucuronidase and Triton X-100 were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

* Present address: Department of Zoology, University of Georgia, Riverbend Research Laboratories, Athens, GA 30602, U.S.A.

Nitrophenol obtained from Fisher Scientific Co. (Waltham, MA, U.S.A.) was sublimed twice before use. The rest of the chemicals used were of analytical grade.

Insects

Non-sexed 5-day-old specimens of the Fc housefly (*Musca domestica*) strain (Morello *et al.*, 1971) were used throughout. Phenobarbital was administered in the food (Capdevila *et al.*, 1973) at 0.5% concentration for 72h before death.

Microsomal fraction

Microsomal fractions were prepared by the mortar procedure of Morello *et al.* (1971) in 0.1M-sodium phosphate buffer, pH 7.5, and used immediately after preparation. The supernatant remaining after centrifugation of the microsomal fraction at 105000g for 90min corresponds to the cytosolic fraction. Centrifugations were carried out in a Beckman L2-65B centrifuge in the no. 40 rotor.

Enzyme assay

Enzyme activity was followed in a reaction mixture containing in a final volume of 10ml the following: 0.167mM-*p*-nitrophenol, 0.833mM-UDP-glucose, 16.7mM- $MgCl_2$, 66mM-sodium phosphate buffer, pH 7.5, and microsomal fraction equivalent to 10g fresh wt. of flies (49mg of protein). Triton X-100 was added as indicated in the Tables and Figures. UDP-glucuronic acid was used at a final concentration of 0.833mM.

The reaction was started by the addition of UDP-glucose and incubation was carried out at 35°C. At zero time and after timed intervals, 0.6ml portions were pipetted into centrifuge tubes containing 2.4ml of 0.1M-trichloroacetic acid. The acid-insoluble material was removed by centrifugation (5000g for 10min), and 0.06ml of 12M-KOH was added to the supernatant (Hollmann & Touster, 1962). The

decrease in A_{400} in the supernatant was determined in a Beckman DU spectrophotometer. All results presented, except those of Fig. 2, were calculated from initial velocities. Protein was determined by the Folin procedure, with albumin as standard (Lowry *et al.*, 1951). All results are the average of five or more closely agreeing experiments.

Results

Intracellular localization

As shown in Table 1, the UDP-glucosyltransferase activity is localized in the microsomal fraction. The microsomal activity corresponds to 0.33 nmol of *p*-nitrophenol conjugated/min per mg of protein.

When β -glucosidase was added to the reaction mixture after 15 min of incubation, 90% of the *p*-nitrophenol conjugated was liberated, whereas the addition of β -glucuronidase did not result in any liberation of *p*-nitrophenol (Table 1). This confirms that the disappearance of *p*-nitrophenol as followed at 400 nm is due to the formation of the corresponding glucoside.

Effect of detergent

Microsomal UDP-glucuronyltransferases are activated by modifications of the membrane structure such as sonication, addition of detergents or digestion by proteolytic enzymes (Dutton & Burchell, 1977). Housefly UDP-glucosyltransferase(s) behaves similarly, as shown in Fig. 1. The enzyme was activated maximally by Triton X-100 concentrations of about 0.8%, the activity remaining high with concentrations up to 1.8%. Maximum activity in the presence of Triton X-100 corresponds to 0.65 nmol of *p*-nitrophenol conjugated/min per mg of protein.

The reaction in the presence or absence of Triton

Fig. 1. Influence of Triton X-100 on the microsomal activity of UDP-glucosyltransferase

Conditions are as described in the Materials and Methods section.

Fig. 2. Time course of *p*-nitrophenol disappearance by housefly microsomal fraction

Conditions are as described in the Materials and Methods section. ●, Control; Δ, + Triton X-100 (0.8%).

Table 1. Intracellular localization of UDP-glucosyltransferase activity in houseflies

Enzyme assay was as described in the Materials and Methods section.

Fraction	<i>p</i> -Nitrophenol conjugated (μmol/15 min per 10 g of flies)
Homogenate	0.200
Cytosolic	0.010
Microsomal	0.240
Microsomal+ β-glucuronidase*	0.235
Microsomal+ β-glucosidase*	0.024

* After 15 min incubation, the reaction mixture was boiled for 2 min and then β -glucuronidase (400 units) or β -glucosidase (1 mg) was added as indicated and incubation was continued for 1 h.

X-100 was linear with time for about 20 min (Fig. 2). After 20 min the reaction continued at a slower rate, presumably owing to the depletion of the acceptor substrate; thus after 60 min of incubation, 70% of the substrate was conjugated and, as a result, the reaction was not under optimal acceptor-substrate conditions. However, a possible product inhibition of the reaction cannot be excluded at present. UDP-glucose

Table 2. *Cofactor requirements for the conjugation of p-nitrophenol by housefly microsomal fraction*

Enzyme assay was as described in the Materials and Methods section. The values in parentheses are percentages of those for the complete reaction mixture without Triton X-100.

Reaction mixture	<i>p</i> -Nitrophenol conjugated ($\mu\text{mol}/15 \text{ min per } 10 \text{ g of flies}$)	
	No Triton X-100	+ Triton X-100 (0.8%)
Complete	0.240 (100)	0.480 (200)
Complete -UDP-glucose	0.020 (8)	0.007 (3)
Complete -MgCl ₂	0.199 (83)	0.370 (154)
Complete -microsomal fraction	0.000 (0)	0.007 (3)
Complete -UDP-glucose+ UDP-glucuronic acid	0.007 (3)	0.000 (0)
Complete -UDP-glucose -microsomal fraction -MgCl ₂	0.000 (0)	0.000 (0)

Table 3. *Induction of UDP-glucosyltransferase by phenobarbital*

The values in parentheses correspond to percentages of the controls. Conditions were as described in the Materials and Methods section. Triton X-100 was added at a final concentration of 0.8% where indicated.

Microsomal fraction	UDP-glucosyltransferase ($\mu\text{mol of } p\text{-nitrophenol}$ conjugated/15 min per 10g of flies)
Control	0.240 (100)
Phenobarbital-treated	0.760 (317)
Control+Triton X-100	0.520 (100)
Phenobarbital-treated+ Triton X-100	1.630 (313)

was not limiting, since doubling its concentration did not result in an increase in activity.

Cofactor requirements

The enzyme is specific for UDP-glucose (Table 2). UDP-glucose could not be replaced by UDP-glucuronic acid, indicating that no measurable amount of UDP-glucuronyltransferase is present. Omission of Mg²⁺ decreased the activity by about 20%. Similar results were obtained in the presence or absence of Triton X-100.

Effect of phenobarbital

Inducers such as phenobarbital and 3-methylcholanthrene have been shown to increase the activity of UDP-glucuronyltransferases in mammals (Bock *et al.*, 1973; see Dutton & Burchell, 1977). Phenobarbital is an inducer of cytochrome *P*-450 in the housefly (Capdevila *et al.*, 1973) and also induces UDP-glucosyltransferase activity in molluscs (Leakey & Dutton, 1975). As shown in Table 3, administration of phenobarbital for 72h increased the activity to over 300% of the controls. That this increase was due not to the activation of the enzyme, but rather to

induction, is evident by the increase observed in microsomal fraction maximally activated by Triton X-100.

Discussion

Conjugation of endogenous and exogenous substrates with glucose *in vivo* is fairly well established (Smith, 1977). Among the exogenous substrates that are excreted as β -glucosides are aminophenol and quinol (Smith, 1977). Our results confirm that in houseflies these conjugations are catalysed by UDP-glucosyltransferase(s), an enzyme of microsomal localization. The activity is of the same order of magnitude as that described for housefly microsomal cytochrome *P*-450-linked hydroxylation of naphthalene, aminopyrine and 2-isopropoxyphenyl *N*-methylcarbamate (Morello *et al.*, 1971; Capdevila *et al.*, 1973). As a result both cytochrome *P*-450 and UDP-glucosyltransferase appear to be closely linked from the functional standpoint. Moreover both enzymes are induced by phenobarbital (Table 3; Capdevila *et al.*, 1973), although induction is higher for UDP-glucosyltransferase. UDP-glucosyltransferase would then be ideally suited to conjugate products of cytochrome *P*-450-linked oxidation without constituting a limiting step in the overall detoxication process of a given substrate.

It is noteworthy that the induction of glucosyltransferases appears to be dependent on the inducer used. Thus 3-methylcholanthrene induces the glucuronidation of *p*-nitrophenol and 1-naphthol in rats, whereas phenobarbital induces preferentially the glucuronidation of chloramphenicol and bilirubin (Bock *et al.*, 1973). In the housefly, phenobarbital induces glucuronidation of *p*-nitrophenol (Table 3), and this observation correlates very well with the type of cytochrome *P*-450 species that are induced by phenobarbital in the housefly (Capdevila *et al.*, 1973). Cytochrome *P*-448 species are preferentially induced by phenobarbital in the *Fc* housefly strain, whereas cytochrome *P*-450 species are induced in mammals.

That the enzyme corresponds to a glycosyltransferase is indicated by the absolute requirement for UDP-glucose (Table 2), by the inability of UDP-glucuronic acid to replace UDP-glucose and by the specific cleavage of the conjugate *in vitro* formed by β -glucosidase and not by β -glucuronidase.

UDP-glucuronyltransferase activity in rat liver appears to correspond to multiple enzymes (Bock *et al.*, 1973; Dutton & Burchell, 1977). Whether or not a similar situation occurs in flies remains to be established.

Appreciation is expressed to Dr. Moises Agosin for his valuable suggestions while we were preparing this manuscript. This work was supported in part by a grant from the Comision de Ayuda a la Investigacion de la Universidad de Chile.

References

- Bock, K. W., Fröhling, W., Remmer, H. & Rexer, B. (1973) *Biochim. Biophys. Acta* **327**, 46–56
- Capdevila, J., Morello, A., Perry, A. & Agosin, M. (1973) *Biochemistry* **12**, 1445–1451
- Dutton, G. J. & Burchell, B. (1977) *Prog. Drug Metab.* **2**, 1–70
- Fukami, J. & Shishido, T. (1966) *J. Econ. Entomol.* **59**, 1338–1346
- Hollmann, S. & Touster, O. (1962) *Biochim. Biophys. Acta* **62**, 338–352
- Leakey, J. E. A. & Dutton, G. J. (1975) *Comp. Biochem. Physiol.* **C51**, 215–217
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Morello, A., Vardanis, A. & Spencer, E. Y. (1968) *Can. J. Biochem.* **46**, 885–892
- Morello, A., Bleecker, W. & Agosin, M. (1971) *Biochem. J.* **124**, 199–206
- Smith, J. N. (1977) in *Drug Metabolism from Microbe to Man* (Parke, D. V. & Smith, R. L., eds.), pp. 219–232, Taylor and Francis, London