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COMMENTARY

UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES

GUIDELINES FOR CONSISTENT INTERIM TERMINOLOGY AND ASSAY CONDITIONS

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Glucuronide formation is a major pathway in the biotransformation and elimination of a wide variety of lipid-soluble endogenous compounds and xenobiotics. The reaction is catalysed by a family of closely related enzyme forms of UDP-glucuronosyltransferase (GT). This enzyme(s) has attracted many scientists because of a variety of interesting features, such as the phospholipid-dependence of its catalytic activity, latency of GT activity in the microsomal membrane and differential inducibility by hormones and xenobiotic inducers. (For recent comprehensive reviews see Refs. 1–3.) In a workshop on 'Glucuronide Formation of Drugs and Endogenous Compounds: Enzymatic Regulation and Implications' held in Göttingen, 24–27 March 1982. Participants from the U.S.A. and eight European countries presented an overview of the state of art on enzyme purification from different species, on the role of the microsomal membrane and of phospholipid–protein interactions in the regulation of enzyme activity *in vitro*, as well as on cellular control of glucuronide formation and its implications in toxicology and clinical pharmacology. In light of recent developments in enzyme isolation and purification (see references in Table 1) and in immunochemical quantitation of the enzyme protein [4] it was felt by most of the participants that an effort should now be made to describe a standardized terminology and assay procedure for GT. An ad hoc committee of the authors suggested the recommendations discussed later.

Terminology

This 'standardised' terminology need not—and in most cases cannot—be the final terminology, but will serve the important interim purpose of giving agreed names to the various GT preparations now being so widely studied.

The enzyme. Because the enzyme requires UDP-glucuronic acid it should be called UDP-glucuronosyltransferase or UDP-glucuronyltransferase to distinguish it from other known glucuronosyltransferase activities [1].

Enzyme forms. Multiple enzyme forms of GT have been physically separated (Table 1); the term *enzyme form* is used in a broad sense recommended by the IUPAC–IUB Commission on Biochemical Nomenclature [12]. Separation of these enzyme forms is still insufficient, their purity being difficult to assess because of their so far similar subunit mol. wts [6, 11]. However limited proteolysis of at least two isolated forms suggests primary structural heterogeneity [11]; i.e. these enzyme forms are probably different gene products.

Substrate-specific forms. If the *enzyme protein* is isolated and purified, and is apparently homogeneous on sodium dodecylsulfate/polyacrylamide gel electrophoresis etc., it should be named after a major substrate, i.e. a substrate that gives the highest turnover number of several tested with the purified enzyme. For example, when the protein is isolated using bilirubin as substrate it should be termed bilirubin-GT (EC 2.4.1.76). Endogenous substrates are preferred for this temporary nomenclature.

Polysubstrate GTs or GT [acceptor-unspecific (EC 2.4.1.17)] for which no major endogenous substrates are known, should at present be named after one of their commonly-used substrates, e.g. p-*nitrophenol-GT* or *GT(*p-*nitrophenol)* (as discussed later). If isolation of such a protein has been followed with *p*-nitrophenol as substrate and if it shows some activity towards oestrone, but at a much lower rate than does the major protein isolated using oestrone as substrate, then this activity should be termed 'oestrone-GT activity of *p*-nitrophenol-GT'.

GT activities. All GT preparations which are not dealing with purified proteins are to be referred to by the term GT activity. Several classifications of GT activities have been published. Although necess-

Table 1. Physical separation of purified hepatic enzyme forms of UDP-glucuronosyltransferase

Species	Enzyme forms of GT	Ref.
Rat	*p*-Nitrophenol-GT/morphine-GT	[5, 6]
	p-Nitrophenol-GT/bilirubin-GT	[7]
	p-Nitrophenol-GT/testosterone-GT	[8]
	Testosterone-GT/oestrone-GT	[9]
Rabbit	*p*-Nitrophenol-GT/morphine-GT	[10]
	p-Nitrophenol-GT/oestrone-GT	[11]

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arily somewhat arbitrary, they illustrate the 'functional heterogeneity' of the enzyme and may be helpful in delineating GT forms. For example, rat liver microsomal GT activities have been classified as *groups* on the basis of their inducibility by two prototype xenobiotic inducers, 3-methylcholanthrene and phenobarbital [13–16]. Two *groups* have been distinguished: group 1 activities are chiefly inducible by 3-methylcholanthrene (three- to six-fold) whereas group 2 activities are chiefly inducible by phenobarbital (two- to five-fold). Among the substrates tested in purified GT preparations, group 1 substrates include 4-nitrophenol, 1-naphthol, 4-methylumbelliferone, 2-aminophenol, 3-hydroxybenzo[*a*]pyrene, i.e. mostly *planar* phenols. Group 2 substrates include morphine and 4-hydroxybiphenyl, i.e. phenols which are *not* planar. Note that, on the basis of GT activities per mg microsomal protein, a third group of GT activities can be distinguished from the two other groups which is only moderately affected by the two inducing agents (< two-fold); it includes substrates such as oestrone, phenolphthalein, paracetamol, oxazepam [Refs 6, 14 and Ullrich and Bock (unpublished)]. As expected, a number of substrates cannot be easily classified, e.g. 2-hydroxybiphenyl which is markedly inducible by both 3-methylcholanthrene and phenobarbital [17]. Moreover it should be realised that on a whole-liver basis all GT activities are moderately inducible by phenobarbital due to the proliferation of endoplasmic reticulum membranes caused by this inducer [18].

Classification has also been carried out on the basis of perinatal development of GT activities [19–21]. For these developmental studies the term *clusters* of GT activities should be reserved, e.g. the late-foetal and neonatal cluster of GT activities which refer to their appearance at specific times. Substrates of the late-foetal cluster, similar to the group 1 substrates, are mostly planar phenols. The change from one cluster to the other is distinct. For example, 4-ethylphenol belongs to the late-foetal cluster, whereas 4-*n*-propylphenol, which differs only by a single CH_2-moiety, belongs to the neonatal cluster [20]. The clusters differ in their experimental inducibility by hormones such as glucucorticoids [19–21].

There is a striking similarity between substrates of group 1 and of the late-foetal cluster. Moreover group 1 activities copurify during GT isolation and purification [6, 22]. It is therefore conceivable that the glucuronidation of group 1 substrates is catalysed by a distinct enzyme form which has been elsewhere termed enzyme 1 or GT_1 [6, 14]. We suggest here that this polysubstrate GT is named at present *p-nitrophenol-GT*, in accordance with the terminology proposed for the more specific enzyme forms accepting endogenous substrates such as bilirubin or steroid hormones. One should always assay a polysubstrate GT protein with this commonly used standard substrate *p*-nitrophenol. When, for special reasons, *p*-nitrophenol-GT activity has not been assayed, the isolated enzyme preparation should be named after the major substrate tested, e.g. 1-naphthol-GT or GT(1-naphthol). Glucuronidation of group 2 and 3 substrates may be catalysed by polysubstrate GTs differing from *p*-nitrophenol-GT. These other, independently regulated, polysubstrate GTs have not yet been sufficiently delineated and characterized.

The assumption of a single *p*-nitrophenol-GT may be oversimplified. However, when the existence of more than one *p*-nitrophenol-GT can be established these forms can be easily indicated by appropriate suffixes. In the discussion about distinct enzyme forms of the phospholipid-dependent GT one should always bear in mind that 'enzyme forms' can be artificially created during enzyme purification which do not exist as independently regulated enzyme forms *in vivo*.

Regulatory properties of an enzyme form such as inducibility by xenobiotics or endogenous inducers, and perinatal development, are expected to be species-dependent and cannot be extrapolated from one species to another. Therefore every publication should state *species, strain, tissue, sex* and *age*, and for non-mammals, *temperature of assay*.

This, we repeat, is a temporary nomenclature until peptide mapping can reasonably ascribe activities to a single protein species which may then take a name, decided upon by an authorized commission, which may include the known substrates. Different gene products (isozymes?), allelozymes (probably responsible for the genetic defect of androsterone-GT activity described for certain Wistar rat strains [23]), as well as covalent posttranslational modifications, have to be envisaged as the molecular basis for the remarkable functional heterogeneity of GT activities. In addition, GTs are likely to exist (in the microsomal membrane or in model micellar and liposomal structures) in different conditions of constraint probably due to different protein–phospholipid interactions which may lead to secondary structural alterations [24]. Primary and secondary structural differences could be distinguished by calling the former *enzyme forms* and the latter *activity states*, e.g. the 'latent' and 'optimally-activated' states in the microsomal membrane.

Assay conditions

To be able to compare results obtained in different laboratories and because of the many variables affecting GT activities it is desirable to have a standardized assay which should give a *reproducible* result with *simple* conditions, not necessarily the conditions for maximal activity. The tissue should be the adult male Wistar rat liver. It should be used in the assay as a liver homogenate (10% w/v) in 0.25 M sucrose or liver microsomes, prepared by centrifugation of the 10,000 *g* (15-min) supernatant of the liver homogenate at 100,000 *g* (60 min). A substrate in common use is *p*-nitrophenol. Its disappearance upon glucuronidation can be followed by a colorimetric procedure [25, 26].

The incubation mixture contains: liver microsomes (1 mg protein/ml) (0.10 ml), Triton X-100 (0.25% w/v) or sodium cholate (0.25% w/v) (0.02 ml), 1 M Tris–HCl (pH 7.4) (0.05 ml), 50 mM $MgCl_2$ (0.05 ml), 5 mM *p*-nitrophenol (0.05 ml), H_2O (0.18 ml) and 30 mM UDP-glucuronic acid (0.05 ml). The total volume is 0.50 ml.

After 2 min preincubation at 37° the reaction is started by the addition of UDP-glucuronic acid. At

0, 5, 10 and 15 min take 0.10 ml from the incubation mixture and precipitate the protein with 1 ml 5% trichloroacetic acid. After centrifugation add 0.25 ml 2 M NaOH to 1 ml of the supernatant and read the O.D. at 405 nm. The extinction coefficient for *p*-nitrophenol at pH > 10 is 18.1×10^3 cm²/mole. When GT activity is assayed in the homogenate 0.05 ml of the liver homogenate (10%, w/v) and 0.15 ml Triton X-100 (0.25% w/v) are suitable in the assay. Typical activities of 28 ± 5 and of 4 ± 1 nmoles/min/mg protein have been found for microsomes and homogenates, respectively.

Some participants recommended a final concentration of 2 mM *p*-nitrophenol. We recommend the use of 0.5 mM *p*-nitrophenol since coloured material is released from microsomes [27] and the kinetic behavior deviates from simple Michaelis–Menten kinetics at the higher substrate concentration [28, 29]. The problem of assaying GT activity in the activated state is well-recognized. However it is the purpose of an enzyme assay to reflect the level of enzyme protein. This is best approximated by assaying GT activity in the activated state because a number of situations modulating or damaging the endoplasmic reticulum membrane *in vivo* will enhance GT activity without increasing the enzyme level [30–32]. Electroimmunochemical quantitation of GT(*p*-nitrophenol) or GT(1-naphthol) suggests that GT activity, determined in the activated state, is a reasonable index of the enzyme protein level [4]. Optimal activation depends upon the particular detergent and the membrane concentration. The concentration of 0.5 mg detergent/mg protein was found to be optimal with a variety of detergents (Triton X-100, Brij 58, Lubrol WX, sodium cholate). Although, for unknown reasons, detergents such as Brij 58 lead to higher GT activity at optimal concentration and to less inhibition at higher concentrations, Triton X-100 was preferred here because of its general availability. Protein should be determined by the method of Lowry *et al.* [33] using bovine serum albumin as standard.

A more accurate and sensitive fluorimetric (Ref. 34, modified as described in Ref. 14 for 4-methylumbelliferone) and radiochemical assay [31] can be carried out with 1-naphthol as substrate in which the formation of 1-naphthol glucuronide is measured. Because of the higher sensitivity of the 1-naphthol-GT assay, optimal conditions are much less dependent upon the proper time and protein concentrations.

In the present state of our knowledge both terminology and assay conditions of GT activities must be tentative. Nevertheless it is hoped that the recommendations may stimulate the discussion and facilitate a comparison of results obtained in different laboratories.

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