Compartmentation of Uridine Diphosphate Glucuronosyltransferase: Effect of Uridine Diphosphate N-Acetylglucosamine on the Apparent V of glucuronidation

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UDP-glucuronosyltransferase, quantitatively the most important enzyme of phase II of mammalian drug metabolism, is strongly bound to hepatic endoplasmic-reticulum membranes and exhibits the phenomenon of latency in fresh microsomal preparations. Latency can be removed and glucuronidation increased by many membrane-lysis procedures and also by UDP-N-acetylglucosamine: this is non-lytic and is a feasible physiological regulator of UDP-glucuronyltransferase (Winsnes, 1972; Zakim & Vessey, 1974, 1976a,b; Otani et al., 1976). Two models have been proposed to explain latency. Model I was suggested by Winsnes (1972) and elaborated by Berry & Hallinan (1976) and assumes that the phospholipid-dependent transferase is embedded in the luminal face of hepatic endoplasmic-reticulum membranes (Fig. 1). Membrane-lysis procedures thus destroy latency by facilitating the access of UDP-glucuronic acid to the active site. It is proposed that in vivo, large, highly charged UDP-glucuronic acid, synthesized in the cytosol, gains access to the transferase via a thiol-dependent permease, which is in turn stimulated by UDP-N-acetylglucosamine + M$^{2+}$ (where M$^{2+}$ is any

Model 1 depicts glucuronosyltransferase (GT) embedded in the luminal face of an endoplasmic-reticulum vesicle. P-SH is a thiol-dependent permease for UDP-glucuronic acid and NDPase-M$^{2+}$ is a bivalent-cation-dependent nucleoside diphosphatase. Model 2(I) depicts glucuronyltransferase in a poorly active, phospholipid-constrained conformation, embedded in the cytoplasmic face of the endoplasmic-reticulum membrane. In model 2(II), UDP-N-acetylglucosamine in the presence of a bivalent cation has converted the transferase into an activated conformation. A family of other active-transferase conformers are depicted in model 2(III): these conformers are generated by treatments that perturb (disrupt) membranes and they are catalytically inefficient.

![Fig. 1. Compartmented and allosteric models for the regulation of UDP-glucuronosyltransferase](image-url)
bivalent metal cation). This explains why UDP-N-acetylglycosamine decreases latency without rendering the transferase susceptible to potent cytosolic inhibitors. Also, while the membrane barrier is still intact, intracisternal nucleoside diphosphatase helps to prevent product inhibition or even reversal of the transferase by an M²⁺-dependent hydrolysis of the UDP co-product of conjugations.

Model 2, proposed by Zakim & Vessey (1974, 1976a), assumes that glucuronosyltransferase is a non-compartmented allosteric enzyme with free access to its substrates and effectors. In fresh native microsomal fraction, the transferase is constrained by phospholipids in a particular conformation so that its catalytic activity is low, but it is resistant to inhibition by cytosolic inhibitors [Model 2(I) in Fig. 1].

In this conformation, many transferases with low affinity for UDP-glucuronic acid (e.g. 4-nitrophenol glucuronosyltransferase) can be converted into more-active conformations by UDP-N-acetylglycosamine + M²⁺ [model 2(II) in Fig. 1]. These agents are considered to act as positive K-type allosteric effectors for the glucuronidation reaction, markedly decreasing the apparent monosubstrate K for UDP-glucuronic acid and as negative K-type effectors for the reverse reaction, increasing the apparent K for UDP. In this model, UDP-N-acetylglycosamine has no effect on monosubstrate V. It is also reportedly without effect on transferases for steroid hormones (but see Wilkinson & Hallinan, 1977), which reportedly already possess high affinities for UDP-glucuronic acid in its absence (Zakim & Vessey, 1976b). Membrane perturbants generate an additional family of activated-glucuronosyltransferase conformers [Model 2(III) in Fig. 1], but these are all susceptible to cytosolic transferase inhibitors and hence are 'catalytically inefficient' (Zakim & Vessey, 1974, 1976a).

Though the allosteric model only predicts K-effects for UDP-N-acetylglycosamine, a likely effect on V is predicted by the compartmented model, by analogy with transferase activation at V max, by detergents and other membrane-lysis agents (e.g. Vessey & Zakim, 1971). A careful study was therefore undertaken to assess the effects of UDP-N-acetylglycosamine on the kinetics of 4-nitrophenol glucuronidation by guinea-pig-liver microsomal fraction.

Microsomal fraction, prepared from starved male guinea pigs (Berry et al., 1975), was used fresh or after freezing for up to 5 days at -10°C. Glucuronidation of 0.4m⁻¹-4-nitrophenol was assayed in 100mM-Tris/HCl buffer, pH7.6, with between five and seven different concentrations of UDP-glucuronic acid from 0.33 to 4.0mM. Six experiments were done in the presence and absence respectively of 0.8mM-UDP-N-acetylglycosamine, with 4mM-MgCl₂ always present. Assays were generally sampled after 3, 5 and 7min and initial rates of glucuronidation graphically assessed. Values for 1/V and 1/[S] were pooled for all six experiments and apparent V and K values were then derived from Lineweaver–Burk regressions, fitted separately to the results obtained in the presence and absence of UDP-N-acetylglycosamine.

With UDP-N-acetylglycosamine present, the apparent V was 12.1nmol of 4-nitrophenol glucuronidated/min per mg of microsomal protein (confidence range 5.8–∞ at P0.02), compared with a value on 2.9nmol/min per mg (confidence range 2.4–3.6 at P0.02) in its absence. Thus UDP-N-acetylglycosamine increased V by 4-fold on average, and despite considerable dispersion in the results, this stimulation was significant at P0.02. By contrast, the apparent K for UDP-glucuronic acid in the presence of 0.14mM-UDP-N-acetylglycosamine (confidence range 0.66–0 at P0.05) was not significantly different from the apparent K of 0.09mM in its absence (confidence range 0.18–0 at P0.05). In two preliminary experiments, 1mM-UDP-N-acetylglycosamine also increased the apparent V of oestrone glucuronidation assayed as described by Wilkinson & Hallinan (1977) and 1mM-ethylmaleimide virtually abolished the increases in V for both 4-nitrophenol and oestrone. This is consistent with the postulated UDP-glucuronic acid permease possessing essential thiol groups.

The findings of this study therefore fit Model 1 for UDP-N-acetylglycosamine action.

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The Respiratory Chain of Bacillus caldolyticus

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Cells grown in batch culture under high aeration in a medium containing 0.8% peptone, 0.4% yeast extract and 0.3% NaCl at pH 7.2 and 72°C were harvested at the end of the exponential phase of growth and washed in 1.2% NaCl. Membranes were prepared by lysis of cells in the presence of lysozyme, deoxyribonuclease and ribonuclease at pH 7.8, collected at 17000g, washed in 50mM-potassium phosphate and 3 mM-MgCl₂ at pH 7.0 and stored at -20°C.

Oxygen-electrode experiments were performed at 50°C; this temperature is judged to be above the melting point of the membrane lipids, since there was a marked break in the plot of NADH-oxidation rate versus temperature at 39°C. NADH, L-malate, succinate and DL-a-glycerophosphate were oxidized by membrane-bound, perhaps flavoprotein, dehydrogenases. Oxidation of NADH can also take place under anaerobic conditions with fumarate as acceptor. The oxidation of NADH, malate and succinate by O₂ and of NADH by fumarate were all equally inhibited by exposure to u.v. light at 360nm (50% inhibition in 20min at 0°C at 4cm from a Mineralite u.v. lamp), whereas ascorbate oxidation, in the presence of tetramethylphenylenediamine, was unaffected; it is claimed that this procedure selectively destroys quinones (Fujita et al., 1966). Methanol/acetone extraction followed by alumina chromatography showed menaquinone as the predominant quinone in Bacillus caldolyticus.

Oxidation of NADH, malate and succinate can also be inhibited by high concentrations of rotenone and antimycin, that of succinate being particularly sensitive to antimycin. Succinate oxidation is also inhibited by very low concentrations of fumarate (approx. K₁ 100μM) and by carbonyl cyanide p-trifluoromethoxyphenylhydrazone (Dawson & Chappell, 1977).

Difference spectra were recorded at 25°C and -196°C. The calculated contents of cytochromes are shown in Table 1. Although in different cultures these concentrations

Table 1. Calculated content of cytochromes in respiratory chain of B. caldolyticus

Wavelengths of λmax. are from reduced-minus-oxidized spectra, from redox titrations. (i) From fourth-derivative analysis this species is composed of two peaks at 555 nm and 565 nm. (ii) Cytochromes b could not be separately resolved within this potential range.

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<th>Cytochrome . . .</th>
<th>c₅₄₉</th>
<th>c₅₄₉</th>
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