Biochimica et Biophysica Acta, 429 (1976) 768—779

BBA 67802

OBSERVATIONS ON THE ACCESSIBILITY OF ACCEPTOR SUBSTRATES TO THE ACTIVE CENTRE OF UDP-GLUCURONOSYLTRANSFERASE IN VITRO

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(Received October 27th, 1975)

Summary

The partition coefficients between octanol and pH 7.4 buffer for eleven substrates of UDP-glucuronosyltransferase (EC 2.4.1.17) have been determined. They range between 1.1 and 690 in the order *p*-aminophenol < phenol < (*o*-aminobenzoic acid = *o*-aminophenol = *p*-aminobenzoic acid) < *p*-nitrophenol < 4-methylumbelliferone < mercaptobenzothiazole < harmol < phenolphthalein < 1-naphthol. The effect of Triton X-100, used as a model membrane pertubant, on the enzyme activity of UDP-glucuronosyltransferase in rat liver homogenates towards these substrates was determined and compared with the partition coefficients. Enzyme activities towards *p*-aminophenol and phenol were decreased by Triton X-100, the enzyme activities towards other acceptor substrates were enhanced maximally with 0.025% (w/v) Triton. 'Native' enzyme activity (except for amino containing compounds) and activation could be related to partition coefficient of the substrate. An increase in lipid solubility resulted in reduced enzyme activity in untreated homogenates and greater activation. These results suggest UDP-glucuronosyltransferase lies behind a partially lipid-impenetrable barrier and it is suggested that this barrier is broken up by membrane perturbants to permit free access of the more lipid-soluble substrates.

In addition, the formation in vitro of a glucuronide from mercaptobenzothiazole was demonstrated.

Introduction

UDP-Glucuronosyltransferase (UDPglucuronate β-glucuronosyltransferase (acceptor-unspecific), EC 2.4.1.17) is the enzyme conjugating steroids, bilirubin

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and xenobiotics by transfer of glucuronic acid from UDP-glucuronic acid [1,2]. UDP-Glucuronosyltransferase is located in the endoplasmic reticulum, which is sedimented as the microsomal fraction of cell homogenates. The enzyme is capable of conjugating ester, ether, amino, thioether and carbodithioic groups in vivo [3,4] and in vitro [1,5,6].

UDP-Glucuronosyltransferase activity in vitro towards many acceptor substrates is increased by the action of membrane perturbants such as phospholipases [7—10], detergents [11—15], trypsin [7], sonication [16], chaotropic agents [17] and alkanes [18]. Phospholipid and/or protein are released from microsomes during these perturbations [7,9,16,18,19]. UDP-*N*-acetylglucosamine also activates UDP-glucuronosyltransferase, though this is thought to be an allosteric effect [20,21].

Octanol-buffer partition coefficients are used to estimate the lipid solubility of compounds [22—25]. The binding and substrate activity of another xenobiotic metabolising enzyme, the mixed-function oxygenase, have been related to the partition coefficients of substrates [26]. The two enzymes, mixed-function oxygenase and UDP-glucuronosyltransferase, are thought to be functionally and topologically related. The mixed-function oxygenase, which is not activated by membrane perturbants, is believed to lie on the surface of the endoplasmic reticulum, while UDP-glucuronosyltransferase, which is activated, is believed to lie below the surface of the membrane [18,27].

The octanol-buffer partition coefficients of eleven acceptor substrates for UDP-glucuronosyltransferase have been measured using conventional assay techniques and compared with UDP-glucuronosyltransferase activity towards those substrates. Enzyme activity was measured in fresh homogenates and in those activated by the detergent Triton X-100; microsomes were avoided to minimise possible interference from 'spontaneous activation' [12]. Acceptor substrates used included phenol and mercaptobenzothiazole, both known to form glucuronides in vivo, for which assay systems were developed.

Materials and Methods

Chemical, animals and substrates

The acceptor substrates *p*-aminophenol, *o*- and *p*-aminobenzoic acids, 1-naphthol, phenol and phenolphthalein were obtained from Fisons, Loughborough, U.K. *o*-Aminophenol, harmol hydrochloride, mercaptobenzothiazole, 4-methylumbelliferone and *o*-aminophenyl glucuronide came from Aldrich Chemical Company, Wembley, U.K. UDPglucuronic acid and β-glucuronidase (from *Helix pomatia*) were from Boehringer Corporation, London, U.K. Potassium hydrogen glucarate and bovine serum albumin were from Sigma London, Norbiton, Surrey, U.K. All other chemicals were of at least reagent grade. The octanol was Fisons' 'specially pure' octanol and the Triton X-100 was from Koch-Light.

Male Charles River Sprague-Dawley rats (350—480 g), housed with access to food and water ad libitum, were used as enzyme source.

All acceptor substrates were dissolved as 2 mM solutions. Harmol, mercaptobenzothiazole, 4-methylumbelliferone and phenolphthalein were dissolved in 0.1 M sodium hydroxide and the solution was titrated to pH 7.4 with hydro-

chloric acid before making up to the required volume. The remaining substrates were dissolved in water. Aminophenol solutions contained 6 mM ascorbic acid and were flushed with nitrogen to prevent oxidation. 1-naphthol and aminophenol solutions were discarded if a yellow colour was observed.

Determination of partition coefficients

Substances were partitioned between buffer-saturated octanol and octanol-saturated buffer as described in ref. 22. Initially 66 mM phosphate buffer was used, later experiments employed 66 mM Tris buffer, both at pH 7.4. No differences between buffers were observed. For partition coefficients below 3, 2 ml octanol to 5 ml buffer was used; for partition coefficients between 3 and 50, 1 ml octanol; and for partition coefficients above 50, 0.1 ml octanol was added to buffer. The substances being partitioned were added to the aqueous layer and the octanol-buffer mixtures shaken at room temperature for 15 min. Longer shaking times did not alter the partition coefficient. Controls included tubes to which no octanol had been added and tubes without test substance. At least three concentrations of test substance were employed and duplicate tubes were shaken at each concentration. Aliquots of aqueous layer were withdrawn and assayed as described below and the partition coefficient calculated as:

$$\frac{(\text{amount of acceptor substrate in control} - \text{amount in test})}{(\text{amount of acceptor substrate in test})} \times \frac{(\text{volume aqueous layer})}{(\text{volume organic layer})}.$$

Assay of the amounts of each acceptor substrate in the aqueous layer was based on the method for estimating UDP-glucuronosyltransferase activity where possible. Phenol was determined by addition of 0.1 ml Folin-Ciocalteu reagent to 1 ml aqueous layer. The E_{750} was measured after standing for 30 min. Aminophenols and aminobenzoic acids were measured by adding 1 ml 1 M trichloracetate/1 M phosphate buffer pH 2.2 to 1 ml aqueous layer and diazotisation and coupling to *N*-1-naphthylethylene diamine dihydrochloride as described below, and measuring E_{360} after standing for 1 h in the dark. *p*-Nitrophenol (E_{400}) and phenolphthalein (E_{555}) were measured after addition of 1 ml glycine buffer (0.27 M glycine, 0.17 M sodium bicarbonate and 0.12 M sodium chloride, pH 10.4) to 1 ml aqueous layer. 1-Naphthol (excitation 325 nm, emission 455 nm) and 4-methylumbelliferone (excitation 375 nm, emission 460 nm) were measured fluorimetrically after adding 1 ml glycine buffer to 1 ml aqueous layer, while harmol (excitation 320 nm, emission 420 nm) was determined after adding an equal volume of 0.2 M HCl. Mercaptobenzothiazole was measured directly using E_{325}. All methods were found to give linear calibration curves under the conditions employed. Absorption and fluorescence maxima were confirmed using a Perkin-Elmer 356 spectrometer or a Perkin-Elmer MPF 3 fluorimeter. The results, where comparable, generally agree with those in ref. 22.

Assay of UDP-glucuronyltransferase

The same incubation system as previously described [5] was used for all

acceptor substrates. The enzyme was prepared by homogenising rat liver (10% w/v) in 0.25 M sucrose/50 mM Tris/5 mM EDTA (pH 7.4) using a teflon-glass homogeniser and centrifuging the homogenate at 2000 × *g* for 10 min to remove cell debris and nuclei.

Incubation vessels contained (final volume 0.3 ml) 0.2 mM acceptor substrate, 2.0 mM UDPglucuronic acid and 50 μl (in activated preparations where 5-fold or more activation was expected 10 μl) of enzyme in 66 mM Tris buffer pH 7.4 containing 10 mM Mg^{2+}. Controls contained no UDPglucuronic acid. Routinely, activated preparations contained 0.025% (v/v) Triton X-100. The vessels were incubated for 10 min at 37°C with shaking at 80 strokes/min aerobically in a water bath. The reaction was stopped and the amout of glucuronidation measured as described below. Conjugation was linear over the time of reaction and not more than 30% of substrate was allowed to react.

o-Aminophenylglucuronide was measured by diazotisation [28]. The reaction was stopped and the pH adjusted to 2.2 with 0.3 ml 1 M phosphate/1 M trichloracetate buffer and centrifuged. A 0.5 ml aliquot of supernatant was mixed with, in turn, 0.1 ml 0.1% (w/v) sodium nitrite, 0.1 ml 0.5% (w/v) ammonium sulphamate and 0.1 ml 0.1% (w/v) *N*-1-naphthylethylenediamine dihydrochloride. The E_{560} was read after 1 h at 25°C in the dark.

Conjugation of other acceptor substrates was measured by substrate disappearance. Aminobenzoic acids were measured by a modification of [28]. The reaction was stopped by adding 1.8 ml 0.22 M trichloracetic acid. After centrifugation 1.6 ml aliquots of supernatant were added to 0.4 ml 0.36 M sodium carbonate and the pH checked to be about 4 with pH paper. Then the free substrate was extracted into 4 ml di-*iso*-propyl ether by inversion shaking for 30 min. Aliquots (3.7 ml) of the ether layer were taken and the free substrate returned to 1 ml 0.1 M sodium hydroxide. Finally 0.5 ml of alkali layer was acidified with 60 μl of 4 M hydrochloric acid and diazotised as described above. Extraction of several concentrations of acceptor substrate added to incubation media, using this technique, indicated that the extraction was consistant. Triplicate determinations were performed for these acceptor substrates. *p*-Aminophenol was determined using a modification of [29]. The incubation was saturated with sodium chloride and the *p*-aminophenol extracted into 2.5 ml di-*iso*-propyl ether by inversion shaking for 15 min. The *p*-aminophenol was returned to 1 ml 0.1 M sodium hydroxide containing 0.1% phenol from 2 ml organic layer. The colour in the aqueous layer was measured at 630 nm after standing for 30 min. Phenol was extracted as for *p*-aminophenol and returned to 0.1 ml 1.0 M sodium hydroxide. Folin-Ciocalteu reagent (0.1 ml) was then added to the aqueous layer and the blue colour estimated at 750 nm after standing for 30 min. Mercaptobenzothiazole was extracted in the same way and the E_{325} measured in the ether extract.

p-Nitrophenol [5] and phenolphthalien were measured after stopping the reaction with 0.7 ml 0.1 M trichloracetic acid and centrifugation. Aliquots (0.8 ml) of supernatant were added to 2 ml glycine buffer (pH 10.4) and the colour measured as described above.

1-Naphthol [30] and 4-methylumbelliferone [12] concentrations were determined fluorimetrically after addition of 2.0 ml glycine buffer pH 10.4. Harmol glucuronidation was measured as in [31]. After addition of 30 μl 5%

(w/v) copper sulphate and centrifugation, aliquots were chromatographed on cellulose in 0.12 M hydrochloric acid. The harmol and harmol glucuronide were eluted into 0.12 M hydrochloric acid and estimated fluorimetrically. The emission and excitation maxima were as described above.

Incubations with β-glucuronidase

As all methods, except those for *o*-aminophenol and harmol, for estimating UDP-glucuronosyltransferase depend on acceptor substrate disappearance, the methods were checked by incubations with β-glucuronidase and its specific inhibitor, glucarolactone. The glucarolactones solution was prepared as described in [32]. Incubations were brought to pH 5.5 with hydrochloric acid and divided into three portions. To one portion water was added, to the second portion 20 μl of β-glucuronidase, and to the third portion β-glucuronidase and 15 mM glucarolactones solution. The vessels were incubated for 1 h at 37°C and the freed substrate measured as described above. At least 80% of the substrate disappearance was restored by β-glucuronidase and 70% of this hydrolysis was prevented by glucarolactone.

Protein determinations

Protein was determined using the Lowry procedure [33] and bovine serum albumin as standard.

Results

Conjugation of phenol and mercaptobenzothiazole

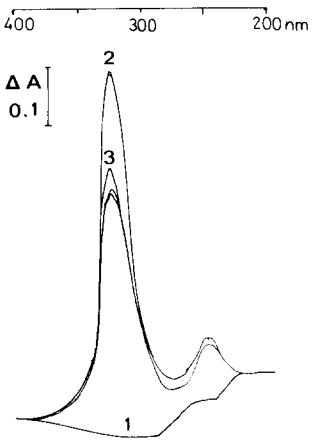
As there appeared to be no published method for estimating phenol and mercaptobenzothazole glucuronidation in vitro, the methods were more carefully evaluated. Incubations, performed as described in Methods, required the presence of UDPglucuronic acid, acceptor substrate and enzyme for activity. If one of these constituents was omitted during incubation and added before extraction, very little acceptor substrate disappearance was observed. Fig. 1 shows the ultraviolet spectra of di-*iso*-propylether extracts from incubations using mercaptobenzothiazole as acceptor. These spectra are similar to those reported in ref. 4 for mercaptobenzothiazole. Essentially complete (91—95%) extraction of the acceptor substrate took place from incubations with enzyme when acceptor was added immediately before extraction solvent. The reproducibility of the technique (from a series of test and control incubations under the same conditions) was ±7% for phenol and ±3% for mercaptobenzothiazole (S.D., 8 determinations).

Confirmation that the colour disappearance was due to glucuronide conjugation was obtained by incubation with β-glucuronidase and the specific inhibitor of β-glucuronidase, glucarolactone, as described in the Methods section.

Table I shows that at least 80% of the conjugate was hydrolysed by β-glucuronidase and about 70% of this hydrolysis was prevented by 15 mM glucarolactones solution. Thus the methods essentially measured glucuronide formation in vitro.

Determination of octanol-buffer partition coefficients

Octanol-buffer partition coefficients of acceptor substrates of UDP-glu-



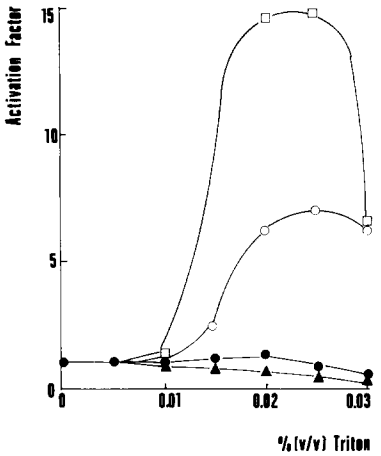


Fig. 1. Spectra of di-*iso*-propyl ether extracts from incubations with mercaptobenzothiazole. Spectra were obtained by scanning using a Perkin-Elmer 356 spectrometer in the split-beam mode. Reference cell contained extracts of an incubation mixture without mercaptobenzothiazole. The baseline [1] was drawn with a second aliquot of reference extract in the test cell. In [2] the test cell contained extracts from incubations with mercaptobenzothiazole to which UDPglucuronic acid had been added after incubation. When mercaptobenzothiazole and UDPglucuronic acid were both present during incubations the extracts produced the scans [3]. Incubations contained (when present) 0.2 mM mercaptobenzothiazole, 2 mM UDPglucuronic acid and 50 μl enzyme in 66 mM Tris buffer pH 7.4 containing 10 mM Mg^{2+} (total volume 0.3 ml), and were for 20 min at 37°C. Extraction with 2.5 ml di-*iso*-propyl ether was as described in the Methods section.

Fig. 2. The effects of various concentrations of Triton X-100 on the conjugation of *p*-aminophenol, harmol, mercaptobenzothiazole and phenol. Incubation were as described in the Methods section except that varying concentrations of Triton X-100 were present. Substrates were (▲———▲) *p*-aminophenol, (○———○) harmol, (□———□) mercaptobenzothiazole and (●———●) phenol.

TABLE I

HYDROLYSIS OF PHENOL AND MERCAPTOBENZOTHIAZOLE CONJUGATES BY β-GLUCURONIDASE

Conditions of incubations are as described in Methods. The synthetic incubation was 10 min in Expt. 1 and 20 min in Expt. 2 and the hydrolytic incubation 1 h in both experiments. Duplicate tubes were incubated under each condition, substrate estimation being as described in Methods.

	Phenol		Mercaptobenzothiazole	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Condition:				
Amount of substrate disappeared (nmol)	9.6	17.0	7.0	20.0
Amount of substrate restored on hydrolysis by β-glucuronidase (nmol)	8.7	13.7	6.3	17.6
Amount of substrate restored in the presence of β-glucuronidase and 15 mM boiled glucarate (nmol)	3.2	3.8	1.6	5.1
% of substrate disappearance restored by β-glucuronidase	91	81	90	88
% inhibition of β-glucuronidase induced substrate reappearance by boiled glucarate	64	72	74	71

TABLE II

OCTANOL-BUFFER PARTITION COEFFICIENTS OF SOME UDP-GLUCURONOSYLTRANSFERASE SUBSTRATES

The partition coefficients between 5 ml of octanol-saturated 66 mM buffer pH 7.4 and varying amounts of buffer-saturated octanol were determined as described in methods over the concentration ranges shown in the table. Results are expressed as mean ± S.D., No. of determinations in parentheses.

Substrate	Concentration Range (μM)	Partition Coefficient
p-Aminophenol	3—30	1.1 ± 0.3(5)
Phenol	21—85	4.2 ± 3.0(3)
o-Aminobenzoate	5—50	5.1 ± 0.4(4)
o-Aminophenol	44—220	5.2 ± 2.4(10)
p-Aminobenzoate	5—50	7.0 ± 1.0(3)
p-Nitrophenol	10—60	24.1 ± 3.1(6)
4-Methylumbelliferone	6—60	37.9 ± 4.1(3)
Mercaptobenzothiazole	3—30	41.2 ± 2.3(3)
Harmol	13—40	154 ± 9(3)
Phenolphtalein	19—125	260 ± 34(4)
1-Naphthol	40—70	690 ± 80(3)

curonosyltransferase were determined at pH 7.4 as described in the Methods section. No differences were observed when phosphate buffer was substituted for Tris buffer in some preliminary experiments or when Triton X-100 (up to 0.03% v/v) was added to the aqueous layer. The results, as given in Table II, show a 600-fold variation in the partition coefficients of these acceptor substrates of UDP-glucuronosyltransferase.

Activity of UDP-glucuronosyltransferase in the presence and absence of Triton X-100

The effect of Triton X-100 on UDP-glucuronosyltransferase activity in homogenates towards the acceptor substrates *o*- and *p*-aminobenzoic acid, *o*- and *p*-aminophenol, harmol, mercaptobenzothaizole, 4-methylumbelliferone, 1-naphthol, *p*-nitrophenol, phenol and phenolphthalein were determined as described in the Methods section. Initially the maximal activating concentration of Triton X-100 was found to be 0.025% (v/v) by incubations with various concentrations of Triton X-100. The results for four acceptor substrates are shown in Fig. 2. Enzyme activity towards two acceptor substrates, *p*-aminophenol and phenol, was not enhanced, only deactivation took place.

Table II shows the comparative enzyme activities and activation achieved for all acceptor substrates in the presence of 0.025% Triton X-100 when assayed in groups of three or four substrates. Fresh and activated enzyme activities were determined in the same preparation. The activation values compare favourably with published data using mouse, male and female rat liver homogenate [12,13,35,36] and male rat liver microsomes [14,15] as enzyme source.

Comparison of partition coefficients, enzyme activity and activation

Hansch et al. [22—24] have demonstrated that, for an effect to be dependant on lipid solubility, the equation

TABLE III

ACTIVITY TOWARDS UDP-GLUCURONYLTRANSFERASE FOR VARIOUS SUBSTRATES IN FRESH AND TRITON X-100 ACTIVATED PREPARATIONS.

Each incubation contained 0.2 mM substrate, 2.0 mM UDPglucuronic acid and 10 or 50 μl of enzyme preparation in 66 mM Tris buffer pH 7.4 containing 10 mM Mg^{2+} (total volume 0.3 ml). The amount of conjugation was assayed by measurements of substrate disappearance as described in methods. Groups of three or four substrates were assayed with and without Triton in the same enzyme preparation. Results are expressed as means ± S.D. No. of determinations in parentheses.

	Activity (nmol · min^{-1} · mg $protein^{-1}$)		Activation factor *
	Fresh	With 0.025% Triton	
Substrate:			
p-aminophenol	2.5 ± 0.7(3)	0.8 ± 0.1(3)	0.33
phenol	19.2 ± 5.6(3)	15.5 ± 8.2(3)	0.8
o-aminobenzoate	0.25 ± 0.09(3)	0.46 ± 0.09(3)	1.6
o-aminophenol	0.16 ± 0.06(3)	0.24 ± 0.06(3)	1.5
p-aminobenzoate	0.63 ± 0.30(3)	1.14 ± 0.06(3)	1.6
p-nitrophenol	5.0 ± 0.5(3)	49 ± 20(3)	9.7
4-methylumbelliferone	1.7 ± 0.6(7)	12 ± 5(3)	9.3
mercaptobenzothiazole	1.5 ± 0.3(3)	16 ± 6(3)	10.4
harmol	1.3 ± 0.5(3)	9.3 ± 2.3(3)	7.2
phenolphtalein	0.9 ± 0.2(3)	7.9 ± 3.3(3)	9.0
1-naphthol	1.1 ± 1.0(9)	21 ± 10(3)	19.8

* Direct comparisons of incubations with and without Triton in the same enzyme preparation were used in calculating the activation factor.

$$\log RBR = \log PC + c \quad (1)$$

holds, where RBR is relative biological response and PC is the octanol buffer partition coefficient.

The activity in unactivated and Triton X-100 activated enzyme preparations (Fig. 3) and activation factor (Fig. 4) for different substrates can be plotted as relative biological responses against their partition coefficients. With unactivated preparations, excluding amino-compounds, the equation

$$\log \text{enzyme activity} = -0.729 \log PC + 1.67 \quad (2)$$

(correlation 0.885, S.D. 0.32, n = 7) holds. It is difficult to describe a line for activated preparations, but increasing lipid solubility did result in some increase in enzyme activity. In a study [36] using equal volumes of octanol and buffer and a series of substituted phenols, likewise an increase in Triton X-100-activated enzyme activity with lipid solubility was visible, though, again no equation can be easily derived. The enzyme activities for acceptor substrates used in that study were estimated by continuous measurement of UDP production in Triton X-100 activated microsomes and that for aminophenols was not unexpectedly low. Aniline forms a labile *N*-glucuronide [37]. Thus, if amino compounds form labile *N*-glucuronides as well as stable *O*-glucuronides, only the *O*-glucuronide formation would be measured by the techniques used in this study and the apparent level of total glucuronidation would be low.

With activation factor as the relative biological response (Fig. 4), and using

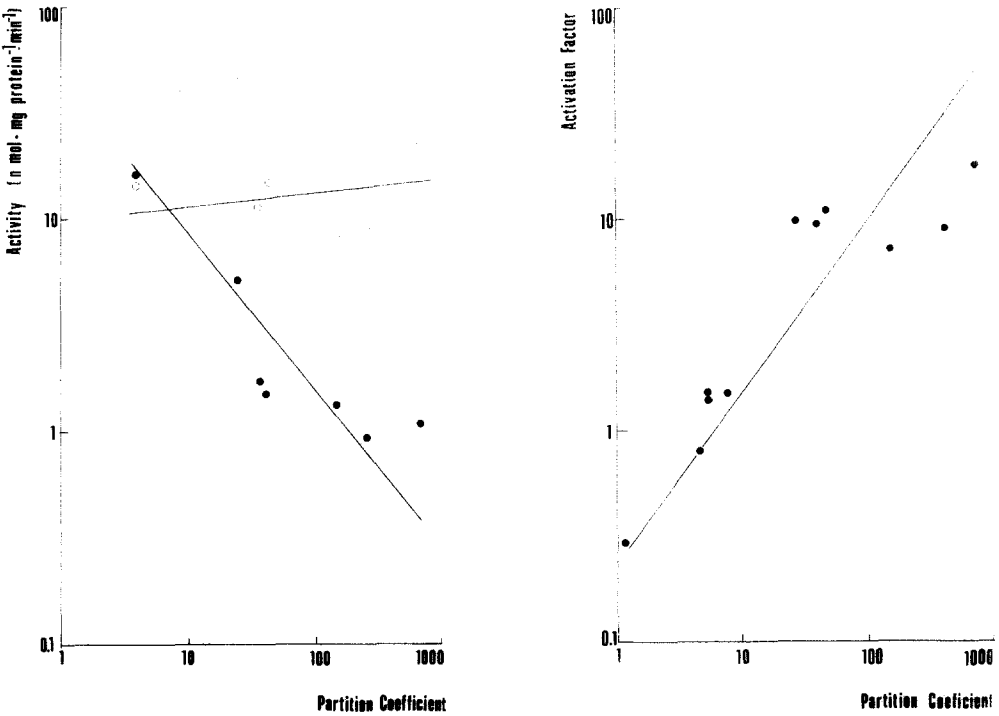


Fig. 3. Comparison of partition coefficients and UDP-glucuronosyltransferase activities in fresh and Triton X-100 activated liver homogenates. The data plotted are derived from Tables II and II and are plotted on logarithmic scales. Resuls from amino-compounds are omitted. The lines are those described in equations (2) and (4). •, Fresh and ○, activated preparations.

Fig. 4. Comparison of partition coefficients and UDP-glucuronosyltransferase activation. The data are derived from Tables II and II and are plotted on logarithmic scales. The line is that described in equation (3).

all the data in Table III, the relationship

$$\log \text{activation} = 0.86 \log PC - 0.68 \quad (3)$$

(correlation 0.92, S.D. 0.34, $n = 11$) can be described.

Thus enzyme activity in unactivated homogenates and the amount of activation depend on lipid solubility of the acceptor substrate. Mathematical combination of equations (2) and (3) gives the equation for Triton X-100 activated preparations

$$\log \text{enzyme activity} = 0.13 \log PC + 1.01 \quad (4)$$

or increased lipid solubility of the substrate results in increased activity in Triton X-100 activated preparations. The same conclusion is arrived at in ref. 36 for a series of substituted phenols.

Discussion

The glucuronides of phenol [3] and mercaptobenzothiazole [4,38] have been found in vivo and this paper describes non-radioactive methods for deter-

mining conjugation of these acceptor substrates in vitro. The glucuronide of mercaptobenzothiazole is presumably, as in vivo, a thioglucuronide. Chemically synthesised benzthiazole-2-thioglucuronide is hydrolysed by β-glucuronidase [39] and the biosynthesis of thioglucuronides in vitro is by the same mechanism as for *o*-glucuronides [5,6].

A wide range of partition coefficients, from the water-soluble to the very lipid-soluble, was observed for the acidic, alcoholic and thiolic UDP-glucuronosyltransferase substrates investigated. As the partition coefficient increases, so the activity of the UDP-glucuronosyltransferase towards that acceptor substrate in homogenate decreases. In Triton X-100-activated preparations, the effect is eliminated and a possible increase in activity of UDP-glucuronosyltransferase for increasing lipid solubility of acceptor substrate is seen. This possibility is also visible in the results from ref. 36.

More clear is the relationship between activation factor and lipid-solubility of the acceptor substrate. Irrespective of acceptor substrate activity and enzyme source the activation factor increases with increased lipid solubility of substrate. This relationship is demonstrated for activation by Triton X-100 but a similar pattern for a limited number of substrates can be seen for spontaneous activation and for activation by digitonin [12] and alkanes [18].

Membranes of the endoplasmic reticulum contain phospholipid and protein. Phospholipases [9,10] and alkanes [18] release phospholipids and activate UDP-glucuronosyltransferase. Detergent [19] and trypsin [7] also release protein, thus either phospholipid or protein or both are released during UDP-glucuronosyltransferase activation. Partial removal of or substitution for phospholipid results in increased accessability of the more lipid soluble acceptor substrate to the active centre of UDP-glucuronosyltransferase. Such free access of acceptor substrate was required to see the full effects of induction of UDP-glucuronosyltransferase activity which otherwise appeared low in both whole cell and broken cell preparations [30,40]. The activity of UDP-glucuronosyltransferase in vivo presumably lies within the endoplasmic reticulum membrane as in vitro UDP-glucuronosyltransferase lies inside the microsomal fraction membranes.

The chief phospholipid affecting UDP-glucuronosyltransferase activity is phosphatidylcholine [9,41] which has a polar head and a non-polar tail. The polar head forms a barrier to the penetration of lipid-soluble, but not relatively water-soluble, substrates (presumably including UDP-glucuronic acid) to the lipid soluble interior of the membrane [42]. Substitution of phospholipid by alkane or removal by membrane perturbants removes this barrier and enables the more lipid-soluble acceptor substrates to gain free access to the active centre of UDP-glucuronosyltransferase. When access is no longer a problem activity of UDP-glucuronosyltransferase for an acceptor substrate increases with increasing lipid solubility as it does for surface enzymes such as the mixed-function oxygenase [26]. Treatment with membrane perturbants above that required for maximum activation of UDP-glucuronosyltransferase often results in a decrease from the maximum value of enzyme activity towards an acceptor substrate [7,8,12,13,17], Fig. 2. This decrease in enzyme activity, for the acceptor substrate *p*-nitrophenol and the perturbants Triton X-100 [12] and phospholipase A [8] was by decrease in V, thus the ability of enzyme to bind

acceptor substrates could be impaired. A reduced ability to bind acceptor substrates might be more marked with the more water-soluble substrates. The optimum amount of perturbation for maximum enzyme activity towards an acceptor could, therefore, depend on a combination of two effects; increased accessability of the enzyme to acceptor substrate caused by perturbing the membrane would be balanced by impairment of the ability of some active centres to bind the substrates.

While accessability of UDPglucuronic acid to UDP-glucuronosyltransferase might be enhanced by Triton X-100 (perhaps by a reduction in apparent K_{UDPGA}, [8]), it would be expected that any change in accessibility would be the same whatever the acceptor substrate. Endogenously generated UDPglucuronic acid appears to give higher rates of glucuronidation of *o*-aminophenol than exogenously-supplied UDPglucuronic acid [43]. Inhibition by detergents of conjugation employing endogenously-generated UDPglucuronic acid would then be because release of the UDPglucuronic acid to the medium was enhanced by membrane perturbation.

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