Factors Modulating the Catalytic Specificity of a Pure Form of UDP-glucuronyltransferase*

Jacques Magdalou‡, Yehosua Hochman, and David Zakim§

From the Liver Studies Unit, Veterans Administration Medical Center, San Francisco, California 94121, the Departments of Medicine and Pharmacology, University of California, San Francisco, California 94143 and ‡ Laboratoire de Biochimie Pharmacologique ERA Centre National de la Recherche Scientifique, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Nancy I, Nancy, France

The activity of a pure, delipidated form of UDP-glucuronyltransferase from pig liver, designated by us as GTz, was assessed as a function of the structure of the aglycone and the nature of the lysophosphatidylcholine used to reconstitute activity. When reconstituted with oleoyl lysophosphatidylcholine, the activity of GTz with para-substituted phenols is related directly to the acidity of the aglycone. The size and shape of the group in the para position have no effect on catalytic rate except via donating or withdrawing electrons from the benzene ring. Thus, a plot of log V* versus the Hammett function (σ) has a slope of +2.2. The relative rates of glucuronidation of different phenols varies, however, as the phospholipid environment of GTz changes. The slope of log V* versus σ decreases when GTz is reconstituted with stearyl lysophosphatidylcholine. For aglycones with relatively high acidity (pK* < 9.2), rates at V* are independent of acidity when GTz is reconstituted with myristoyl lysophosphatidylcholine. By contrast, the activity at V* for GTz reconstituted with myristoyl lysophosphatidylcholine depends on acidity for phenolic aglycones with pK* values greater than 9.2. For the lipids tested, reconstitution with oleoyl lysophosphatidylcholine yields enzyme with highest activity, and reconstitution with myristoyl lysophosphatidylcholine yields enzyme with lowest activity. The data can be interpreted as indicating that the lipid environment of the enzyme influences the rate of bond breaking between the UDP and glucuronic acid moieties of UDP-glucuronic acid. For GTz in oleoyl lysophosphatidylcholine, this rate is so rapid that the attack of the phenolate ion is rate-limiting and, hence, the observed dependence of activity at V* on acidity of the phenol. As the rate of bond breaking becomes slower, as for enzyme reconstituted with myristoyl lysophosphatidylcholine, the acidity of the aglycone affects reaction rate only for aglycones with values of pK* above 9.2.

UDPG-glucuronoltransferases in the hepatic endoplasmic reticulum are important enzymes for detoxifying endogenously synthesized compounds and xenobiotics. The reactions catalyzed by these enzymes alter the physiologic and pharmacologic activities of many compounds, and at the same time, facilitate their excretion into bile and urine. Recent advances in techniques for separating different forms of these enzymes suggest that there are several proteins with activity as UDP-glucuronoltransferase (1–3), which may account for the glucuronidation of a broad variety of structures containing a diversity of functional groups (4). There are, however, no data on the basis for the specificity of different forms of UDP-glucuronoltransferases. It is unknown, for example, whether different UDP-glucuronoltransferases catalyze the conjugation of more than a single functional group, or why there are large variations in the rates of glucuronidation of homologous compounds, e.g. a series of phenols. In addition, the activities and regulatory properties of UDP-glucuronoltransferases within intact, untreated microsomes seem to depend on interactions between these enzymes and the phospholipids of the microsomal membrane (5, 6). The availability of pure forms of UDP-glucuronoltransferase, at this time, makes it possible to examine in detail the kinetic properties of single forms and, thus, to resolve significant questions as to (a) factors that determine specificity, and (b) the precise manner in which the phospholipid environments of these enzymes influence their functions. In this paper, we present data on the basis for variable rates of glucuronidation of phenolic aglycones by a single pure form of UDP-glucuronoltransferase. One of the important conclusions to be drawn from these data is that the ratios of rates of glucuronidation of a series of phenolic compounds is not a static property of the enzyme studied, but can be modulated by the structure of the phospholipid in the enzyme’s environment.

EXPERIMENTAL PROCEDURES

Preparation of UDP-glucuronoltransferase—UDP-glucuronoltransferase was partially purified from pig liver as in Ref. 7. Enzyme purified to the hydroxylapatite step in Ref. 7 (12–15 mg of protein) was applied to a hydroxylapatite column (Bio-Rad, Bio-Gel HT), 1.5 x 25 cm, equilibrated with 5 mM Tris, pH 8 (0°C), 0.1 mM dithioerythritol, 2 mM EDTA, 0.5% (w/v) cholate, and 10% (v/v) glycerol. The column was washed with 100 ml of 90 mM P, in the same buffer and then with a gradient of 90 to 350 mM P, (100 x 100 ml) in the same buffer. Fractions of 5 ml were collected. Two peaks of UDP-glucuronoltransferase were eluted. Peak 1 contained residual phospholipids, but Peak 2 had no detectable phospholipid phosphorus. This fraction of enzyme was used in all studies reported in this paper. We have designated this form of the enzyme as GTz, to indicate that it is one of two forms of UDP-glucuronoltransferase purified to date from pig liver. GTz, after elution from hydroxylapatite, was dialyzed extensively (48 h with four changes of 30 volumes of 5 mM Tris, pH 8 (0°C), 0.1 mM dithioerythritol, 2 mM EDTA, and 10% (v/v) glycerol) in order to reduce the concentrations of cholate and P,. Results were unaffected by small, residual amounts of cholate in the preparation of this enzyme in that more prolonged dialysis did not alter activities of reconstituted enzyme (8). Enzyme was stable for at least 2 months.
Specificity of UDP-glucuronyltransferase

when stored at -20 °C, GTzp was pure, based on analysis of enzyme electrophoresed on 7.5% polyacrylamide gels after treatment with sodium dodecyl sulfate (9), which yielded a single band of protein. Protein was measured by the method of Lowry et al. (10).

Assay of Enzyme Activities—All assays were carried out in 50 mM Tris- HCl, pH 7.5, at 30 °C. Activities with p-nitrophenol were assayed by measuring the change in absorbance at 400 nm. Activity with estrone as aglycone was measured radiochemically using [14C]estrone (Amersham) as in Ref. 11. Activities with all other aglycones were measured by coupling the release of UDP from UDP-glucuronic acid or UDP-glucose to reduction of pyruvate to lactate (12). This was achieved by adding 20 units of purified pyruvate kinase (Sigma), 1 unit of purified lactic dehydrogenase (Sigma), 0.5 μmol of phosphoenolpyruvate, and 0.1 μmol of NADH to each reaction mixture (final volume of 1 ml). The course of the reaction was monitored at 340 nm. This method was validated by comparing the rates of glucuronidation of p-nitrophenol as measured in the coupled system with rates measured by the change in absorbance at 400 nm, which reflects directly the glucuronidation of substrate. Rates were identical when measured by these two techniques.

The preparation of GTzp and the coupling enzymes contained no NADH-oxidase activity so that blank rates measured in the absence of aglycone and/or UDP-glucuronic acid were negligible. Aglycones that were poorly soluble in water were dissolved in ethanol. No more than 10 μl of ethanol were added to a reaction mixture. It was determined in separate experiments that this amount of ethanol did not affect the activity of GTzp measured with p-nitrophenol and other water-soluble phenolic aglycones, nor did it affect the blank rate when the coupling enzymes were present in the assay system.

For each aglycone, rates of glucuronidation were measured as a function of aglycone concentration at different fixed concentrations of UDP-glucuronic acid as in Ref. 7. Activities at Vm and other relevant kinetic constants were determined as in Ref. 7 for an enzyme with a rapid equilibrium, random order kinetic mechanism (13, 14).

Reconstitution of GTzp—All phospholipids (sn-1-O-acyl lysophosphatidylcholine) were pure, synthetic compounds obtained from P-L Biochemicals. These were used without further purification. Phospholipids were dispersed in water (25 mg/ml) at 0 °C and kept under argon until further use. Enzyme at 0 °C was mixed with the indicated phospholipids in a ratio of 1 to 10 (w/w). Mixing was achieved by agitation by hand of the protein and phospholipid. This method and the ratio of protein to lipid yield maximal activation when enzyme is reconstituted with lysophosphatidylcholine. Maximal activation occurs essentially instantaneously under these conditions. The mixture of enzyme and lipid was kept at 0 °C under a stream of argon until aliquots were removed for assay. The activity of the enzyme-phospholipid was stable for at least 8 h, which was the longest interval tested.

RESULTS

Factors Modulating Rates of Glucuronidation at Vm of Phenolic Aglycones—Variations in the activity of a single, pure form of UDP-glucuronyltransferase as a function of aglycone structure could be due to differences in rates of catalytic steps during glucuronidation and/or to variations in the affinity of the enzyme for different aglycones. Either of the above effects could depend, in turn, on the steric or electronic properties of the aglycone. The relationship between structure and rates of the catalytic steps was examined by measuring activity at Vm with a series of para-substituted phenols having different acidities. The GTzp used in these experiments was reconstituted with oleoyl lysophosphatidylcholine. The data from these experiments indicated that rates at Vm increased as the pK value of the phenolic aglycone decreased. The data, therefore, were plotted (closed circles, Fig. 1) as log Vm versus σ, which is derived from the Hammett equation for para-substituted phenols (Equation 1). The value of ρ used was 2.23 (15).

\[ pK_{\text{phenol}} - pK_{\text{phenol}^{+}} = \rho \sigma \] (1)

A plot of the data as log Vm (substituted phenol) - Vm (phenol) versus σ yields a line with the same slope as shown in Fig. 1. As presented, the data indicate directly the individual values of Vm. The experimental value of ρ, from the slope of the plot in Fig. 1, is 2.1. Given the close correspondence between the value of ρ for the dissociation of para-substituted phenols and the experimentally determined value of ρ for Equation 2,

\[ \log V_m^{\text{phenol}^{+}} = \log V_m^{\text{phenol}} + \rho \sigma \] (2)

it is reasonable to conclude that the relative rate of glucuronidation of para-substituted phenols depends on their acidities. The effect of para substitution on reactivity is due to donation or withdrawal of electrons from the benzene ring with attendant effects on the stability of the phenolate ion. Thus, for two phenols, A and B,

\[ \log V_m^{A} - \log V_m^{B} = pK_{A}^{+} - pK_{B}^{+} \] (3)

The rate of glucuronidation for each para-substituted phenol, therefore, appears to be a function of the concentrations of the complex GTzp-phenolate ion, relative to GTzp-phenol. We can draw the following conclusions about the function of the GTzp form of UDP-glucuronyltransferase when the enzyme is reconstituted with oleoyl lysophosphatidylcholine. With respect to the factors important for binding of phenols, GTzp does not distinguish between the phenol and phenolate forms of aglycones. With respect to the catalytic mechanism of GTzp, protonated forms of the phenols are not substrates for glucuronidation, even though they bind to the enzyme.

To extend the conclusions drawn from the data for para-substituted phenols, rates of glucuronidation at Vm were measured with 1-naphthol and estrone. These are known to be

FIG. 1. Activities at Vm of GTzp reconstituted with oleoyl lysophosphatidylcholine as a function of the acidity of phenolic aglycones. Enzyme was reconstituted and activities assayed as under "Experimental Procedures." The data are plotted as Vm (micromoles/min/mg of protein) on the log scale versus σ, which is derived from the Hammett equation for para-substituted phenols (Equation 1). Closed circles are activities at Vm for the indicated aglycone. Open circles are activities at Vm for the indicated aglycone.

\[ \log V_m^{\text{phenol}^{+}} = \log V_m^{\text{phenol}} + \rho \sigma \] (2)

\[ \log V_m^{A} - \log V_m^{B} = pK_{A}^{+} - pK_{B}^{+} \] (3)
substrates for UDP-glucuronyltransferase. The Hammett equation usually is not used in studying the reactivity of these compounds in organic reactions. Nevertheless, we calculated values of $\sigma$ from Equation 1 for 1-naphthol and estrone in order to determine whether rates of glucuronidation could be related to the acidity of these compounds. As for the series of para-substituted phenols, rates of glucuronidation at $V_{n}$ for estrone and 1-naphthol (Fig. 1, open circles) are predicted with a high degree of accuracy by the $pK_a$ values of these compounds. The data are not shown, but activities at $V_{n}$ were nearly identical for 1- and 2-naphthol.

An interesting and potentially useful observation for predicting rates of glucuronidation catalyzed by GT$_3P$ reconstituted with oleoyl lysophosphatidylcholine is that the substitutions in the benzene ring have no detectable effect on rates of glucuronidation despite considerable variation in their structures. On the other hand, steric factors are not completely unimportant for determining the rate of glucuronidation of phenols (Table I). A nitro group at either the ortho or the meta position appears to interfere with glucuronidation. It is likely that there are different reasons for the low rates of glucuronidation of o-nitro and 3,4-dinitrophenols. The nitro group in the former compound could interfere with attack of the phenolate ion on UDP-glucuronic acid, but a meta nitro is sufficiently far away from the attacking group not to interfere on this basis. The $pK_a$ value of the hydroxy group in o-aminophenol (9.97) is considerably lower than that for p-aminophenol, probably because of internal H-bonding between the o-aminophenol and the phenolate ion. Despite this fact, the rate at $V_{n}$ for glucuronidation of o-aminophenol is 0.22 pmol/min/mg of protein, which is what one would predict from Equation 1 for 1-naphthol and estrone in pH 7.5, as under "Experimental Procedures." Activities at $V_{n}$ for glucuronidation of o-aminophenol (9.97) is considerably lower than that for p-nitrophenol. These data are compatible with the conclusion that the binding energy of phenolic aglycones to GT$_3P$ is due primarily to interactions between the enzyme and the benzene ring of the aglycone. Benzene itself, however, was not an inhibitor of GT$_3P$. We found, too, that pyridine and aniline did not bond to the active site of the enzyme, as evidenced by their inability at concentrations as high as 0.5 mM to inhibit the glucuronidation of 0.1 mM p-nitrophenol. These data are compatible with the conclusion that the binding energy of phenolic aglycones to GT$_3P$ is due primarily to interactions between the active site and the phenolic function itself.

### Table I

| Aglycone     | $V_{n}$ |
|--------------|---------|
| o-Nitrophenol| 0.107   |
| 3,4-Dinitrophenol | 0.170 |
| o-Aminophenol | 0.22   |

### Table II

Table II provides the determined values of $K_{aglycone}$ for GT$_3P$ reconstituted with oleoyl lysophosphatidylcholine.

| Aglycone   | $K_{aglycone}$ |
|------------|----------------|
| p-Nitrophenol | 0.08          |
| p-Bromophenol  | 0.005        |
| 1-Naphthol       | 0.0222      |
| Phenol           | 0.13         |
| p-Hydroxyphenyl   | 0.033        |
| o-Aminophenol     | 0.08         |
| o-Nitrophenol     | 0.014        |
| p-Aminophenol     | 0.022        |
| 3,4-Dinitrophenol | 0.042        |

The Influence of Lipid Environment on Rates of Glucuronidation of Phenols by UDP-glucuronyltransferase—Prior work from this laboratory has shown that the kinetic properties of UDP-glucuronyltransferase are determined by the chain length and unsaturation of the acyl portion of the lysophosphatidylcholine added to delipidated preparations of the enzyme (7). The data in Fig. 1 suggest that this effect of the acyl group on the activity of UDP-glucuronyltransferase could be due to alterations in the reactivity of the phenolate ion within the active site of the enzyme. As shown by the data in Table IV, varying the lipid environment of GT$_3P$ does not simply affect the reactivity of the phenolate ion bound to the active site of the enzyme. For, if this were the case, one would expect parallel changes in the rates of glucuronidation of...
of the  phenol  has  no  influence  on  the  rate of glucuronidation of phenols  with pKₐ values of less than  about 9.3. As acidity
after  reconstitution of GTp  with myristoyl lysophosphatidyl-
ment  and  after  reconstitution  with  stearoyl  lysophosphatidyl-
with p-nitrophenol  as glycone was the  same  in  this  environ-
ment  and  after  reconstitution  with  stearoyl  lysophosphatidyl-
with the enzyme is reconstituted with oleoyl lysophosphati-
dylcholine as a function  of  the  lipid  environment of
lanyl  lysophosphatidylcholine to  stearoyl  lysophosphatidyl-
are of the various substitutions  that  are  at  the para position to the
phenol-hydroxy group and  for 1-naphthol.
In fact, as the acyl chain of the lyso derivative is shortened, the  activities at
Vₐ for glucuronidation of p-nitrophenol  and  1-naphthal as a function of the  lipid  environment of GTzp.
Enzyme  was reconstituted with the indicated lipid, as under  "Experimental Procedures." Assays were carried out at 30 °C as a function of variable concentrations of p-nitrophenol at several different concentrations of UDP-glucuronic acid. Activities at Vₐ were calculated as under  "Experimental Procedures"  and  are micromoles of substrate conjugated/min/mg of protein.

| Aglycone                  | Activity |
|---------------------------|----------|
| p-Nitrothiophenol         | 0.095    |
| Aniline                   | Not detectable |
| Pyridine                  | Not detectable |
| p-Nitrobenzoic acid       | 0.019    |
| o-Aminobenzoic acid       | 0.017    |
| Benzeneic acid            | 0.019    |

**TABLE III**

Glucuronidation of compounds other than phenols

GT₂p  was reconstituted with oleoyl lysophosphatidylcholine and activities were measured as under "Experimental Procedures" at a single set of substrate concentrations. The concentrations of benzoic acid derivatives and p-nitrothiophenol were 0.1 and 0.02 mm, respectively. The concentration of UDP-glucuronic acid was 2 mm. Activities are micromoles of substrate glucuronidated/min/mg of protein.

**TABLE IV**

Variation of activity at Vₐ for glucuronidation of p-nitrophenol and 1-naphthol as a function of the lipid environment of GTzp

Different phenolic aglycones as the lipid environment of GT₂p is changed. What is observed instead is a 20-fold decline in the rate of conjugation of p-nitrophenol, but essentially no change in the rate of conjugation of 1-naphthol when the lipid environment of GT₂p is changed from oleoyl lysophosphatidylcholine to stearoyl lysophosphatidylcholine. In fact, as the acyl chain of the lyso derivative is shortened, the activities at Vₐ of GTzp become identical with p-nitrophenol and 1-naphthol as substrates. These results indicate that changing the lipid environment of GT₂p can lead to apparently selective effects on activity of the enzyme with various phenolic aglycones. This point is amplified by the data in Fig. 2. Changing from oleoyl to stearoyl lysophosphatidylcholine affects most significantly only the rate of conjugation of p-nitrophenol, lowers by 3-fold the rate of conjugation of p-bromophenol, but has almost no effect on the rates of glucuronidation of 1-naphthol and phenol. There still is a relationship between rates of glucuronidation and acidity of the aglycone for GT₂p reconstituted with stearoyl lysophosphatidylcholine, but the slope of the plot in Fig. 2 for values of σ are between 0 and 1.24 is +0.8 as compared with a theoretical value of +2.23. The rate of conjugation of p-aminophenol is the same for enzyme reconstituted with either oleoyl or stearoyl lysophosphatidylcholine.

Data were not collected for GT₂p reconstituted with palmitoyl lysophosphatidylcholine because the activity at Vₐ with p-nitrophenol as glycone was the same in this environment and after reconstitution with stearoyl lysophosphatidylcholine (Table IV). Activities at Vₐ were measured, however, after reconstitution of GT₂p with myristoyl lysophosphatidylcholine. These data, shown in Fig. 3, indicate that the acidity of the phenol has no influence on the rate of glucuronidation of phenols with pKₐ values of less than about 9.3. As acidity
Rates of glucuronidation vary by about 200-fold over this range of pK_a values (Fig. 1) for GT_{wp} reconstituted with oleoyl lysophosphatidylcholine. We tried to assess rates of glucosylation catalyzed by GT_{wp} reconstituted with other lipids, but activities were too small to measure except for enzyme reconstituted with oleoyl lysophosphatidylcholine. The data in Table V are not activities at V_max, but rather, are rates measured at high concentrations of UDP-glucose and aglycone. That measurements of activities at V_max would not change the significance to be drawn from the data in Table V is indicated by the fact that the rate at V_max for glucosylation of p-nitrophenol is 1.1 nmol/min/mg of GT_{wp} reconstituted with oleoyl lysophosphatidylcholine.

### DISCUSSION

The data in Figs. 1–3 indicate that the rate of glucuronidation of phenolic aglycones, catalyzed by GT_{wp}, is a function of the acidity of the aglycone and the enzyme’s lipid environment. By knowing the structure of the aglycone and the lipid used to reconstitute GT_{wp}, rates of glucuronidation of phenols may be predicted accurately. It is also possible to predict whether two compounds will compete with each other for metabolism by this form of UDP-glucurononyltransferase. In addition, the data provide new ideas about the catalytic mechanism of this enzyme and the manner in which phospholipids modulate activity. With regard to phenolic aglycones, there are two species of enzyme-substrate complex. These are E-phenol and E-phenolate ion. Since the former is not a reactive species, the rate at V_max can be expressed in Equation 4, under the conditions existing in Fig. 1.

\[
V_{\text{max}} = k_1[E] \times [\text{UDP-glucuronic acid}] \quad (4)
\]

For a given amount of GT_{wp}, Equation 4 can be rewritten as

\[
V_{\text{max}} = k_1[E] \times [\text{phenolate ion}] \quad (4a)
\]

The concentration of the complex E-phenolate ion will depend on the pK_a value of the phenolic aglycone and the pH of the assay system, assuming that the pK_a value of the phenol is the same in the bulk phase and when bound at the active site. Equation 4a states, therefore, that the activity at V_max is a function of the fraction of active sites occupied by phenolate ion when enzyme is saturated with aglycone. Since the enzyme does not appear to distinguish between phenol and phenolate in terms of binding of these forms to the active site, V_max for different phenolic aglycones is a function of the fraction of substrate in the phenolate form. Equation 4a applies not only to the data in Fig. 1 for enzyme reconstituted with oleoyl lysophosphatidylcholine, but also for weakly acidic phenols glucuronidated by enzyme in other lipid environments (Figs. 2 and 3).

It is not possible to decide, on the basis of the data, the mechanism of catalysis. The data, however, seem to be helpful in this regard. Thus, Equation 4a is compatible with an SN_2 reaction, that is, a reaction in which there is simultaneous bond making and bond breaking at C-1 of glucuronic acid during attack on UDP-glucuronic acid by the nucleophilic aglycone. Phospholipids could influence the rate of glucuronidation by an effect on k_1, which can be considered to be the reactivity of phenolate ion within the active site of GT_{wp}. To evaluate this possibility, one needs to compare rates of glucuronidation for a given phenol as a function of phospholipid environment under the condition that Equation 4a applies. This is true for rates at V_max for the glucuronidation of phenol and p-aminophenol by GT_{wp} reconstituted with either oleoyl or myristoyl lysophosphatidylcholine. Comparisons of the appropriate data in Figs. 1 and 3 indicate that k_1 falls by a factor of about 3 to 4 when the lipid environment is changed from oleoyl lysophosphatidylcholine to myristoyl lysophosphatidylcholine. It seems, therefore, that the phospholipid environments studied so far have only a small influence on the reactivity of phenolate ions within the active site of GT_{wp}.

Rates of glucuronidation are independent of [phenolate ion] when GT_{wp} is reconstituted with myristoyl lysophosphatidylcholine and the aglycone is relatively acidic (Fig. 3). Hence, Equation 4a does not apply under all conditions. The rate of glucuronidation at V_max for GT_{wp} reconstituted with myristoyl lysophosphatidylcholine can be described as Equation 5 or 5a, when relatively acidic phenols are the substrates.

\[
V_{\text{max}} = k_3[E] \quad (5)
\]

Since, for a given aglycone, V_max is smaller when Equation 5a and not Equation 4a is the applicable expression for rate, the step for which k_3 is the rate constant is slower than the product k_1[E-phenolate ion]. The data in Figs. 1–3 can be explained, therefore, by the idea that the values of k_1 and especially k_3 are affected by the lipid environment of GT_{wp}. When the step for which k_3 is the rate constant is a facile process, k_3[E-phenolate ion] determines rate. When k_3 is smaller than k_1[E-phenolate ion], the process associated with k_3 is rate-determining and activity at V_max is independent of [phenolate ion]. The mechanism seems to shift under the influence of a changing phospholipid environment from one that is compatible with an SN_2 mechanism to one that appears to be SN_1. We propose, therefore, that when Equation 5a applies, the slow step in the catalytic process is bond breaking between C-1 of glucuronic acid and UDP. Certain phospholipid environments, such as oleoyl lysophosphatidylcholine, appear to facilitate this step sufficiently that bond breaking and bond making may become simultaneous.

One would expect GT_{wp} to catalyze the breakdown of UDP-glucuronic acid to UDP and glucuronic acid. We have been unable to detect a measurable rate for this reaction with the techniques used to date. Thus, we believe the energy barrier to formation of an intermediate carbonium ion is exceedingly high in the absence of aglycone under all conditions of reconstitution of GT_{wp}.

A Possible Mechanism for Phospholipid-induced Facilitation of the Bond-breaking Step—There is reason to believe that the process of binding of UDP-glucuronic acid to GT_{wp} can destabilize the bond between UDP and C-1 of glucuronic acid. The basis for this idea is that considerable inherent binding energy for interactions between GT_{wp} and UDP and glucuronic acid (7) disappears when UDP-glucuronic acid binds to GT_{wp}. There is, in fact, a good correlation between the amounts of binding energy that disappear during binding of UDP-glucuronic acid to enzyme reconstituted with different lipids and the effect of a given lipid on activity at V_max (measured with p-nitrophenol) (7). Moreover, the amount of inherent binding energy that disappears during binding of UDP-glucuronic acid is large as compared with the free energy of activation needed to destabilize the bond between UDP and

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**Table V**

Rates of glucosylation catalyzed by GT_{wp}

| Aglycone  | Activity |
|-----------|----------|
| Phenol    | 1.20     |
| 1-Naphthol| 1.25     |
| p-Nitrophenol | 0.60     |

GT_{wp} was reconstituted with an amount of oleoyl lysophosphatidylcholine that activates enzyme maximally. Activities were measured at 30 °C in 50 mM Tris, pH 7.5, with 10 mM UDP-glucose and 0.1 mM aglycone. Rates are nanomoles of aglycone metabolized/min/mg of enzyme.

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**Specificity of UDP-glucurononyltransferase**

The data in Figs. 1-3 can be expressed in Equation 4. The slow step in the catalytic process is bond breaking, and Equation 4 applies, the slow step in the catalytic process is bond breaking. The mechanism seems to shift under the influence of a changing phospholipid environment from one that is compatible with an SN_2 mechanism to one that appears to be SN_1. We propose, therefore, that when Equation 5a applies, the slow step in the catalytic process is bond breaking between C-1 of glucuronic acid and UDP. Certain phospholipid environments, such as oleoyl lysophosphatidylcholine, appear to facilitate this step sufficiently that bond breaking and bond making may become simultaneous.
Specificity of UDP-glucuronyltransferase

glucuronic acid and account, thereby, for the observed rate enhancement (conjugation of \( p \)-nitrophenol) on going from myristoyl to oleoyl lysophosphatidylcholine. The rate enhancement as a function of phospholipid-protein interactions hence could be due to variable induction of "strain" in the bond between UDP and glucuronic acid.

An interesting observation is that rates of glucosylation of phenols catalyzed by GT2p reconstituted with oleoyl lysophosphatidylcholine as compared with glucuronidation are exceedingly slow and do not depend on the acidity of the aglycone. Since the differences between UDP-glucuronic acid and UDP-glucose are limited to a COO\(^{-}\) at C-6 of the sugar of the former versus -CH\(_2\)OH at C-6 of the latter, it is possible that the negative charge in the sugar moiety of UDP-glucuronic acid has an important function in facilitating catalysis.

Significance for Further Work of the Interdependence of Rates of Glucuronidation on the Structure of the Aglycone and the Lipid Environment of UDP-glucuronyltransferase—The data presented above indicate that the effects of lipid-protein interactions must be considered carefully in evaluating the number and specificities of UDP-glucuronyltransferase. Since a single protein may have different properties in different lipid environments, comparing the kinetic properties of two purified or partially purified proteins that are part of lipoprotein complexes is not sufficient for establishing the uniqueness of the two proteins. We think it is important, therefore, to remove all lipids and detergents from purified forms of UDP-glucuronyltransferase, and to compare the properties of apparently different forms of the enzyme after reconstitution in chemically defined phospholipids. This approach would seem not only to provide considerable insight into the function of these enzymes, but also should avoid confusion in the designation of uniqueness for a form of the enzyme. These observations on the significance of complete delipidation and removal of detergents, especially nonionic detergents (8), for accurate studies of UDP-glucuronyltransferase may apply, too, to investigations of the number of different forms of microsomal cytochrome P-450 which, like UDP-glucuronyltransferase, are integral proteins of the membrane that can be modulated functionally by interactions with their lipid environments (18, 19).

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