A Kinetic Mechanism for Modulation of the Activity of Microsomal UDP-Glucuronyltransferase by Phospholipids

EFFECTS OF LYSOPHOSPHATIDYLCHOLINES

(Received for publication, November 12, 1980, and in revised form, February 9, 1981)

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The affinity of delipidated microsomal UDP-glucuronyltransferase (EC 2.3.1.17) for UDP is greater than that for UDP-glucuronic acid. Measurement of $K_a$ for glucuronic acid reveals that glucuronic acid binds to the enzyme. Hence, the difference in affinity of the enzyme for UDP versus UDP-glucuronic acid indicates that inherent binding energy for interactions between enzyme and this substrate is used for purposes other than enhancing binding. A reasonable interpretation of these data is that the binding of UDP-glucuronic acid to enzyme requires distortion of the substrate and/or the enzyme. Inherent binding energy due to interactions between enzyme and UDP and glucuronic acid is utilized to effect such distortions. This type of mechanism can cause significant rate enhancement. Phospholipid activators of UDP-glucuronyltransferase activate by amplifying this basic mechanism. Thus, addition of various species of lysophosphatidylcholine to the delipidated enzyme increase the activity at $V_{max}$ and enhance the affinity for UDP, glucuronic acid, and UDP-glucuronic acid. However, activators enhance the affinity of the enzyme for UDP-glucuronic acid to a significantly smaller extent than they enhance affinity for the UDP and glucuronic acid portions of the substrate. Calculations of the amount of binding energy for interactions between enzyme and UDP-glucuronic acid that can be used for stimulating activities at $V_{max}$ yield values in agreement with the observed enhancement of activities at $V_{max}$ for enzyme reconstituted with various types of lysophosphatidylcholine.

UDP-glucuronyltransferase (EC 2.4.1.17) is an integral component of the microsomal membrane. We have proposed previously that the kinetic properties of this enzyme are regulated via interactions between it and the phospholipids of the membrane (1-3). The experimental basis for this idea is that altering the composition and/or structure of the lipid portion of the microsomal membrane changes the kinetic properties of UDP-glucuronyltransferase (1-7). Moreover, delipidation of a partially purified preparation of enzyme leads to a marked diminution of activity, which can be restored by adding selected types of phospholipids (8, 9). Reconstitution of the delipidated form of the enzyme appears to require a phospholipid containing a phospholylcholine moiety (8). In addition, the specific activity of reconstituted enzyme is influenced by the length and unsaturation of the acyl groups of added phospholipids. UDP-glucuronyltransferase thus is one of several membrane-bound enzymes regulated by lipid-protein interactions. The problem in defining further how lipids affect the properties of UDP-glucuronyltransferase is 2-fold. It is essential to understand how lipid-protein interactions affect the observed kinetic changes and to determine the chemistry of the interactions between each of these components. We report, in this paper, observations on the kinetic mechanism for activation of delipidated UDP-glucuronyltransferase by a series of lysophosphatidylcholines. The data also reveal how the chain length of the acyl groups of the lysophosphatidylcholine determines the activity at $V_{max}$ of reconstituted enzyme.

MATERIALS AND METHODS

Phospholipase $A_2$, from Naja naja venom, was purified by the method of Cremona and Kearney (10). Egg lysolecithin was prepared by treating purified lecithin from eggs (11) with phospholipase $A_2$ (12, 13). Lysolecithin used in the reconstitution experiments was purchased from Applied Science Laboratories, State College, PA, or P-L Biochemicals, Milwaukee, WI. They were used without further purification. Phospholipid phosphorus was determined after digestion of CHCl$_3$/CH$_3$OH extracts of different forms of UDP-glucuronyltransferase (8). Protein was measured by the biuret method (14) except when glycerol was present. In this instance protein was determined with the method of Lowry et al. after precipitation of the protein with trichloracetic acid (15).

Partial Purification and Delipidation of UDP-Glucuronyltransferase—Microsomes were prepared from fresh swine liver as previously described (16). Microsomes (30-40 mg of protein/ml) in 0.1 M Tris (pH 8.0, 0 °C), 10 mM EDTA, were frozen at -80 °C until used. Microsomes were diluted with 0.1 M Tris (pH 8.0, 0 °C), to 15-20 mg of protein/ml (total protein, 7,400 mg). Triton X-100, 10% (w/v), was added with stirring to a final concentration of 0.2% (w/v). The microsomes were centrifuged for 2 h at 100,000 × g. The pellet, which contained essentially all the activity of UDP-glucuronyltransferase, was resuspended in 0.1 M Tris, pH 8.0 (0 °C), to a volume of 500 ml. Deoxycholate (recrystallized, 10% w/v solution) was added with stirring to a final concentration of 0.18% (w/v). The mixture was centrifuged at 100,000 × g for 2 h. The supernatant contained the measurable activity of the enzyme. CaCl$_2$ and purified phospholipase $A_2$ were added to it to a final concentration of 1 mM and 0.01 ml/ml, respectively. The mixture was allowed to stand for 1 h at 4 °C. The activity of phospholipase $A_2$ then was inhibited by adding EDTA, to a final concentration of 5 mM. The solution (~400 ml) was dialyzed overnight against 40 volumes of 20 mM Tris, pH 8.0 (0 °C). Egg lysolecithin was added to the dialyze to a final concentration of 1 mg/ml. The mixture was sonicated in an ice bath for 200 s (Heat Systems, Ultrasoniins, Inc., Model W 150F, at maximum power) and then loaded onto a 400-ml bed of DEAE-Cl-6B gel in a Buchner funnel. The DEAE had been equilibrated with 20 mM Tris, pH 8.0 (0 °C), and

*This work was supported in part by Grants PCM-7817706 from the National Science Foundation and by Grant GH-30316 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Activity of UDP-Glucuronyltransferase

0.02% (w/v) egg lysolecithin. The gel was washed with 400 ml of 40 mM NaCl in 20 mM Tris, pH 8.0 (0 °C), and the enzyme was eluted with 1 liter of 120 mM NaCl in 20 mM Tris, pH 8.0 (0 °C). Fractions with high enzyme activity were pooled (600-800 ml) and dialyzed, overnight, against 15 volumes of 10 mM Tris, pH 8.0 (0 °C). The DEAE-Cl-4B dialyze was added to the DEAE-Cl-4B dialyze to a final concentration of 2 mg/ml. The mixture was sonicated for 200 s, as described above, on ice. The solution was applied to a DE52 gel (300-400 ml) prewashed with 10 mM Tris, pH 8.0 (0 °C), in a Buchner funnel. The gel was washed with 10 mM Tris, pH 8.0 (0 °C), and fractions of 100 ml were collected. The fractions with high enzyme activity were pooled (~700 ml). The DE52 eluate was sonicated, as previously described, for 80 s on ice. The solution was poured through 60 ml of CM-25 gel (prewashed with 20 mM PIPES, pH 7.0 (0 °C), in a sintered glass funnel). The CM-25 gel was washed with 100 ml of 40 mM NaCl in 20 mM PIPES, pH 7.0 (0 °C), and the enzyme was eluted with 100 ml of 250 mM NaCl in 20 mM PIPES, pH 7.0 (0 °C). Fractions of 20 ml were collected. The fractions with high activity (60-80 ml) were pooled and dialyzed against 35 volumes of 20 mM Tris, pH 8.0 (0 °C), overnight. The enzyme solution was frozen at -80 °C and could be stored for several months without appreciable loss of activity.

Fifty ml of the CM-25 dialysate were concentrated 10-fold on an Amicon XM-50 membrane (total protein, 5-10 mg). The dialysate (pH 8.0, 0 °C), dithioerythritol, EDTA, sodium cholate, and glycerol were added to the concentrated dialysate after chromatography on CM-25. Final concentrations were 50 mM Tris, 0.1 mM dithioerythritol, 2 mM EDTA, 0.5% (w/v) cholate, and 10% (v/v) glycerol. The enzyme was applied to a hydroxyapatite column, washed with 50 ml of the same buffer. The enzyme was eluted from the column with a 0-0.5 M K2HPO4 gradient (100 X 100 ml) in the same buffer. Fractions of 0.5 ml were collected. The fractions with maximal enzyme activity were pooled and dialyzed twice against 30 volumes of 0.1 mM dithioerythritol, 2 mM EDTA, 10% (v/v) glycerol and 5 mM Tris, pH 8.0 (0 °C) for 5 and 5 h. The enzyme solution (2-3 mg) was frozen at -20 °C and could be stored for several months without loss of activity.

Based on activities at Vmax, and after correction for detergent-induced activations, the purification procedure described above yields an enzyme preparation that is enriched 140-fold as compared with microsomes. The yield of microsomal protein in the final preparation is about 0.1%. Enzyme eluted from the hydroxyapatite column contained no detectable phospholipids.

Enzyme Assays—All enzyme activities were determined at 30 °C, in 50 mM Tris, pH 7.5, by following the change in optical density of p-nitrophenol at 400 nm (8). For determination of kinetic constants, activities were measured at eight different concentrations of UDP-glucuronic acid and six different fixed concentrations of p-nitrophenol. Best straight lines were fitted to the data and to the secondary plots, according to the scheme for kinetic analysis of a bisubstrate enzyme with a random, rapid equilibrium kinetic mechanism (17). Initially, all experiments were carried out in duplicate. We found that replicate determinations of kinetic constants gave values differing by no more than approximately 15%. Analysis of inhibition studies and calculation of values for Ki were carried out according to the kinetic scheme outlined in Segal (18). Initial rates of formation of p-nitrophenylglucuronic acid were difficult to measure with highly active enzyme because of product inhibition by UDP. Addition of 1 mM Mg2+ to assays prevented this product inhibition. Careful estimates of initial rates of activity at several different concentrations of substrates in the absence and presence of Mg2+ indicated, however, that Mg2+ had no effect on the rate of glucuronidation. This is true because the tendency of UDP for formation of chelates with divalent metal ions is much greater than for chelates between UDP-glucuronic acid and metal ions (19). Hence, 1 mM Mg2+ was added to assays except in studies with UDP added as inhibitor. When glucuronic acid was added to assays, the total concentration of Tris plus glucuronic acid, preadjusted to pH 7.5, was maintained at 50 mM. This was done because evidence from separate experiments suggested that salt concentrations above 100 mM inhibited the activity of reconstituted enzyme.

Reconstitution—Lysophosphatidylcholines were added in 10-fold excess (on a weight basis) to delipidated enzyme. This amount yielded maximal activities. Activation was instantaneous on the time scale of the experiment, and the mixture of lysophosphatidylcholines and enzyme was stable at 0 °C for at least 8 h. pH optima for activities at Vmax and KpNP were determined, and were close to 7.5 for all forms of UDP-glucuronyltransferase studied.

RESULTS AND DISCUSSION

Effects of Lysophosphatidylcholine on the Activity at Vmax of Delipidated UDP-glucuronyltransferase—The partially purified, delipidated preparation of UDP-glucuronyltransferase has measurable activity. The presence of residual activity in the delipidated preparation of enzyme stimulated careful reanalysis for contamination with small amounts of lipid phosphorus. None was found. The activity of the delipidated preparations also could not be attributed to small amounts of cholate because complete removal of trace amounts of cholate did not reduce activity. We believe, therefore, that the low residual activity of delipidated UDP-glucuronyltransferase in our preparations reflects self-association between molecules of this enzyme as well as between it and other integral components of the microsomal membranes. These associations must satisfy the requirements of the enzyme for interaction with amphipathic materials, which accounts for the complete dispersal in water of the partially purified preparation.

Addition of lysophosphatidylcholine enhances the activity at Vmax of delipidated UDP-glucuronyltransferase (Table I). The importance of chain length and unsaturation in stimulating catalysis is evident in the 25-fold variation between enzyme reconstituted with stearoyl lysophosphatidylcholine as compared with oleoyl lysophosphatidylcholine.

Effects of Lysophosphatidylcholine on Kinetically Derived Binding Constants for Interactions of UDP-glucuronyltransferase with Its Substrates—The kinetic mechanism of UDP-glucuronyltransferase is rapid equilibrium, random order (20). Thus, there are two binding constants for each substrate, one for binding of substrate to free enzyme and the other for binding to enzyme already saturated with the other substrate. The constants KUDP-GA and KpNP are for the binding of each substrate to free enzyme. The constants KUDP-GA versus KUDP-GA and KpNP versus KpNP indicates that there are significant substrate-induced effects on the properties of the enzyme. Prior binding of UDP-glucuronic acid alters the affinity of the E-S complex, enzyme·UDP-glucuronic acid, for p-nitrophenol as compared with the affinity of free enzyme for p-nitrophenol. The reverse also occurs. The presence and type of phospholipid appears to determine whether binding of the first substrate enhances or diminishes affinity of an E-S complex for the binding of the second substrate.

Table I

| Addition to enzyme | Activity at Vmax |
|--------------------|-----------------|
| None               | 0.096           |
| Myristoyl lysophosphatidylcholine | 0.90 |
| Palmitoyl lysophosphatidylcholine | 0.97 |
| Stearoyl lysophosphatidylcholine | 0.40 |
| Oleoyl lysophosphatidylcholine | 10.1 |

1 The abbreviations used are: PIPES, 1,4-piperazinediethanesulfonic acid; UDP-GA, UDP-glucuronic acid; pNP, p-nitrophenol.
substrate. For example, prior binding of UDP-glucuronic acid to delipidated UDP-glucuronyltransferase enhances subsequent binding of p-nitrophenol as compared with the binding of p-nitrophenol to the enzyme in the absence of UDP-glucuronic acid. By contrast, prior binding of UDP-glucuronic acid to enzyme reconstituted with myristoyl, palmitoyl, and oleoyl lysophosphatidylcholines diminishes the affinity of the enzyme for p-nitrophenol. Since the binding of UDP-glucuronic acid alters the active site of UDP-glucuronyltransferase, as reflected by differences between $K_{\text{UDP-GA}}$ and $K'_{\text{UDP-GA}}$, the inherent binding energies for the interaction $E_+ + \text{UDP-glucuronic acid} \rightleftharpoons E_+ \cdot \text{UDP-glucuronic acid}$ are greater than the observed binding energies calculated from the values of $K_{\text{UDP-GA}}$ in Table II by using the equation $\Delta G = RT \ln K_a$. This must be so because the energy required to produce the observed substrate-induced effects on the properties of UDP-glucuronyltransferase (the difference between $K_{\text{UDP-GA}}$ and $K'_{\text{UDP-GA}}$) comes at the expense of the inherent binding energy of the enzyme-substrate interactions. The observed binding energy of UDP-glucuronic acid will be reduced as compared with its inherent binding energy by an amount equal to the energy used to alter the $E\cdot S$ complex. The data in Table II indicate that the amount of inherent binding energy of UDP-glucuronic acid utilized to alter the affinity of the $E\cdot S$ complex for p-nitrophenol ($K_{\text{SNP}}$ versus $K'_{\text{SNP}}$) depends on the species of lysophosphatidylcholine added to the enzyme. The nature of the acyl chain of the lysophosphatidylcholine added to UDP-glucuronyltransferase thus has a significant effect on the inherent binding energies of substrates, but this is not obvious from the values of $K_{\text{UDP-GA}}$ for different forms of the enzyme.

Binding of UDP and Glucuronic Acid to UDP-glucuronyltransferase—Although the data in Table II indicate that there are substrate-induced effects on the properties of UDP-glucuronyltransferase and that the inherent binding energy for interactions between enzyme and substrate is used to effect such changes, they do not provide an estimate of how much of the inherent energy is used for purposes other than binding. This is so because inherent binding energy can be used directly to facilitate the catalytic steps as well as to alter affinity for ligands. In order to estimate the amount of inherent energy used for purposes other than enhancing affinity for ligands, one must know the difference between the inherent binding energies and the observed binding energies. In order to make some estimates of the former quantities, we determined the binding constants for binding of UDP and glucuronic acid to UDP-glucuronyltransferase. These binding constants were determined via inhibition studies. The applicable scheme for inhibition is described in the Methods section. The procedure is given in Fig. 1 in which $A$ is UDP-glucuronic acid and $B$ is p-nitrophenol (18). The terms $aK_A$ and $aK_B$, respectively, correspond to the designations $K'_{\text{UDP-GA}}$ and $K'_{\text{SNP}}$. The value of $\beta$ in Fig. 1 reflects changes in the enzyme induced by binding of inhibitor.

Addition of lysophosphatidylcholines to delipidated UDP-glucuronyltransferase enhances the binding of UDP to the enzyme (Table III). The most interesting aspect of these data, however, is that the affinity of enzyme for UDP is considerably greater than that for UDP-glucuronic acid for all forms of UDP-glucuronyltransferase. Moreover, the differences in affinities for UDP $versus$ UDP-glucuronic acid are larger than the amount of binding energy utilized to alter the affinity of UDP-glucuronyltransferase for p-nitrophenol. For example, on calculating these energies (from the expression $\Delta G = RT \ln K_a$) the difference in the binding of UDP and UDP-glucuronic acid to delipidated enzyme is about 2900 calories/mol. The difference between $K_{\text{SNP}}$ and $K'_{\text{SNP}}$ for this form of UDP-glucuronyltransferase is about 1500 calories. Corresponding values for enzyme reconstituted with oleoyl lysophosphatidylcholine are 2100 calories/mol (difference between $K_{\text{UDP-GA}}$ and $K'_{\text{UDP-GA}}$) and 1500 calories/mol (difference between $K_{\text{SNP}}$ and $K'_{\text{SNP}}$). Moreover, binding of UDP alone is sufficient to alter the properties of the active site of UDP-glucuronyltransferase as reflected by differences between $K_{\text{SNP}}$ and $\beta K_{\text{SNP}}$ in Table III. Thus, the observed binding energies for UDP (as calculated from the values of $K'_{\text{UDP}}$) also must be less than the inherent binding energy for the interaction between en-

**TABLE II**

| Addition to enzyme       | $K_{\text{UDP-GA}}$ | $K'_{\text{UDP-GA}}$ | $K_{\text{SNP}}$ | $K'_{\text{SNP}}$ |
|--------------------------|----------------------|-----------------------|------------------|------------------|
| None                     | 0.46                 | 0.034                 | 0.628            | 0.046            |
| Myristoyl lysophosphatidylcholine | 0.053               | 0.117                 | 0.085            | 0.183            |
| Palmitoyl lysophosphatidylcholine | 0.051               | 0.125                 | 0.067            | 0.159            |
| Stearoyl lysophosphatidylcholine | 0.015               | 0.008                 | 0.077            | 0.041            |
| Oleoyl lysophosphatidylcholine | 0.050               | 0.059                 | 0.061            | 0.70             |

**Fig. 1.** Kinetic scheme for inhibition of an enzyme with a rapid equilibrium, random order mechanism. $I$ is competitive with $A$. 

**Table:**

| Activity of UDP-Glucuronyltransferase | 4785 |
|--------------------------------------|------|
| **Effect of selected lysophosphatidyicholines on the binding constants of partially purified, delipidated UDP-glucuronyltransferase** |      |
| Enzyme was prepared and assayed at 30 °C as under “Materials and Methods.” Binding constants were determined from kinetic data for a random order, rapid equilibrium kinetic mechanism, according to Cleland (17). Units are millimolar. The terms $K_{\text{UDP-GA}}$ and $K_{\text{SNP}}$ are for binding of substrate to enzyme in the absence of the second substrate. The terms $K_{\text{UDP-GA}}$ and $K_{\text{SNP}}$ are for binding of substrate to enzyme already saturated with the alternate substrate. |      |
| **Addition to enzyme** | $K_{\text{UDP-GA}}$ | $K'_{\text{UDP-GA}}$ | $K_{\text{SNP}}$ | $K'_{\text{SNP}}$ |
| None | 0.46 | 0.034 | 0.628 | 0.046 |
| Myristoyl lysophosphatidylcholine | 0.053 | 0.117 | 0.085 | 0.183 |
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| Stearoyl lysophosphatidylcholine | 0.015 | 0.008 | 0.077 | 0.041 |
| Oleoyl lysophosphatidylcholine | 0.050 | 0.059 | 0.061 | 0.70 |
zyme and this ligand. These findings suggest one of two possibilities. The binding of the glucuronic acid moiety of UDP-glucuronic acid has a large positive free energy. Alternatively, the inherent binding energy of this part of the substrate is utilized for purposes other than enhancing binding of UDP-glucuronic acid. If the latter is a valid explanation for the differences between $K_{UDP-GA}$ and $K_{UDP}^*$, binding of the glucuronic acid portion of UDP-glucuronic acid also must lead to the “disappearance” of inherent binding energy due to the interaction between enzyme and the UDP portion of the substrate. The possible explanations for the differences between $K_{UDP-GA}$ and $K_{UDP}^*$ were investigated by measuring $K_{UDP}^{glucuronic acid}$.

The data in Table IV indicate that glucuronic acid is an effective inhibitor of UDP-glucuronyltransferase. The differences in the affinity of UDP-glucuronyltransferase for UDP as compared with UDP-glucuronic acid cannot be attributed, therefore, simply to a positive free energy for the binding of the glucuronic acid moiety of the latter compound. They suggest, instead, that the inherent binding energy of the glucuronic acid part of the substrate is utilized for purposes other than enhancing binding. Also, binding of the glucuronic acid occurs in such a way as to decrease the binding energy of the UDP portion of UDP-glucuronic acid.

By analogy with data for several enzymes (cf. review by Jencks (21)), but especially lysozyme (22), one can consider the UDP-glucuronic acid binding site of UDP-glucuronyltransferase to be composed of several subsites. Binding energies for UDP and glucuronic acid would be greater than that for UDP-glucuronic acid if there were some hindrance to binding at the subsite interacting with the region of UDP-glucuronic acid that takes part in the catalytic step. For example, binding of UDP-glucuronic acid to the enzyme could require distortion of the bond linking the pyrophosphate of UDP to the glucuronic acid. Another possibility is that binding of UDP-glucuronic acid distorts the enzyme, but binding by UDP and glucuronic acid do not. The inherent binding energy of the UDP and glucuronic acid moieties would be needed to provide energy for these distortions, which would lead to apparent disappearance of inherent binding energy and account thereby for the observed differences between $K_{UDP-GA}$ and $K_{UDP}^*$. The energy used to distort the $E-S$ complex in this manner could enhance catalysis.

Estimates of the Maximal (Inherent) Binding Energy Due to Interactions between UDP-glucuronyltransferase and UDP-glucuronic Acid—The maximal potential binding energy, or inherent binding energy, for the interaction between UDP-glucuronyltransferase and UDP-glucuronic acid is not a simple sum of the maximal potential binding energies for the UDP and glucuronic acid portions of the molecule. The principal reason for this discrepancy is that the loss of entropy on the binding of free glucuronic acid to enzyme will be greater as compared to the loss of entropy on binding to the enzyme of glucuronic acid already attached covalently to UDP (19). The same reasoning applies to the entropy loss associated with the binding of free UDP to enzyme. Since there is no way to measure these quantities, it is not possible to determine exactly the enthalpy changes associated with the binding of these ligands. On the other hand, the maximal potential enthalpy change for the binding of glucuronic acid to enzyme will be greater for the glucuronic acid portion of UDP-glucuronic acid as compared with free glucuronic acid. The same is true for the enthalpy change associated with the binding of UDP. It is possible, at least, to make minimal estimates of the inherent binding energy available for enhancing catalysis. Moreover, we think it is reasonable to expect that the entropy changes associated with the binding of UDP and glucuronic acid to UDP-glucuronyltransferase will be similar for delipidated and phospholipid-activated forms. Comparing the differences in the amounts of inherent binding energy that disappear on activation with various lysophosphatidylcholines with the energy required to account for the observed activations at $V$ is especially useful, therefore, for evaluating whether inherent binding energy of UDP-glucuronic acid is used to facilitate catalysis.

The inherent or maximal potential binding energy for UDP and glucuronic acid can be estimated from the values of $K_{UDP}^*$, $K_{UDP}^{glucuronic acid}$, and $\beta$ in Tables III and IV. The latter are important because the inherent binding energy is different from $RT \ln K_j$ by the amount of energy used to change $K_{NP}^*$ to $K_{NP}$, which equals $RT \ln \beta$. The maximal potential binding energies for UDP and glucuronic acid, calculated in the above manner, are tabulated in Table V. The differences between these values and the observed binding energies of UDP-glucuronic acid are tabulated in Table VI. The second column in Table VI is the inherent binding energy that is used for other purposes. The data in Table V indicate that there is positive correlation between the inherent binding energy for the binding of glucuronic acid to various forms of UDP-glucuronyltransferase and the activity at $V_{max}$ of the different forms of the enzyme. Additionally, there is a positive correlation between the amount of binding energy that “disappears” (Table VI) and activities at $V_{max}$ of UDP-glucuronyltransferase activated with different lysophosphatidylcholines.

Estimates of Amounts of Inherent Binding Energy That Can Be Used to Facilitate Catalysis—A minimal estimate (for the reasons given above) of the inherent or maximal

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**Table III**

| Addition to enzyme              | $K_{UDP}^*$ | $K_{NP}$ | $\beta K_{NP}$ |
|---------------------------------|-------------|----------|---------------|
| None                            | 3.6         | 0.63     | 0.25          |
| Myristoyl lysophosphatidylcholine| 1.4         | 0.085    | 0.23          |
| Palmitoyl lysophosphatidylcholine| 1.10       | 0.067    | 0.23          |
| Stearoyl lysophosphatidylcholine | 0.12        | 0.077    | 0.30          |
| Oleyl lysophosphatidylcholine    | 1.6         | 0.061    | 0.01          |

**Table IV**

| Addition to enzyme | $K_{UDP}^{glucuronic acid}$ | $K_{NP}$ | $\beta K_{NP}$ |
|--------------------|-----------------------------|----------|---------------|
| None               | 17.1                        | 0.63     | 0.45          |
| Myristoyl          | 3.5                         | 0.085    | 0.21          |
| Palmitoyl          | 6.0                         | 0.067    | 0.127         |
| Stearoyl          | 14.0                        | 0.077    | 0.031         |
| Oleyl             | 5.3                         | 0.061    | 0.76          |
TABLE V
Maximal potential binding energies of UDP and glucuronic acid for binding to various forms of UDP-glucuronyltransferase

Binding energies are calculated from the expression $\Delta G = RT \ln K_e$. The maximal potential binding energy is the observed binding energy for each ligand plus any energy that is used to alter the interaction between enzyme and p-nitrophenol, that is, the difference between $K_{sNP}$ and $K_{sNP}^*$. Energies are in calories/mol at 30 °C.

| Addition to enzyme | Maximal potential binding energy for ligand |
|--------------------|--------------------------------------------|
|                    | UDP | Glucuronic acid |
| None               |     |                 |
| Myristoyl lysophosphatidylcholine | -8100 | -2600 |
| Palmitoyl lysophosphatidylcholine  | -9700 | -4200 |
| Stearoyl lysophosphatidylcholine   | -10600 | -2800 |
| Oleoyl lysophosphatidylcholine     | -9100 | -4700 |

Table VI
Calculated amount of potential binding energy not used to enhance the affinity of UDP-glucuronyltransferase for UDP-glucuronic acid

Binding energies are calculated as in Table V. The binding energy that disappears is the difference in binding energy between the total potential binding energies for UDP and glucuronic acid and the observed binding energies for UDP-glucuronic acid. Energies are calories/mol at 30 °C.

| Treatment of enzyme | Potential binding energy that disappears |
|--------------------|------------------------------------------|
| None               | -6100                                    |
| Myristoyl lysophosphatidylcholine | -6900                                    |
| Palmitoyl lysophosphatidylcholine  | -6900                                    |
| Stearoyl lysophosphatidylcholine   | -6800                                    |
| Oleoyl lysophosphatidylcholine     | -7800                                    |

Potential binding energy for the interaction between delipidated enzyme and UDP-glucuronic acid is $-10,800$ calories. The observed binding energy of UDP-glucuronic acid to delipidated UDP-glucuronyltransferase is $-4,600$ calories/mol. Hence, a minimal estimate of the binding energy that can be used to facilitate catalysis by the delipidated form of UDP-glucuronyltransferase is $-6,100$, calories/mol. We know from Table II that 1,504 calories of potential binding energy are used to enhance the affinity for binding of p-nitrophenol to the enzyme·UDP-glucuronic acid complex ($K_{sNP}$ versus $K_{sNP}^*$). The energy utilized in this way will not facilitate catalysis. Thus, the amount of binding energy available for facilitation of catalysis by delipidated enzyme is $4,600$ calories/mol. This amount of energy would increase the catalytic rate by $2.1 \times 10^3$-fold. Utilization of binding energy from the interactions between substrates and delipidated enzyme, therefore, can provide significant enhancement of the catalytic rate of the delipidated enzyme. The real enhancement of catalysis due to utilization of potential binding energy could be much greater. In order to compare the amounts of inherent binding energy that can be made available for facilitating the catalytic rate of enzyme treated with lysophosphatidylcholine versus delipidated enzyme, one must correct the data in Table VI for differences in the energy level of p-nitrophenol when it binds to the various forms of UDP-glucuronyltransferase previously saturated with UDP-glucuronic acid. The calculated amounts of potential or inherent binding energy that disappear and can be used to enhance catalysis in activated, as compared with delipidated, enzyme are listed in Table VII. Also tabulated are the observed differences in catalytic rate, expressed as calories, between reconstituted and delipidated enzyme. There is excellent agreement between the calculated amounts of energy available for enhancing catalysis by phospholipid-activated UDP-glucuronyltransferase versus delipidated enzyme and the observed differences in rates of catalysis. Some of the disparity between calculated and observed energies could be due to differences in the energy level of enzyme-bound UDP-glucuronic acid. There are two interrelated reasons for concluding, however, that corrections due to this factor will be small. First, enhanced binding of UDP-glucuronic acid to lipid-treated as compared with delipidated enzyme can be accounted for almost completely, in all cases, by enhanced affinity of the enzyme for the UDP portion of the substrate. Second, UDP does not take part in the catalytic steps.

Table VII
Binding energy utilized to enhance catalysis

Amounts of potential binding energy utilized to facilitate catalysis were calculated as outlined in the text. The observed differences in activities at $V_{max}$ for the pair of enzyme forms compared were used to calculate the difference in free energy of activation for glucuronidation according to the equation $\Delta G = -RT \ln (V_i/V_o)$. Energies are calories/mol at 30 °C.

| Enzyme forms compared                        | Calculated difference in binding energy available for enhancing catalysis | Observed difference in free energy of activation |
|----------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------|
| Delipidated versus myristoyl lysophosphatidylcholine | 1600                                                                      | 1400                                              |
| Delipidated versus palmityl lysophosphatidylcholine     | 1500                                                                      | 1400                                              |
| Delipidated versus stearoyl lysophosphatidylcholine    | 630                                                                       | 860                                               |
| Delipidated versus oleoyl lysophosphatidylcholine      | 3300                                                                      | 2800                                              |
except for the fact that binding of one substrate changes the affinity of the enzyme for the second substrate. The nature of this distortion in the enzyme depends on the presence of lysophosphatidylcholines and on the chain length and unsaturation of the attached acyl groups. For, whereas some of the binding energy due to interactions between delipidated enzyme and substrate can be used to enhance affinity for binding of the second substrate and not for facilitation of catalysis, lysophosphatidylcholines that are good activators of UDP-glucuronyltransferase do not allow this to happen. In the presence of a good activator such as oleoyl lysophosphatidylcholine, all the binding energy that disappears is used to facilitate catalysis. The enzyme’s lipid environment influences the inherent binding energy of ligands, and can determine how this energy is used, as for example, to facilitate binding of a second ligand or to facilitate catalysis. The property of the lipid environment that is important in determining the structure of UDP-glucuronyltransferase remains to be elucidated.

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J. Biol. Chem. 1981, 256:4783-4788.

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