Effect of Phospholipids on the Thermal Stability of Microsomal UDP-Glucuronosyltransferase†

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ABSTRACT: The GT_{2P} isoform of microsomal UDP-glucuronosyltransferase from pig liver is a lipid-dependent enzyme. The data in the present work indicate that, in addition to regulation of activity, the thermal stability of the enzyme also is modulated by the acyl chain composition of phosphatidylcholines (PC) used to reconstitute the activity of pure enzyme. There was a reversible, temperature-dependent change in the state of the pure enzyme to an inactive form with onset at $T > 38$ °C, depending on the environment of the enzyme. The midpoint for the transition shifted from 39.8 °C for enzyme in a bilayer of distearoylphosphatidylcholine (DSPC) to 47.5 °C for enzyme in a bilayer of 1-stearoyl-2-oleoylphosphatidylcholine (SOPC). For all lipids, the transition from a catalytically active to an inactive form of the enzyme was associated with large compensating changes in H and S. Lipid-induced stabilization of the active form of UDP-glucuronosyltransferase at $T > 37$ °C was associated with decreases in ΔH and ΔS, but the decreases in ΔS were larger, indicating that lipid-induced stabilization of the active form of the enzyme was entropic. The transition between the active and inactive forms of the enzyme was too rapid in either direction to measure in a standard spectrophotometer. In addition to reversible inactivation of the enzyme, there was a slower irreversible, temperature-dependent inactivation. The rate of this process depended on the acyl chains of the phosphocholines interacting with the enzyme. However, there was no obvious correlation between the structures of lipids that stabilized the different inactivation reactions.

As compared with water-soluble proteins, integral membrane proteins interact with an extremely complex environment; the apolar interior of the membrane, which has variable properties depending on distance from the membrane–water interface; the polar region of the phospholipids at the membrane–water interface; and bulk water. The function of integral membrane enzymes can be modulated by interactions between the surface of the protein and each of these environments (Churchill et al., 1983; Kimelberg, 1977; Kovatchev et al., 1981; McElhaney, 1982; Noel and Pande, 1986; Sandermann, 1978; Yuli et al., 1981). The influence on enzyme function of the bulk aqueous phase or the polar groups at the membrane–water interface can be rationalized with what is known about water-soluble enzymes. However, the basis for modulation of function by the apolar region of the membrane is less certain, especially because membrane proteins have not been shown to have selective avidities for lipids with specific types of acyl chains (DeVaux & Seigneuret, 1985; Marsh, 1987; Oldfield et al., 1978; Paddy et al., 1981). Interactions between the surface of an integral membrane protein and the acyl chains of membrane lipids thus are unlikely to influence enzyme function via specific binding to sites on proteins or because of chemical changes in the environment, e.g., a change analagous to an increase or decrease in $[H^+]$.

Our interest in the problem of lipid-induced regulation of integral membrane enzymes has been lipid-induced changes in the catalytic activity of microsomal UDP-glucuronosyltransferase (EC 2.4.1.108). The isoform of UDP-glucuronosyltransferase designated as GT_{2P} (Hochman et al., 1981; Hochman & Zakim, 1983a), when pure and delipidated, has a small residual activity, but the catalytic rate constant of the enzyme is increased as much as 700-fold when appropriate lipids are added to it (Hochman et al., 1981; Magdalou et al., 1982; Hochman & Zakim, 1984). Avidity for substrates (Hochman et al., 1981), catalytic specificity (Magdalou et al., 1982), and allosteric properties are modulated by the acyl chain composition of the phospholipids used to reconstitute activity (Hochman et al., 1983; Hochman & Zakim, 1983b; Zakim et al., 1988). In addition, there are data to suggest that the thermal stability of UDP-glucuronosyltransferase is a lipid-dependent property. For example, for enzyme in intact, untreated microsomes, the activity of UDP-glucuronosyltransferase increases with temperature at least to 48 °C, but for enzyme in microsomes treated with detergents, activity declines for $T > 37$ °C (Dannenberg et al., 1989). The pure enzyme also undergoes a reversible transition to a less active state at $T > 37$ °C (Dannenberg et al., 1988; Hochman et al., 1983). We report in this paper that the thermal stability of UDP-glucuronosyltransferase is a lipid-dependent property in that the acyl chain composition of the lipid environment regulates the reversible change in state of pure, delipidated UDP-glucuronosyltransferase to an inactive form and modulates as well irreversible, thermal inactivation of this form of the enzyme.

Materials and Methods

All lipids were purchased from Avanti Polar Lipids. The GT_{2P} type of UDP-glucuronosyltransferase was purified from pig liver microsomes as described previously (Hochman et al., 1981; Hochman & Zakim, 1983). The enzyme was delipidated on hydroxyapatite during the last step of purification (Hochman et al., 1981). When residual lipids are measured, no lipid phosphorus is found in the delipidated enzyme using an assay and amount of enzyme that can detect about 1 mol of lipid/mol of enzyme, which is far less than the amount required to reconstitute the activity of the delipidated preparation (Zakim et al., 1988). Proteins were measured by the

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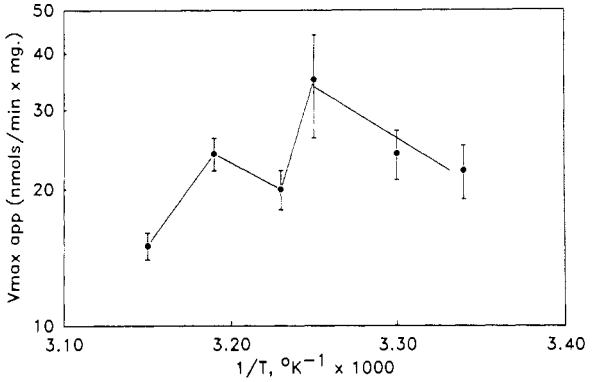

FIGURE 1: Temperature dependence of the activity of pure, delipidated GT_{2P}. Enzyme activities were assayed as described under Materials and Methods and extrapolated to infinite concentrations of UDP-glucuronic acid. V_{max}^{app} values are expressed in nanomoles per minute per milligram of protein and are plotted on a logarithmic scale. Bars represent the standard error of the mean.

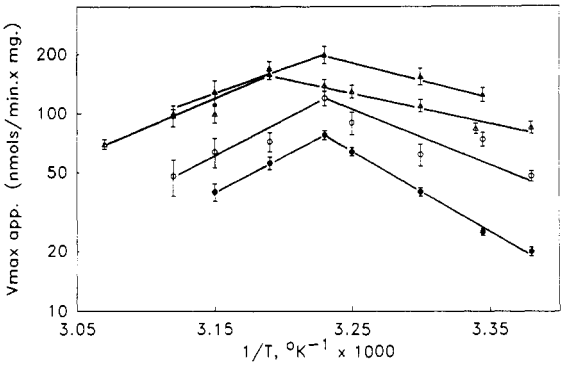

FIGURE 2: Temperature dependence of the activity of GT_{2P} reconstituted in lipid vesicles. Enzyme was reconstituted into ULVs of DSPC (●), DPPC (○), DOPC (▲), or SOPC (△) as described under Materials and Methods. Activities were plotted versus substrate concentrations in double-reciprocal plots to obtain V_{max}^{app} (nanomoles per minute per milligram of protein). The maximal activities thus obtained are plotted according to the Arrhenius equation. The bars are standard errors of the mean for each point.

method of Lowry et al. (1951). Small, unlilamellar lipid vesicles (ULVs)[1] were made by sonication of hydrated suspensions of lipids as in Scotto and Zakim (1985). The lipid content of ULVs was quantitated by measurement of inorganic phosphate (P_i) (Scotto & Zakim, 1985). Pure UDP-glucuronosyltransferase was reconstituted into ULVs of DSPC, DPPC, and SOPC as in Scotto and Zakim (1988). Briefly, ULVs of DSPC or DPPC, prepared by sonication, were mixed with enzyme at room temperature for 2 h. ULVs of SOPC were resonicated below the phase transition temperature and mixed with enzyme at 2 °C for 2 h. As demonstrated in Scotto and Zakim (1988), membrane proteins including UDP-glucuronosyltransferase become irreversibly associated with lipid vesicles under these conditions. Reconstitution into ULVs of DOPC was effected by repeated freeze–thawing of a mixture of ULVs and pure protein (Kassahara & Hinkle, 1977). Density gradient centrifugation of lipid–protein mixtures confirmed that enzyme was associated with the vesicles (Scotto & Zakim, 1985). The ratio of lipid to protein in the reconstitution experiments was 10/1 (w/w). The lipid/protein ratios in reconstituted liposomes isolated from density gradients were about 400/1 (mol/mol).

Enzyme activities were assayed with *p*-nitrophenol as aglycon by measuring the disappearance of color at 400 nm. Assays contained 0.05 mM *p*-nitrophenol, 1 mM $MgCl_2$, 50 mM Tris, pH 7.5, and varying amounts of UDP-glucuronic acid in the range 0.1–5.0 mM. Activities at each temperature were measured for at least six different concentrations of UDP-glucuronic acid and extrapolated graphically to infinite concentrations of this substrate. Unless otherwise noted in the legends, all activities are values at V_{max}. Measurements of thermal stability were carried out by treating enzyme at 44 °C for different intervals and then assaying for residual activity, as above, at 37 °C in the presence of 3.0 mM UDP-glucuronic acid.

RESULTS

Effect of Symmetrical Phospholipids on the Thermotropic Properties of UDP-Glucuronosyltransferase. The data in Figure 1 are a plot of activity at V_{max} as a function of temperature for pure, delipidated UDP-glucuronosyltransferase. The data, plotted according to the Arrhenius equation, show that the delipidated enzyme underwent a change of state at 35 °C to a form with less activity than at lower temperatures. In addition, the data suggest complex, temperature-dependent changes at T <35 °C. Activity at 40 °C was higher than at 37 °C, but activity then fell again as the temperature was raised to 44 °C. The scatter of the data in Figure 1 suggests that the points at T >35 °C can be connected by a single straight line, but in each experiment, activity at 37 °C was less than at 40 °C.

Insertion of enzyme into ULVs increased activity to an extent that depended on the acyl chains of the lipids (Figure 2). Activity increased as the viscosity of the bilayers decreased. The mechanism of this effect will be addressed in subsequent work. The most important feature in Figure 2, in the context of the current experiments, however, was that insertion of the enzyme into bilayers of DSPC, DPPC, or DOPC altered the thermotropic properties of UDP-glucuronosyltransferase. The Arrhenius plots in Figure 2 were linear in the range of 30–37 °C but had discontinuities in slopes above 37 °C. Thus, the temperature for the onset of the change in state seen in Figure 1 for delipidated enzyme was raised by all the lipids used in Figure 2 by +2 °C. In addition, for enzyme embedded in ULVs, only one temperature-dependent change appeared to occur at T >35 °C.

The fall-off in activities at temperatures above 37 °C did not reflect irreversible, thermal denaturation of the enzyme. For example, the activity of enzyme in DOPC assayed at 44 °C was 40% of the activity measured at 37 °C, but the activity of this enzyme when treated for 3 min at 44 °C and then assayed at 37 °C was 95% of the activity of enzyme assayed at 37 °C without prior heating at 44 °C. Similar results were obtained for heating of enzyme in other lipids. The failure to recover activity completely at 37 °C reflected some loss at 44 °C due to irreversible inactivation (see below). Therefore, the discontinuities in the plots in Figure 2 can be attributed to the mechanism in reaction 1, in which the activity of E′ was less than that of E.

$$E \leftrightarrow E' \quad (1)$$

The energy of activation for reaction 1 must have been quite small because equilibrium in reaction 1 was reached in the mixing time for the experiments. When enzyme at 37 °C was added to an assay at 40 °C, there was no time delay in reaching the steady-state rate of glucuronidation, and activity measured at 37 °C reached the steady-state value within the mixing time for the system no matter what the temperature of the prior treatment of the enzyme. On the other hand, the

[1] Abbreviations: DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; SOPC, 1-stearoyl-2-oleoylphosphatidylcholine; ULVs, unilamellar lipid vesicles.

Table I: Stabilization of the Reversible Temperature-Induced Denaturation of Pure UDP-Glucuronosyltransferase by Different Lipid Bilayers[a]

lipid	K_{eq} (44 °C)	T_m (°C)	ΔG(44 °C) (cal/mol)	ΔH (kcal/mol)	ΔS(44 °C) [cal/(mol·°C)]
DSPC	3.21	39.8	−739	50.4	161
DPPC	2.25	41.0	−514	47.1	150
DOPC	1.04	44.2	−25	44.9	142
SOPC	0.54	47.5	+400	38.4	120

[a] Pure GT_{2P} was incorporated into preformed ULVs of the indicated lipids as described under Materials and Methods. Equilibrium constants at 44 °C were obtained from Figure 2, as described in the text. The thermodynamic parameters were calculated from the data in Figure 3, all of which were for pH 7.5. T_m is the temperature at which half of the total enzyme was in the active form, i.e., the midpoint of the denaturation reaction in eq 1.

thermodynamic parameters for reaction 1 (see below) suggested that this reaction was associated with a substantial change in UDP-glucuronosyltransferase. Hence, we tried to demonstrate changes in the spectral properties of the enzyme in association with the temperature-induced changes in function. Absorption spectra in the range 260–350 nm and fluorescence spectra (excitation 280 nm, emission 320–420 nm) of the enzyme did not change in association with the transition in reaction 1. This finding does not rule out a structural perturbation of the enzyme but shows only that there were no measurable changes in regions adjacent to tyrosine or tryptophan residues.

The discontinuities in Figure 2 did not coincide with temperatures for phase changes for the ULVs in which the enzyme was reconstituted. Thus, the transition temperature for ULVs of DOPC is −20 °C, and that for DSPC is 65 °C (Silvius, 1982). Although DPPC melts from the gel to liquid-crystal phase at about 42 °C (Silvius, 1982), the discontinuity in the Arrhenius plot occurred below this temperature. Data published elsewhere suggest that UDP-glucuronosyltransferase, in the concentrations present in Figure 2, does not lower the transition of DPPC (Hochman et al., 1983), but we do not have calorimetric data on this point.

If the change of state of UDP-glucuronosyltransferase observed at $T > 37$ °C (Figure 2) were due to complete conversion of the enzyme from E to E′ at 40 °C, then activity at $T > 40$ °C should have increased, which was not seen. We interpret this result, along with the smooth decline in activities at $T > 37$ °C (Figure 2), to mean that there was a progressive, temperature-dependent conversion of E to E′ as the temperature was continually increased. By measuring the activity of UDP-glucuronosyltransferase at increasingly higher temperatures, we found that the E′ form of the enzyme had no measurable activity (data not shown). Even under this condition, however, i.e, complete conversion of active enzyme (E) to inactivate enzyme (E′), the activity of E could be restored when the temperature was lowered to 37 °C or less. These results indicate that the effect of the lipid environment on the equilibrium position of reaction 1 can be determined from measurements of enzyme activity because activity at $T > 37$ °C was the activity due only to enzyme in the E form. The fraction of total enzyme present as form E, at any $T > 37$ °C, hence could be calculated from the activity measured at that temperature divided by the activity extrapolated to that temperature from the Arrenhius plot over the range 30–37 °C. Equilibrium constants for reaction 1, calculated from the amounts of E and E′ determined as above, are presented in Table I at 44 °C. These data show that the equilibrium position of reaction 1 was determined by the lipids comprising the environment of UDP-glucuronosyltransferase. The active

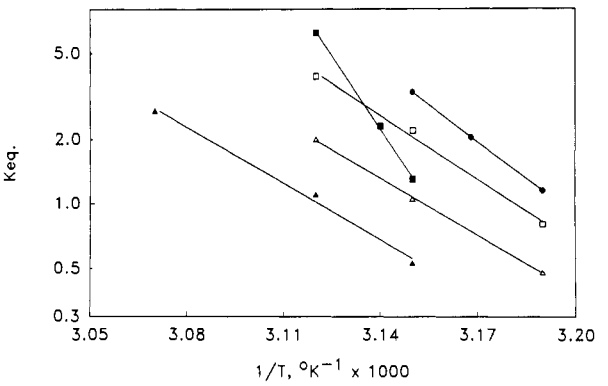

FIGURE 3: Temperature dependence of the equilibrium constant of reaction 1. The equilibrium constants for the transition E ↔ E′ at several temperatures were obtained as described in the text for GT_{2P} incorporated into ULVs of DSPC (●), DPPC (□), DOPC (▲), or SOPC (▲), or into micelles of oleoyllyso-PC (■). The calculated equilibrium constants are plotted versus temperature as van't Hoff isochores.

form of the enzyme (E) was stabilized by the lipid in the liquid-crystal phase at 37 °C (DOPC) whereas the inactive form (E′) was stabilized by lipids in the gel phase (DSPC and DPPC).

The effect of temperature on the equilibrium position of reaction 1 is shown in Figure 3 in the form of van't Hoff plots for each lipid. The thermodynamic parameters calculated from the data in Figure 3 are shown in Table I. Although each line in Figure 3 is based on only three data points, it is unlikely that additional data points would change the main and most important conclusions to be drawn from the data. Thus, the data indicate that the differences in free energies between E and E′ in different environments reflected small net differences in the balances between an unfavorable change in enthalpy and a favorable change in entropy for the transition to inactive enzyme in reaction 1, as is typical for the folding → unfolding transition of water-soluble proteins (Privalov, 1979; Tanford, 1968).

The slopes of the van't Hoff plots for ULVs of DSPC, DPPC, or DOPC appear to be nearly the same, but the data are not sufficient to be certain that the small differences in ΔH for reaction 1 calculated from van't Hoff plots are real. Nevertheless, the data were consistent in that ΔH was smaller for lipids that tended to stabilize the active form of UDP-glucuronosyltransferase. Whether ΔH for reaction 1 was smaller in less viscous lipid environments or was unchanged, stability of the E form of the enzyme in some lipid environments was not due to increased enthalpy for the transition to the E′ form. It seems clear, therefore, that reaction 1 was driven by entropy changes and that the variable influence of DSPC, DPPC, and DOPC on the equilibrium for reaction 1 was due to the effects of these lipids on the entropy change for this reaction, not the enthalpy change.

Thermotropic Properties of UDP-Glucuronosyltransferase in Asymmetrical Phospholipids. Because almost all the phospholipids in liver microsomes are asymmetric (Montfoort et al., 1971; van Golde et al., 1968) and because enzyme in untreated microsomes does not undergo a temperature-induced transition in the range of 37–48 °C (Dannenberg et al., 1989), we examined the thermotropic properties of UDP-glucuronosyltransferase after reconstition into ULVs of an asymmetric lipid. The data for enzyme embedded in ULVs of SOPC are displayed in Figure 2. The Arrenhius plot was linear and continuous up to 40 °C, indicating that SOPC stabilized the active form of the enzyme better than symmetrical phospholipids. As for the other diacylphospholipids, the

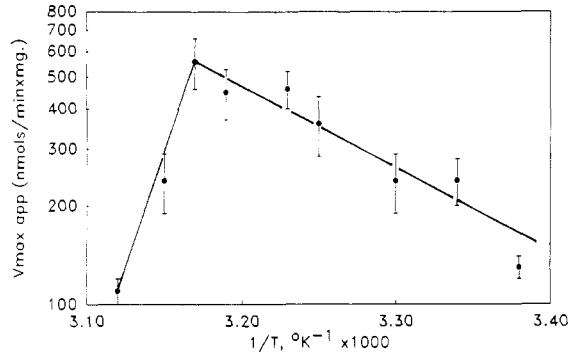

FIGURE 4: Arrhenius plot for GT_{2P} reconstituted in micelles of oleoyllyso-PC. Enzyme was treated for 60 min at 30 °C with a suspension of oleoyllyso-PC and buffer. Activities at V_{max}^{app} were obtained from double-reciprocal plots of reaction rates as a function of substrate concentration at different temperatures. Conditions for the assays are described under Materials and Methods.

transition from E to E′ was reversible for enzyme in SOPC, and E′ was inactive (data not shown). The temperature dependence of the equilibrium constant for reaction 1, for enzyme in SOPC, is shown in Figure 3. The thermodynamic constants calculated from the data in Figure 3 are presented in Table I. These data fit with the effect of the bilayers of DSPC, DPPC, and DOPC in that there was a progressive increase in the midpoint for the transition E ↔ E′ (T_m) and progressive decreased in ΔH and ΔS in the series DSPC, DPPC, DOPC, SOPC. Thus, for all the bilayer systems, stability of the active form of the enzyme increased as the ΔH for reaction 1 decreased because there was an associated greater fall in ΔS. The results for the asymmetric SOPC seemed anomalous, however, because the temperature for the gel to liquid-crystal transition in bilayers of SOPC is about 15 °C, i.e., between those for DPPC and DOPC, whereas for the other lipids there was an inverse correlation between T_m for reaction 1 and the temperature for the phase transition of the lipid.

Thermotropic Properties of UDP-Glucuronosyltransferase in Micelles of Lysophosphatidylcholine. The data in Figure 2 suggest that the active form of UDP-glucuronosyltransferase (E) is stabilized relative to E′ by putting the enzyme into more disordered lipid environments than bilayers in a gel phase and that increasing the acyl chain disorder beyond that in the liquid-crystal phase might enhance the thermal stability of the E form to a greater extent than bilayers of DOPC or SOPC. This idea was tested by determining whether reconstituting the enzyme into micelles of oleoyllysophosphatidylcholine, which have more acyl chain disorder than bilayers, increased the temperature for the onset of inactivation or increased the T_m for the transition in reaction 1 as compared with ULVs of DOPC (Figure 2). The activities at V_{max} for enzyme in micelles of oleoyllysophosphatidylcholine are shown in Figure 4, as a function of temperature. As compared with the bilayers, the Arrenhius plot for enzyme in micelles indicated that the lyso derivative did in fact stabilize the active form of UDP-glucuronosyltransferase at $T > 37$ °C. The Arrenhius plot was linear and continuous up to 42 °C. There was a discontinuity in the slope between 42 and 44 °C. As for the systems in Figure 2, the fall off in enzyme activity at $T > 42$ °C was reversible. Also, the activity of the inactive form of the enzyme at $T > 42$ °C was zero. The effects of the micelles of oleoyllysophosphatidylcholine on the thermal stability of UDP-glucuronosyltransferase were considerably different, however, from those of the bilayer systems. For example, although the onset of the transition from E to E′ was at a higher temperature in micelles versus ULVs of SOPC, the T_m for the transition in SOPC was at a higher temperature than for the micellar system (T_m of 44.2 °C). The van't Hoff plot for enzyme reconstituted into oleoyllysophosphatidylcholine is shown in Figure 3. On the basis of the data in Table I, we anticipated that ΔH and ΔS for reaction 1 would be smaller for enzyme in lysophosphatidylcholine than for enzyme in SOPC, but the thermodynamic parameters for enzyme in micelles of oleoyllysophosphatidylcholine were ΔH, 103.5 kcal/mol; ΔS, 328 eu; and ΔG, −639 cal/mol at 44 °C. These data for the micellar system suggest that reaction 1 probably does not describe the thermal inactivation of E in the presence of oleoyllysophosphatidylcholine.

Effect of Phospholipids on the Irreversible Inactivation of UDP-Glucuronosyltranserase. UDP-glucuronosyltransferase is an unusually stable enzyme. Delipidated enzyme decayed irreversibly to inactive enzyme at 35 °C with a half-time of about 24 h ($k = 4.8 \times 10^{-4}$/min). The rate constant for irreversible denaturation of delipidated enzyme at 44 °C was 4×10^{-2}/min, which was measured by warming the enzyme to 44 °C, removing aliquots serially, and assaying at 35 °C. Irreversible, thermal inactivation depended on the lipids in the environment of the enzyme. Enzyme–lipid complexes were warmed to 44 °C; aliquots were removed serially and assayed at 37 °C for residual activity of UDP-glucuronosyltransferase. The rate constants in these experiments were 3.9×10^{-2}, 1.2×10^{-3}, and 8.4×10^{-3} per minute for ULVs of DSPC, DOPC, and SOPC, respectively. The rate constant in oleoyllyso-PC micelles was 2.1×10^{-3}/min.

An important question is whether irreversible inactivation was due to inactivation of E or E′. The lack of correlation between lipid-induced stabilization of the E form relative to E′, the rapid equilibration between these forms especially as compared with the rates of irreversible thermal inactivation, and the effect of different lipids on the rates of irreversible inactivation suggested that the observed irreversible inactivation was due predominantly to denaturation of the E′ form, not the E form. For example, DOPC was more effective in stabilizing UDP-glucuronosyltransferase against irreversible thermal inactivation than was SOPC whereas the latter lipid was more effective than the former in stabilizing the active form E relative to E′.

Discussion

What Properties of the Lipid Environment Determine the Properties of UDP-Glucuronosyltransferase? The modifications of the environment that affected the function of UDP-glucuronosyltransferase in the above experiments were limited to variations of the acyl chains of the phospholipids. It is difficult to propose a mechanism by which substitution of one hydrophobic surface for another will alter the energetics of the interactions between the apolar interior of membranes and the apolar surface of the protein. Specific interactions between the enzyme and the −C=C− bond at C9–10, if they occurred, might explain the data, but different lipids with a double bond at C9–10 had differential effects on thermal stability. Possibly, the active form of the enzyme could be stabilized by a mismatch between the spans of the apolar regions of the bilayer and UDP-glucuronosyltransferase because the mismatch leads to aggregation of the protein, and the extent of aggregation could depend on the mismatch in different acyl chains (Mouritsen & Bloom, 1984; Riegler & Mohwald, 1986; Peschke et al., 1987). This mechanism seems unlikely, however, because bilayers of DPPC and DOPC had differential effects on function but have essentially the same span for the apolar regions (Lewis & Engelman, 1983). Also, we would expect that regulation of stability secondary to lipid-dependent aggregation of the enzyme in the bilayers would be due to

changes in ΔH, which is the opposite of what was found. We believe, therefore, that modulation of the functional state of UDP-glucuronosyltransferase by acyl chains does not have a chemical basis. We propose instead that the acyl chains of the lipids stabilized the active form of UDP-glucuronosyltransferase by a nonspecific mechanism.

Permissive Role for Lipids in the Stabilization of UDP-Glucuronosyltransferase and in the Modulation of Its Kinetic Properties. There is a similarity between lipid-induced stabilization of UDP-glucuronosyltransferase and stabilization of water-soluble proteins by glycerol (Gekko & Timasheff, 1981a) in that stabilization in the latter setting, like that for the effects of lipids on UDP-glucuronosyltransferase, was associated with negative changes in ΔH but larger negative changes in ΔS. Stabilization of water-soluble proteins by glycerol was a nonspecific consequence of exclusion of glycerol from the surfaces of proteins (Gekko & Timasheff, 1981b), which were larger for the denatured than for the native states. The acyl chains of lipids could have stabilized UDP-glucuronosyltransferase by a similar nonspecific mechanism.

The thermodynamics for reaction 1 are extremely difficult to unravel because there are contributions to ΔH and ΔS from the protein and from interactions between the surface of the protein and water, the water–bilayer interace, and the acyl chains within the hydrophobic interior of the bilayer. If, however, we assume that E and E′ in reaction 1 were the same for enzyme in all bilayers, the analysis is simplified because contributions of the enzyme per se to ΔH and ΔS in reaction 1 would be the same for enzyme in any bilayer and changes in ΔH and ΔS would be due only to changes within the lipids. Since changes in ΔH could not have been the cause of lipid-dependent stabilization of the active form of UDP-glucuronosyltransferase, we will limit the discussion to consideration of the entropy changes of the acyl chains accompanying reaction 1.

Consider that unfolding of E during the transition E → E′ leads to an expansion of the protein within the bilayer. This will cause more molecules of lipid to interact with the surface of E′ than with E. Transfer of a lipid from the liquid-crystal state to the surface of a protein restricts the motion of acyl chains and decreases slightly the order parameter of the acyl chains (Marsh, 1985; Seelig et al., 1982). Transfer of a lipid from the gel state to the surface of a protein will increase acyl chain motion and decrease the order parameter of the acyl chains (Bloom & Smith, 1985). The effects of the transition E → E′ on the entropies of the acyl chains interacting with the surface of the protein thus will depend on the specific acyl chains in the environment of the enzyme. For example, ΔS for transfer of DOPC from the bulk lipid phase (liquid crystal) to the surface of UDP-glucuronosyltransferase would be smaller than for transfer of DSPC. An environment of stearoyl chains as compared with oleoyl chains thus would tend to stabilize the E′ form of the enzyme and favor the transition E → E′. The data are consistent with this idea, except those for SOPC. However, ΔS for the melting of bilayers of SOPC is quite small as compared with other bilayers of PC (Coolbear et al., 1983). Possibly, for reasons that are unclear now, ΔS for transfer of SOPC from the liquid-crystal phase to the surface of a protein is smaller than for DOPC.

The data suggest that reaction 1 does not apply to UDP-glucuronosyltransferase in oleoyllysophosphatidylcholine but the explanation for stabilization of the active form of the enzyme by relatively disordered bilayers could still apply to the enzyme in micelles. Stabilization of the E form of the enzyme in the micellar system could have prevented completely the thermal inactivation of the enzyme by the pathway in reaction 1 even at 44 °C, but not have prevented inactivation by an alternative pathway, e.g., reaction 2, in which E is the same as in eq 1 but E′ and E″ are different.

$$E \leftrightarrow E'' \quad (2)$$

It appears that UDP-glucuronosyltransferase might be indifferent to the apolar environment until heat is added to the system and that the changes within the enzyme that are driven by heat, or possibly by the energy of enzyme–ligand interactions, depend in part on the consequences of a change in the enzyme for the structure/packing of the apolar region of the lipid bilayer. That is, varying the lipid component of the lipid–protein interface does not promote changes in the enzyme from its ground state, but the lipid component determines in part what changes are allowed in UDP-glucuronosyltransferase when energy is added from outside the membrane. The idea that lipids are passive in determining the functional state of UDP-glucuronosyltransferase also can explain the effects of different lipids on its catalytic activity (Hochman et al., 1981; Hochman & Zakim, 1984) and on the activities of other integral membrane enzymes (Jahnig & Bramhall, 1982).

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Registry No. DSPC, 4539-70-2; DPPC, 2644-64-6; DOPC, 10015-85-7; SOPC, 6753-56-6; UDP-glucuronsyltransferase, 9030-08-4.

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Unusual Redox Properties of Electron-Transfer Flavoprotein from *Methylophilus methylotrophus*†

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ABSTRACT: The most positive redox potential ever recorded for a flavin adenine dinucleotide (FAD) containing protein has been measured for an electron-transfer flavoprotein (ETF) synthesized by *Methylophilus methylotrophus*. This potential value, 0.196 V versus the standard hydrogen electrode (vs SHE), was measured at pH 7.0 for the one-electron reduction of fully oxidized ETF (ETF_{ox}) to the red anionic semiquinone form of ETF ($ETF^{\bullet-}$). Quantitative formation of $ETF^{\bullet-}$ was observed. The first successful reduction of ETF from *M. methylotrophus* to its two-electron fully reduced form was also achieved. Although addition of the second electron to $ETF^{\bullet-}$ was extremely slow, the potential value measured for this reduction was −0.197 V vs SHE, suggesting a kinetic rather than thermodynamic barrier to two-electron reduction. These data are believed to be consistent with the postulated catalytic function of ETF to accept one electron from the iron–sulfur cluster of trimethylamine dehydrogenase (TMADH). The second electron reduction appears to have no catalytic function. The very positive potential measured for this ETF and the wide separation of potentials for the two electron reduction steps show that this ETF is a unique and interesting flavoprotein. In addition, this work highlights that while ETFs exhibit similar structural and spectral properties, they display wide variations in redox properties.

Electron-transfer flavoproteins (ETFs)[1] are FAD-containing enzymes that exist as biological electron-transfer links between enzymes of mitochondrial and bacterial degradation pathways and their respective electron-transport chains. As outlined by Davidson et al. (1986), it is of interest to compare and contrast the properties of ETFs from taxonomically diverse sources to ascertain those properties that are conserved among the ETFs and to characterize those properties that render ETF specific for its respective electron donor. The most fundamental properties to be evaluated in such a survey are the oxida-

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[1] Abbreviations: $E^{0\prime}_1$, midpoint potential of first electron reduction; $E^{0\prime}_2$, midpoint potential of second electron reduction; ETF, electron-transfer flavoprotein; ETF_{ox}, oxidized ETF; ETF_{red}, two-electron-reduced ETF; $ETF^{\bullet-}$, anionic, one-electron-reduced ETF; $ETF_{red}H^-$, anionic, hydroquinone form of ETF_{red}; $FADH^-$, anionic form of FAD hydroquinone; FMN^-, anionic, one-electron-reduced form of FMN; $FMNH_2$, FMN hydroquinone; MV^{2+}, oxidized methylviologen; $MV^{\bullet+}$ one-electron-reduced MV; TMADH, trimethylamine dehydrogenase; TMPD, tetramethyl-1,4-phenylenediamine dihydrochloride.

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