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EFFECTS OF OLEOYL-CoA ON THE ACTIVITY AND FUNCTIONAL STATE OF UDP-GLUCURONOSYLTRANSFERASE

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Abstract—Addition of oleoyl-CoA to microsomes inhibited UDP-glucuronosyltransferase (assayed with 1-naphthol or *p*-nitrophenol) at concentrations within the physiologic range of total long-chain acyl-CoAs in liver. Inhibition of activity was associated with changes in the regulatory properties of the enzyme indicating that oleoyl-CoA altered the functional state of UDP-glucuronosyltransferase. The effect of oleoyl-CoA on the state of UDP-glucuronosyltransferase depended on the concentration of oleoyl-CoA, whether oleoyl-CoA was added in the presence or absence of substrates, the duration of treatment with oleoyl-CoA, and the aglycone with which activity was assayed. When oleoyl-CoA was added to microsomes in the presence of aglycones or UDP-glucuronic acid, inhibition by oleoyl-CoA was reversed by albumin, which by itself had no effect on activity. But UDP-glucuronosyltransferase, assayed with either aglycone, did not revert to the native state on removing oleoyl-CoA. Instead sequential treatment with oleoyl-CoA and albumin, in the presence of at least one substrate, produced a form of UDP-glucuronosyltransferase that was more active than the native state. When oleoyl-CoA was added to microsomes in the absence of aglycones or UDP-glucuronic acid, the activity of enzymes assayed with 1-naphthol decayed irreversibly to zero. Similar treatment followed by assay with *p*-nitrophenol as aglycone led to an active form of the enzyme that was inhibited further by albumin. The data are compatible with the idea that long-chain acyl-CoAs could regulate the functional state of UDP-glucuronosyltransferase.

UDP-glucuronosyltransferases detoxify a broad array of endogenous and exogenous compounds [1]. Glucuronidation is an especially important pathway for detoxifying intermediates in the pathways between unreactive parent compounds and reactive, genotoxic products [2–4], which are carcinogenic. Any factor that modulates the rates of glucuronidation reactions is likely, therefore, to have an impact on long-term health [5, 6]. It was reported recently that fatty acids, added to perfused livers, cause efflux from liver to perfusate of hydroxylated metabolites of benzo[*a*]pyrene, suggesting that fatty acids inhibit glucuronidation of these intermediates [7]. Further study of this phenomenon showed, however, that fatty acids *per se* do not inhibit UDP-glucuronosyltransferase, but that long-chain acyl-CoAs, which are obligatory intermediates in such fatty acid metabolizing pathways as oxidation and esterification, are excellent inhibitors [8]. Inhibition was reported to be irreversible and to occur at concentrations of oleoyl-CoA [8] in the range of total long-chain acyl-CoAs in intact liver [8]. The basis for inhibition of UDP-glucuronosyltransferase by oleoyl-CoA was not pursued, however, until now. The data presented in this paper confirm that oleoyl-CoA is a potent inhibitor of UDP-glucuronosyltransferase and that inhibition occurs at concentrations within the range of acyl-CoAs occurring *in vivo*. Our data also provide additional evidence for the basis of the effects of oleoyl-CoA on UDP-glucuronosyltransferases and show that, depending on the conditions in microsomes when oleoyl-CoA is added, inhibition can be reversed.

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MATERIALS AND METHODS

Oleoyl-CoA and bovine serum albumin were purchased from Sigma. Microsomes were prepared from male Wistar rat liver as in Ref. 10 and were stored at −20° until used. Assays of UDP-glucuronosyltransferase were carried out with 1-naphthol as aglycone [11] or with *p*-nitrophenol [12]. Detailed conditions for assays are given in the text and legends to the figures. Protein was measured with the biuret method [13].

RESULTS

Reversible inhibition of UDP-glucuronosyltransferase by oleoyl-CoA. The data in Fig. 1 show initial rates of activity for UDP-glucuronosyltransferase assayed with 1-naphthol. There was a progressive decrease in activity as the concentration of oleoyl-CoA was increased. Inhibition of UDP-glucuronosyltransferase occurred at the lowest concentration of oleoyl-CoA tested, which was 2.5 μM. At the highest concentration of oleoyl-

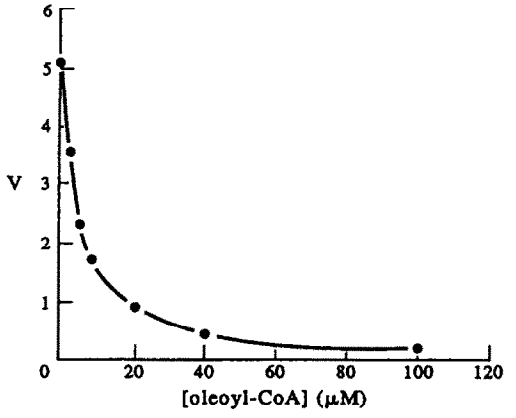

Fig. 1. Inhibition of the glucuronidation of 1-naphthol by oleoyl-CoA. Microsomes (0.23 mg protein) were added to a complete assay system at 30° containing 20 μM 1-naphthol and 10.0 mM UDP-glucuronic acid in a final volume of 1.0 mL. Immediately after adding microsomes to the cuvette, the indicated amounts of oleoyl-CoA were added and recording was begun. The decrease in the concentration of 1-naphthol was followed as described in Materials and Methods. Each data point is the mean of two separate determinations. Activities are expressed in nmol/min/mg protein.

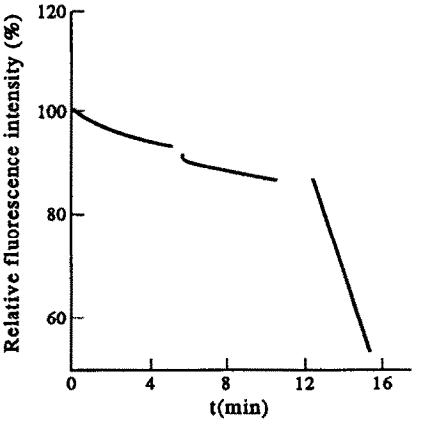

Fig. 2. Reversal of inhibition of UDP-glucuronosyltransferase by removing oleoyl-CoA from microsomes. Microsomes (0.23 mg protein) were added at 30° to an assay system containing 20 μM 1-naphthol but no UDP-glucuronic acid. Immediately after adding microsomes, oleoyl-CoA (50 μM final concentration) was added. Recording was begun, and 5 min later UDP-glucuronic acid (1 mM final concentration) was added. After an additional 5 min, albumin (3.5 mg) was added to the cuvette. The final volume was 1.0 mL. Data are shown as relative fluorescence.

CoA tested (100 μM), UDP-glucuronosyltransferase activity was not detected. If we assume that all the oleoyl-CoA was distributed within the microsomes in the experiment in Fig. 1, then significant inhibition of UDP-glucuronosyltransferase occurred for a concentration of oleoyl-CoA in the range of 2.5 mol/100 mol membrane phospholipid. This calculation is based on an average molecular weight of 800 per phospholipid and a ratio of microsomal protein/phospholipid (w/w) of 1.0/0.30 [14].

The effect of small concentrations of oleoyl-CoA on the activity of UDP-glucuronosyltransferase is an important observation in the context of the well-known biphasic effect of detergents, in general, on the activity of the enzyme. Thus, the effects of bile salts, Triton, and phenothiazines on the activity of UDP-glucuronosyltransferase occur at concentrations on the order of 100 mol detergent/100 mol phospholipid [15–17]. Obviously, oleoyl-CoA inhibited UDP-glucuronosyltransferase at concentrations far below this range. Moreover, in contrast with other detergents, oleoyl-CoA did not activate the enzyme at any concentration.

Albumin has high avidity for long-chain acyl-CoAs and can remove these compounds from lipid bilayers (cf. Ref. 18, for example). We therefore used albumin as a binding protein for oleoyl-CoA to investigate possible reversal of the effects of this molecule on the activity of UDP-glucuronosyltransferase. The data in Fig. 2 show that addition of albumin did, in fact, reverse oleoyl-CoA-dependent inhibition of UDP-glucuronosyltransferase. Microsomes in this experiment were added to an assay system containing 1-naphthol and oleoyl-CoA (50 nmol) but no UDP-glucuronic acid. Quenching of the fluorescence of 1-naphthol between 0 and 5 min in Fig. 2, which was prior to adding UDP-glucuronic acid, was due to photo-oxidation of 1-naphthol [19]. UDP-glucuronic acid (1 mM) was added to the assay at 5 min. But the rate of quenching of the fluorescence of 1-naphthol did not change until albumin was added 10 min after starting the assay. The rate of glucuronidation after 10 min was 9.45 nmol/min/mg protein. The activity of UDP-glucuronosyltransferase measured in the absence of oleoyl-CoA was 2.2 nmol/min/mg protein. Albumin added in the absence of oleoyl-CoA had no effect on the activity of UDP-glucuronosyltransferase. Addition of albumin and oleoyl-CoA to assays prior to adding microsomes also was without effect on the activity of UDP-glucuronosyltransferase (data not shown). These last experiments establish that sequential treatment of microsomes with oleoyl-CoA and then albumin was required for albumin-induced activation of the enzyme in Fig. 2 and that removing oleoyl-CoA from microsomes reversed inhibition but not by returning the enzyme to its original state.

The data in Table 1 show that the extent of activation of UDP-glucuronosyltransferase, on adding albumin to microsomes treated previously with oleoyl-CoA, depended on the amount of oleoyl-CoA added initially. There was an indirect relationship between the activity of UDP-glucuronosyltransferase and the concentration of oleoyl-CoA present during the assay, but a direct relationship between the amount of oleoyl-CoA added to assays and enzyme activity after removing oleoyl-CoA.

In all the experiments in Table 1, microsomes were added to assays (at 30°) containing oleoyl-CoA

Table 1. Effect of albumin on reactivation of UDP-glucuronosyltransferase, assayed with 1-naphthol, after treatment with variable amounts of oleoyl-CoA

Oleoyl-CoA (μM)	Activity before adding albumin (nmol/min/mg)	Activity after adding albumin (nmol/min/mg)
0	1.9	
10.0	0.87	3.30
20.0	0.62	6.30
50.0	ND	8.70
100.0	ND	4.7

Microsomes (0.23 mg protein/mL) were treated with the indicated concentration of oleoyl-CoA in a final volume of 1 mL in the presence of a complete assay system. Activities were assayed as described in Materials and Methods using 20 μM 1-naphthol and 1.0 mM UDP-glucuronic acid at 30°. After 10 min, albumin (3.5 mg) was added to the assay cuvettes, and rate data were collected for an additional 10 min. All data are initial rates of activity (mean of two determinations); ND indicates that the rate was too small to measure accurately.

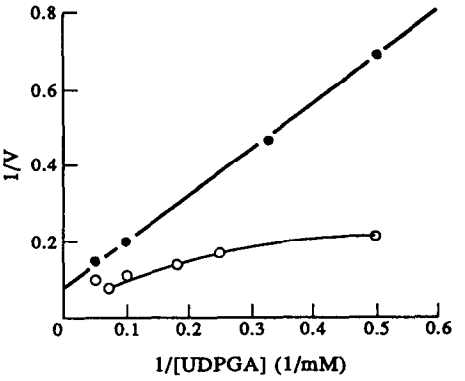

Fig. 3. Kinetic pattern of UDP-glucuronosyltransferase, assayed with 1-naphthol, in the presence and absence of oleoyl-CoA. Microsomes were added to complete assay systems at 30°. The concentration of 1-naphthol was 20 μM in all assays. The concentrations of UDP-glucuronic acid are indicated in the Fig. For data depicted by closed circles (●), 50 μM oleoyl-CoA was added immediately after adding microsomes to the assays. Data in open circles (○) are for untreated microsomes. Each data point is the mean of two separate determinations. Activities are expressed in nmol/min/mg protein.

and 1-naphthol. Reactions were started by adding UDP-glucuronic acid. When microsomes were mixed with oleoyl-CoA in the absence of either 1-naphthol or UDP-glucuronic acid, oleoyl-CoA inactivated UDP-glucuronosyltransferase irreversibly (data not shown). The ratio of oleoyl-CoA to microsomal phospholipid in these experiments was 5 mol oleoyl-CoA/100 mol phospholipid, which corresponded to the conditions in Fig. 1 for assays containing 20 μM oleoyl-CoA. Assays of UDP-glucuronosyltransferase in Table 1, however, were carried out using 1.0 mM UDP-glucuronic acid. In contrast, assays in the experiment in Fig. 1 contained 10.0 mM UDP-glucuronic acid.

To provide further insight into the effects of oleoyl-CoA on the enzyme, we determined the effect of oleoyl-CoA on the regulatory properties of the enzyme. In untreated microsomes, for example, the kinetics of UDP-glucuronosyltransferase are not Michaelis–Menten. Double-reciprocal plots of activity as a function of the concentration of UDP-glucuronic acid are concave upward [20, 21]. The basis for the non-Michaelis–Menten kinetics of the enzyme in microsomes cannot be established directly. But pure enzyme, reconstituted into gel phase lipid bilayers, also displays non-Michaelis–Menten kinetics [22]. Binding studies of enzyme in this system have shown two binding sites for UDP-glucuronic acid per molecule enzyme and that the sites appear to be negatively cooperative [21]. Another allosteric property of the enzyme is activation by UDP-*N*-Ac-glc [20, 21]. The data in Fig. 3 show the non-Michaelis–Menten kinetics of enzyme in untreated microsomes (open circles). The kinetic pattern of UDP-glucuronosyltransferase in microsomes treated with oleoyl-CoA, at a concentration at which inhibition was less than complete, was Michaelis–Menten (closed circles). Shown in Table 2 are the effects of oleoyl-CoA on the response of UDP-glucuronosyltransferase to

Table 2. Effect of UDP-*N*-Ac-glc on the activity of UDP-glucuronosyltransferase in the presence and absence of oleoyl-CoA

Additions to assay			Activity
UDP-*N*-Ac-glc	Oleoyl-CoA	Albumin	(nmol/min/mg)
–	–	–	2.05
+	–	–	6.75
–	+	–	0.54
+	+	–	0.50
–	+	+	9.02
+	+	+	6.89

Microsomes were treated with oleoyl-CoA as in Fig. 1. When present, the concentration of oleoyl-CoA was 20 μM. The concentration of 1-naphthol was 20 μM. The concentrations of UDP-*N*-Ac-glc and UDP-glucuronic acid were 1.0 and 0.5 mM, respectively. Albumin was added to assays as in Table 1. Activities (means of two determinations) were measured at 30°.

UDP-*N*-Ac-glc. UDP-*N*-Ac-glc did not activate enzyme in the presence of oleoyl-CoA. When oleoyl-CoA was removed by adding albumin to microsomes, UDP-*N*-Ac-glc was an inhibitor of UDP-glucuronosyltransferase. Albumin itself had no effect on the activation of UDP-glucuronosyltransferase by UDP-*N*-Ac-glc (data not shown). The data in Fig. 3 and Table 2 show, therefore, that oleoyl-CoA did not simply inhibit the native state of UDP-glucuronosyltransferase but effected a transition to a state(s) with modified function.

Note that the data in Fig. 3 depicted by the open circles show substrate inhibition of enzyme in untreated microsomes at concentrations of UDP-

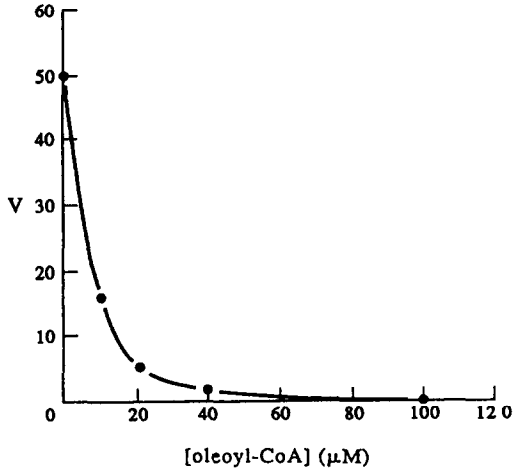

Fig. 4. Effect of oleoyl-CoA on the glucuronidation of 1-naphthol by microsomes treated previously with cholate. Microsomes (19.4 mg/mL) were treated with cholate at 0° at a ratio (w/w) of 2/1 (microsomal protein/cholate), which gave a maximal activation of UDP-glucuronosyltransferase. The cholate-treated microsomes (0.23 mg protein) were then added to complete assay systems at 30°. Immediately after adding microsomes, the indicated amounts of oleoyl-CoA were added and recording was begun. Each data point is the mean of two separate determinations. Activities are expressed in nmol/min/mg protein.

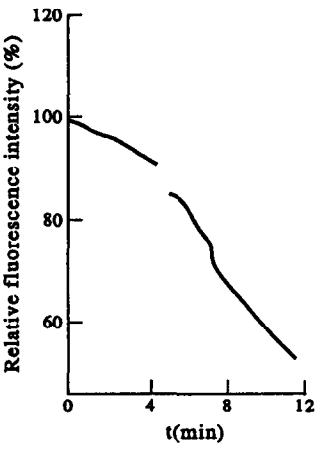

Fig. 5. Reversal of oleoyl-CoA-induced inhibition of cholate-treated microsomes for assays with 1-naphthol as aglycone. Microsomes (0.23 mg protein) treated with cholate as in Fig. 4 were added to a complete assay system at 30°. The concentrations of 1-naphthol and UDP-glucuronic acid, respectively, were 20 μM and 1.0 mM. Oleoyl-CoA (20 μM final concentration) was added and recording was begun. After 5 min, albumin (3.5 mg) was added. Data are shown as relative fluorescence.

glucuronic acid greater than 15 mM. We also found that UDP-glucuronosyltransferase in untreated microsomes was inhibited at high concentrations of 1-naphthol. It was not possible, for these reasons, to determine the kinetic constants for enzyme in untreated microsomes and thus to provide an analysis of the detailed effects of oleoyl-CoA on these constants.

Combined effects of oleoyl-CoA and other detergents on the activity of UDP-glucuronosyltransferase. We determined the effect of oleoyl-CoA on the activity of UDP-glucuronosyltransferase in microsomes treated with an amount of cholate that led to a maximal activation of UDP-glucuronosyltransferase. Comparison of activities in the absence of added oleoyl-CoA for experiments in Figs. 1 and 4 show that cholate had a large activating effect on UDP-glucuronosyltransferase and that oleoyl-CoA was as effective an inhibitor of UDP-glucuronosyltransferase in cholate-treated microsomes as in untreated microsomes. Independent of arguments about the topology of UDP-glucuronosyltransferase in microsomes (cf. Ref. 23), the results of experiments in cholate-treated microsomes make it highly likely that the effect of oleoyl-CoA on the function of UDP-glucuronosyltransferase was mediated via effects on the putative microsomal transport protein for UDP-glucuronic acid (cf. Ref. 23).

As for otherwise untreated microsomes, the effect of oleoyl-CoA on the activity of UDP-glucuronosyltransferase in cholate-treated microsomes was reversed by binding oleoyl-CoA to albumin (Fig. 5). Reversal of the oleoyl-CoA-dependent inhibition was not complete, however, in that activities after removing oleoyl-CoA by binding to albumin did not reach levels in microsomes treated with cholate only. The basis for lack of complete reversal of the inhibitory effect of oleoyl-CoA was not investigated further. We found too that cholate activated UDP-glucuronosyltransferase in microsomes treated with oleoyl-CoA (in the presence of substrates) and then albumin (data not shown); but the specific activity of UDP-glucuronosyltransferase in these experiments did not reach that in microsomes treated with cholate only.

Effect of oleoyl-CoA on the activity of UDP-glucuronosyltransferase assayed with p-*nitrophenol.* The data in Fig. 6 show that oleoyl-CoA inhibited the rate of glucuronidation of *p*-nitrophenol. There was, however, a significant difference between the effects of oleoyl-CoA on the glucuronidation of *p*-nitrophenol and 1-naphthol in that oleoyl-CoA did not inhibit completely the glucuronidation of *p*-nitrophenol (compare Figs. 1 and 6 and Tables 1 and 3). On the other hand, as shown by the data in Table 3, the mechanism of inhibition of the glucuronidation of *p*-nitrophenol was the same as that for the glucuronidation of 1-naphthol. For assays with both aglycones, UDP-glucuronosyltransferase was converted by oleoyl-CoA to functional states with less activity than the native states. And in both cases the modified states did not revert to the native states on removing oleoyl-CoA.

Differential effects of oleoyl-CoA on the stability of UDP-glucuronosyltransferase assayed with 1-*naphthol or* p-*nitrophenol.* As mentioned above, treatment of microsomes with oleoyl-CoA in the absence of either UDP-glucuronic acid or 1-naphthol led to irreversible inactivation of UDP-

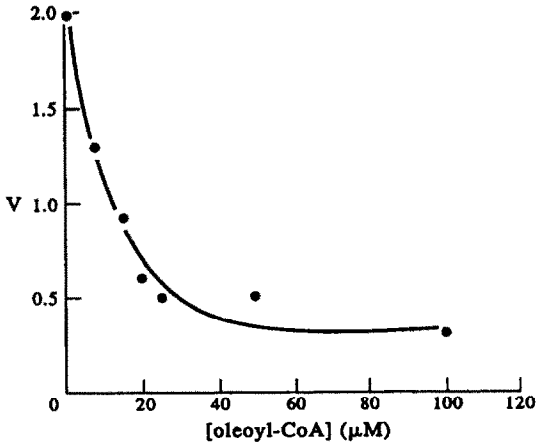

Fig. 6. Oleoyl-CoA-induced inhibition of UDP-glucuronosyltransferase assayed with *p*-nitrophenol as aglycone. The experiments were carried out as in Fig. 1 at the indicated concentrations of oleoyl-CoA. The concentrations of *p*-nitrophenol and UDP-glucuronic acid, respectively, were 0.05 and 1.0 mM. Each data point is the mean of two separate determinations. Activities are expressed in nmol/min/mg protein.

Table 3. Effect of albumin on reactivation of UDP-glucuronosyltransferase, assayed with *p*-nitrophenol, after treatment with variable amounts of oleoyl-CoA

Oleoyl-CoA (μM)	Activity before adding albumin (nmol/min/mg)	Activity after adding albumin (nmol/min/mg)
0	1.97	1.97
5.0	1.24	1.93
10.0	0.74	2.54
15.0	0.51	3.44
20.0	0.44	4.37
25.0	0.37	6.06
50.0	0.41	10.8
100.0	0.23	5.35

Microsomes were treated with the indicated amount of oleoyl-CoA in a final volume of 1 mL in the presence of a complete assay system. Activity was assayed as described in Materials and Methods using 0.05 mM *p*-nitrophenol and 1.0 mM UDP-glucuronic acid at 30°. After 10 min, albumin (3.5 mg) was added to the assay cuvettes, and rate data were collected for an additional 10 min. All data are initial rates of activity.

glucuronosyltransferase assayed with this aglycone. However, under the same conditions, the enzyme was not inactivated when assayed with *p*-nitrophenol as aglycone. Thus, when 1 mg of microsomal protein was mixed with 100 μM oleoyl-CoA and the mixture treated at 20° for 30 min, activity measured after adding *p*-nitrophenol and UDP-glucuronic acid was 2.62 nmol/min/mg protein. This rate was 13-fold greater than the initial rate measured immediately after adding the same amount of oleoyl-CoA to microsomes. It was 30% greater than the activity of

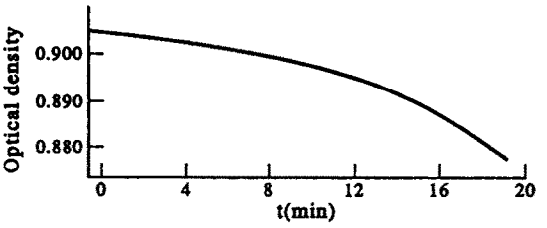

Fig. 7. Spontaneous reversal of oleoyl-CoA-induced inhibition of UDP-glucuronosyltransferase assayed with *p*-nitrophenol. Microsomes (0.23 mg protein) and oleoyl-CoA (100 μM final concentration) were added to an assay system minus substrates. After 16 min at 20°, 0.05 mM *p*-nitrophenol and 1.0 mM UDP-glucuronic acid were added, the temperature was raised to 30°, and recording was begun.

UDP-glucuronosyltransferase in untreated microsomes but less than the activity in microsomes treated sequentially, in the presence of substrates, with oleoyl-CoA and then albumin (Table 3). Whereas oleoyl-CoA, in the absence of substrates, irreversibly inactivated UDP-glucuronosyltransferase for assays with 1-naphthol, there was spontaneous activation, under these conditions, of the enzyme for assays with *p*-nitrophenol as aglycone.

The basis for this apparently spontaneous activation was examined in the experiment in Fig. 7, which shows the time course of glucuronidation (reflected by decreasing optical density) of *p*-nitrophenol in microsomes treated with 100 μM oleoyl-CoA in the absence of substrates for 16 min at 20°. Recording in Fig. 7 was begun after adding UDP-glucuronic acid and *p*-nitrophenol. The data show a continually increasing rate of glucuronidation of *p*-nitrophenol. The initial rate (e.g. between 0 and 4 min) was 0.27 nmol/min/mg protein. The rate of glucuronidation between 16 and 20 min was 1.30 nmol/min/mg protein. We found in addition to the spontaneous activation of the enzyme assayed with *p*-nitrophenol that the properties of UDP-glucuronosyltransferase, treated with oleoyl-CoA at 20° for about 20 min, were not the same as those of enzyme produced by sequential treatment of microsomes with oleoyl-CoA and albumin. For example, cholate activated the UDP-glucuronosyltransferase for assays with *p*-nitrophenol as aglycone when added to microsomes treated sequentially with oleoyl-CoA and then albumin in the presence of substrates (data not shown). By contrast, cholate inhibited the enzyme in microsomes in which oleoyl-CoA was removed (in the absence of substrates) by spontaneous hydrolysis (closed circles, Fig. 8). Albumin also inhibited enzyme in this preparation of microsomes, and inhibition by cholate and albumin was additive (open circles, Fig. 8). These last results are like those reported by Zhong *et al.* [8] for the effect of oleoyl-CoA on the activity of UDP-glucuronosyltransferase assayed with *p*-nitrophenol as aglycone. The differences between the effects of oleoyl-CoA on the glucuronidation of 1-naphthol and *p*-nitrophenol in microsomes to which oleoyl-CoA was added in the

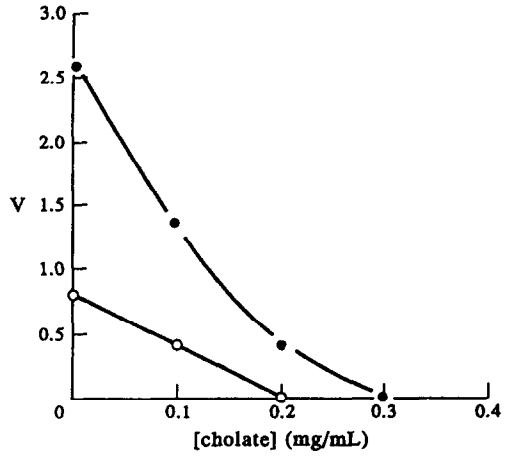

Fig. 8. Effect of cholate and albumin on the activity of UDP-glucuronosyltransferase (assayed with *p*-nitrophenol as aglycone) treated with oleoyl-CoA in the absence of UDP-glucuronic acid of *p*-nitrophenol. Microsomes (0.23 mg protein) were treated with 100 μM oleoyl-CoA at 20° for 30 min. UDP-glucuronic acid (1.0 mM) and *p*-nitrophenol (0.05 mM) were added and activity was measured at 30°. Data in closed circles (●) are activities in the presence of the indicated concentrations of cholate. Data in open circles (○) are for assays in the presence of the indicated amounts of cholate and 3.5 mg albumin. Each data point is the mean of two separate determinations. Activities are expressed in nmol/min/mg protein.

absence of substrates reflect irreversible inactivation of the activity with 1-naphthol as substrate but reversible inhibition of activity with *p*-nitrophenol as substrate. We noted, however, that the form of the *p*-nitrophenol-conjugating enzyme generated by relatively presumed slow, spontaneous removal of oleoyl-CoA secondary to hydrolysis was different from the form generated by rapid removal of oleoyl-CoA by binding to albumin. The mechanism for the spontaneous alteration in the properties of UDP-glucuronosyltransferase (assayed with *p*-nitrophenol) was not examined further. Note, however, that microsomes contain an active acyl-CoA hydrolase [24]. Calculations based on the specific activity of this enzyme in rat liver microsomes [24] show that the activity is large enough to hydrolyze about half the added oleoyl-CoA under the conditions of the experiment in Fig. 7. A possible explanation for the spontaneous activation of the *p*-nitrophenol conjugation then, after adding oleoyl-CoA, is the acyl-CoA hydrolase activity of microsomes.

DISCUSSION

Basis for inhibition of UDP-glucuronosyltransferase by oleoyl-CoA. The data presented above show that oleoyl-CoA inhibited UDP-glucuronosyltransferase by converting the native state of the enzyme to a modified state with less catalytic activity and modified regulatory properties. Although detergents, in general, modify the functional state of UDP-glucuronosyltransferase, other detergents activate the enzyme in microsomes and inhibit it only at quite high concentrations. Hence, oleoyl-CoA appears to be a unique detergent in this context. It has been shown, however, that UDP-glucuronosyltransferase is inhibited reversibly by phosphorolipids bearing a net negative charge in the polar region [25, 26] and that the extent of this inhibition increases with negative charge, e.g. phosphatidic acid is a more potent inhibitor on a mole basis than phosphatidylinositol [26]. In fact, 1 mol% phosphatidic acid in the membrane matrix inhibits pure enzyme completely [26]. We think these last results provide a basis for understanding the effects of oleoyl-CoA on the UDP-glucuronosyltransferase because the terminal triphosphate region of CoA has a large negative charge.

Oleoyl-CoA interacts with lipid bilayers via the polymethylene moiety of the compound, which inserts into the bilayer [18]. The CoA moiety extends into the aqueous phase. The topology of the interactions between UDP-glucuronosyltransferase and the polar and apolar regions of oleoyl-CoA is expected to be similar then to that for interactions between the enzyme and phospholipids. Since phospholipids with an oleoyl group as the polymethylene chain of the matrix are especially effective in reconstituting an enzyme with high specific activity and avidity for substrates [22, 27, 28], and since the enzyme is not inhibited by oleate [8], the inhibitory effect of oleoyl-CoA can be attributed to interactions between UDP-glucuronosyltransferase and the negatively charged CoA moiety of oleoyl-CoA. But CoA itself does not inhibit UDP-glucuronosyltransferase [8], probably because, when free in water, it is not present at the membrane-water interface. We interpret these data to mean that the oleate moiety anchors the CoA moiety to the membrane-water interface and that CoA in this location destabilizes the native state of UDP-glucuronosyltransferase. Depending on the isoform of the enzyme and whether substrates are present, the states stabilized by oleoyl-CoA may be active or inactive; and they may relax, on removing oleoyl-CoA, to a variety of functional states that differ from the native state and those stabilized by oleoyl-CoA.

A final interesting point with regard to the mechanism of the effects of oleoyl-CoA is that inhibition of the enzyme was reversed upon removing oleoyl-CoA but that this event did not regenerate the native state of UDP-glucuronosyltransferase. Instead a new, modified state was produced. This effect is not unlike the non-specific activations of UDP-glucuronosyltransferase by a variety of techniques, such as treatment with detergents that activate directly [15, 23] or high hydrostatic pressure [11, 19]. In these instances too, removing the perturbation that has altered the enzyme does not allow it to relax to the native state. Clearly then the native state of UDP-glucuronosyltransferase in microsomes is metastable [19], i.e. the native enzyme is trapped kinetically in a conformation that is not the lowest free energy state available to the catalytically active enzyme.

Possible physiologic implications of the data. Since the presence of UDP-glucuronic acid, which is present constantly *in situ*, determines in part the

effects of oleoyl-CoA on the rate of glucuronidation of 1-naphthol and *p*-nitrophenol, the data presented above suggest that oleoyl-CoA, and perhaps other long-chain acyl-CoAs, are modulators of rates of glucuronidation in intact cells. This idea is supported by the work of Zhong *et al.* [8], which showed that glucuronidation reactions are inhibited in perfused livers when rates of fatty acid uptake are high, i.e. when the concentration of fatty acid in perfusate is high. There is a basis, therefore, for proposing that attention be given to defining the functional states of UDP-glucuronosyltransferases in response to manipulations that modify the metabolism of long-chain fatty acids. Indeed, the amount and type of fat in the diet do lead to alterations in the functional state of these enzymes [29]. That adding albumin to otherwise untreated microsomes had no effect on the activity of UDP-glucuronosyltransferase might suggest that microsomes, as isolated from liver, do not contain sufficient amounts of long-chain acyl-CoAs to inhibit UDP-glucuronosyltransferase. But this result does not exclude that the nutritional state of an animal can modulate the function of UDP-glucuronosyltransferases via effects on the concentrations of long-chain acyl-CoAs in liver, which will be diluted out during the homogenization of liver followed by isolation and washing of the microsomal fraction of the cell.

Are there separate isoforms of UDP-glucuronosyltransferase for the metabolism of 1*-naphthol and* p*-nitrophenol?* Several investigators have shown that 1-naphthol and *p*-nitrophenol are metabolized by the same isoforms of UDP-glucuronosyltransferase [30–32]. By contrast, the present data show that oleoyl-CoA has selective effects on the metabolism of these aglycones. The basis for conclusions about the substrate specificity of UDP-glucuronosyltransferase in Refs. 30–32 is quite different from the insight provided in this context by the current experiments. Thus, there is no way to extrapolate from the published data with pure enzymes [30, 31] or assays of a cloned transfected enzyme [32] to the problem of the number and substrate specificities of the UDP-glucuronosyltransferases in liver microsomes from uninduced animals. Obviously, assays of enzymes in microsomes cannot provide this sort of information either. On the other hand, our experiments with microsomes show that the effect of oleoyl-CoA on properties of UDP-glucuronosyltransferase depends on which substrate is used as aglycone. The simplest interpretation of these data is that there is an isoform(s) of UDP-glucuronosyltransferase that predominantly conjugates 1-naphthol and there are others that predominantly conjugate *p*-nitrophenol. We have also found, in this regard, that *p*-nitrophenol did not inhibit the rate of conjugation of 1-naphthol in liver microsomes (Kavekansky J and Zakim D, unpublished data), which further supports this idea.

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