(1984a) *Arch. Biochem. Biophys. 229*, 560–567.
Slomiany, A., Liau, Y. H., Takagi, A., Jozwiak, Z., & Slomiany, B. L. (1984b) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 43*, 581.
Takagi, A., Slomiany, A., Murty, V. L. N., & Slomiany, B. L. (1984a) *Gastroenterology 86*, 1273.
Takagi, A., Liau, Y. H., Jozwiak, Z., Slomiany, B. L., & Slomiany, A. (1984b) *Fed. Proc., Fed Am. Soc. Exp. Biol. 43*, 582.
Witas, H., Slomiany, B. L., Zdebska, E., Kojima, K., Liau, Y. H., & Slomiany, A. (1983) *J. Appl. Biochem. 5*, 16–24.
Wu, A. M., & Pigman, W. (1977) *Biochem. J. 161*, 37–47.

Fatty Acids Bound to Unilamellar Lipid Vesicles as Substrates for Microsomal Acyl-CoA Ligase†

Noa Noy and David Zakim*
Division of Digestive Diseases, Department of Medicine, Cornell University Medical College, New York, New York 10021
Received November 16, 1984

ABSTRACT: Palmitate incorporated into single-layered vesicles of phosphatidylcholine was used as a substrate for palmitoyl coenzyme A ligase (palmitoyl-CoA ligase) in microsomes from rat liver. This was done in order to avoid the use of detergents for dispersal of the water-insoluble palmitate and the possibility of precipitating palmitate added to the aqueous assay as a salt suspension. The activity of the ligase measured when palmitate was added to assays as a component of phospholipid vesicles was 10–40-fold greater vs. activities reported in the literature using other methods for adding fatty acids to the assay system. Phospholipids, however, had no direct effect on the activity of palmitoyl-CoA ligase. The data indicate, therefore, that the activity of this enzyme has been underestimated because of the manner in which fatty acid was added to the assay, which has a significant effect on the activity of the ligase. It is shown too that the rate of spontaneous transfer of palmitate from unilamellar vesicles of phosphatidylcholine to microsomes via a hydrated intermediate is far more rapid than the inherent catalytic activity of the fatty acyl-CoA ligase. The data also suggest that the membrane-associated pool of fatty acid and not fatty acid in the aqueous phase of the assay is the pool of substrate interacting with the ligase.

The substrates of many enzymes that are integral components of biological membranes have exceedingly limited solubility in water. These substrates will partition selectively into the lipid phases of membranes, and concentrations in membranes will be high whereas concentrations in water will be low. The implications of the insolubility in water of substrates for the function of membrane-bound enzymes have not been examined in a systematic manner. It is unknown, therefore, whether the selective partitioning of substrates into membranes is important for function of membrane-bound enzymes generally or in certain specific cases. Lack of knowledge on this point seems related in part to the manner in which assays for the activity of membrane-bound enzymes are carried out when the substrates are insoluble in water. These substrates usually are added to assays as complexes with proteins containing nonspecific sites for binding of hydrophobic compounds, or they are added as salt suspensions [e.g., see Aas (1971), Farstad et al. (1967), Pande & Mead (1968), and Pande (1972)]. Detergents are added to assays in some instances for the purpose of dispersing the water-insoluble substrates (Bar-Tana et al., 1971; Tanaka et al., 1979; Suzue & Marcel, 1972). The data are treated as if substrates added by these methods distribute homogeneously in the assay system.

The first reaction in the utilization of fatty acids in cells is synthesis of coenzyme A (CoA) derivatives, which is catalyzed by a long-chain acyl-CoA ligase (EC 6.2.1.3). This enzyme is an integral component of the endoplasmic reticulum of cells. The natural substrates for this enzyme have exceedingly low solubilities in water. The fatty acyl-CoA ligase is assayed routinely by adding fatty acid substrates as salt suspensions to a suspension of microsomes in aqueous buffers (Bar-Tana et al., 1971). Assay systems usually contain detergents, presumably to aid in dispersing fatty acids, even though it is reported that detergents inhibit the activity of the fatty acid acyl-CoA ligase (Pande & Meade, 1968). In the present experiments, we describe an assay for this enzyme in which fatty acids are added to the assay as integral components of unilamellar vesicles of phosphatidylcholine. The data presented show that there are several advantages to an assay system in which fatty acids are introduced in this manner as compared with previous systems. The data appear also to have important implications for thinking about mechanisms for the intracellular movement of water-insoluble substrates of membrane-bound enzymes.

Materials and Methods

Scintillation liquid (Liquescint) was purchased from National Diagnostics. All other chemicals were obtained from Sigma Chemical Co. [^{14}C]Palmitate and [^{14}C]stearate were obtained from New England Nuclear. Microsomes were prepared from male Wistar rat livers (3–4 months old) (Pande & Meade, 1968). Microsomes were suspended in 0.25 M sucrose and stored at −80 °C until used. Fatty acid binding protein was partially purified from male Sprague-Dawley rats weighing 300–400 g (Ockner & Manning, 1982). Microsomal protein was determined by the Lowry method (Lowry & Lopez, 1946). Liposomes were made from egg phosphatidylcholine (Sigma, type III in hexane). After removal of

† This work was supported in part by a grant from the National Institutes of Health (GM33142).

0006-2960/85/0424-3521$01.50/0

hexane by evaporation under argon, the lipid was suspended in a buffer containing 100 mM KCl, 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5, and 1 mM ascorbate. The suspension was sonicated for 20 min at 4 °C and then centrifuged at 100000*g* for 20 min to pellet undispersed lipid. Liposomes containing [^{14}C]palmitate or [^{14}C]stearate were made in the same way as were liposomes, after mixing the fatty acid in ethanol and egg phosphatidylcholine in hexane at mole ratios of 1:10 to 1:50. Microsomal palmitoyl-CoA ligase was assayed at 37 °C according to Bar-Tana et al. (1971). The assay system included, in a final volume of 0.25 mL, 150 mM Tris, pH 7.5, 2 mM ethylenediaminetetraacetic acid (EDTA), 50 mM $MgCl_2$, 20 mM ATP, and 0.6 mM CoA. These concentrations of ATP and CoA approximate what is required to saturate the enzyme with these two substrates (data not shown). Rat liver microsomes in 0.25 M sucrose were added to a final concentration of about 10 μg of microsomal protein per assay. ^{14}C-Labeled fatty acids were added as a complex with liposomes of egg phosphatidylcholine. No detergent was present. The reaction was usually started by the addition of 15 μL of CoA solution (10 mM CoA in 50 mM dithiotreitol), since it was found that the enzyme is unstable in the absence of ATP. The reaction was terminated by the addition of 1 mL of Dole's reagent (isopropyl alcohol/heptane/1 N H_2SO_4, 40:10:1), 0.35 mL of water, and 0.6 mL of heptane (Dole, 1956). The heptane layer, containing the unreacted palmitate, was removed, and the lower phase was washed 5 times with 0.6 mL of heptane; 0.4 mL of the lower phase was counted for ^{14}C-fatty acyl CoA.

The distribution constants of palmitate and stearate between egg phosphatidylcholine bilayers and water were measured by equilibrium dialysis. One milliliter of unilamellar vesicle suspension containing 5 mg of egg phosphatidylcholine and 0.6 μmol of either [^{14}C]palmitate or [^{14}C]stearate was placed in a dialysis bag. The bag was placed in 20 mL of 2 mM EDTA and stirred at room temperature until equilibrium was reached (about 30 h). The distribution of fatty acid between lipid bilayers and the water phase was determined from the counts of ^{14}C-fatty acids and calculated as [(moles of fatty acid)$_{lipid}$/moles of lipid]/[(moles of fatty acid)$_{water}$/moles of water].

Results

Synthesis of Palmitoyl-CoA from Fatty Acids Bound Initially to a Phospholipid Bilayer. Palmitate was incorporated into single-layered vesicles of phosphatidylcholine from egg by cosonication of palmitate and the phospholipids. When added to a mixture of liver microsomes, ATP, and CoA, palmitate bound to unilamellar vesicles was a good substrate for fatty acyl-CoA ligase. A typical time course for synthesis of palmitoyl-CoA from palmitate bound to unilamellar bilayers of phosphatidylcholine is shown in Figure 1. Synthesis was not truly linear with time except for the first few seconds. Thus, 10% of the palmitate was utilized within the first 30 s of reaction, which was first order. Of importance in Figure 1 is that all the palmitate added to the reaction was metabolized. By contrast, when acyl-CoA ligase was assayed as in Pande (1972), in which palmitate was added to the assay as the potassium salt, the reaction stopped after utilization of only 20% of added palmitate (data not shown). The reason for this problem was not investigated further, but failure to utilize all the added fatty acid is apparent in the literature for assays in which fatty acids were added to an aqueous system as salt suspensions (Pande, 1972).

The initial activity of the ligase, calculated from the utilization of palmitate in the first 30 s of the reaction shown in Figure 1, is 875 nmol min^{-1} (mg of microsomal protein)$^{-1}$. This rate is several times greater than rates reported previously for the activity of the microsomal ligase assayed with palmitate. Addition of palmitate to an assay mixture containing Triton X-100 in order to disperse the fatty acid (1 mg of Triton X-100/mL of assay mixture) resulted in an initial rate of activity that was 50% lower vs. the rate observed when palmitate was added as a complex with ligand vesicles.

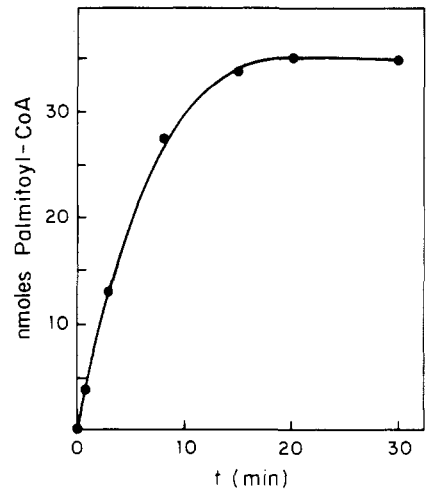

FIGURE 1: Time course of synthesis of palmitoyl-CoA catalyzed by rat liver microsomal palmitoyl-CoA ligase. The assay mixture contained 35 nmol of [^{14}C]palmitate, 175 μg of egg phosphatidylcholine, and 8 μg of microsomal proteins. EDTA, $MgCl_2$, ATP, and Tris, pH 7.5, were added as described under Materials and Methods. The reaction was started by adding palmitate as the complex liposomes–palmitate.

The rate of reaction in Figure 1 and its first-order time course could reflect a rate-limiting transfer of palmitate from the lipid vesicles to the active site of acyl-CoA ligase. This possibility was excluded by two observations. First, the reaction in Figure 1 was initiated by adding palmitate bound to lipid vesicles. The rate of metabolism of palmitate over the initial 30 s of reaction and subsequently was identical with the rate in Figure 1 when microsomes were allowed to "equilibrate" at 37 °C with the complex liposomes–palmitate for variable times prior to starting the reaction by adding CoA. The mixing time for the data in Figure 1 was sufficiently long, therefore, for palmitate to equilibrate between the active site of the liase and all phases of the system. In addition, the first-order rate constant for the reaction in Figure 1 is 0.0033 s^{-1}. In separate experiments, we have measured the rate of reaction 1. The

$$\text{vesicles–palmitate} + n\text{H}_2\text{O} \rightarrow \text{vesicles} + \text{palmitate–(H}_2\text{O)}_n \quad (1)$$

first-order rate constant for this reaction at 37 °C is 8.64 s^{-1} (Daniels et al., 1985). Transfer of palmitate from the unilamellar vesicles to the active site of the ligase must have occurred, therefore, at a rate on the order of 10^3 times faster than the rate of the reaction in Figure 1.

Effect of Added Phospholipids on the Activity of Microsomal Acyl-CoA Ligase. The data in Figure 1 suggest that the kinetic pattern of the assay system fits the Michaelis–Menten model when palmitate bound initially to unilamellar lipid vesicles is the substrate. This is confirmed by the data in Figure 2. The data in this figure are plotted with the assumption that there is a homogeneous distribution of palmitate throughout all the phases of the assay system. Obviously, this is not the case, but the basis for expressing substrate concentrations will not influence the kinetic pattern of the enzymatic reaction. The rates of the reaction in Figure 2 are

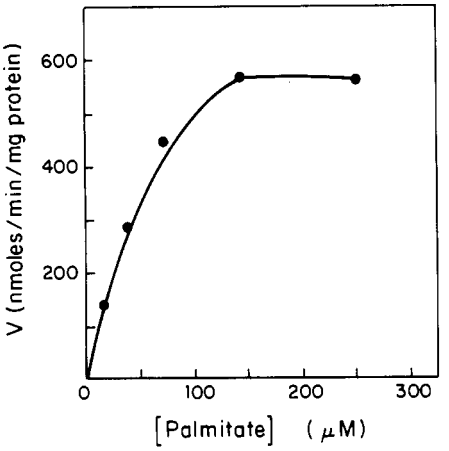

FIGURE 2: Initial rates of activity of palmitoyl-CoA ligase as a function of the total concentration of palmitate in the assay mixture. Each assay contained 174.3 μg of egg phosphatidylcholine added as single-layered liposomes, 8 μg of microsomal protein, and variable amounts of [^{14}C]palmitate. Palmitate was added as the complex liposome–palmitate.

Table I: Effect of Varying Concentrations of Liposomes on Activity of Palmitoyl-CoA Ligase[a]

amount of lipid added to assay	activity	amount of lipid added to assay	activity
160	771	1760	280
640	390	3200	166

[a] Initial rates of enzyme activity were determined as described under Materials and Methods. Assays contained a total of 12.57 nmol of palmitate and 10 μg of microsomal protein in a volume of 0.25 mL. Palmitate was added as the complex liposomes–palmitate, which was used to start the reaction. Liposomes were prepared, as described under Materials and Methods, from phosphatidylcholine from egg yolk. Activities are nanomoles of palmitoyl-CoA synthesized per minute per milligram of microsomal protein. The amounts of lipid added per assay are in micrograms.

based on amounts of palmitate metabolized in the first 30 s of reaction, as in Figure 1.

The data in Figure 2 were obtained in a system with a constant total amount of lipid phase (microsomes and liposomes). The concentration of palmitate was varied by adding a constant amount of unilamellar vesicles that contained variable amounts of palmitate. The rate of synthesis of palmitoyl-CoA was related in this setting directly to the total amount of fatty acid in the assay. By contrast, the specific activity of palmitoyl-CoA ligase decreased when the total amount of palmitate was kept constant, but the total amount of lipid in the assay increased (Table I). A similar effect was observed when the amount of microsomes per assay was increased. These last results are expected since increasing the volume of the lipid phases will lead to a decrease in the concentration of fatty acid in both the lipid and aqueous phases of the assay system. In other words, the decrease in the specific activity of ligase in response to increasing amounts of total lipids in the assay does not represent inhibition by phospholipids of the catalytic rate of the ligase or an alteration of the avidity of the ligase for palmitate. The apparent inhibition of ligase by added phospholipids (Table I) is due to dilution of the fatty acid substrate as the volume of the lipid phase is enlarged. This line of reasoning predicts that added lipids will not appear to influence the activity of acyl-CoA ligase if activities in the presence of different amounts of lipid phase are related to concentrations of palmitate in the lipid phase instead of to the total amount of fatty acid in the assay system.

The data in Figure 3 show rates of metabolism of palmitate as a function of the mole fraction of palmitate in the phospholipids of the membrane compartments of the assay system. The data are plotted in double-reciprocal form. Each assay contained a different amount of added phospholipid. The concentrations of fatty acid in the lipid phases of the assay were calculated from the measured partition coefficient for palmitate in unilamellar vesicles of phosphatidylcholine suspended in buffer (as described under Materials and Methods). We have assumed that palmitate partitions equally between microsomes and added vesicles of phospholipids. This assumption may not be completely valid but introduces, if incorrect, only a small error in the estimated value of K_m^{app} for palmitate. All the data points in Figure 3 fall on a single line. This result confirms that variable amounts of phospholipid added to the assay system affect enzyme activity only because they alter the concentration of palmitate in the lipid phases and/or the aqueous phase of the assay. It follows from the result in Figure 3 that added phospholipids do not influence the ligase except by modifying the concentration of fatty acids.

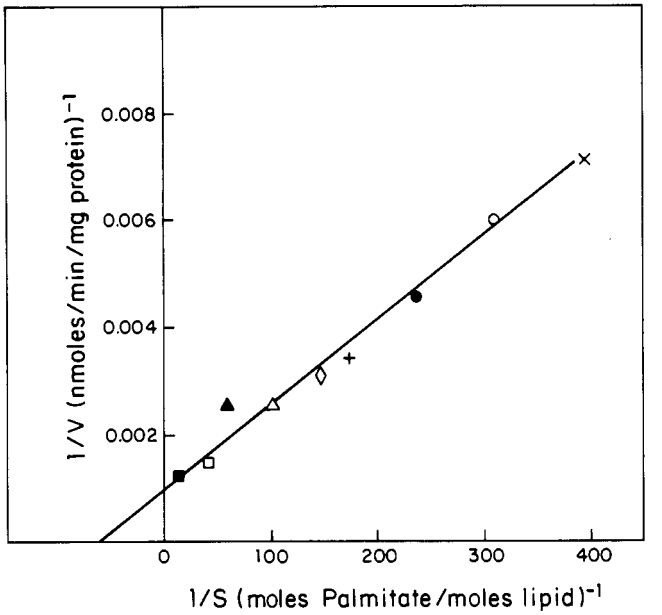

FIGURE 3: Activity of palmitoyl-CoA ligase as a function of the concentration of palmitate in the lipid phase of the assay system. Enzyme activity was measured as described under Materials and Methods in the presence of variable amounts of added liposomes (in nanomoles) per assay: 4000 (○); 3000 (●); 2200 (+); 1874 (◇); 1467 (×); 1468 (□); 1431 (△); 800 (▲); 220 (■). The concentrations of palmitate are expressed as the mole fraction in the total lipid phase of the assay. Data are plotted in double-reciprocal form. Activities are nanomoles of palmitoyl-CoA synthesized per minute per milligram of microsomal protein.

Effect of Fatty Acid Binding Protein and Albumin on Activity of Palmitoyl-CoA Ligase. Liver and intestinal mucosa contain a small protein with high avidity for fatty acids. This protein, which is called fatty acid binding protein, has been observed to enhance the activity of acyl-CoA ligase under the condition that fatty acids are added to assays as salt suspensions (Burnett et al., 1979). It is proposed that the protein facilitates access of fatty acid to the active site of the ligase (Burnett et al., 1979). Albumin, which also enhances the activity of the ligase, is believed to do so via the same mechanism (Burnett et al., 1979). These results have been interpreted as support for the idea that fatty acid binding protein is important for metabolism of fatty acids in cells (Burnett et al., 1979; Mishkin et al., 1972). It was of interest, therefore, to determine the effect of fatty acid binding protein and albumin on acyl-CoA ligase activity in the system described by Figure 1. Both of these proteins inhibit the rate of metabolism

Table II: Reported Activities for Microsomal Palmitoyl-CoA Ligase

ref	act. [nmol min^{-1} (mg of protein)$^{-1}$]	comment
Suzue & Marcel (1972)	222	V_{max} for palmitate, CoA, and ATP concn optimal; palmitate added in suspension of Triton WR-1339
Pande & Meade (1968)	113.3	all substrate concn optimal; palmitate added as salt solution
Aas (1971)	120	all substrate concn optimal; assays included 0.83% albumin and 0.1% Triton X-100
Tanaka et al. (1979)	340	all substrate concn optimal; palmitate added in suspension of Triton X-100
Farstad et al. (1967)	22	all substrate concn optimal; palmitate added complexed with albumin at 6:1 mol/mol ratio
present work	990	V_{max} for palmitate, CoA, and ATP concn optimal; palmitate added incorpd in liposomal bilayer

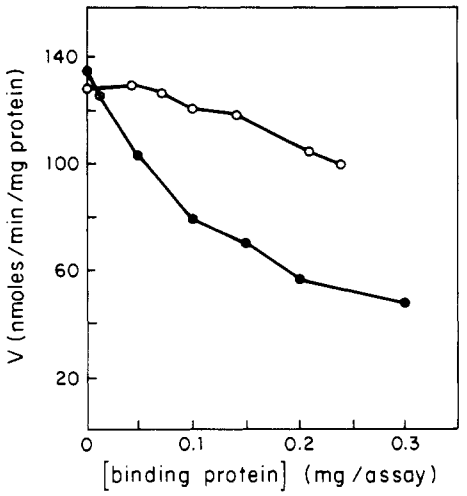

FIGURE 4: Effect of fatty acid binding protein and albumin on the activity of palmitoyl-CoA ligase. Variable amounts of partially purified fatty acid binding protein (○) or albumin (●) were added to an assay system containing 14 μg of microsomal protein and 1.5 nmol of [^{14}C]palmitate in 85.5 μg of liposomes made from egg phosphatidylcholine.

of palmitate when this fatty acid is present initially as an integral component of a unilamellar vesicle of phosphatidylcholine (Figure 4). The difference in the extent of inhibition due to the fatty acid binding protein vs. albumin reflects that a given amount of albumin vs. fatty acid binding protein binds more fatty acids.

Fatty Acid in the Aqueous Phase of the Assay System Is Not the Substrate for Acyl-CoA Ligase. The data presented above and in Daniels et al. (1985) indicate that the rate of movement of palmitate between liposomes and water and between water and microsomes is rapid as compared with the catalytic activity of palmitoyl-CoA ligase. This makes it impossible to determine directly whether the active site of this enzyme equilibrates with the pool of palmitate in the membranes or in the aqueous phases. We attempted to resolve this question in the following way. Since fatty acids have limited solubility in water but are miscible completely in lipid bilayers, one can establish conditions in which the concentration of fatty acid in the aqueous phase of the assay system not only is small but also is smaller than the concentration of the acyl-CoA ligase. If the fatty acids in the aqueous phase represented the pool of substrate interacting directly with the enzyme, the assumptions of Michaelis–Menten kinetics would not be valid under this set of conditions, that is, when concentration of enzyme exceeded concentration of substrate.

It was not possible technically to add more than 6 mg of microsomal protein per milliliter of assay mixture, which placed an upper limit on the concentration of acyl-CoA ligase in the assay. The molecular weight of purified acyl-CoA ligase is reported as 66 000–75 000, and the abundance of this enzyme in microsomal proteins is between 1% and 5% (Bar-Tana et al., 1971; Tanaka et al., 1979). If we take the molecular weight as 75 000 and the abundance as 1% of microsomal protein, then 6 mg of microsomal protein contains about 0.8 nmol of active sites of the ligase. Assays of acyl-CoA ligase were conducted under conditions in which the concentration of fatty acid in the aqueous phase of the system was less than or about equal to the concentration of the ligase. It was not possible to arrive at assay conditions in which the concentraiton of active sites of the ligase was of the same order as the aqueous phase concentration of palmitate. This condition could be achieved with stearate as the fatty acid because this fatty acid partitions more favorably into bilayers (partition coefficient of 4×10^6 in favor of bilayers for palmitate vs. 10^7 for stearate). Initial rates of synthesis of stearoyl-CoA were determined as in Figure 1 and 2 as a function of variable amounts of stearate per assay but constant amounts of phospholipid. The concentration of stearate in the aqueous phase of each assay was calculated from the measured partition coefficient and known amounts of lipid and aqueous phases. Rates of synthesis of stearoyl-CoA were plotted in double-reciprocal form as a function of the calculated concentrations of stearate in the aqueous phase. These data indicated a rate of synthesis of stearoyl-CoA at V_{max} of 500 nmol min^{-1} (mg of microsomal protein)$^{-1}$. The rate was $0.5V_{max}$ at 0.15 μM stearate in the aqueous phase. Since the concentration of ligase was 0.8 μM in these assays, this result indicates that the pool of stearate within the microsomal membrane interacted directly with the active sites of the ligase. Thus, it is a physical impossibility for 0.15 nmol of stearate to occupy half the binding sites of 0.8 nmol of acyl-CoA ligase.

Discussion

The data reported above indicate that the activity of microsomal fatty acyl-CoA ligase has been underestimated by all previous investigators by about 1 order of magnitude (Table II). The reasons for the large differences between rates reported here and those recorded previously in the literature are not clear, but there are several possibilities. We believe the most significant difference between previous assay systems and the one utilized herein is that there is considerable uncertainty with the former systems as to the physical state of the added fatty acids. It can be demonstrated, for example, that all the fatty acids added to an assay as a salt suspension are not utilized by acyl-CoA ligase. This result suggests that fatty acids added in this manner precipitated out of the aqueous phase. Hence, it is possible that rates of acyl-CoA ligase measured previously were rates of solvation of fatty acids precipitated out of the aqueous phase. It has been shown recently, for example, that microcrystals of benzo[*a*]pyrene dissolve slowly in water (Li et al., 1983) even after absorption of the microcrystals to phospholipid vesicles. A similar mechanism could be the basis for slow rates of metabolism of palmitate to palmitoyl-CoA when a salt suspension of palmitate is added to microsomes suspended in aqueous buffer. As compared with addition of salt suspensions of palmitate to an aqueous suspension of microsomes, palmitate added as a component of a single-layered vesicle of phospholipid is readily available to the active site of acyl-CoA ligase. The

data are consistent with the conclusion that palmitate reaches the latter sites via rapid desorption from the vesicles because this process is known to proceed far more rapidly (half-times on the order of 50 ms) than the rate of metabolism of palmitate (Daniels et al., 1985; Doody et al., 1980).

There are other possible reasons for past failure to measure the full catalytic activity of fatty acyl-CoA ligase. It has been reported that detergents used previously to aid in dispersing fatty acids are inhibitors of the ligase (Pande, 1972). This is interesting because albumin and fatty acid binding protein enhance the activity of acyl-CoA ligase in assay systems containing detergent (Burnett et al., 1979) but not in the system used herein. Activation of ligase by these proteins could reflect removal of detergent that otherwise would interact with the ligase.

Irrespective of the exact mechanism for differences in the activity of the ligase reported here and previously, the data in Figure 1 show that adding palmitate as a component of a lipid bilayer has significant advantages for assays of the ligase. This type of assay system may prove to have general applicability for assay of other membrane-bound enzymes that have water-insoluble substrates.

Significance of Membrane-Bound Substrate. The data support the idea that fatty acids or at least stearate in the aqueous phase of the reaction is not the substrate for the ligase. Instead, the fatty acid within the microsomal membrane itself appears to be the proximate pool of substrate for the enzyme. This result is interesting because the membrane not only contains the enzyme but also concentrates the substrate. We have pointed out previously that this feature of membrane-bound enzymes is important in the glucuronidation of estrone by microsomal UDP-glucuronyltransferase (Zakim & Vessey, 1980; Boyer et al., 1980). It seems as if the localization of membrane-bound enzymes in membranes can be understood in many instances in terms of the substrates with which they interact. The interaction of at least two membrane-bound enzymes with substrates within the membrane also is important in the context of the intracellular movement of the water-insoluble substrates for the enzymes. For the case of fatty acids, for example, the interaction between the ligase and microsomal palmitate suggests that transport of fatty acids from the inner half of the plasma membrane of cells to the active site of palmitoyl-CoA ligase in the endoplasmic reticulum depends primarily on transport of fatty acids to the endoplasmic reticulum and not directly via the cytosol to the active site of the enzyme.

Implications of the Data for the Mechanism of Intracellular Transport of Fatty Acids. Since naturally occurring long-chain fatty acids are poorly soluble in water, a special mechanism has been postulated to account for the transport of fatty acids through the aqueous phase of the cell. By analogy with established mechanisms for transport of water-insoluble compounds in blood, it is believed that fatty acids are transported between intracellular compartments via binding to carrier proteins (Ockner & Manning, 1982; Burnett et al., 1979; Mishkin et al., 1972; Trutzch & Atias, 1981; Ockner et al., 1972). The identification in cytosol of a low molecular weight protein that binds fatty acids has been taken as support for the idea that fatty acids are transported within cells as complexes with carrier proteins (Ockner & Manning, 1982; Mishkin et al., 1972; Ockner et al., 1972). Another observation considered as support for the concept of carrier-mediated, intracellular transport of fatty acids is that the so-called fatty acid binding protein purified from the cytosol of cells enhances the rate of synthesis of fatty acyl-CoA compounds when added to an assay mixture containing fatty acids, microsomes, detergent, ATP, and CoA (Burnett et al., 1979). There is, however, no direct evidence that fatty acid binding protein or any other protein actually enhances the rate of transport of fatty acids between hydrophobic compartments that are separated by water. The data presented above and in Daniels et al. (1985) provide, we believe, further clues as to the possible mechanism of intracellular transport of long-chain fatty acids.

The assay system utilized in Figure 1 depends on transfer of palmitate between lipid vesicles separated by water. The data presented indicate that this transfer occurs spontaneously at a rate that exceeds by far the catalytic capacity of the acyl-CoA ligase. Thus, although fatty acids are poorly soluble in water, they can move rapidly from a hydrophobic to an aqueous phase. It seems, then, that the dissociation of fatty acids from the plasma membrane of cells will not be a rate-determining step in their transfer to the site of metabolism in the endoplasmic reticulum membranes. The assay conditions described here allow for a fatty acid flux from vesicles to microsomes that can easily keep up with the rate of catalysis when the enzyme is saturated with fatty acid. In vivo, the flux will depend on the concentration gradient of fatty acid between the outer membrane of the cells and the endoplasmic reticulum membranes and on the distance between those membranes. The magnitude of the gradient will depend in turn on the supply of fatty acids to the plasma membrane on one hand and on the rate of depletion by metabolizing enzymes on the other hand.

Registry No. EC 6.2.1.3, 9013-18-7; palmitic acid, 57-10-3.

REFERENCES

Aas, M. (1971) *Biochim. Biophys. Acta 231*, 32–47.

Bar-Tana, J., Rose, G., & Shapiro, B. (1971) *Biochem. J. 122*, 353–362.

Boyer, T. D., Zakim, D., & Vessey, D. A. (1980) *J. Biol. Chem. 255*, 627–631.

Burnett, D. A., Lysenko, N., Manning, J., & Ockner, R. K. (1979) *Gastroenterology 77*, 241–249.

Daniels, C. K., Noy, N., & Zakim, D. (1985) *Biochemistry* (in press).

Dole, V. P. (1956) *J. Clin. Invest. 35*, 15–154.

Doody, M. C., Pownall, H. J., Kao, Y. J., & Smith, L. C. (1980) *Biochemistry 19*, 108–116.

Farstad, M., Bremer, J., & Norum, K. R. (1967) *Biochim. Biophys. Acta 132*, 492–502.

Li, K.-P., Li, Y.-Y., & Boley, L. (1983) *Biochem. Biophys. Res. Commun. 112*, 1069–1076.

Lowry, O. H., & Lopez, J. A. (1946) *J. Biol. Chem. 162*, 421.

Mishkin, S., Stein, L., Gatmaitan, Z., & Arias, I. M. (1972) *Biochem. Biophys. Res. Commun. 47*, 997–1003.

Ockner, R. K., & Manning, J. A. (1972) *J. Biol. Chem. 257*, 7872–7878.

Ockner, R. K., Manning, J. A., Poppenhausen, R. B., & Ho, W. K. L. (1972) *Science (Washington, D.C.) 172*, 56–58.

Pande, S. V. (1972) *Biochim. Biophys. Acta 270*, 197–208.

Pande, S. V., & Meade, J. F. (1968) *J. Biol. Chem. 243*, 352–361.

Suzue, G., & Marcel, Y. L. (1972) *J. Biol. Chem. 247*, 6781–6783.

Tanaka, T., Hosaka, D., Hoshimaru, M., & Numa, S. (1970) *Eur. J. Biochem. 98*, 165–172.

Trulzch, D., & Arias, I. M. (1981) *Arch. Biochem. Biophys. 209*, 433–330.

Zakim, D., & Vessey, D. A. (1977) *J. Biol. Chem. 252*, 7534–7537.