Effect of liver fatty acid binding protein on fatty acid movement between liposomes and rat liver microsomes

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Although movement of fatty acids between bilayers can occur spontaneously, it has been postulated that intracellular movement is facilitated by a class of proteins named fatty acid binding proteins (FABP). In this study we have incorporated long chain fatty acids into multilamellar liposomes made of phosphatidylcholine, incubated them with rat liver microsomes containing an active acyl-CoA synthetase, and measured formation of acyl-CoA in the absence or presence of FABP purified from rat liver. FABP increased about 2-fold the accumulation of acyl-CoA when liposomes were the fatty acid donor. Using fatty acid incorporated into liposomes made either of egg yolk lecithin or of dipalmitoylphosphatidylcholine, it was found that the temperature dependence of acyl-CoA accumulation in the presence of FABP correlated with both the physical state of phospholipid molecules in the liposomes and the binding of fatty acid to FABP, suggesting that fatty acid must first desorb from the liposomes before FABP can have an effect. An FABP–fatty acid complex incubated with microsomes, in the absence of liposomes, resulted in greater acyl-CoA formation than when liposomes were present, suggesting that desorption of fatty acid from the membrane is rate-limiting in the accumulation of acyl-CoA by this system. Finally, an equilibrium dialysis cell separating liposomes from microsomes on opposite sides of a Nuclepore filter was used to show that liver FABP was required for the movement and activation of fatty acid between the compartments. These studies show that liver FABP interacts with fatty acid that desorbs from phospholipid bilayers, and promotes movement to a membrane-bound enzyme, suggesting that FABP may act intracellularly by increasing net desorption of fatty acid from cell membranes.

INTRODUCTION

Fatty acid binding protein (FABP) is the name given to any of a class of proteins which occur in several cell types (Bass, 1985; Ockner *et al.*, 1972). The binding of long-chain fatty acids by these proteins (Fournier *et al.*, 1983; Ockner & Manning, 1974; Ockner *et al.*, 1972), as well as accumulated circumstantial evidence implicating liver FABP with fatty acid metabolism (Renaud *et al.*, 1978; Wu-Ridout *et al.*, 1976; Mishkin *et al.*, 1975; Levi *et al.*, 1969), are the main reasons for the prevalent opinion that their function is to mediate intracellular fatty acid movement. Of all the FABP proteins reported to date, perhaps the most thoroughly studied is rat liver FABP. This protein has been purified and characterized (Dempsey *et al.*, 1981; Ockner *et al.*, 1982; Takahashi *et al.*, 1983) and recently the cloning, sequencing and bacterial expression of the gene has been reported (Gordon *et al.*, 1983; Lowe *et al.*, 1985; Chan *et al.*, 1985).

The above studies have provided information on the structure of liver FABP, but data related to its function are less extensive. It has been suggested that an FABP–acyl-CoA interaction prevented acyl-CoA from inhibiting a variety of intracellular enzymes (Lunzer *et al.*, 1977; Barbour & Chan, 1979; Grinstead *et al.*, 1983; Rhoads *et al.*, 1983). Data from other studies have reported liver FABP to either stimulate or inhibit microsomal and mitochondrial acyl-CoA synthetase activity (Wu-Ridout *et al.*, 1976; Burnett *et al.*, 1979; Noy

& Zakim, 1985). Effects FABP may have on the activity of acyl-CoA synthetase are of interest, relative to a functional role for FABP, since fatty acid activation is the initial step for most metabolic pathways involving intracellular fatty acids.

Using an acyl-CoA ligase associated with rat liver microsomes, Noy & Zakim (1985) have recently shown that palmitate incorporated in unilamellar vesicles is a convenient substrate for this enzyme, since fatty acid transferred rapidly and spontaneously from vesicles to microsomes via a hydrated intermediate. Addition of either FABP or albumin to the assay actually reduced acyl-CoA formation. The results led the authors to suggest that the flux of fatty acid from plasma membrane to endoplasmic reticulum could be determined mainly by the supply of fatty acids to the plasma membrane and their depletion by metabolizing enzymes on the endoplasmic reticulum. They implied that FABP may not have a major influence on fatty acid flux (Noy & Zakim, 1985).

In a previous study (Brecher *et al.*, 1984), we developed an assay *in vitro* for measuring the movement of fatty acids from multilamellar liposomes to binding proteins such as liver FABP and albumin, and showed that liposomes could readily donate much of the incorporated fatty acid for binding by either albumin or liver FABP. In the present study we used rat liver microsomes as a source of acid:CoA ligase (AMP-forming), EC 6.2.1.3 (acyl-CoA synthetase), and showed that liver FABP promoted the movement of fatty acid

Abbreviation used: FABP, fatty acid binding protein.

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from liposomes to the microsomal enzyme, suggesting that FABP may influence fatty acid flux within the cell.

EXPERIMENTAL

Materials

Chemicals. [$1\text{-}^{14}\text{C}$]Oleic acid (56 mCi/mmol), [$2\text{-}^{14}\text{C}$]palmitic acid (54 mCi/mmol), [$2\text{-palmitoyl-9,10-}^3\text{H}$]dipalmitoylphosphatidylcholine (60 Ci/mmol) and [$1\text{-}^{14}\text{C}$]oleoyl-CoA (56 mCi/mmol) were purchased from New England Nuclear. Egg yolk lecithin (Grade 1) (i.e. phosphatidylcholine of unspecified fatty acid composition) was obtained from Lipid Products (Surrey, U.K.). All unlabelled lipids and bovine serum albumin (crystallized and lyophilized) were from Sigma. Fluorinert was purchased from ISCO.

Animals. Male Wistar rats were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) and used at body weights ranging from 250 to 350 g.

Methods

Preparation of microsomal fraction. Rat liver microsomes were prepared from tissue homogenized in 4 vol. of a solution containing 0.25 M-sucrose, 1 mM-EDTA and 10 mM-Hepes, pH 7.4. The homogenate was centrifuged at 22000 g for 15 min and the resulting supernatant was centrifuged for 60 min at 150000 g. The microsomal pellet was washed once in buffer without EDTA, resuspended in the same buffer at a concentration of 6–10 mg/ml and stored in portions at -70°C .

Preparation of rat liver FABP. The protein was purified by minor modifications of the procedure of Dempsey *et al.* (1981) as described recently by us (Offner *et al.*, 1986). The final product was routinely characterized by u.v. spectroscopy and by SDS/polyacrylamide-gel electrophoresis to ensure a single band of about 14000 M_r . FABP was stored at a final concentration of 2–2.5 mg/ml in a buffer containing 30 mM-Tris and 50 mM-NaCl, pH 8.5.

Preparation of liposomes. Liposomes were prepared as described previously (Brecher *et al.*, 1984). Routinely, 1 ml of liposomes were made containing 40 mM-lecithin, 0.4–1.1 mM-fatty acid, 1.0 μCi of [^3H]dipalmitoylphosphatidylcholine and 1.0 μCi of ^{14}C -labelled fatty acid. Liposomes were stored at 4°C .

Preparation of vesicles. Unilamellar vesicles were made from liposome preparations by sonication using methodology previously established in our laboratory (Burrier & Brecher, 1983). Vesicles were characterized on a BioGel A-15 column (2.5 cm $\times$ 35 cm) to ensure that more than 95% of the labelled phospholipid and fatty acid co-eluted in a region corresponding to particles having a diameter of about 22.5 nm. Vesicles were stored at 4°C .

Acyl-CoA synthetase assay. The reaction mixture typically contained 2 mM-ATP, 0.75 mM-CoA, 0.5 mM- MgCl_2 , 100 mM-NaCl, 10 mM-Tris, pH 7.4, the designated amount of liposomes or vesicles containing labelled fatty acid, and 2–6 μg of microsomal protein, all in a total volume of 100 μl . Reactions were initiated by

adding the substrate to tubes that had been preincubated for 2 min at 37°C , then, after incubation for the designated time, the reaction was terminated by adding 2 ml of chloroform:methanol (2:1, v/v). A two-phase system was formed by adding 0.4 ml of saline and labelled acyl-CoA was determined by measuring radioactivity in the upper phase (Brecher & Chobanian, 1974). Assays were expressed as nmol of acyl-CoA formed per mg of microsomal protein.

Binding assay. Binding of fatty acids to FABP was accomplished by incubating liposomes containing labelled fatty acid with FABP, allowing the fatty acid to partition between liposomes and protein, and then separating liposomes from protein by centrifugation. The procedure used was similar to that described recently (Brecher *et al.*, 1984).

Preparation of fatty acid-FABP complex. FABP (20 μM) was incubated with liposomes containing egg lecithin/cholesterol/fatty acid (molar proportions 20:7:1). The fatty acid concentration in the reaction mixture was at least twice that of the FABP present and the specific activity was between 2000 and 5000 d.p.m./nmol. Following incubation for 60 min at 25°C the complex was separated from liposomes by centrifugation at 100000 g.

Equilibrium dialysis experiments. The two compartments of an equilibrium dialysis cell were separated by a polycarbonate disc membrane (Nuclepore capillary pore membrane; pore diameter 0.2 μm) that was moistened in water prior to use. The indicated amounts of liposome, protein, buffer, enzyme cofactors and microsomes were added to the appropriate side of the cell in a final volume of 1.5 ml. The cells were incubated at room temperature on an orbital rotating platform for the designated times. In most cases 100 μl aliquots were withdrawn and extracted according to the procedure of Folch *et al.* (1957) to separate acyl-CoA from fatty acid.

Sucrose density gradient centrifugation. Aliquots (200 μl) of reaction mixtures containing microsomes and either vesicles or liposomes were layered onto 4.0 ml of a linear density gradient (25–45% sucrose) in a buffer containing 0.1 M-NaCl and 0.01 M-Tris, pH 7.4. Centrifugation was for 18 h using a Beckman SW60 Ti rotor at 50000 rev./min and 4°C in a Beckman L265B ultracentrifuge. Gradients were fractionated by piercing the bottom of the tube and pumping in Fluorinert. Aliquots (200 μl) were collected and assayed for protein and radioactivity.

Analytical procedures. Protein concentrations were determined by the method of Lowry *et al.* (1951), using bovine serum albumin as standard. In our laboratory this procedure overestimates the true amount of rat liver FABP by 1.45 (Offner *et al.*, 1986); appropriate corrections were made.

RESULTS

Effect of FABP on accumulation of oleoyl-CoA generated by microsomal acyl-CoA synthetase

Fig. 1 is a comparison of acyl-CoA formation with time when either unilamellar vesicles or multilamellar

Fig. 1. Effect of liver FABP on microsomal acyl-CoA synthetase activity using as substrate oleic acid contained in either micelles, unilamellar vesicles or multilamellar liposomes

Reaction mixtures contained 10 μg of microsomal protein and 24 μM-oleic acid added in either Triton X-100:oleic acid micelles (0.1% Triton) (□), egg lecithin vesicles (molar ratio 32:1) (△), or egg lecithin liposomes (molar ratio 32:1) (○). FABP was added to a final concentration of 50 μM in the presence of either vesicles (▲) or liposomes (●).

liposomes (both consisting of egg lecithin and oleic acid, molar ratio 35:1) were used to supply an equimolar amount of fatty acid to the microsomal acyl-CoA synthetase assay. Use of vesicles to introduce substrate to the assay resulted in the rate of acyl CoA formation being 10-fold greater and the amount of acyl-CoA formed being 4-fold greater than when liposomes were used. Use of mixed micelles of Triton X-100 and fatty acid as substrate resulted in rates of acyl-CoA formation even more rapid than when vesicles were used. Micelles also resulted in a greater amount of acyl-CoA being formed, approx. 100% substrate conversion compared with about 70% for vesicles. Liposomes, when used to introduce fatty acid into the assay, resulted in less than 50% of substrate being converted to product. Addition of FABP to either the vesicle or liposomal system resulted in a decrease or increase, respectively, of the rates of acyl-CoA formation.

Fig. 2 shows that microsomal acyl-CoA synthetase activity was dependent upon oleic acid concentration when fatty acid was introduced to the assay incorporated in either vesicles or liposomes. The effect of FABP, to increase formation of oleoyl-CoA when fatty acid is supplied via liposomes and decrease acyl-CoA formation

Fig. 2. Effect of substrate and FABP on acyl-CoA synthetase activity

Reaction mixtures contained either varying amounts of egg lecithin:oleic acid vesicles (molar ratio 32:1) and 0.5 μg of microsomal protein, or varying amounts of egg lecithin liposomes (molar ratio 32:1) and 6 μg of microsomal protein. Incubations were for 3 min or 10 min using vesicles and liposomes, respectively. Concentration of FABP was 15 μM. △, Vesicles alone; ▲, vesicles + FABP; ○, liposomes alone; ●, liposomes + FABP.

Fig. 3. Sucrose density gradient centrifugation of liver microsomes mixed with either egg lecithin vesicles or liposomes

Microsomes (300 μg of protein) were mixed with 200 μg of either phospholipid preparation in a total volume of 200 μl of buffer, incubated for 5 min at ambient temperature and applied to preformed sucrose gradients. Protein distribution throughout the gradient was determined following fractionation of the tube. ●, Microsomes alone; ○, microsomes plus vesicles; △, microsomes plus liposomes.

when supplied via vesicles, was consistent over the substrate concentration range used.

Interaction of vesicles with microsomes

Fig. 3 shows a sucrose density gradient ultracentrifugation sedimentation profile of microsomes which were

incubated in the absence or presence of vesicles. Addition of vesicles caused the sedimentation properties of the microsomes to change, resulting in a less-dense microsomal particle population. When microsomes were incubated in the presence of liposomes, an appreciable shift in density of the microsomes was not observed. Additional data (not shown), tracing the distribution throughout the gradient of radiolabelled phospholipid from either liposomes or vesicles in the presence of microsomes, revealed that about 15% of the total vesicle phospholipid was associated with microsomal protein whereas almost none of the liposomal phospholipid migrated to that region. These data, indicative of an interaction between microsomes and vesicles, presented complicating variables in the use of vesicles; for this reason we focused attention on the enhancing effect of FABP when liposomes were the donor of fatty acid.

Characteristics of liposome assay system with FABP

In experiments not shown we established that the dependency of the reaction on ATP, CoA, and Mg^{2+} was consistent with previous studies of microsomal acyl-CoA synthetase (Bar-Tana *et al.*, 1971; Normann *et al.*, 1981), and the effect of FABP was not revealed to be any more dependent on the cofactors than the reaction itself. These data suggest that neither the presence of FABP nor the use of liposomes to introduce fatty acid to the assay caused any change in cofactor requirements of the microsomal enzyme.

FABP-oleic acid complex as fatty acid donor

Experiments were performed to test for acyl-CoA formation when an FABP-fatty acid complex was used to deliver fatty acid to the microsomal acyl-CoA synthetase. An FABP-fatty acid complex was made by incubating FABP with liposomes that contained fatty acid, followed by separation of the liposomes by centrifugation. The supernatant was used as substrate source for microsomal acyl-CoA synthetase. Table 1 is a comparison of reaction rates for oleoyl-CoA formation. When an FABP-oleic acid complex was used as substrate, the rate of acyl-CoA formation was almost 7-fold greater than when liposomes were used, but only 60% of that observed when vesicles were used. The

FABP-oleic acid complex also resulted in a reaction rate intermediate between that of liposomes plus FABP and vesicles plus FABP.

The physical state of liposomes influences their effectiveness as fatty acid donors

Fig. 4 shows a temperature dependence for both formation of acyl-CoA and binding of fatty acid by FABP. Dipalmitoylphosphatidylcholine undergoes a transition from a gel to a more loosely packed liquid crystalline state at about 42 °C. Using dipalmitoylphosphatidylcholine liposomes containing fatty acid as substrate for the synthetase assay or donors for a binding assay, the temperature-dependence of both binding and activation were shown to be correlated with the physical state of the liposomes. When liposomes were in the liquid crystalline state both activation and binding were greater than when in the gel state, with the most marked change occurring several degrees below the transition temperature for pure dipalmitoylphosphatidylcholine liposomes. This probably reflects physical chemical changes caused both by fatty acid in the bilayer,

Fig. 4. Effect of temperature on acyl-CoA formation using dipalmitoylphosphatidylcholine liposomes and an FABP-fatty acid complex

All reaction mixtures contained 20 μ M-fatty acid. Microsomes, added at a concentration of 60 μ g/ml when acyl-CoA was measured, were not included in the binding assay. Palmitic acid was incorporated into dipalmitoylphosphatidylcholine liposomes (molar ratio 100:1). FABP, when added to reaction mixtures containing liposomes, was present at 20 μ M. Fatty acid-FABP complexes were added as equimolar mixtures of either oleic or palmitic acid and FABP (20 μ M). Reactions containing liposomes were incubated for 10 min and those with fatty acid-FABP complex were incubated for 2 min. $\circ$, Dipalmitoylphosphatidylcholine liposomes alone; $\bullet$, dipalmitoylphosphatidylcholine liposomes + FABP; $\triangle$, oleate-FABP complex; $\blacktriangle$, palmitate-FABP complex. Binding assay: $\blacksquare$, dipalmitoylphosphatidylcholine liposomes + FABP.

Table 1. Comparison of rates of oleoyl-CoA formation using liposomes, vesicles, and FABP as fatty donors

Oleic acid was added at a concentration of 20 μ M when liposomes or vesicles (both with a lecithin:oleic acid molar ratio of 100:1) acid were used, and 12 μ M when the FABP-oleic acid complex (molar ratio 5:3) was used. FABP, when present, was 20 μ M. All reaction mixtures contained microsomal protein (60 μ g/ml) and appropriate cofactors, and were incubated for 2 min at 37 °C. Data represent the mean $\pm$ S.E.M. for seven or eight separate determinations.

Substance added	Reaction rate (nmol/min per mg)
Vesicles	57 $\pm$ 2
Vesicles + FABP	50 $\pm$ 2
FABP-oleic acid complex	35 $\pm$ 1
Liposomes + FABP	10 $\pm$ 1
Liposomes	5 $\pm$ 0.5

Fig. 5. Effect of liver FABP on the movement of fatty acid from liposomes to microsomes using a Nuclepore filter to separate the bilayer systems

Liposomes containing 20 μM -oleic acid (molar ratio 100:1) were on one side of the equilibrium dialysis cell and microsomes (600 $\mu\text{g}/\text{ml}$) were on the opposite side. Liver FABP was added to the liposome side at a final concentration of 20 μM . Aliquots (100 μl) were removed at designated times and acyl-CoA and free fatty acid were measured. ●, Liver FABP added, oleoyle-CoA measured; ○, liver FABP added, oleate measured; ▲, no FABP, oleoyle-CoA measured; △, no FABP, oleate measured.

reactants and possibly products of the reaction mixtures, as well as properties of the enzyme.

FABP-fatty acid complexes, using either oleate or palmitate, were assayed under comparable conditions. Acyl-CoA formation was greater than with liposomes as fatty acid donor, and the temperature dependence observed with dipalmitoylphosphatidylcholine liposomes was not found. Comparable experiments were carried out using both palmitic and oleic acid incorporated into either egg lecithin or dipalmitoylphosphatidylcholine liposomes, and no appreciable differences were found (results not shown).

Transport properties of FABP

The ability of FABP to transport fatty acids was shown by adding fatty-acid-containing liposomes from rat liver microsomes to opposite sides of a Nuclepore filter in an equilibrium dialysis apparatus. Control experiments established that microsomal acyl-CoA synthetase activity did not traverse the filter, nor did fatty acid or phospholipid from liposomes. As shown in Fig. 5, there was neither acyl-CoA formation nor movement of fatty acid from the side containing liposomes to the side containing microsomes. Addition of liver FABP to the side containing fatty acid-containing liposomes resulted in a time-dependent increase in the concentrations of both fatty acid and acyl-CoA on the microsomal side. Comparable results (not shown) were obtained using the following conditions: addition of liver FABP originally to the microsomal side (indication that protein moved readily between compartments), use of palmitate rather than oleate, and use of different concentrations of FABP and fatty acid while keeping the molar ratio of fatty acid to FABP approximately the same. Separate experiments, where radioactivity was

Table 2. Effect of different proteins on acyl-CoA synthetase activity

Reaction mixtures contained 20 μM -oleic acid in egg lecithin liposomes (molar ratio 32:1) and 6 μg of microsomal protein, the necessary cofactors, and 50 μM of the designated soluble protein.

Protein added	Oleoyle-CoA formed	
	(nmol/min per mg)	(% of control)
None	3.8	100
Lysozyme	3.3	87
Ribonuclease	3.7	97
Myoglobin	3.8	100
Ovalbumin	4.3	113
Albumin	7.5	197
Liver FABP	7.2	189

analysed by t.l.c., showed virtually all radioactivity present on the microsomal side to be either acyl-CoA or fatty acid. No radioactivity was detectable in any of the major phospholipid classes, triacylglycerol or lysolecithin.

Specificity of FABP effect

Myoglobin and other proteins not capable of binding fatty acids were each tested for the property of facilitating fatty acid movement and subsequent activation by using the equilibrium dialysis procedure. None of these proteins appreciably facilitated either fatty acid movement across the membrane or activation (results not shown). In a similar manner, myoglobin, lysozyme, ribonuclease, and ovalbumin were each added separately to the assay *in vitro*. All of these proteins, except ovalbumin, resulted in acyl-CoA formation being nearly equal to control values (no protein added). Addition of ovalbumin resulted in a 13% increase over control (Table 2). Albumin, when added to the assay *in vitro*, resulted in a 97% increase over control, an increase similar to that resulting from addition of liver FABP.

DISCUSSION

In this study we made use of two types of synthetic phospholipid bilayers, unilamellar vesicles and multilamellar liposomes, as a method of supplying long chain fatty acid to assay microsomal acyl-CoA synthetase. Previous studies had shown liposomes to be a convenient donor of fatty acid in an aqueous assay, since they provided a means of adding relatively high concentrations of fatty acid which was accessible to binding by either albumin or liver FABP (Brecher *et al.*, 1984).

Vesicles have also been shown by Noy & Zakim (1984) to be effective donors of fatty acid; those authors used them to introduce fatty acid into an assay for microsomal acyl-CoA synthetase. Observations made by those authors, regarding both the rate of fatty acid desorption from vesicles and a decrease in acyl CoA production following addition of FABP, led them to conclude that FABP is unnecessary for fatty acid

movement throughout the cell (Noy & Zakim, 1984). Contrary to their conclusions, properties of FABP emphasized by our use of liposomes as fatty acid donors lend credence to the involvement of FABP in fatty acid movement throughout the cell.

Use of liposomes resulted in rates of acyl CoA formation that were approx. 9–10-fold slower than when vesicles were used. In these experiments both fatty acid concentration and the fatty acid:lecithin molar ratio were identical for liposomes and vesicles. Assuming fatty acid concentration to be equal in all phospholipid bilayers of vesicles and liposomes, then a possible explanation for the difference in rates may be due to there being a 7-fold greater amount of fatty acid located on the outermost leaflets of vesicles than on outermost leaflets of liposomes. In unilamellar vesicles, the outermost leaflet comprises 67% of their total phospholipid (Mason & Huang, 1978), while in liposomes about 10% of the total phospholipid is on the outer leaflets (Bangham *et al.*, 1967). If it is assumed that desorbed fatty acid constitutes a pool that is the most accessible substrate to membrane-bound enzyme, and that desorption is proportional to fatty acid concentration at the lipid-water interface, then the concentration of desorbed fatty acid will be greater when vesicles are donor particles.

Alternatively, if the same assumption about desorbed fatty acid being the most accessible substrate is made, then the substrate concentration will also be greater when hydration rates are faster. Rapid desorption of fatty acid from vesicles has been indicated by studies of Doody *et al.* (1980), Daniels *et al.* (1985), and Sengupta *et al.* (1976), the last having employed fluorescent techniques to show that exchange of fatty acids between unilamellar structures was far more rapid than between multilamellar liposomes. Greater rates of fatty acid desorption from vesicles also could result from the greater bilayer curvature in vesicles, as documented in studies on cholesterol exchange between bilayers where, by using vesicles of different sizes, it was shown that a more rapid transfer of cholesterol into the aqueous phase resulted when curvature was increased (McLean & Phillips, 1984; Fugler *et al.*, 1985).

To examine the possibility that FABP is regulating the accessibility of fatty acid to the membrane-bound enzyme, an FABP-fatty acid complex was used as substrate in the synthetase assay. Rates of acyl-CoA accumulation obtained using the complex were intermediate to those obtained using vesicles or liposomes as the donor of fatty acid. They were also intermediate to those obtained when FABP was present in assays where vesicles or liposomes were the donor of fatty acid, suggesting that FABP binds fatty acid originally associated with either vesicle or liposome and subsequently acts as fatty acid donor for the synthetase.

The observation that vesicles interact (possibly by either adsorption or fusion) with microsomes, is a complication in analysing the effect of FABP on the vesicle system. DiCorleto & Zilversmit (1977) noted that fusion or adsorption between unilamellar vesicles and mitochondria occurred, complicating interpretation of phospholipid exchange studies between sonicated bilayer and mitochondria, but multilamellar particles did not interact with mitochondria and were the preferable synthetic bilayer for those studies.

Our data suggest that fatty acid is probably desorbed from the liposome before it can be either bound by FABP

or activated by enzyme. Binding of fatty acid showed a temperature dependency correlating with the physical states of liposomes. More fatty acid was bound when it was added using liposomes in the liquid-crystalline state, and a marked difference in binding was observed using dipalmitoylphosphatidylcholine liposomes above and below their transition temperature. Activation of fatty acid also showed a similar temperature dependency when liposomes were the fatty acid donor, but not when FABP was the fatty acid donor.

Experiments conducted with equilibrium dialysis cells showed that in the absence of FABP the filter provided an effective barrier for fatty acid transfer between compartments, presumably because any fatty acid molecule that desorbed from liposomes has a greater tendency to reassociate with the bilayer than to diffuse through the aqueous medium to microsomes. When FABP was added to dialysis systems, there occurred a fatty acid flux into the compartment containing microsomes, resulting in accumulation of acyl-CoA. The most probable explanation is that fatty acid desorbed from liposomes, associated with FABP rather than recombining with the bilayer, and the fatty acid-FABP complex diffused across the filter to the microsomal compartment. Thus, liver FABP, like any protein capable of binding fatty acids, can promote net desorption of fatty acid from liposomes by slowing the rate of the reverse process. Fatty acid when complexed to FABP may be in a more effective form for diffusion or transport across unstirred water layers within the cell, with FABP having a role analogous to that proposed for bile salt micelles or albumin in controlling extracellular fatty acid transport (Dietschy, 1978).

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