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Interaction of rat liver microsomes containing saturated or unsaturated fatty acids with fatty acid binding protein: Peroxidation effect

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Abstract

In the studies described here rat liver microsomes containing labeled palmitic, stearic, oleic or linoleic acids were incubated with fatty acid binding protein (FABP) and the rate of removal of ^{14}C-labeled fatty acids from the membrane by the soluble protein was measured using a model system. More unsaturated than saturated fatty acids were removed from native liver mircrosomes incubated with similar amounts of FABP. The *in vitro* peroxidation of microsomal membranes mediated by ascorbate-Fe^{++}, modified its fatty acid composition with a considerable decrease of the peroxidizability index. These changes in the microsomes facilitated the removal of oleic and linoeic acids by FABP, but the removal of palmitic and stearic acids was not modified. This effect is proposed to result from a perturbation of membrane structure following peroxidation with release of free fatty acids from susceptible domains. (Mol Cell Biochem **137:** 135–139, 1994)

Key words: fatty acids, microsomes, peroxidation, chemiluminescence, fatty acid binding protein

Abbreviations: BSA – bovine serum albumin, FABP – fatty acid binding protein

Introduction

Like many other compounds that are poorly soluble in water, free fatty acids circulate in plasma tightly bound to albumin. At the intracellular level a low molecular weight protein (15 KDa), named fatty acid binding protein (FABP) is thought to be involved in the transport of fatty acids and other organic anions [1,2,3].

Since long chain fatty acids are used mainly by the endoplasmic reticulum for the synthesis of acyl CoA derivatives [4], it was our interest to study the interaction of rat liver microsomes loaded with saturated or unsaturated long chain fatty acids with fatty acid binding protein. The present results demonstrate that the removal of free fatty acids from microsomal membranes is influenced by the structure of the free fatty acid and the integrity of the membrane.

Materials and methods

[1-^{14}C] palmitic acid, 8.4 mCi/mmol; [1-^{14}C] steraric acid, 58.0 mCi/mmol; [1-^{14}C] oleic acid, 56.0 mCi/mmol and [1-^{14}C] linoleic acid 53.0 mCi/mmol were purchased from Du Pont Co. Biotechnology Systems. The corresponding non-labelled fatty acids (approx 99% by capillary GC) and BSA (fraction V) were obtained from Wako Pure Chemicals Industries Ltd., Japan. Standards of fatty acids methyl esters were generously supplied by NU Chek Prep, Inc, Elysian, MN, USA.

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Microsomes

Rat liver was cut into small pieces and washed extensively with 0.15M NaCl. An homogenate 30% (w/v) was prepared in soln. 0.25 M sucrose, 10 mM Tris-HCl pH 7.4, 10 mM EDTA, using a potter-Elvejhem homogenizer. The homogenate was spun at 10,000 g for 10 min., 3 ml of the result supernatant was applied to a Sepharose 4B column (1.6 × 12 cm) equilibrated and eluted with 10 mM Tris-HCl (pH 7.4), 0.01 % NaN^3. The microsomal fraction appearing in the void volume (10–16 ml) was brought to 0.25 M sucrose by addition of solid sucrose. All operations waere realized at 4°C. The quality of this microsomal preparation is similar in composition as regards concentrations and activities of certain microsomal enzymes to that obtained by ultracentrifugation[5].

Incorporation of fatty acids into microsomes

A trace of [1-^{14}C] fatty acid and 150 nmol of unlabelled fatty acid (amonium salt) were added to 1 ml of microsomal suspension containing 5 mg of protein and vortexed vigorously. Individual batches of microsomes were prepared separately with only one of the unlabelled free fatty acids types.

Peroxidation of microsomes containing [1-^{14}C] fatty acids

Chemiluminescence and lipid peroxidation were initiated by adding ascorbate to microsomes containing [1-^{14}C] fatty acids. Microsomes at a concentration of 1 mg of protein were incubated at 37°C with 0.01 M phosphate buffer pH (7.4), 0.4 mM ascorbate, final volume 2 ml. Phosphate buffer is contaminated with sufficient iron to provide the necessary ferrous or ferric iron for lipoperoxidation. Microsomal preparations which lacked ascorbate were carried out simultaneously. Chemiluminescence was measured as counts per minute in a liquid scintillation analyzer packard 1900 TR.

Measurement of fatty acid composition

Microsomal lipis were extracted with chloroform/methanol (2:1 v/v) [6]. Fatty acids were transmethylated with 5% HCl in methanol at 80°C for 60 min. Fatty acid methyl esters were analyzed with GC-14A gas chromatograph (Zhimadzu, Kyoto, Japan) equipped with a DB-225 megabore column (30 m x 0.32 mm i.d., J & V Scientific, Folson, CA, USA). Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 250°C, the column temperature was held at 90°C for 1 min, 90–180°C at 15°/min, 180–200° at 3°/min, 200–220°C at 3°/min, 220°C for 7 min. Fatty acid methyl esters peaks were identified by comparison of retention times with those of standards.

Fatty acid binding protein preparation

Faty acid binding protein was prepared as previously described [7]. FABP delipidation was done as described by Lester's procedure [8].

Removal of fatty acids from microsomes

Rat liver microsomes, native or peroxidized (0.5 mg protein, containing 15 nmol [1-^{14}C] fatty acid) were added to plastic microfuge tubes and incubated with various amounts of FABP, in a final volume of 1 ml of 0.01 M Tris-HCl (pH 7.4). After 10 min at room temperature with stirring, 40 µl of 2,5 M acetic–sodium acetate (pH 5.0) were added; the samples were centrifuged at 10,000 × 5 min in an Ecys micro centrifuge. The precipitate microsomes were resuspended with 0.5 ml of NaOH 0.05 M. Aliquots of the supernatants and precipitates were then removed and radioactivity measured as described previously [9].

Protein determination

Proteins were determined by the method of Lowry *et al.* [10] using BSA as standard.

Results

Fatty acid composition of native and peroxidized microsomes

The times courses of the chemiluminescence resulting from the addition of ascorbic acid to rat liver microsomes are shown in Fig. 1. The response became maximal 15–20 min after the addition of ascorbic acid. The fatty acid composition of total lipids of native and peroxidized microsomes is presented in (Table 1). There was a significant decrease in C18:3 and C20:4 fatty acids in peroxidized microsomes. As a result of the relative decrease in fatty acid unsaturation of peroxidized microsomes, the PI (Peroxidizability index), a parameter based on the maximal rates of oxidation *in vitro* of specific fatty acids, decreased a 60% compared to native microsomes.

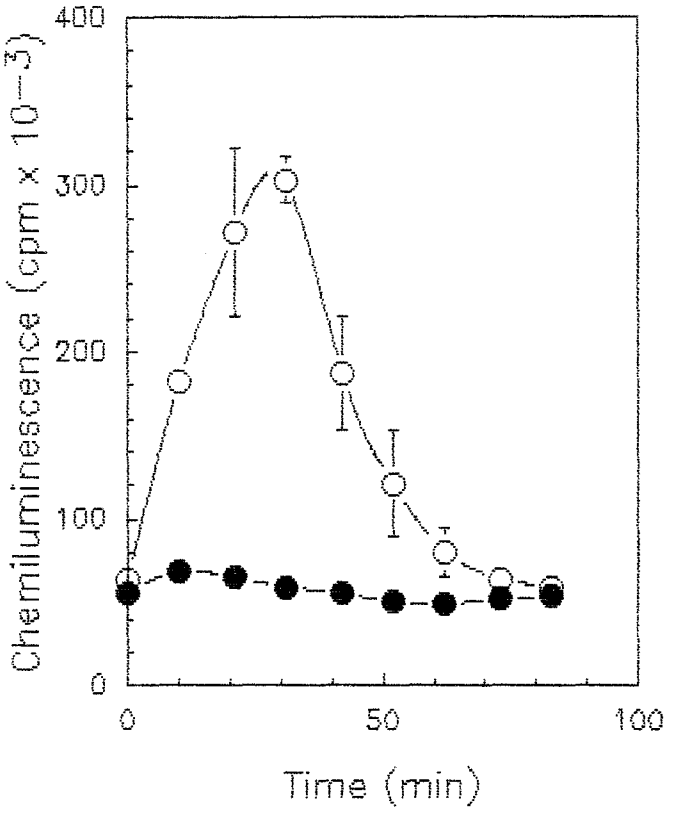

Fig. 1. Chemiluminescence of rat liver microsomes in the presence O or in the absence of ascorbic acid ●. Each point is the mean value from three experiment and the bars represent the standard errors of the means.

Table 1. Effect of peroxidation on fatty a cid composition of microsomal lipids

	% of fatty acids	
Fatty acid	Native microsomes	Peroxidized microsomes
C16:0	20.31 ± 1.15	26.84 ± 2.38
C16.1	4.27 ± 1.04	4.87 ± 2.31
C18:0	19.99 ± 1.19	18.80 ± 0.94
C18:1	15.46 ± 0.23	16.52 ± 0.85
C18:2	15.47 ± 0.79	12.85 ± 0.76
C18:3	3.94 ± 1.04	tr *
C20:4	12.02 ± 1.05	4.14 ± 0.24 *
Peroxidizability index	71.91 ± 5.09	28.78 ± 1.64

Peroxidizability index was calculated according to the formula PI= (percent of monoenoic acids × 0.025) + (percent of dienoic acids × 1) + (percent of trienoic acids x 2) + (percent of tetraenoic acids × 4). Data are given as the mean ± SE of 3 experiments. Statistically significant differences in fatty acid concentration between native and peroxidized microsomes are indicated by * , $P < 0.01$ using Student's *t* test.

Effect of fatty acid structure on removal rate

The effect of fatty acid chain length on the removal of labeled fatty acid from native rat liver microsomes by FABP was examined by comparing palmitic and stearic acids. Fig. 2 shows that stearic acid removal is higher than that of palmitic acid, indicating that longer chain free fatty acid is removed better than shorter chain free fatty acid. A comparison of oleate and linoleate was used to evaluate the effect of acyl chain unsaturation on fatty acid removal from microsomes by FABP. The results of Fig. 2 showed that the removal of

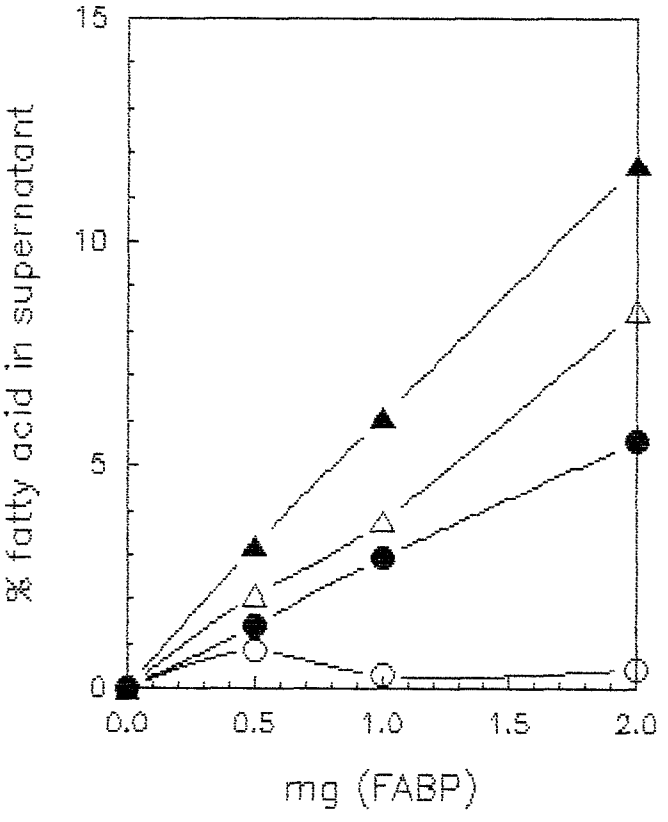

Fig. 2. Removal of saturated and unsaturated fatty acids from native rat liver microsomes by fatty acid binding protein. Palmitic acid O. stearic acid ●, oleic acid Δ, linoleic acids ▲. Each value represents the mean of two separate experiments.

linoleic acid was almost 60% greater than that of oleic acid. The amount of fatty acid removed from native microsomes was proportional to FABP concentration, excepting the values for palmitic acid. A two carbon increase in fatty acyl chain length or the addition of double bands results in an increase in removal by FABP. The percentages of fatty acid removed by mg of FABP were as follows: plamitic acid 0.31, stearic acid 2.94, oleic acid 3.77, and linoleic acid 6.08.

Effect of peroxidation of microsomes on fatty acid removal by FABP

To determine if peroxidation of microsomal membranes influences the removal of saturated and unsaturated long chain fatty acids by FABP, microsomes containing [1-^{14}C] fatty acids were peroxidized by ascorbate-Fe at 37°C during 15 min and an aliquot taken to perform the removal assay in the presence of rat liver FABP. Whereas the removal of palmitic and stearic acids by FABP was not affected after microsomal peroxidation oleic and linoleic acid removal in the presence of 1 mg of FABP was 1.6 and 2.24 times higher when compared with native microsomes, Fig. 3.

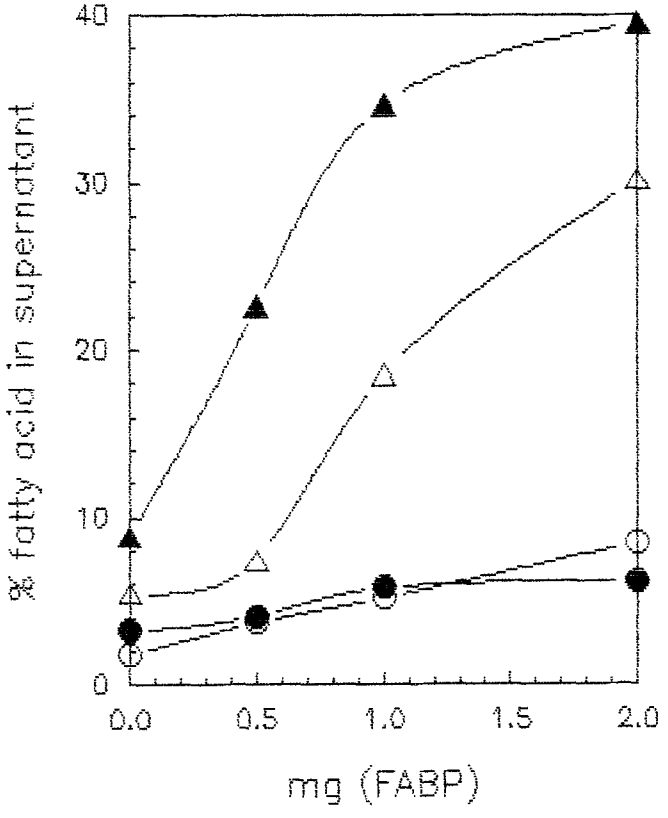

Fig. 3. Removal of fatty acids from peroxidized rat liver microsomes by fatty acid binding protein. Palmitic acid O, stearic acid ●, oleic acid Δ, linoleic acid ▲. Each value represents the mean of two separate experiments.

Effect of delipidation of FABP on fatty acid removal from microsomes

Delipidated FABP was more effective than native FABP on the removal of oleic acid from microsomes, thus the percentage of oleic acid removed by 1 mg of native and delipidated FABP was 11.5 and 40.9% respectively. When peroxidized microsomes containing [1-^{14}C] oleic acid were incubated with delipidated FABP the percent of fatty acid removed by mg of soluble protein was 68.9, Fig. 4.

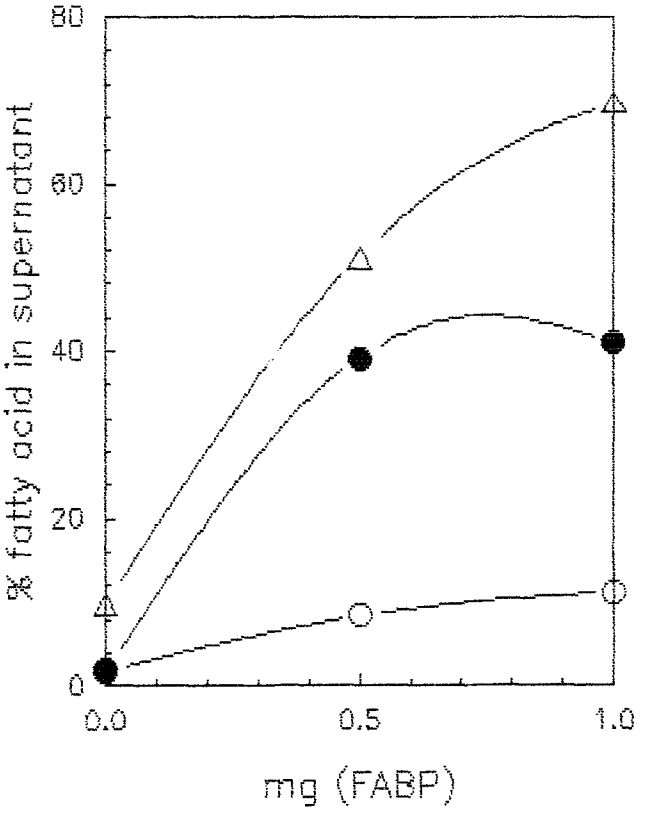

Fig. 4. Oleic acid removal from microsomal membranes by fatty acid binding protein O : native microsomes + native FABP, ● : native microsomes + delipidated FABP, Δ : peroxidized microsomes + delipidated FABP. Each value represent the mean of two separate experiments.

Discussion

In the past few years, evidences accumulated indicated that fatty acid binding protein is intimately involved in the intracellular trafficking of fatty acids [11]. Although movement of long chain fatty acids may occur spontaneously, it has been postulated that intracellular movement is facilitated by FABP. Many laboratories have investigated the possible participation of this soluble protein as a long chain fatty acid carrier [3]. We have recently described the use of a model system for monitoring the removal of radioactive long chain fatty acids and retinoids from microsomes [12]. Initial studies found that albumin when incubated with [^{14}C]-palmitic acid microsomes, showed radioactivity in the supernatant following centrifugation, suggesting that the fatty acid was bound to soluble protein.

In this report we demonstrate that the removal of radioactive fatty acids from microsomes by rat liver FABP is influenced by both the structure of the fatty acid and the

integrity of the membrane in which it is included. Our results are similar to those reported in studies on the free fatty acid transfer from rat liver FABP to phospholipid vesicles [13]. It has been demonstrated using solution theory that the cis-unsaturated fatty acids partition into fluid domains while the trans-unsaturated and saturated fatty acids preferentially partition into solid like domains in membranes [14]. Since lipid peroxidation produce a decrease of fluidity on the lipid bilayer, this fact could explain why peroxidation of microsomal membranes, facilitates the removal of oleic and linoleic acids by FABP but not that of palmitic and stearic acids. It has been determined that rates of hydration of fatty acids bound to lipid vesicles appear to be rapid enough to account for intracellular movement between compartments in the absence of carrier proteins. Increasing fatty acid chain length diminishes the rate of hydration whereas increasing unsaturation increases this rate [15].

Vorum *et al.* [16] have recently demonstrated using highly purified samples of radioactively labelled fatty acids that the solubility of monomeric laurate is greater than 500 μM, whereas the solubility of monomeric myristate is 20–30 μM. Palmitate, stearate and oleate solutions showed tendency to aggregation even at concentrations below 1 μM. Due to the extremely low solubility of long chain fatty acids in aqueous media it has been proposed [17] that compartmental exchange of fatty acids involves a contact mechanism which results in direct transfer of fatty acids between complexes and membrane components.

Using a carbon-13 NMR study it has been demonstrated that when the first binding site of FABP is filled the excess fatty acid is partitioned into phospholipid membranes which can hold appreciably greater quantitites of fatty acid per mass than FABP [18].

We do not know yet how the fatty acid composition of FABP affect the fatty acid partition between this important cytoplasmic protein and microsomal membranes. Studies in this direction should contribute to a better understanding of the role of fatty acid binding protein.

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