Relation of lung fatty acid binding protein to the biosynthesis of pulmonary phosphatidic acid and phosphatidylcholine

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Abstract The activities of glycerophosphate and lysophosphatidylcholine (LPC) acyltransferases were determined using lung microsomes in the presence of lung fatty acid binding protein (FABP). The synthesis of phosphatidic acid (PA) was increased two- to fourfold in the presence of FABP as compared to albumin. Lung FABP did not increase the incorporation of palmitoyl CoA into phosphatidylcholine. The results indicate that FABP-bound fatty acyl CoA may be a preferred substrate for glycerophosphate acyltransferase. —**Haq, R. U., F. Tsao, and E. Shrago.** Relation of lung fatty acid binding protein to the biosynthesis of pulmonary phosphatidic acid and phosphatidylcholine. *J. Lipid Res.* 1987. **28:** 216–220.

Supplementary key words fatty acid binding protein • phosphatidic acid • phosphatidylcholine • glycerophosphate acyltransferase • lysophosphatidylcholine acyltransferase

In lung tissue, lipids function as a component of surfactant as well as maintaining the structural integrity of membranes. Lung phosphatidylcholine (PC), a major component of pulmonary surfactant, is primarily synthesized via CDP-choline pathway (1). Phosphatidic acid (PA) is not only a precursor of de novo synthesized PC, but also of other glycerolipids and phospholipids. An important route for the synthesis of PA involves stepwise acylation of *sn*-glycerol-3-phosphate by two molecules of fatty acyl CoA, catalyzed by the PA synthesizing enzyme system (2–4). By virtue of PA being positioned at a critical branch point, an alteration in its synthesis could have a profound effect on the whole scheme of pulmonary lipid metabolism.

Fatty acid binding protein (FABP) is a major cytosolic protein present in intestinal mucosa, liver, myocardium, kidney, and adipose tissue (5–7). The protein, which has been well characterized, exhibits a high binding affinity for long chain fatty acids and their acyl CoA thioesters (8–10). Recently, lung FABP having high affinity for long chain acyl CoAs has been purified and characterized in this laboratory (11). As a result of this binding property, acyl CoA esterification by glycerophosphate and lysophosphatidylcholine (LPC) acyltransferases was tested in the presence of lung FABP. In this study, we reprot for the first time the effect of FABP on PA and PC formation in lung.

MATERIALS AND METHODS

Male Sprague-Dawley rats were purchased from King's Animals, Oregon, WI and maintained on standard Purina chow. [^{14}C]glycerol-3-phosphate, [^{14}C]palmitoyl CoA, and [1-^{14}C]dipalmitoyl phosphatidylcholine were purchased from New England Nuclear Corp., Boston, MA. Palmitoyl CoA and other lipid standards were purchased from Pharmacia P-L Biochemicals, Piscataway, NJ. Precoated silica gel 60 plates were obtained from American Scientific Products, Chicago, IL. All other chemicals were of the highest grade commercially available.

Abbreviations: FABP, fatty acid binding protein; PC, phosphatidylcholine; PA, phosphatidic acid; LPC, lysophosphatidylcholine.

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Microsomal preparation

Rat lungs were perfused with a 0.15 M NaCl solution via the right ventricle to remove blood. The perfused lungs were excised, homogenized in 0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol solution, pH 7.4, to obtain a 66% homogenate, and subjected to differential centrifugation. The microsomal fraction was prepared by centrifugation of the 20,000 *g* supernatant at 105,000 *g* for 1 hr. The pellet was resuspended in the sucrose solution and centrifuged for 30 min at 105,000 *g*. The washed microsomes were suspended in the same solution, quickly frozen in dry ice–methanol, and then stored at −20°C for future use. The enzyme activities remained unchanged for 3 months under these conditions.

Lung FABP

Lung FABP was prepared as described previously (11) with the exception that an anion exchange column was used at the final step, and the protein was eluted with 0.5 M NaCl.

Glycerophosphate acyltransferase assay

The enzymatic activity of glycerophosphate acyltransferase was measured by a modification of the method of Fallon and Lamb (12). Unless otherwise stated, the reaction mixture at 37°C contained 70 mM Tris–HCl, 1.5 mM DTT, 114 μM palmitoyl CoA, 1.5 mM [^{14}C]glycerol-3-phosphate (sp act 1450 dpm/nmol), 200 μg of microsomal protein, and different concentrations of FABP or albumin in a total volume of 0.35 ml. The reaction was started by the addition of microsomal protein, and stopped after 2 min by the addition of 3 ml of chloroform–methanol 2:1. The rate of reaction was linear in terms of time and enzyme protein. Lipids were extracted and washed in a modified medium described by Bligh and Dyer (13). All the washes contained 0.15 M HCl for the quantitative recovery of lysophosphatidic acid (14). The lower chloroform phase was dried under nitrogen, and then applied to silica gel 60 TLC plates. Lipids were separated by development of plates in chloroform–methanol–acetic acid–water 170:30:20:7 (by volume) (15). Chromatographed lipids were visualized by staining in an iodine chamber and spots were compared to authentic lipid standards. Lipid areas were scraped from plates and assayed for radioactivity after mixing with Aquasol.

Lysophosphatidylcholine acyltransferase assay

The conversion of endogenous lysophosphatidylcholine into phosphatidylcholine was measured by a modified method of Stymne and Stobart (16). The incubation medium at 37°C contained 65 mM Tris–HCl, pH 7.4, 1 mM CoASH, 40 μM [^{14}C]palmitoyl CoA (sp act 3600 dpm/nmole), 200 μg of microsomal protein, and different concentrations of albumin or FABP in a final volume of 0.40 ml. The reaction was started by the addition of microsomal protein and terminated after 10 min by the addition of 3 ml of chloroform–methanol 2:1. Lipids were extracted as stated above. Phosphatidylcholine was subsequently separated by development of silica gel 60 plates in chloroform–methanol–water 65:45:5 (by volume) and assayed for radioactivity.

Preparation of [1-^{14}C]dipalmitoyl phosphatidic acid

[1-^{14}C]dipalmitoyl phosphatidic acid was prepared by hydrolyzing [1-^{14}C]dipalmitoyl phosphatidylcholine (5 μCi/0.05 mmol) by phospholipase D (17). The free form of phosphatidic acid was extracted and isolated by silicic acid column chromatography.

RESULTS AND DISCUSSION

The liver enzyme system catalyzing the biosynthesis of PA from glycerol-3-phosphate proceeds in two successive acylation steps (18). However, in the earlier studies there has been some controversy as to whether the major product is lysoPA and PA (12, 19, 20). Hence, attention was focused on the identification of synthesized products in lung microsomes.

Among different solvent systems tested, the solvent system used in these experiments produced the best separation of PA and lysoPA. This was also confirmed by chromatographing [1-^{14}C]dipalmitoyl phosphatidic acid in an adjacent lane along with the samples. More than 95% of the radioactivity was present in PA, indicating no accumulation of an intermediate product under these conditions. These data are similar to those reported in an earlier study showing PA as the sole product of the lung PA synthesizing enzyme system (16). As both the transferases, i.e., glycerophosphate and monoglycerophosphate, are involved in the synthesis of the final product (4, 21), the results are expressed as glycerophosphate incorporation into PA. The effects of lung FABP and bovine serum albumin (fatty acid-free) on the synthesis of PA are shown in **Fig. 1.** It is clear that, at equimolar concentrations, FABP was superior to albumin in the stimulation of PA formation. The addition of 2 nmol of FABP enhanced the esterification of glycerol-3-phosphate by a factor of fourfold over that of albumin. In an earlier study (22), liver FABP was shown to stimulate the incorporation of glycerol-3-phosphate by liver microsomes into lysoPA instead of PA. The difference could be due to different assay conditions or misidentification of the product. It appears that a second acylation step is more rapid than the first, so that the intermediate product (lysoPA) does not accumulate in detectable amounts. Unlike the liver study, the experiments reported here also utilized "physiological" concentrations of FABP.

Fig. 2 shows the effect of different concentrations of palmitoyl CoA in the presence of 5 nmol of albumin or

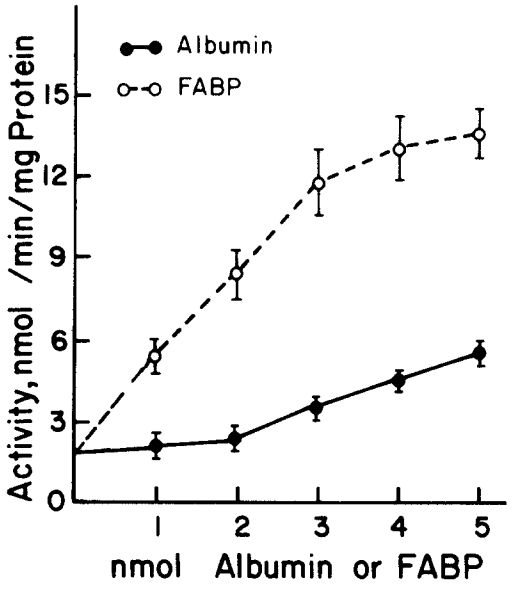

Fig. 1. Effects of various concentrations of albumin and lung FABP in incorporation of [^{14}C]glycerol-3-phosphate into phosphatidic acid. The incubation mixtures contained 114 μM palmitoyl CoA. Each point represents the mean ± SEM of duplicate determinations in three separate experiments.

FABP. The important observation is that with 50 nmol of palmitoyl CoA, FABP is a more effective promoter of PA synthesis. It seems that palmitoyl CoA at the higher concentrations may be inhibitory to enzyme activity (Fig. 2), as observed from the experiments without added albumin or FABP. Comparison of these data with enzyme activity in the presence of FABP, indicates that the protein might protect the enzyme against the detrimental effects of long chain fatty acyl CoAs, present in the range of 25 nmol/g wet weight in the lung (23). It has been reported that inhibitory effects of palmitoyl CoA on liver acetyl coenzyme A carboxylase (24) and adenine nucleotide translocase (25) activity is reversed by liver FABP. It is very unlikely that the observed inhibition of glycerophosphate incorporation into PA is due to detergent effects of palmitoyl CoA (114 μM). Recent studies show that in 0.1 M Tris–HCl buffer, critical micelle concentrations of palmitoyl CoA are in the neighborhood of 200 μM (26). Moreover, membranes also bind fatty acyl CoA substrates with high affinity (27). Therefore, the concentration of free palmitoyl CoA in these assays containing microsomes (200 μg of protein) is well below the critical micelle concentration. The physiological significance of FABP is apparent in view of the fact that albumin is present in only trace amounts within the cell (28, 29). Hepatic and lung FABP, which comprise about 4–5% and 1.5–2.5%, respectively, of the soluble protein of these tissues (11, 30), might be the "physiological proteins" to modulate the effect of fatty acids and their esters on the activity of membrane-bound enzymes. Polyclonal antibodies against hepatic FABP crossreact weakly with lung FABP, implying partial homology (11). Moreover, hepatic FABP can replace the lung FABP for the increased stimulation of PA formation with lung microsomes (data not shown). This observation is similar to lung fatty acyl CoA synthetase enzyme, which has been reported to be affected by both hepatic and lung FABP (11).

The observations reported here support the hypothesis that, in the intact cell, an FABP-bound fatty acyl CoA, rather than free acyl CoA, is the preferred acylating agent for certain enzymes. To test the hypothesis as to whether this is a phenomenon for membranous enzymes in general, the deacylation–reacylation pathway enzyme (LPC acyltransferase), which utilizes palmitoyl CoA as a substrate, was assayed in the presence of lung FABP. It is evident from **Table 1** that there is no significant increase in the incorporation of [^{14}C]palmitoyl CoA into PC in the presence of albumin or FABP. The results indicate that lung FABP does not affect the activity of all membrane-bound enzymes that utilize fatty acyl CoAs as a substrate. It is of interest to compare these results with a recent study showing increased incorporation of [^{14}C]linolenate into PC in the presence of albumin (16). Two points have to be considered: first the concentration of albumin used is very high (227 nmol) and not found inside the cell; second, fatty acyl CoA concentration (250 μM) is well above the critical micelle concentration. It is clear that lung FABP, an intracellular protein, does not stimulate the synthesis of PC via the reacylation pathway. On the other hand, lung FABP may play a vital role in the stereo-

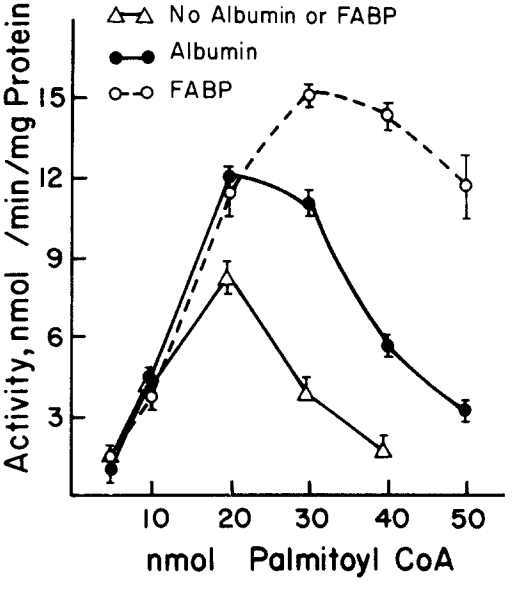

Fig. 2. Effects of palmitoyl CoA concentration on the incorporation of [^{14}C]glycerol-3-phosphate into phosphatidic acid. Experiments were performed in the presence and absence of 5 nmol of albumin or lung FABP. Each point represents the mean ± SEM of duplicate determinations in three separate experiments.

TABLE 1. Incorporation of [^{14}C]palmitoyl CoA into phosphatidylcholine[a]

Albumin or FABP	FABP	Albumin
nmol		
0	0.81 ± 0.02[b]	0.71 ± 0.1[b]
1	0.84 ± 0.06	0.70 ± 0.07
2	0.89 ± 0.06	0.72 ± 0.01
3	0.89 ± 0.02	0.75 ± 0.12
4	0.89 ± 0.02	0.83 ± 0.12
5	0.92 ± 0.04	0.90 ± 0.13

[a] LPC acyltransferase activity expressed as nmol/min per mg protein.
[b] The results are mean ± SEM of three different experiments.

chemical positioning of acyl groups in PA. It is not known whether one or both of the acylation steps, i.e., glycerophosphate acyltransferase or monoglycerophosphate acyltransferase, is stimulated by lung FABP. Also, at the present time, the mechanism of increased PA synthesis in the presence of lung FABP is not clear. However, observations reported here imply that somehow FABP forms a complex with the substrate and the enzyme. It does not seem to be interacting only with the substrate since, if that was the case, the activity of LPC acyltransferase, which employs palmitoyl CoA as one of the substrates, would have increased in the presence of lung FABP. It is clear that the FABP effect cannot be explained in simple terms in that it "delivers" the substrate to membrane-bound enzymes. Lung FABP specifically recognizes this PA-synthesizing enzyme system for the interaction along with the substrate. Further studies with purified and separated components of the PA-synthesizing enzyme system are required to delineate the mechanistic understanding of PA formation in the presence of lung FABP.

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