Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3484–3488, May 1990
Biochemistry

Plasma membrane fatty acid-binding protein and mitochondrial glutamic-oxaloacetic transaminase of rat liver are related

(hepatocyte/transport/amino acid sequence)

P. D. BERK*†‡, H. WADA§, Y. HORIO§, B. J. POTTER*, D. SORRENTINO*, S.-L. ZHOU*, L. M. ISOLA*, D. STUMP*, C.-L. KIANG*, AND S. THUNG¶

Departments of *Medicine (Hepatic Research Group), †Biochemistry, and ¶Pathology, Mount Sinai School of Medicine, New York, NY 10029; and §Department of Pharmacology II, University of Osaka, Osaka, Japan

Communicated by Esmond E. Snell, February 20, 1990

ABSTRACT **The hepatic plasma membrane fatty acid-binding protein (h-FABP$_{PM}$) and the mitochondrial isoenzyme of glutamic-oxaloacetic transaminase (mGOT) of rat liver have similar amino acid compositions and identical amino acid sequences for residues 3–24. Both proteins migrate with an apparent molecular mass of 43 kDa on SDS/polyacrylamide gel electrophoresis, have a similar pattern of basic charge isomers on isoelectric focusing, are eluted similarly from four different high-performance liquid chromatographic columns, have absorption maxima at 435 nm under acid conditions and 354 nm at pH 8.3, and bind oleate with a $K_a \approx 1.2–1.4 \times 10^7$ M^{-1}. Sinusoidally enriched liver plasma membranes and purified h-FABP$_{PM}$ have GOT enzymatic activity; the relative specific activities (units/mg) of the membranes and purified protein suggest that h-FABP$_{PM}$ constitutes 1–2% of plasma membrane protein in the rat hepatocyte. Monospecific rabbit antiserum against h-FABP$_{PM}$ reacts on Western blotting with mGOT, and vice versa. Antisera against both proteins produce plasma membrane immunofluorescence in rat hepatocytes and selectively inhibit the hepatocellular uptake of [^{3}H]oleate but not that of [^{35}S]sulfobromophthalein or [^{14}C]taurocholate. The inhibition of oleate uptake produced by anti-h-FABP$_{PM}$ can be eliminated by preincubation of the antiserum with mGOT; similarly, the plasma membrane immunofluorescence produced by either antiserum can be eliminated by preincubation with the other antigen. These data suggest that h-FABP$_{PM}$ and mGOT are closely related.**

Glutamic-oxaloacetic transaminase (GOT; L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) is a well studied enzyme that plays a major role in amino acid (1) and energy (2) metabolism. Within eukaryotic cells it exists as distinct cytoplasmic (3) and mitochondrial (4, 5) isoenzymes (sGOT and mGOT, respectively) that catalyze essentially the same reactions (6). Neither isoenzyme has any known role in the cellular uptake of long-chain nonesterified fatty acids. By contrast, hepatic plasma membrane fatty acid-binding protein (h-FABP$_{PM}$), a plasma membrane protein of ≈40 kDa that plays an as yet only partially defined role in cellular fatty acid uptake, was described by our laboratory in 1985 (7). Analogous plasma membrane FABPs were subsequently isolated from the major tissues with high transmembrane fatty acid fluxes (8), including jejunal mucosa (9), adipose tissue (10), and cardiac muscle (11, 12). These proteins are structurally distinct from and immunologically unrelated to the smaller, cytoplasmic FABPs, designated FABP$_C$ (10, 13), previously isolated from these same tissues (13).

Here we present data suggesting that h-FABP$_{PM}$ of rat liver is closely related to mGOT. The similarities observed between mGOT and h-FABP$_{PM}$ raise interesting questions in cellular and molecular biology.

MATERIALS AND METHODS

Protein Purification. mGOT was prepared from whole rat liver homogenates (14). h-FABP$_{PM}$ was isolated from sinusoidally enriched liver plasma membranes (15, 16). Initially (procedure A), proteins extractable from the membranes with 2 M NaCl were subjected to preparative isoelectric focusing. Fractions with isoelectric points ≥9.0 were further purified by oleate-agarose affinity chromatography followed by gel permeation HPLC (8). More recently (procedure B), improved recoveries were obtained with an alternative procedure, in which salt-extracted membrane proteins were separated by HPLC over, sequentially, hydroxyapatite (Bio-Gel HPHT) and hydrophobic-interaction (TSK phenyl 5 PW) columns. Fractions containing h-FABP$_{PM}$ were collected and examined for purity by gel permeation HPLC (Superose 12, 10/30 column, 300 mm × 10.0 mm i.d.), SDS/PAGE, and isoelectric focusing. Details of this isolation procedure will be published elsewhere (D. Stump, B.J.P., and P.D.B.).

Measurement of GOT Activity. Activity of this enzyme was determined with a commercially available kit (Sigma Diagnostics; procedure 58-UV) (17). When mGOT activity in plasma membranes or liver homogenates was measured, Triton X-100 was used to solubilize the proteins (18).

Analytical Procedures. Amino acid analysis (performed at the Protein Sequencing Facility, Rockefeller University) employed HCl hydrolysis and subsequent quantitation by a Waters Picotag HPLC system. Sequencing (two samples were sequenced, at the Protein Sequencing Facilities of Yale and Rockefeller Universities) was performed on an Applied Biosystems model 470A gas-phase sequencer, with on-line phenylthiohydantoin determination by HPLC on an Applied Biosystems model 120 analyzer. SDS/PAGE–Western blotting and analytical isoelectric focusing were performed as previously reported (8).

Preparation of Antibodies. Monospecific polyclonal antibodies were produced in female New Zealand White rabbits by intradermal injection of antigen in Freund's complete adjuvant, followed by biweekly intramuscular booster injections of antigen emulsified in Freund's incomplete adjuvant, as previously reported (7). Monoclonal antibodies against h-FABP$_{PM}$ were obtained from hybridoma cell lines produced by the fusion of spleen cells from immunized BALB/c mice with NS1 mouse myeloma cells (19). Hybridoma su-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FABP, fatty acid-binding protein; h-FABP$_{PM}$, hepatic plasma membrane FABP; GOT, glutamic-oxaloacetic transaminase; mGOT, mitochondrial isoenzyme of GOT.

‡To whom reprint requests should be addressed at: Box 1079, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029.

pernatants were screened and assayed for antibody activity by an ELISA technique employing an alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Sigma) as the second antibody.

Immunofluorescence and Immuno Electron Microscopy. The distribution of mGOT and h-FABP$_{PM}$ in acetone-fixed cryostat sections of snap-frozen rat liver was determined by the indirect fluorescence antibody technique, using rabbit antisera against the two proteins as primary antibodies and fluorescein-conjugated sheep anti-rabbit gamma globulin as the second antibody (20). Immuno electron microscopy of rat liver sections was performed by standard techniques (21), employing the same primary antibodies and a colloidal gold-conjugated second antibody. ELISA-positive monoclonal antibodies were further evaluated by indirect immunofluorescence, employing single-cell suspensions of rat hepatocytes isolated by collagenase perfusion (22). For some immunofluorescence studies, employing both rabbit antisera and monoclonal antibodies, antibodies were incubated with antigen prior to their use.

Cellular Fatty Acid Uptake and Antibody Inhibition. The uptake of [^{3}H]oleate by isolated hepatocytes was studied by a rapid filtration technique (23). Sulfobromophthalein and taurocholate uptake studies employed [^{35}S]sulfobromophthalein (a gift of Allan W. Wolkoff, Albert Einstein College of Medicine, Bronx, NY) and [^{14}C]taurocholate and were conducted under the same conditions as those employed for oleate (24). Antibody inhibition studies were performed with cells preincubated for 1 hr on ice with the appropriate antibody, washed, and resuspended in Hanks' Hepes buffer, pH 7.4, as previously described (24). Finally, the effect on oleate uptake of preincubating the antibody with antigen was tested by adding 150 μg of mGOT to 300 μl of anti-h-FABP$_{PM}$ immune rabbit serum at room temperature for 30 min before antiserum was added to the cells. The appropriate control (preimmune serum) and a separate aliquot of the antiserum were incubated at room temperature with an equivalent volume of buffer. In all cases, uptake of ligands was estimated from the slope of the initial, linear portion of their cumulative uptake curves. These techniques afford an accurate measure of initial influx rates for a variety of ligands and in various cell types, including hepatocytes (10, 11, 25, 26).

Labeling of Cell Surface Proteins *in Vitro*. Hepatocytes in overnight monolayer cultures were radiolabeled (^{125}I) with lactoperoxidase at 4°C, conditions under which only cell surface components would be expected to be labeled (27). Specific immunoadsorbents were prepared by mixing 0.75 ml of protein A-Sepharose suspension (containing 2 mg of protein A per ml of swollen gel) with 0.25 ml of rabbit anti-rat mGOT, h-FABP$_{PM}$, plasma membrane sulfobromophthalein/bilirubin-binding protein (26), albumin, transferrin, or ligandin antiserum. This mixture was diluted to a final volume of 2 ml with IP buffer (0.5% Nonidet P-40/0.1% SDS/0.15 M NaCl/1 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride/10 mM Tris·HCl, pH 7.5) and incubated at 4°C on a roller mixer. Each gel was then washed four times in IP buffer and resuspended to a total volume of 2 ml for use. At the termination of the iodination reaction, the cells were scraped from the culture dishes and resuspended in 4 ml of IP buffer, briefly sonicated in a cup horn (Branson sonicator), and centrifuged at 50 × *g* to remove unbroken cells and debris; the pellet was discarded. The supernatant was mixed with 20 μg each of rat transferrin and albumin and an equal volume of IP buffer, incubated on ice for 15 min, and then centrifuged at 15,000 × *g* for 15 min. Duplicate 0.2-ml aliquots were mixed with 0.1 ml of the appropriate protein A immunoadsorbent in 1.5-ml screw-cap tubes, and a further 1 ml of IP buffer was added to each tube. The tubes were incubated at 4°C overnight on a roller mixer. The protein A-Sepharose was pelleted by centrifugation at 10,000 × *g* for 5 min and washed three times with IP buffer, prior to measurement of radioactivity in a γ-counting spectrometer. Total counts were determined from 0.2-ml aliquots of the sonicated lysates. Counts due to nonspecific binding, determined using a protein A–nonimmune rabbit IgG complex, were subtracted from the counts in the specific immunoprecipitates.

RESULTS

h-FABP$_{PM}$ Preparations. h-FABP$_{PM}$ samples purified either by procedure A, by procedure B, or by the one-step affinity chromatography procedure reported earlier (7) were electrophoretically indistinguishable and gave a single precipitin line of identity in Ouchterlony studies employing a polyclonal rabbit antiserum raised against an antigen prepared by procedure A. Purity of h-FABP$_{PM}$ prepared by procedure A or B invariably exceeded 98%. For procedure B, recovery, as assessed by GOT enzymatic activity (see below), was 48 ± 6% of the GOT activity measured in the starting membranes.

Amino Acid Composition and Sequence. The experimentally determined amino acid composition of highly purified h-FABP$_{PM}$ is similar to that reported previously (14, 28, 29) for rat liver mGOT or deduced from cDNA sequences (30, 31) (Table 1). With respect to amino acid sequencing, neither sequencing facility was able to define with certainty the N-terminal amino acids at positions 1 and 2, due to the presence of free amino acids in the submitted samples. However, the 22 subsequent residues were identical to the corresponding amino acids of mGOT (14, 30).

Electrophoretic and Chromatographic Behavior. Purified mGOT and plasma membrane FABPs from rat liver (8), jejunum (9), adipose tissue (10), and cardiac muscle (8, 11) migrated identically during SDS/PAGE (Fig. 1), with an estimated molecular mass of 43 kDa. Both mGOT and h-FABP$_{PM}$ were highly basic, and each demonstrated a similar pattern of charge isomers, with an average isoelectric point centered at pH 9.1 (Fig. 1). The two proteins also demonstrated identical behavior in four distinctly different HPLC columns: hydroxyapatite, reverse phase, hydrophobic interaction, and gel permeation (Fig. 2).

Enzymatic Activity of h-FABP$_{PM}$. The plasma membrane preparation from which h-FABP$_{PM}$ was purified possessed

Table 1. Amino acid (AA) composition of h-FABP$_{PM}$ and mGOT

	No. of residues per mol of protein*			
		mGOT		
AA	h-FABP$_{PM}$ AA analysis	From cDNA (ref. 30)	AA analysis (ref. 14)	AA analysis (ref. 29)
Asx	35	36	36	32
Glx	39	39	43	45
Ser	23	23	22	22
Gly	34	35	36	32
His	9	9	8	8
Arg	23	20	19	17
Thr	17	19	17	16
Ala	33	33	33	29
Pro	21	19	19	14
Tyr	12	12	12	11
Val	29	28	29	28
Met	10	10	10	13
Cys	ND	7	8	ND
Ile	18	19	19	15
Leu	33	32	31	30
Phe	19	19	19	24
Lys	29	30	27	43
Trp	ND	7	9	ND

*Calculation based on M_r = 44,000; ND, not determined.

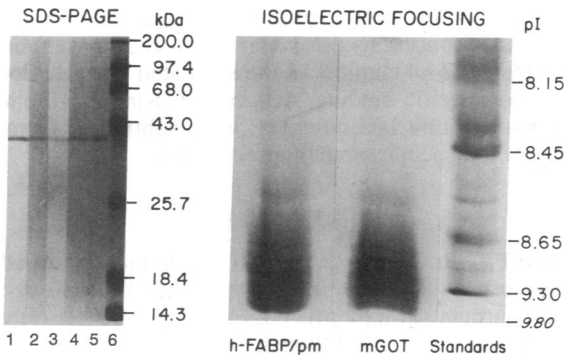

FIG. 1. (*Left*) SDS/PAGE of purified mGOT (lane 1) and plasma membrane FABPs from rat hepatocytes (lane 2), jejunal enterocytes (lane 3), adipocytes (lane 4), and cardiomyocytes (lane 5). Size standards are shown in lane 6. (*Right*) Analytical isoelectric focusing of h-FABP$_{PM}$ and mGOT. Proteins were visualized by silver staining (8).

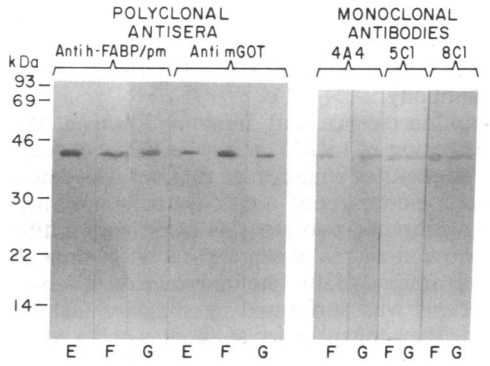

FIG. 3. Western blots probed with rabbit antisera against h-FABP$_{PM}$ and mGOT and three monoclonal antibodies raised against h-FABP$_{PM}$. Lanes: E, extract of liver plasma membrane proteins (250 μg); F, purified h-FABP$_{PM}$ (3–5 μg); G, purified mGOT (5 μg). Both polyclonal antisera and monoclonal antibodies reacted with both h-FABP$_{PM}$ and mGOT.

GOT enzymatic activity (mean 2.8 units/mg of membrane protein) that was enriched 3- to 4-fold compared to that of whole rat liver homogenate; conversely, the activity in the same membranes of the conventional mitochondrial marker succinate dehydrogenase was consistently <25% of its value in the homogenate. During chromatographic purification of h-FABP$_{PM}$ by HPLC, a peak of GOT activity was recovered from each of the columns and corresponded in each instance to the elution profile of the h-FABP$_{PM}$. The specific activity of the purified protein, 140–180 units/mg of protein, is similar to reported values for mGOT of 100–230 units/mg (14, 32). From the relative GOT specific activities of h-FABP$_{PM}$ and liver plasma membranes, we estimate that h-FABP$_{PM}$ constitutes 1–2% of plasma membrane protein in rat hepatocytes. mGOT was retained on and eluted from oleate-agarose affinity chromatography columns similarly to h-FABP$_{PM}$ (data not shown). In addition, studies of the binding of [^{3}H]oleate to h-FABP$_{PM}$ and mGOT were carried out according to Glatz and Veerkamp (33). When analyzed according to Scatchard (34), each protein exhibited a single class of binding sites, with similar association constants (mGOT, $K_a = 1.2 \times 10^7$ M^{-1}; h-FABP$_{PM}$, $K_a = 1.4 \times 10^7$ M^{-1}).

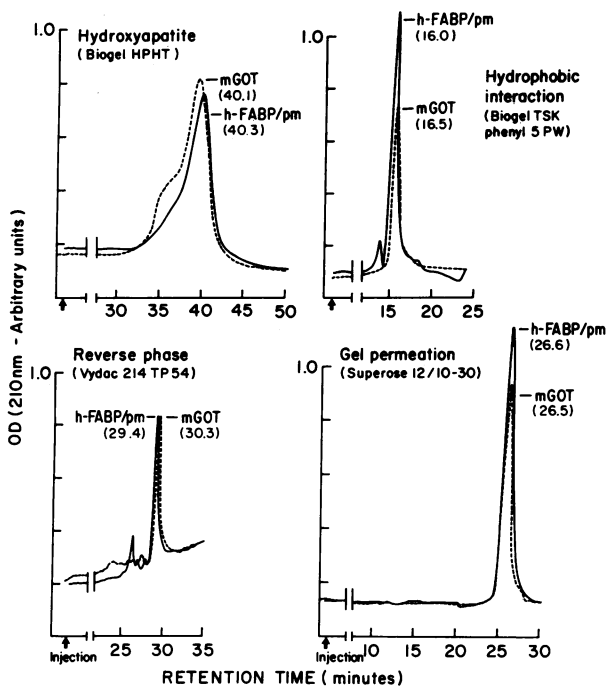

FIG. 2. Elution of h-FABP$_{PM}$ and mGOT from four different HPLC columns. In each case the proteins were run in immediate succession under identical conditions with respect to flow rates and solvent and salt gradients.

Absorption Spectra. The absorption spectra of the two proteins were strikingly similar. In acid (pH 4.8) each had an absorption maximum at 435 nm (mGOT, $\varepsilon_0 = 3.8 \times 10^3$ $M^{-1}\cdot cm^{-1}$; h-FABP$_{PM}$, $\varepsilon_0 = 4.5 \times 10^3$ $M^{-1}\cdot cm^{-1}$); at pH $\geq$ 8.3, the absorption maximum shifted to 354 nm as previously reported for mGOT (35) (mGOT, $\varepsilon_0 = 8.6 \times 10^3$ $M^{-1}\cdot cm^{-1}$; h-FABP$_{PM}$, $\varepsilon_0 = 8.0 \times 10^3$ $M^{-1}\cdot cm^{-1}$).

Immunologic Relationships. Monospecific rabbit antiserum raised against rat mGOT also reacted by both immunodiffusion and immunoblot against h-FABP$_{PM}$, and vice versa (Fig. 3). A total of 13 monoclonal cell lines producing an anti-h-FABP$_{PM}$ have been identified thus far by ELISA screening. Of the monoclonal antibodies evaluated in more detail, three (4A4, 5C1, and 8C1) gave positive plasma membrane immunofluorescence when freshly isolated hepatocytes were used as targets. Each of these three monoclonal antibodies identified both h-FABP$_{PM}$ and mGOT in Western blots (Fig. 3).

Immunofluorescence Studies. Both rabbit anti-h-FABP$_{PM}$ and anti-mGOT produced marked, specific plasma membrane immunofluorescence in slices of freshly frozen rat liver (Fig. 4), which was lacking in similar studies performed with the preimmune sera. The presence of anti-mGOT bound to

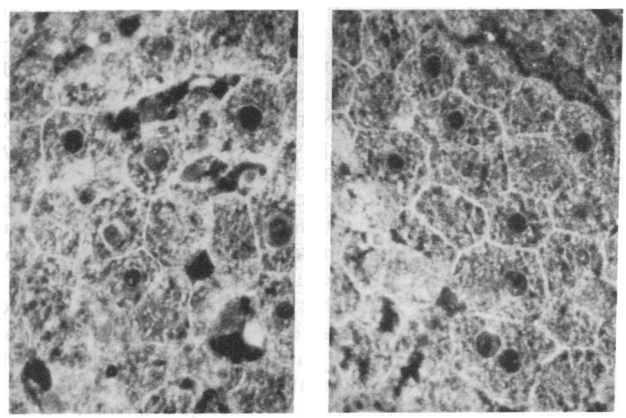

FIG. 4. Immunofluorescence of rat liver sections with rabbit anti-rat mGOT (*Left*) or anti-h-FABP$_{PM}$ (*Right*) as primary antibody and fluoresceinated anti-rabbit immunoglobulins as secondary antibody. Both antisera gave specific plasma membrane staining as well as punctate cytoplasmic staining, which were lacking in studies with preimmune sera.

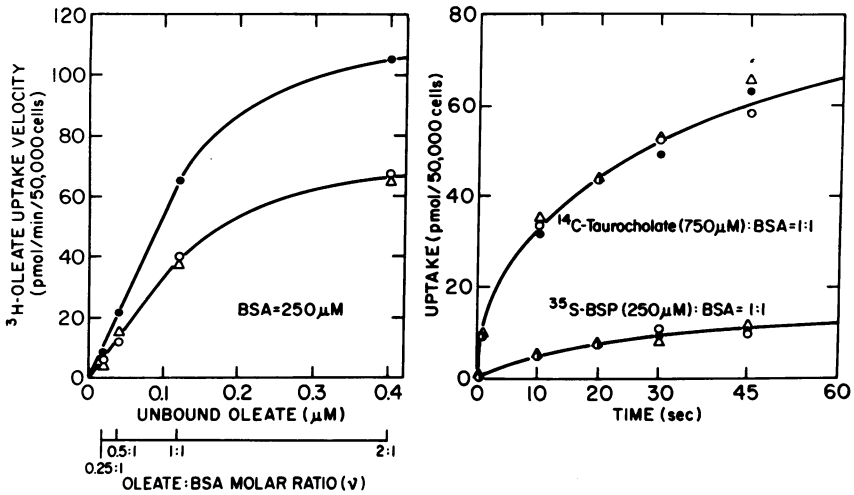

FIG. 5. Effects of anti-h-FABP$_{PM}$ (△), anti-mGOT (○), and preimmune serum (●) on hepatocellular uptake of [^{3}H]oleate (*Left*) and [^{35}S]sulfobromophthalein (^{35}S-BSP) and [^{14}C]taurocholate (*Right*). Both antisera noncompetitively inhibited oleate uptake but had no effect on the uptake of the other two organic anions studied. Compared to the control study of specific oleate uptake [V_{max} = 100 ± 6 pmol/min per 50,000 cells (mean ± SD, n = 3), K_m = 100 ± 16 nM], anti-h-FABP$_{PM}$ and anti-mGOT reduced V_{max} to 66 ± 5 ($P < 0.002$) and 69 ± 5 ($P < 0.005$), respectively. Corresponding K_m values were 125 ± 12 and 113 ± 12 (P not significant). BSA, bovine serum albumin.

plasma membranes and of anti-h-FABP$_{PM}$ bound to mitochondria was confirmed by immuno electron microscopy using a colloidal gold-conjugated second antibody. The plasma membrane immunofluorescence produced with anti-mGOT could be eliminated by preincubation of the antiserum for 30 min at room temperature with purified h-FABP$_{PM}$. Similarly, the plasma membrane immunofluorescence produced with anti h-FABP$_{PM}$ was eliminated when the antiserum had been absorbed with mGOT (data not shown).

Cellular Fatty Acid Uptake Studies. When diluted to an identical IgG concentration of 1 mg/ml, anti-h-FABP$_{PM}$ and anti-mGOT produced similar inhibitory effects on the hepatocellular uptake of [^{3}H]oleate but had no effect on the uptake of [^{35}S]sulfobromophthalein or [^{14}C]taurocholate (Fig. 5). The inhibitory effect of both antisera on oleate uptake was noncompetitive. In the particular study illustrated, each produced an ≈35% reduction in V_{max} but had no effect on K_m, compared to control studies employing an equivalent concentration of preimmune serum. As with the membrane immunofluorescence, the ability of anti-h-FABP$_{PM}$ to inhibit [^{3}H]oleate uptake could be blocked by prior incubation of the antibody with mGOT.

Cell Surface Labeling Studies. Immunoprecipitates of three plasma membrane proteins, h-FABP$_{PM}$, plasma membrane sulfobromophthalein/bilirubin-binding protein, and transferrin receptor (bound to transferrin and precipitated with anti-transferrin) contained 0.39 ± 0.06%, 0.57 ± 0.06%, and 0.63 ± 0.16% of the input radioactivity, respectively (n = 10). By contrast, only 0.02 ± 0.01% of the input radioactivity was recovered with the protein A–anti-albumin immunoadsorbent. Radioactivity recovered in the protein A–anti-ligandin immunoadsorbent was only 4–7% of that recovered in the three membrane protein immunoprecipitates and did not differ from the nonspecifically bound radioactivity recovered with the protein A–anti-IgG adsorbent (Student's t test; $P >$ 0.2) confirming that intracellular proteins were not labeled under the conditions of this experiment. Comparable results were obtained in a separate study employing hepatocyte single-cell suspensions in lieu of hepatocyte monolayer cultures.

DISCUSSION

The paragraphs above present data on amino acid composition, N-terminal sequence, electrophoretic and chromatographic behavior, absorption spectra, enzymatic activity, fatty acid binding characteristics, immunologic relationships, and subcellular distribution all pointing to the conclusion that the h-FABP$_{PM}$ isolated from liver plasma membranes and mGOT are very similar. This conclusion is supported by the covalent labeling of both proteins with a [^{3}H]labeled C_{18} diazarino fatty acid derivative synthesized as a photoaffinity probe in the laboratory of G. Kurz (G. Stoll and G. Kurz, personal communication). Although mGOT, once released from mitochondria, may bind nonspecifically to a variety of lipid structures including cell membranes (36–38), four lines of evidence suggest that the h-FABP$_{PM}$ isolated from plasma membranes is indigenous to that subcellular location and is not merely the result of mitochondrial contamination. (*i*) The plasma membranes from which h-FABP$_{PM}$ was extracted were enriched in GOT activity, whereas the activity in the membranes of the conventional mitochondrial marker enzyme succinate dehydrogenase was reduced. (*ii*) Immunohistologic studies demonstrated the presence of h-FABP$_{PM}$/mGOT antigens in hepatocyte plasma membranes as well as intracellularly. (*iii*) Cell surface labeling studies with freshly isolated hepatocyte suspensions and monolayer cultures, under conditions that do not label intracellular proteins, led to appreciable labeling of h-FABP$_{PM}$. (*iv*) Rabbit anti-rat h-FABP$_{PM}$ and anti-rat mGOT selectively inhibited the plasma membrane transport system for free fatty acid uptake but had no effect on the analogous but distinct membrane transport systems for sulfobromophthalein and taurocholate. Since taurocholate uptake is a sodium-coupled secondary active process critically dependent on maintenance of an adequate intracellular ATP supply (reviewed in ref. 39), the latter data suggest that the antibody-mediated inhibition of fatty acid uptake was not the result of mitochondrial injury.

Nuclear-encoded mitochondrial proteins typically are synthesized on free cytosolic ribosomes as larger precursors containing N-terminal mitochondrial presequences or signal peptides (40, 41). mGOT is a well studied example of such a mitochondrial protein (42–50), and cDNAs for both the mature rat enzyme (31) and its larger precursor (30, 51) have been cloned. For mGOT to be identical to a plasma membrane protein would pose interesting questions in cell and molecular biology. A close similarity, if not identity, between another plasma membrane protein, organic anion-binding protein, and the β subunit of the mitochondrial F_1 ATP synthase has been suggested (52).

Despite the obvious similarities noted above, mGOT and h-$FABP_{PM}$ may not be identical. One of our monoclonal antibodies, 5C2, reacts strongly on Western blots with h-$FABP_{PM}$, but only weakly and inconsistently with mGOT. In nuclease protection assays with rat liver mRNA, employing as a probe a 252-base-pair fragment at the 5′ end of an authentic mGOT cDNA (the cDNA clone was kindly provided by J. R. Mattingly; ref. 30), two bands are seen: one corresponding to the intact probe and one of ≈125 base pairs. This suggests that, in addition to an mGOT mRNA, rat liver contains an mRNA that is identical over a region sufficient to code for ≤40 amino acids but then contains a mismatch of thus far undetermined extent. Finally, immunoscreening of rat liver cDNA libraries has identified, in addition to cDNA clones for mGOT, a crossreacting pUC8 clone, designated pm-1. The ≈800-base-pair pm-1 cDNA insert includes a poly(A) region and thus represents the 3′ end of the 2.4-kilobase mRNA in rat liver to which it hybridizes on Northern blots. Although this is the same size as mGOT mRNA, the restriction maps of pm-1 and mGOT cDNA differ. Because pm-1 encodes for the C-terminal end of a protein and amino acid sequence data for h-$FABP_{PM}$ are thus far available only for an N-terminal region, their relationship remains to be established.

At the present time, available data indicate that h-$FABP_{PM}$ and mGOT are strikingly similar. Clarification of the basis for these similarities will require further studies at both the protein and the nucleic acid level.

This work was supported in part by Grant DK26438 from the National Institutes of Health and by a generous gift from the Monique Weil-Caulier Bequest.

1. Bender, D. A. (1975) *Amino Acid Metabolism* (Wiley, New York).
2. Williamson, J. R. (1979) *Annu. Rev. Physiol.* **41**, 485–506.
3. Boyd, J. W. (1961) *Biochem. J.* **81**, 434–441.
4. Katsunuma, N., Matsuzawa, T. & Fujino, A. (1962) *J. Vitaminol.* **8**, 74–79.
5. Morino, Y. & Wada, H. (1963) in *Proceedings of the Symposium on Chemical and Biological Aspects of Pyridoxal Catalysis*, eds. Snell, E. E., Fasella, P. M. & Rossi-Fannelli, A. (Pergamon, New York), pp. 175–184.
6. Braunstein, A. E. (1973) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 9, 3rd Ed., pp. 377–481.
7. Stremmel, W., Strohmeyer, G., Borchard, F., Kochwa, S. & Berk, P. D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4–8.
8. Potter, B. J., Stump, D., Schwieterman, W., Sorrentino, D., Jacobs, L. N., Kiang, C.-L., Rand, J. & Berk, P. D. (1987) *Biochem. Biophys. Res. Commun.* **148**, 1370–1376.
9. Stremmel, W., Lotz, G., Strohmeyer, G. & Berk, P. D. (1985) *J. Clin. Invest.* **75**, 1068–1076.
10. Schwieterman, W., Sorrentino, D., Potter, B. J., Rand, J., Kiang, C.-L., Stump, D. & Berk, P. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 359–363.
11. Sorrentino, D., Stump, D., Potter, B. J., Robinson, R. B., White, R., Kiang, C.-L. & Berk, P. D. (1988) *J. Clin. Invest.* **82**, 928–935.
12. Stremmel, W. (1988) *J. Clin. Invest.* **81**, 844–852.
13. Bass, N. M. (1985) *Chem. Phys. Lipids* **38**, 95–114.
14. Huynh, Q. K., Sakakibara, R., Watanabe, T. & Wada, H. (1980) *J. Biochem. (Tokyo)* **88**, 231–239.
15. Reichen, J., Blitzer, B. L. & Berk, P. D. (1981) *Biochim. Biophys. Acta* **640**, 298–312.
16. Fisher, M. M., Bloxam, D. L., Oda, M., Phillips, M. J. & Yousef, Y. M. (1975) *Proc. Soc. Exp. Biol. Med.* **150**, 177–184.
17. Rej, R. & Hørder, M. (1983) in *Methods of Enzymatic Analysis*, ed. Bergmeyer, H. U. (Verlag Chemie, Weinheim, F.R.G.), 3rd Ed., pp. 416–424.
18. Baumer, M. E. & Doonan, S. (1976) *Int. J. Biochem.* **7**, 119–124.
19. Galfre, E. & Milstein, C. (1981) *Methods Enzymol.* **73**, 1–46.
20. Thung, S. N., Gerber, M. A., Kasambalides, E. J., Gilja, B. K., Keh, W. G. & Gerlich, W. H. (1986) *Hepatology* **6**, 1315–1318.
21. Aoki, N., Gerber, M. A., Thung, S. N., Chen, M. L., Christman, J. K., Price, P. M., Flordellis, C. S. & Acs, G. (1984) *Hepatology* **4**, 84–89.
22. Beloqui, O., Nunes, R. M., Blades, B., Berk, P. D. & Potter, B. J. (1986) *Alcohol. Clin. Exp. Res.* **10**, 463–470.
23. Stremmel, W. & Berk, P. D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3086–3090.
24. Stremmel, W., Strohmeyer, G. & Berk, P. D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3584–3588.
25. Okuda, H., Nunes, R. M., Vallabhajosula, S., Strashun, A., Goldsmith, S. H. & Berk, P. D. (1986) *J. Hepatol.* **3**, 251–259.
26. Stremmel, W., Gerber, M. A., Glezerov, V., Thung, S. N., Kochwa, S. & Berk, P. D. (1983) *J. Clin. Invest.* **71**, 1796–1805.
27. Watts, C. (1985) *J. Cell Biol.* **100**, 633–637.
28. Huynh, Q. K., Sakakibara, R., Watanabe, T. & Wada, H. (1980) *Biochem. Biophys. Res. Commun.* **97**, 474–479.
29. Doonan, S., Barra, D., Bossa, F., Porter, P. B. & Wilkinson, S. M. (1981) *Comp. Biochem. Physiol. B* **69**, 747–752.
30. Mattingly, J. R., Jr., Rodriguez-Berrocal, F. J., Gordon, J., Iriarte, A. & Martinez-Carrion, M. (1987) *Biochem. Biophys. Res. Commun.* **149**, 859–865.
31. Horio, Y., Sakakibara, R., Tanaka, T., Taketoshi, M., Obaru, K., Shimada, K., Morino, Y. & Wada, H. (1986) *Biochem. Biophys. Res. Commun.* **134**, 803–811.
32. Cremel, G., Filliol, D. & Waksman, A. (1985) *Anal. Biochem.* **150**, 332–336.
33. Glatz, J. F. C. & Veerkamp, J. H. (1983) *Anal. Biochem.* **132**, 89–95.
34. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672.
35. Kallen, R. G., Korpela, T., Martel, A. E., Matushima, Y., Metzler, C. M., Metzler, D. E., Morozov, Y. V., Ralston, I. M., Savin, F. A., Torchinsky, Y. M. & Hiroshi, U. (1985) in *Transaminases*, eds. Christen P. & Metzler, D. E. (Wiley, New York), pp. 91–99.
36. Marra, E., Doonan, S., Saccone, C. & Quagliariello, E. (1977) in *Bioenergetics of Membranes*, eds. Packer, L., Papageorgiou, G. C. & Trebst, A. (Elsevier/North Holland, Amsterdam), pp. 415–424.
37. Furuya, E., Yoshida, Y. & Tagawa, K. (1979) *J. Biochem. (Tokyo)* **85**, 1157–1163.
38. Marra, E., Doonan, S., Saccone, C. & Quagliariello, E. (1978) *Eur. J. Biochem.* **83**, 427–435.
39. Scharschmidt, B. F. (1982) in *Hepatology*, eds. Zakim, D. & Boyer, T. D. (Saunders, Philadelphia), pp. 297–351.
40. Hay, R., Bohni, P. & Gasser, S. (1984) *Biochim. Biophys. Acta* **779**, 65–87.
41. Roise, D. & Schatz, G. (1988) *J. Biol. Chem.* **263**, 4509–4511.
42. Sondereger, P., Jaussi, R. & Christen, P. (1980) *Biochem. Biophys. Res. Commun.* **94**, 1256–1260.
43. Sakakibara, R., Huynh, Q. K., Nishida, Y., Watanabe, T. & Wada, H. (1980) *Biochem. Biophys. Res. Commun.* **95**, 1781–1788.
44. Sakakibara, R., Kamisaki, Y. & Wada, H. (1981) *Biochem. Biophys. Res. Commun.* **102**, 235–242.
45. Sannia, G., Abrescia, P., Columbo, M., Giardina, P. & Marino, G. (1982) *Biochem. Biophys. Res. Commun.* **105**, 444–449.
46. Behra, R. & Christen, P. (1986) *J. Biol. Chem.* **261**, 257–263.
47. Jaussi, R., Cotton, B., Juretic, N., Christen, P. & Schumperli, D. (1985) *J. Biol. Chem.* **260**, 16060–16063.
48. Jaussi, R., Behra, R., Giannattasio, S., Flura, T. & Christen, P. (1987) *J. Biol. Chem.* **262**, 12434–12437.
49. Obaru, K., Nomiyama, H., Shimada, K., Nagashima, F. & Morino, Y. (1986) *J. Biol. Chem.* **261**, 16976–16983.
50. Joh, T., Nomiyama, H., Maeda, S., Shimada, K. & Morino, Y. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6065–6069.
51. Pave-Preux, M., Ferry, N., Bouguet, J., Hanoune, J. & Barouki, R. (1988) *J. Biol. Chem.* **263**, 17459–17466.
52. Wolkoff, A. W., Goeser, T. & Novikoff, P. M. (1989) *Hepatology* **10**, 630 (abstr.).