Eur. J. Biochem. 211, 725–730 (1993)

Characterization of cardiac fatty-acid-binding protein from human placenta

Comparison with placenta hepatic types

Tanya DAS, Gaurisankar SA and Manju MUKHERJEA
Department of Biochemistry, University of Calcutta, India

(Received September 29, 1992) – EJB 92 1378

When a 105000×*g* supernatant of human placenta was incubated with [1-^{14}C]oleate and subjected to Sephadex G-75 gel filtration and HPLC, two fatty-acid-binding protein (FABP) peaks were obtained. One of these, when further purified by carboxymethyl-cellulose, gave one 15.3-kDa FABP with p*I* 5.3. The other, when chromatographed on DEAE cellulose, separated into two 14.2-kDa FABP with p*I* 6.9 and 5.4. Purity of the proteins was checked by SDS/PAGE. Molecular mass, p*I*, immunochemical properties and amino acid compositions all indicated that 15.3-kDa FABP was of the cardiac type, whereas both 14.2-kDa FABP were of the hepatic type. Cardiac FABP did not cross-react with hepatic proteins. When tested for the acceptor/donor properties of these FABP, hepatic types were found to be better candidates than cardiac in uptaking fatty acids from liposomes. Cardiac FABP, on the other hand, acted in a more efficient way as a donor, indicating a distinct role of these proteins in human placenta, which furnishes a multiorgan system for the developing fetus.

Fatty-acid-binding proteins (FABP) are found in extraordinary abundance in various mammalian tissues that are involved in uptake and/utilization of fatty acids [1, 2]. These proteins belong to a new superfamily of 14–16 kDa nonenzymic cytosolic proteins which are characterized by structural similarity, indicating a common ancestral gene [3, 4]. In addition to FABP, this protein family includes the various cellular retinoid-binding proteins, the p2 protein of periferal nerve myelin and the p422 adipocyte protein [5–7]. Recently, two new proteins, bovine mammary-derived growth inhibitor, a proposed growth inhibitor and gastrotropin, a putative stimulator of gastric acid and pepsinogen secretion, have been shown to belong to this family, extending the similarity in an unexpected way [8, 9].

Of all FABP reported so far, perhaps the most extensively studied are hepatic (h), cardiac (c) and gut (g) types [1]. Although the precise physiological role of these proteins is yet to be elucidated, the proposed functions include promoting the cellular uptake and intracellular utilization of fatty acids, targeting fatty acids and acyl-CoA to different metabolic pathways, modulating enzyme activities and protecting enzymes and cellular structures from detergent effects. FABP have tissue-specific expression with some tissues containing more than one type [10, 11]. Each of these proteins may thus be adapted to serve a unique physiological role in one organ according to tissue needs.

During embryogenesis, free fatty acids are transported across the placenta from mother to fetus and vice versa [12, 13]. In addition, both fatty acid synthesis [14] and oxidation [15] occur in placenta. FABP may therefore have an important role in this unique tissue which is the sole purveyor of all fetal needs. The presence of cFABP mRNA in late gestation has been demonstrated in rat placenta [16]. We have earlier isolated two isoforms of FABP from developing human placenta and studied their role in regulating fatty acid metabolism [14, 17]. This paper reports the purification and characterization of a cFABP from human placenta and its comparison with the placenta hFABP, which were isolated previously.

Correspondence to M. Mukherjea, Department of Biochemistry, University of Calcutta, 35, Ballygunge Circular Road, Calcutta, India 700 019

Abbreviations. FABP, fatty-acid-binding proteins; h, hepatic; c, cardiac; g, gut.

MATERIALS AND METHODS

Amino acid kits, Sephadex G-75, DEAE-cellulose, carboxymethyl-cellulose, α-lactalbumin, soybean trypsin inhibitor, trypsinogen, carbonic anhydrase, cytochrome *c*, SDS, Tris, Lipidex 1000, agarose and phenyl isothiocyanate were purchased from Sigma Chemical Co., St. Louis, USA. Pharmalyte was obtained from Pharmacia Fine Chemicals, Sweden. Egg yolk phosphatidylcholine (PtdCho) was from Lipid Products, Surry, UK. [1-^{14}C]Oleic and [1-^{14}C]stearic acids were gifts from Prof. K. D. Mukherjee, Federal Centre for Lipid Research, Münster, FRG. All other chemicals were of HPLC or analytical grade and were purchased from local dealers.

Human placentas of 5–30 weeks gestation were collected from patients undergoing legal abortion either by suction or via hysterotomy from the Department of Obstetrics and Gynecology, National Medical College and Hospital, Calcutta. Placentas above 30 weeks were collected from patients delivering stillborn babies from Medical Termination of Pregnancy Clinics in and around Calcutta. Term placentas were also obtained after normal delivery or via Cesarian section. Tissues were collected within 15 min of delivery/oper-

ation and kept in ice. Gestational ages were calculated from the period of amenorrhea and by crown-rump length of the fetus [18].

Preparation of human placenta supernatant

Human placenta tissues were washed in cold saline, minced, suspended in 10 mM potassium phosphate, pH 7.5, containing 1 mM mercaptoethanol and 0.154 M KCl (buffer A) and homogenized using a Teflon glass homogenizer. Homogenate thus obtained was centrifuged at 105 000 × *g* for 1 h, lyophilised and used for further studies.

Isolation of placenta FABP

Lyophilised powder was dissolved in minimum volume of buffer A and delipidated with Lipidex 1000 [19]. To this supernatant, [1-^{14}C]oleate was added prior to gel filtration on Sephadex G-75 (2.5 cm × 80 cm) equilibrated and developed in the same buffer. A protein peak at molecular mass 10–20 kDa with affinity for labelled fatty acids was collected, dialyzed against buffer A and subjected to HPLC (Hewlett Packard Series HP 1050) on a gel-filtration column (TSK-3000 SW XL, 7.8 mm × 300 mm, Hewlett Packard). The eluted fractions were assayed for FABP activity, and those showing positive response were subjected to further purification.

Purification of placenta cFABP

The fractions containing FABP activity and having relatively higher molecular mass (14–16 kDa) were dialyzed against 10 mM sodium acetate, pH 5.0 (buffer B), and applied to a carboxymethyl-cellulose column (1 cm × 5 cm) previously equilibrated with buffer B. The column was eluted by a linear gradient of sodium acetate (10–200 mM). Fractions containing FABP activity were pooled, concentrated, adjusted to pH 7.5 and stored at −20°C.

Purification of placenta hFABP

After HPLC elution, the protein peak of lower molecular mass (14–16 kDa), showing affinity for added ligand, was dialysed against 10 mM Tris/Cl, pH 8.5, and further purified by DEAE-cellulose anion-exchange chromatography following our previously published method [17]. FABP eluted from the column were concentrated and stored at −20°C.

Measurement of FABP

Protein determinations were performed following the method of Lowry et al. [20] using bovine serum albumin as standard. Error was corrected by binding assay using the method of Glatz and Veerkamp [19].

Electrophoretic experiments and isoelectric focusing

Purity of the proteins was judged by SDS/PAGE according to the method of Laemmli [21] using a 12.5% resolving gel. Molecular mass markers and FABP were solubilized at 100°C in SDS/PAGE sample buffer. The markers were α-lactalbumin, soybean trypsin inhibitor, trypsinogen, carbonic anhydrase and cytochrome *c*. Proteins were detected by staining with Coomassie brilliant blue R250.

Isoelectric focusing on polyacrylamide gels was performed by the method of Wrighly [22] using 5% gel columns. Carrier ampholines of pH 3–10 were used at a final concentration of 20% (by vol.).

Immunological methods

Human anti-cFABP serum was a gift from Prof. F. Spener, University of Münster, FRG. Human fetal c-FABP, antibodies against human placenta p*I*-6.9 FABP and human fetal hFABP were prepared in our laboratory [17, 23, 24]. Immunological cross-reactivity of human placenta FABP was tested by Ouchterlony double-immunodiffusion test [25].

Amino acid analysis

Lyophilyzed FABP were hydrolysed with 6 M HCl at 110°C for 24 h. For determination of Trp, protein samples were hydrolysed with 0.1% phenol. Phenyl thiocyanate derivatives of amino acids were prepared by coupling the amino acids with phenyl isothiocyanate and detected in HPLC (Hewlett Packard) using ODS 2 µm column (125 mm × 4 mm). A gradient of 50% acetonitrile in 12.5 mM phosphate, pH 6.4, was used as the mobile phase.

Lipid analysis

Lipids were extracted from the proteins by the method of Folch et al. [26] and separated by TLC. Individual lipids were identified using standards.

Preparation of liposomes

The designated amount of PtdCho for the liposomes was dissolved in chloroform/methanol (2:1, by vol.). The solvent was evaporated with N_2, and the lipid was further dried in vacuum for 1 h. To prepare the liposomes, a procedure was followed described by Brecher et al. [27]. [1-^{14}C]Oleate and [1-^{14}C]stearate were routinely included in the liposome preparations.

Transfer of fatty acids from liposome to FABP

The incubation medium (total volume 500 µl) contained 400 µl fatty-acid-containing liposomes diluted to the appropriate concentration and 100 µl FABP solution. After addition of binding protein and gentle mixing, the samples were incubated for 60 min at room temperature and centrifuged at 105 000 × *g* for 30 min at 4°C. Radioactivity in the supernatant represented the FABP-bound ligand. A correction was made for blank incubated in the absence of protein.

Movement of fatty acids from FABP to vesicles

To examine the donor property of FABP, the proteins were incubated with [1-^{14}C]oleate for 30 min at ambient temperature. The fatty acid was added from the concentrated ethanol stock solution, with final ethanol concentration less than 1% (by vol.). It was assumed that at a 1:10 ratio of FABP/fatty acid, all the FABP was saturated. Unbound fatty acids were removed by treatment with Lipidex 1000 for 10 min at 0°C [19]. After removal of Lipidex 1000 by a brief centrifugation amount of bound fatty acid was assayed in supernatant. An equal volume of donor FABP and acceptor vesicles were mixed and incubated for 60 min. Liposomes

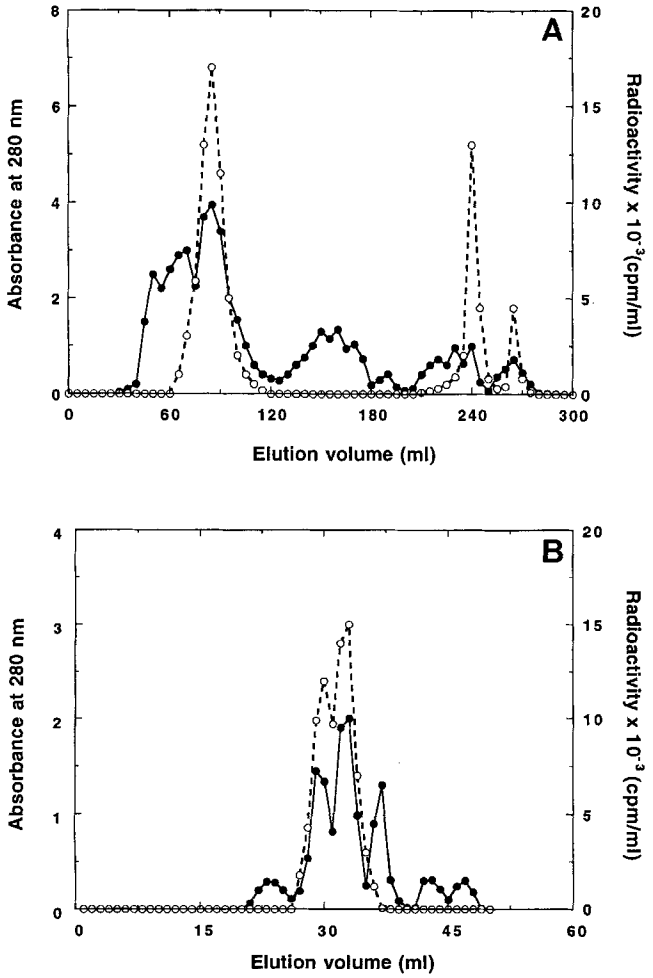

Fig. 1. Purification of human placenta FABP by gel filtration. (A) Delipidated placenta cytosol, charged with [1-^{14}C]oleate, was applied to a Sephadex G-75 column (2.5 cm × 80 cm) and eluted with 10 mM phosphate, pH 7.5. (B) The second peak from (A) was further chromatographed on an HPLC gel-filtration column (7.8 mm × 300 mm) using the same buffer. (●) Protein; (○) [1-^{14}C]oleate bound.

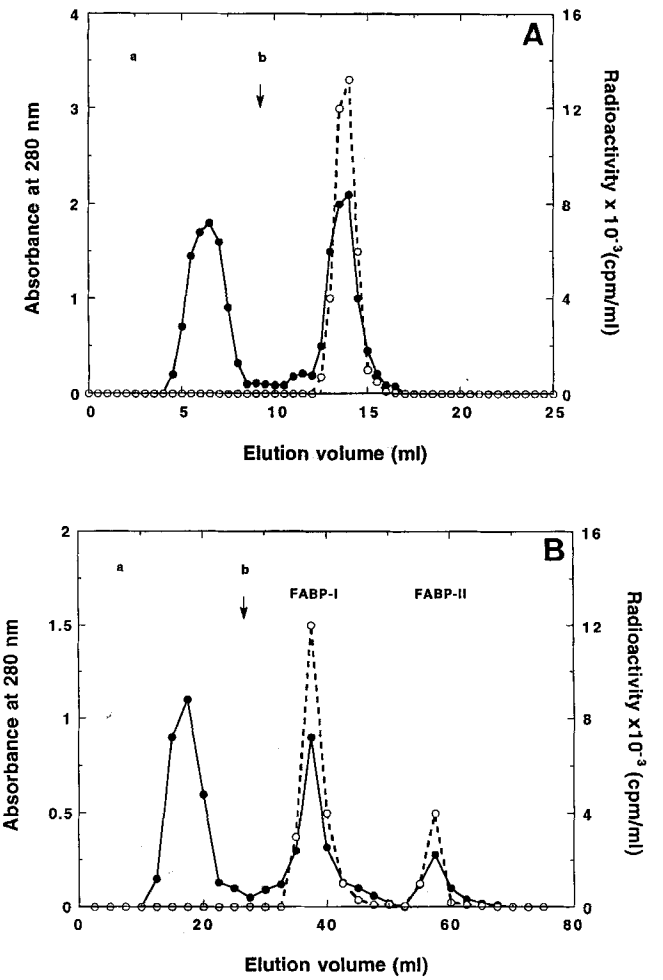

Fig. 2. Ion-exchange chromatography of placenta FABP. (A) The first peak from Fig. 1B was loaded onto a carboxymethyl-cellulose column (1 cm × 5 cm). (a) Elution by 10 mM sodium acetate, pH 5.0. (b) Elution by a linear gradient of sodium acetate (10–200 mM). (B) The second peak from Fig. 1B was applied to a DE-AE-cellulose column (1 cm × 10 cm). (a) Elution by 10 mM Tris/Cl, pH 8.5. (b) Elution by a linear gradient of NaCl up to 300 mM (●) Protein; (○) [1-^{14}C]oleate bound.

were then separated by centrifugation at 105000 × *g* for 30 min. Radioactivity of supernatant was used for calculating the amount of remaining ligand.

RESULTS

Purification of FABP from the cytosol of human placenta

Incubation of 105000 × *g* supernatant from human placenta with [1-^{14}C]oleate and subsequent separation over Sephadex G-75 revealed two protein peaks containing ^{14}C-labelled fatty acid (Fig. 1A). The first peak (70 kDa) corresponded to the added ligand bound to albumin from contaminating blood proteins. The second peak represented [1-^{14}C]oleate complexed by one or more 12–16-kDa protein. Since in human blood no low-molecular-mass FABP has been detected, the placenta origin of the binding protein(s) of the second peak was ascertained. This fraction, when subjected to HPLC, again resolved into three close peaks, of which the first two were able to bind [1-^{14}C]oleate (Fig. 1B). The first fraction having relatively higher molecular mass was further purified by carboxymethyl-cellulose. When eluted with a gradient of sodium acetate (10–200 mM), a single protein peak was obtained (Fig. 2A) which retained the fatty acid. The second peak after HPLC was chromatographed on a DEAE-cellulose column. As previously reported [17], using a NaCl gradient of 0–300 mM, two fatty-acid-binding peaks, FABP-I and FABP-II, were recovered (Fig. 2B).

Table 1. Determination of molecular mass of placenta FABP.

FAB type	p*I*	Molecular mass from	
		gel filtration	SDS/PAGE
		kDa	
cFABP	5.3	15.5	15.3
hFABP	5.4	14.5	14.2
hFABP	6.9	14.5	14.2

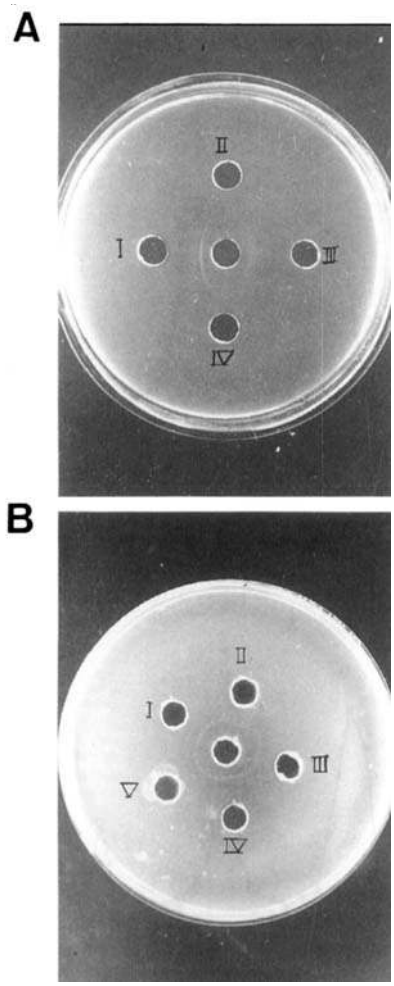

Fig. 3. Ouchterlony double-immunodiffusion of placenta FABP. (A) Cross-reactivity with cFABP. The central well contained 20 ml anti-cFABP serum, peripheral wells contained placenta p*I*-5.3 cFABP (I), p*I*-5.4 hFABP (II), fetal cFABP (III) and p*I*-6.9 hFABP (IV), 5 mg each. (B) Cross-reactivity with hFABP. The central well contained 20 ml anti-hFABP serum, peripheral wells contained placenta cytosol (I), p*I*-5.4 hFABP (II), p*I*-6.9 hFABP (III), fetal hFABP (IV) and liver cytosol (V), 5 mg each.

Determination of molecular mass

Over the year, the molecular masses of different FABP have been reported to be 14–16 kDa. 14.2 kDa has been described for hFABP, 15.3 kDa for cFABP and 15.1 kDa for gFABP [1]. We used several approaches to obtain a good estimate of the molecular masses of three FABP obtained. As can be seen from Table 1, values obtained via gel chromatography and electrophoresis agreed quite well. Interestingly, the molecular mass of the FABP obtained by carboxymethylcellulose was 15.3 kDa, and those of both FABP-I and FABP-II were 14.2 kDa [17]. Carbohydrate covalently linked to FABP that may substantially add to the molecular mass was not detected when assayed with the Schiff reagent [28].

Isoelectric focussing

The p*I* of 15.3-kDa FABP was found to be 5.3. The p*I* values of FABP-I and FABP-II (14.2 kDa each) were already obtained as 6.9 and 5.4, respectively (figure not shown).

Table 2. Amino acid composition of human placenta FABP. Molecular masses of both hFABP are 14.2 kDa and that of cFABP is 15.3 kDa. Values are given to the nearest integer.

Amino acid	Amino acid content				
	hFABP		cFABP	hFABP [3]	cFABP [31]
	p*I* 6.9	p*I* 5.4	p*I* 5.3		
	mol/mol				
Ala	4	4	5	3	6
Arg	2	2	4	2	4
Asx	11	11	15	11	14
Cys	1	1	2	1	1
Glx	20	20	16	20	11
Gly	11	11	10	11	10
His	1	1	4	1	3
Ile	13	13	7	12	7
Leu	7	7	13	8	14
Lys	15	15	19	15	14
Met	3	3	2	4	2
Phe	8	8	6	8	6
Pro	1	1	2	1	2
Ser	7	7	7	7	6
Thr	12	12	17	12	19
Trp	0	0	1	0	2
Tyr	1	1	3	1	2
Val	10	10	11	10	10
Total	127	127	140	127	132

Immunological cross-reactivity

In double-immunodiffusion assay, anti-(human cFABP) serum precipitated only with placenta p*I*-5.3 FABP and fetal c-FABP (Fig. 3A), the pattern revealing complete cross-reactivity. On the other hand, placenta p*I*-6.9 and p*I*-5.4 FABP were both precipitated with anti-(fetal hFABP) serum (Fig. 3B). It was observed that there were reactions of identity between these two lines and those formed with placenta cytosol, fetal hFABP and fetal liver cytosol. Anti-(human cFABP) serum was unable to precipitate placenta p*I*-6.9 and p*I*-5.4 FABP and anti-(p*I*-6.9 FABP) serum did not react with placenta p*I*-5.3 FABP (figure not shown).

Amino acid composition

The amino acid composition of p*I*-6.9 and p*I*-5.4 FABP, revealed by amino acid analysis and individual determinations, were almost identical (Table 2). In each of these proteins, the most prominent feature was the absence of Trp. The compositions were notably distinct from that of p*I*-5.3 FABP. Amino acid compositions of the formar two showed similarity to that of human hFABP [3], whereas similarity could be observed between the amino acid composition of p*I*-5.3 FABP and human cFABP [29, 30]. Moreover, our finding that p*I*-5.3 FABP contains Cys is in line with that reported by Borcher et al. [31].

Lipid analysis

Presence of endogenous lipids was detected in all three placenta FABP by TLC. However, fatty acids were the only detectable lipid component in these fractions and were extracted completely by organic solvents indicating non-covalent binding.

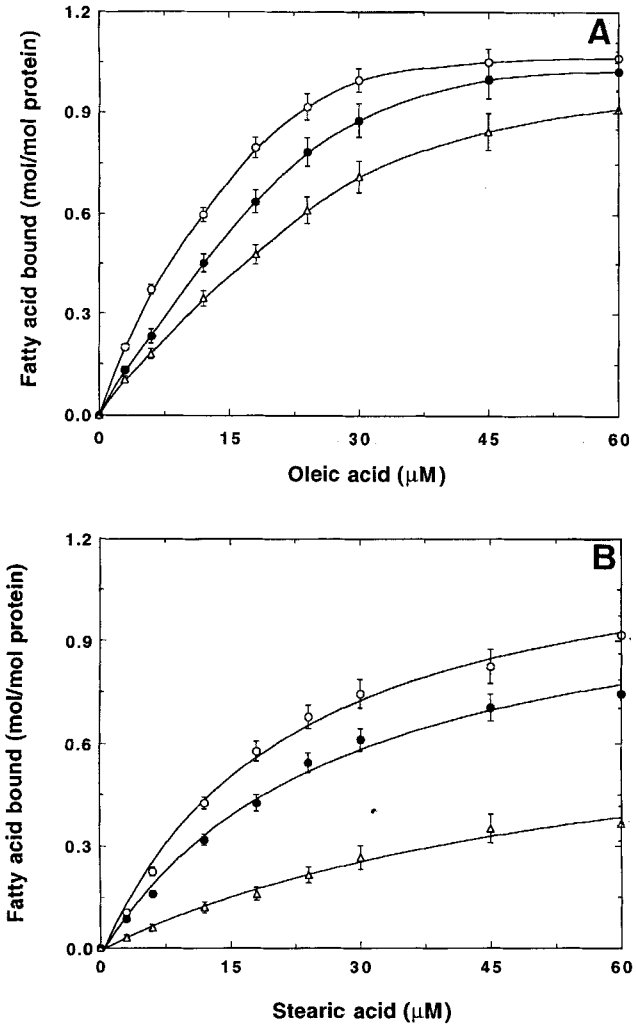

Fig. 4. Transfer of fatty acid from liposome to FABP. Varying amounts of liposomes containing labelled oleic acid (A) or stearic acid (B) were incubated with 10 mM of either p*I*-6.9 (○) or p*I*-5.4 (●) or p*I*-5.3 (△) FABP for 60 min at 37°C. Reaction mixtures were centrifuged and the labelled ligands in the supernatant was assayed for bound fatty acid. Values are means ± SEM of three sets of experiments.

Transfer of fatty acid from liposome to FABP

The transfer of [1-^{14}C]oleate and [1-^{14}C]stearate was monitored as described under Materials and Methods. Fig. 4A shows that all the three FABP participated in the uptake of [1-^{14}C]oleic acid, although the transferring ability is higher for p*I*-6.9 and p*I*-5.4 hFABP than p*I*-5.3 FABP. Highest efficiency was observed with p*I*-6.9 hFABP. When [1-^{14}C]stearic acid was included inside the liposome, p*I*-5.3 cFABP showed almost no uptake (Fig. 4B). In this case also a dominance of p*I*-6.9 hFABP over p*I*-5.4 FABP was observed.

Reversibility of fatty acid movement

In agreement with previous reports [32, 33], the net movement of long-chain fatty acid from all three FABP to membranes was evident (Fig. 5). In this report, we also showed a striking difference in the amount of fatty acid transfer from p*I*-5.3 cFABP versus p*I*-6.9 or p*I*-5.4 hFABP to liposomes. p*I*-5.3 cFABP has been found to be more efficient

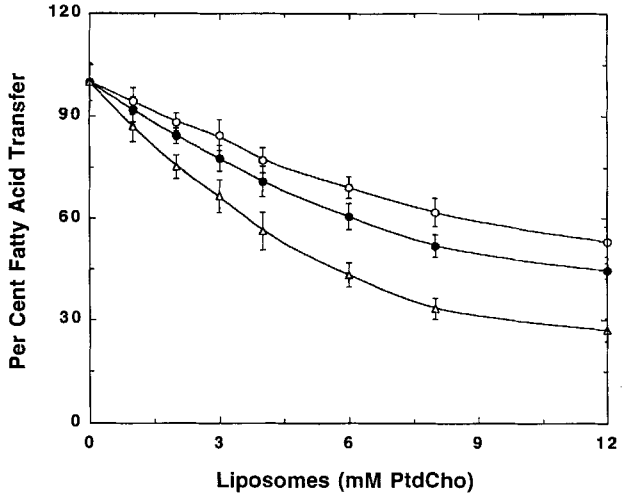

Fig. 5. Reversibility of fatty acid movement. Different amount of liposomes were incubated with ^{14}C-labelled oleic acid bound either p*I*-6.9 (○), or p*I*-5.4 (●), or p*I*-5.3 (△) FABP for 60 min at 37°C. Radioactivity of the supernatant was used to calculate the amount of remaining ligand. Values are means ± SEM of three sets of experiments.

than both p*I*-6.9 and p*I*-5.4 hFABP in donating its fatty acid to phospholipid vesicles.

DISCUSSION

The present investigation has shown that human placenta contains two types of FABP, hFABP and cFABP, on the basis of molecular mass, amino acid composition, immunochemical behavior, p*I* and binding affinities. In our study, one cFABP, with 15.3 kDa and p*I* 5.3, and two isoforms of hFABP, each with 14.2 kDa and p*I* values 6.9 and 5.4, have been obtained. These results conform with those obtained for cFABP and hFABP of adult human heart [29–31] and liver [3], respectively.

Amino acid compositions and immunochemical properties of human placenta FABP also support the presence of both cFABP and hFABP in this tissue. Ouchterlony double-immunodiffusion studies revealed that immune sera directed against cFABP or hFABP do not cross-react with the opposite protein. This observation is in agreement with the results of Said and Schultz [34] and Offner et al. [35] with rat liver and cFABP.

It is known that intestine also contains two types of FABP, hFABP and gFABP. Cistola et al. postulated that these two FABP may form a multifunctional transport system in the cytosol to transport fatty acids and a wide variety of amphiphiles [36]. Unlike enterocytes, where two different types of FABP are expressed in one cell type, placenta is a multiorgan system, therefore, the reasons for the presence of two types of FABP in this tissue may be different. We therefore searched for specific functions of cFABP and hFABP in this unique tissue. When oleic acid was incorporated into liposomes and equilibrated with placenta FABP, both cFABP and hFABP bound the fatty acid. Under our study conditions, hFABP showed dominance over cFABP in accepting fatty acid from model membranes. When stearic acid was used, cFABP showed almost no binding. The results are in line with the fact that cFABP does not bind stearate [37]. When reversibility of fatty acid movement was studied, it was ob-

served that cFABP donates oleic acid to liposomes with higher efficiency than hFABP. All the findings indicate different roles of cFABP and hFABP in human placenta fatty acid uptake and utilization.

A number of data suggest that although hFABP promote fatty acid activation and utilization both by mitochondrial and microsomal pathways, it in fact favors partition towards the latter [38, 39]. Lunzer et al. [40] have implicated hFABP as the principal acceptor of fatty acids. hFABP may also modulate the inhibitory effects of long-chain fatty acids and acyl-CoA on enzymes, but reduction in fatty acid oxidation has not been found to be associated with the change in hFABP mRNA levels [11]. On the other hand, tissue distribution of cFABP is generally, but not entirely, consistent with a role for this protein as an intracellular carrier of fatty acids destined predominantly for utilization as a fuel source via β-oxidation [41]. Since human placenta serves as a multiorgan system until the fetal organs have developed, presence of both cFABP and hFABP may be of great importance from the functional aspect of this tissue. Further work is in progress to find the exact functions of these two types of FABP in relation to fetal development.

We thank Prof. A. K. Ghosh, National Medical College and Hospital, Calcutta, for clinical materials, Dr. K. D. Mukherjee, Federal Centre of Lipid Research, Münster, FRG, for the gifts of [1-^{14}C]oleate and [1-^{14}C]stearate. Grateful acknowledgement is due to Prof. F. Spener, University of Münster, FRG, for the generous gift of anti-(human cFABP) serum. This work was supported by a grant from the Council of Scientific and Industrial Research, New Delhi, India.

REFERENCES

1. Veerkamp, J. H., Peeters, R. A. & Maatman, R. G. H. J. (1991) *Biochim. Biophys. Acta 1081*, 1–24.
2. Kaikaus, R. M., Bass, N. M. & Ockner, R. K. (1990) *Experientia (Basel) 46*, 617–633.
3. Chan, L., Wei, C. F., Li, W. H., Yang, C. Y., Ranter, P., Pownall, H., Goto, A. M. Jr & Smith, L. C. (1985) *J. Biol. Chem. 260*, 2629–2632.
4. Spener, F. & Mukherjea, M. (1990) in *Subcellular biochemistry* (Hilderson, H. J., ed.) vol. 16, pp. 1–19, Plenum Press, New York.
5. Chytil, F. & Ong, D. E. (1987) *Annu. Rev. Nutr. 7*, 321–335.
6. Hunt, C. R., Ro, J. H. S., Dobson, D. E., Min, H. Y. & Spiegelman, B. M. (1986) *Proc. Natl Acad. Sci. USA 83*, 3786–3790.
7. Bernlohr, D. A., Angus, C. W., Lane, M. D., Bolanowski, M. A. & Kelly, T. J. Jr (1984) *Proc. Natl Acad. Sci. USA 81*, 5468–5472.
8. Bohmer, F. D., Kraft, R., Otto, A., Wernstedt, C., Hellmann, U., Kurtz, A., Muller, T., Rohde, K., Etzold, G., Lehmann, W., Langen, P., Heldin, C. H. & Grosse, R. (1987) *J. Biol. Chem. 262*, 15137–15143.
9. Walz, D. A., Wider, M. D., Snow, J. W., Dass, C. & Desiderio, D. M. (1988) *J. Biol. Chem. 263*, 14189–14195.
10. Kimura, H., Odoni, S., Nishi, S., Sato, H., Arakawa, M. & Ono, T. (1991) *J. Biol. Chem. 266*, 5963–5972.
11. Gordon, J. I., Elshourbagy, N., Lowe, J. B., Liao, S., Alpers, D. H. & Taylor, J. M. (1985) *J. Biol. Chem. 260*, 1995–1998.
12. Hull, D. (1975) *Br. Med. Bull. 31*, 32–36.
13. Wolf, H., Stave, U., Novak, M. & Monkus, E. F. (1974) *J. Perinat. Med. 2*, 75–87.
14. Das, T., Sa, G., Bandyopadhyay, A. & Mukherjea, M. (1991) *J. Biosci. 16*, 235–242.
15. Zimmerman, T., Hummel, L., Moller, U. & Kinzi, U. (1979) *Biol. Neonat. 36*, 109–112.
16. Heukeroth, R. O., Birkenmeier, E. H., Levin, M. S. & Gordon, J. I. (1987) *J. Biol. Chem. 262*, 9709–9717.
17. Das, T., Sa, G. & Mukherjea, M. (1988) *Lipids 23*, 528–533.
18. Chaudhury, D., Kushari, J. & Mukherjea, M. (1982) *Eur. J. Obstet. Gynecol. Reprod. Biol. 13*, 309–316.
19. Glatz, J. F. C. & Veerkamp, J. H. (1983) *Anal. Biochem. 132*, 89–95.
20. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randal, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
21. Laemmli, U. K. (1970) *Nature 227*, 680–685.
22. Wrighley, C. W. (1971) *Methods Enzymol. 22*, 559–564.
23. Das, T., Sa, G. & Mukherjea, M. (1989) *Biochim. Biophys. Acta 1002*, 164–172.
24. Das, T., Sa, G., Ghosh, P. K., Mukhopadhyay, D. & Mukherjea, M. (1989) *Med. Sci. Res. 17*, 985–986.
25. Ouchterlony, O. (1962) *Prog. Allergy 6*, 30–154.
26. Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem. 226*, 497–509.
27. Brecher, P., Saouaf, R., Sugarman, J. M., Eisenberg, D. & LaRosa, K. (1984) *J. Biol. Chem. 259*, 13395–13340.
28. Zacharias, R. M., Zell, T. E., Morrison, J. H. & Woodlock, J. J. (1969) *Anal. Biochem. 30*, 148–152.
29. Unterberg, C., Heidl, G., von Bassewitz, D. B. & Spener, F. (1986) *J. Lipid Res. 27*, 1287–1293.
30. Offner, G. D., Brecher, P., Sawlivich, W. B., Costello, C. E. & Troxler, R. F. (1988) *Biochem. J. 252*, 191–198.
31. Borchers, T., Hojrup, P., Neilsen, S. U., Roepstorff, P., Spener, F. & Knudsen, J. (1990) *Mol. Cell. Biochem. 98*, 127–133.
32. McCormack, M. & Brecher, P. (1990) *Biochem. J. 244*, 717–723.
33. Storch, J. & Bass, N. M. (1990) *J. Biol. Chem. 265*, 7827–7831.
34. Said, B. & Schulz, H. (1984) *J. Biol. Chem. 259*, 1155–1159.
35. Offner, G. D., Troxler, R. F., Brecher, P. (1986) *J. Biol. Chem. 261*, 5584–5589.
36. Cistola, D. P., Scchettini, J. C., Banaszak, L. J., Walsh, M. T. & Gordon, J. I. (1989) *J. Biol. Chem. 264*, 2700–2710.
37. Bass, N. M. (1985) *Chem. Phys. Lipids 38*, 95–114.
38. Paulussen, R. J. A. & Veerkamp, J. H. (1990) in *Subcellular biochemistry* (Hilderson, H. J., ed.) vol. 16, pp. 175–226, Plenum Press, New York.
39. Iritani, N., Fukuda, E. & Inoguchi, K., (1980) *J. Nutr. Sci. Vitaminol.* 26, 271–277.
40. Lunzer, M. A., Manning, J. A. & Ockner, R. K. (1977) *J. Biol. Chem. 252*, 5483–5487.
41. Fournier, N. C. & Rahim, M. (1985) *Biochemistry 24*, 2387–2396.