J. Biosci., Vol. 14, Number 4, December 1991, pp. 235-242. © Printed in India.

Relationship between fatty acid binding proteins, acetyl-CoA formation and fatty acid synthesis in developing human placenta

TANYA DAS, GAURISANKAR SA, ANUP K BANDYOPADHYAY and MANJU MUKHERJEA*

Department of Biochemistry, University College of Science, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India

MS received 22 February 1991; revised 31 July 1991

Abstract. The relationship between fatty acid binding proteins, ATP citrate lyase activity and fatty acid synthesis in developing human placenta has been studied. Fatty acid binding proteins reverse the inhibitory efect of palmitoyl-CoA and oleate on ATP citrate lyase and fatty acid synthesis. In the absence of these inhibitors fatty acid binding proteins activate ATP citrate lyase and stimulate [1–^{14} C] acetate incorporation into placental fatty acids indicating binding of endogenous inhibitors by these proteins. Thus these proteins regulate the supply of acetyl-CoA as well as the synthesis of fatty acids from that substrates. As gestation proceeds and more lipids are required by the developing placenta fatty acid binding protein content, activity of ATP citrate lyase and rate of fatty acid synthesis increase indicating a cause and efect relationship between the demand of lipids and supply of precursor fatty acids during human placental development.

Keywords. ATP citrate lyase; fatty acid binding proteins; fatty acid synthesis; placenta; correlation.

1. Introduction

During embryogenesis when demand of lipid is very high to supply energy and to synthesize cellular membranes for the developing placenta, more lipids and precursor fatty acids are to be synthesized. The rate of fatty acid synthesis and total activities of the synthesizing enzymes are positively correlated in several animal systems. In chicken liver, fatty acid synthesis and the activities of acetyl-CoA carboxylase (Goodridge 1973), malic enzyme and ATP citrate lyase (Goodridge 1968; Silpananta and Goodridge 1971) are correlated when neonatal chicks are fed. Activities of these enzymes are determined largely by the relative concentrations of enzyme activators and inhibitors as well as by the composition of long chain fatty acids and acyl-CoA pool. The long chain fatty acids and their CoA esters may be compartmentalized in the cell and changes in their distribution might affect lipogenesis. It has been suggested that intracellular trafficking of these inhibitors might be affected partly by their binding to fatty acid binding proteins (FABPs) (Spener and Mukherjea 1990). These proteins belong to a class of low molecular mass (14–15 kDa) non-enzymic proteins which bind hydrophobic ligands and are abundantly present in the cytosol of many mammalian cells (Bass 1985; Sweetser *et al* 1987; Das *et al* 1989). FABPs are distinct from the recently discovered 10 kDa

*Corresponding author.

Abbreviations used: FABPs, fatty acid binding proteins; G6PD, glucose-6-phosphate dehydrogenase; PAL-CoA, palmitoyl-CoA.

acyl-CoA binding protein which binds medium chain acyl-CoAs but not fatty acids (Mogensen *et al* 1988). Besides having an important role in the cellular transport and metabolism of fatty acids, these proteins have been reported to regulate many fatty acid synthesizing enzymes (Paulussen and Veerkamp 1990; Spener *et al* 1989) either by directly enhancing delivery of substrates in a usable form, targeting substrates to particular metabolic fates or eliminating inhibitory effects of long chain fatty acids.

It is known that synthesis of palmitic acid from malonyl-CoA and acetyl-CoA is catalyzed by fatty acid synthase, a multienzyme complex. The extra mitochondrial acetyl-CoA is supplied by ATP citrate lyase and the reducing power required for fatty acid synthesis is furnished mainly by glucose-6-phosphate dehydrogenase (G6PD). All these enzymes get inhibited by fatty acids and their CoA esters (Glatz and Veerkamp 1985; Kawaguchi and Bloch 1974). Earlier reports from this laboratory show that FABPs protect G6PD from the detrimental efect of fatty acids and fatty acyl-CoA esters in human placenta and fetal tissues (Das *et al* 1988, 1989; Sa *et al* 1989). The present work aims at studying the relationship between FABPs, ATP citrate lyase activity and fatty acid synthesis in developing human placenta.

2. Materials and methods

ATP, bovine serum albumin (BSA), DEAE-cellulose, malate dehydrogenase, malonyl-CoA, NADH, oleic acid, palmitoyl-CoA (PAL-CoA) and Sephacryl S-200 were purchased from Sigma Chemical Co., St. Louis, Mo, USA. [1-^{14}C] acetate was a gift from Dr K D Mukherjee, Federal Centre of Lipid Research, Munster, Germany. All other chemicals used were of analytical grade and were purchased locally.

Human placentas of gestational ages between 5-30 weeks 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 obtained from patients delivering still born babies and term placentas were collected at the time of parturition or *via* caesarean section from different hospitals in Calcutta. Tissues were collected within 15 min of operation/delivery and kept in ice. Gestational ages were calculated from the period of amenorrhea and by crown-rump length of the fetus (Chaudhuri *et al* 1982).

2.1 *Preparation of human placental supernatant*

Placentas were excised, fragmented and washed with 0·9% NaCl to remove blood. Fragments were homogenized in 10 mM Tris-HCI buffer (pH 8·5) in a Teflon glass homogenizer and centrifuged at 105,000 *g* for 1 h. The supernatant was heated at 50°C for 20 min, shaken vigorously with 25% butanol (v/v) for 1 min and centrifuged at 36,000 *g* for 30 min to remove lipids and denatured proteins. The delipidated supernatant was lyophilized for complete removal of butanol.

2.2 *Preparation of human placental FABPs*

Isoforms of human placental FABPs (DE-I, DE-II and DE-III) were purified by the

procedure as described by Das *et al* (1988). The proteins were routinely characterized by UV spectroscopy and by SDS-PAGE to ensure single band of about 14,000 molecular weight and were stored at 0–4°C as lyophilized powder.

2.3 *Effect of PAL-CoA, oleate and FABPs on ATP citrate lyase*

Human placental cytosol (105,000 *g* supernatant) was prepared in 0·25 M sucrose. ATP citrate lyase activity was measured in a Hitachi spectrophotometer, Model U 3210, following the decrease in extinction at 340 nm according to the method of Takeda *et al* (1969). Inhibition of the enzyme was studied in the reaction mixture in presence of different concentrations of PAL-CoA or oleate. The isoforms of FABPs were added separately in the reaction mixture containing the inhibitors.

2.4 *Fatty acid synthesis*

Tissue slices of human placenta were incubated with 0·25 μCi of [1–^{14}C] acetate at 37°C for 3h and homogenized with 8 ml of chloroform/methanol (2:1) mixture. The homogenate was kept at room temperature for 4–6 h under N_2 with occasional shaking. After centrifugation the chloroform layer containing lipids was washed with 0·7% NaCl and concentrated in the presence of N_2. Individual lipids were separated by thin layer chromatography (TLC) and identified by using standard lipids. Fatty acid synthesis was studied by observing the amount of radioactivity incorporated throughout the gestation. Role of FABPs, PAL-CoA and oleate on fatty acid synthesis were examined.

2.5 *Estimation of protein*

Protein was estimated according to the method of Lowry *et al* (1951) using bovine serum albumin as standard.

2.6 *Statistical analysis*

Data were treated statistically using student's *t* test. In order to study the extent of correlation between any two of the relevant variables involved, correlation coefficients were computed. The variability of the data was presented as mean ± SEM. Differences at $P < 0{\cdot}05$ were considered to be significant.

3. Results

3.1 *Modulation of ATP citrate lyase activity by PAL-CoA, oleate, and FABPs*

Results of figure 1 indicate that human placental ATP citrate lyase is sensitive to PAL-CoA and oleate. Less than 75 μM PAL-CoA or 100 μM oleate completely inhibit the enzyme. Inhibition of this enzyme has been found to be a function of PAL-CoA and oleate concentrations. DE-II and DE-III fractions of FABPs protect the placental ATP citrate lyase against such inhibitions (figure 1). To assess the

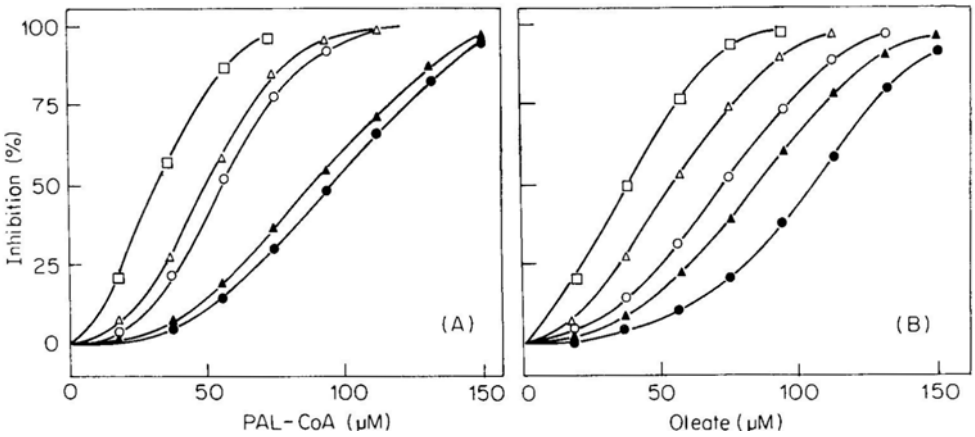

Figure 1. Protection of human placental ATP citrate lyase against PAL-CoA **(A)** and oleate **(B)** inhibition by various amounts of FABPs.(□)Control; (O) 15 μg DE-II; (Δ) 15 μg DE-III; (●)25 μg DE-II;(▲) 25 μg DE-III. Assay conditions are those described under § 2.

relative protection afforded by FABPs, concentrations of PAL-CoA or oleate required for 50 % inhibition of this enzyme in the presence and absence of FABPs have been calculated. In the presence of 25 μg of DE-II, 2·25 and 1·8 times higher amounts of PAL-CoA and oleate, respectively were required for 50% inhibition. To achieve the same protection, about the same amount of DE-III was required in the case of PAL-CoA inhibition but more than one and half times of DE-III was needed in the case of oleate. DE-I fraction of FABP showed no such protective effect.

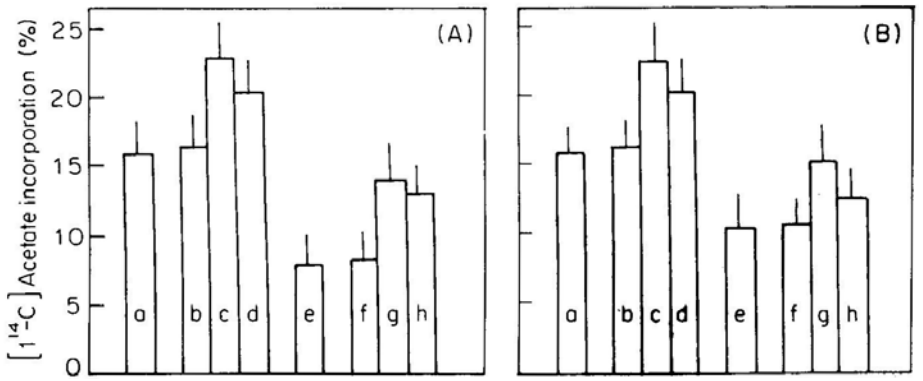

Figure 2. Role of FABPs in regulating the inhibition of human placental fatty acid synthesis by PAL-CoA **(A)** and oleate **(B).** Control (a); with 15 μg DE-I (b)/DE-II (c)/DE-III (d) per g tissue; with 25 μM PAL-CoA/oleate (e); with 25 μM PAL-CoA/oleate and 15 μg DE-I (f)/DE-II (g)/DE-III (h) per g tissue. Values are mean ± SEM of 3 sets of experiments.

3.2 *Role of FABPs in regulating fatty acid synthesis*

PAL-CoA and oleate (figure 2) inhibit fatty acid synthesis. In the presence of a fixed

concentration (25 μg each) of DE-II/DE-III, this inhibition is released. DE-I has no such effect. DE-II has been found to be more effective than DE-III in releasing the inhibition in the case of oleate. When no inhibitor is present, FABPs increase fatty acid synthesis.

3.3 *Ontogeny of human placental FABPs*

Table 1 shows the ontogenic profiles of FABPs in human placenta. Concentration of DE-II remains maximum throughout the gestation, while that of DE-III is minimum. From early stage of intrauterine development, levels of all these FABPs increase sharply up to 25-30 weeks of gestation. The rate of increase slows down afterwards.

3.4 *Development of ATP citrate lyase activity and fatty acid synthesis in human placenta*

Table 2 indicates that ATP citrate lyase activity is discernable in human placenta as early as at 5-10 weeks of gestation. Enzyme activity increases in parallel with gestationup to 25-30 weeks but shows a decreasing trend at term. The amount of [1–^{14}C] acetate incorporation into placental fatty acids increases steadily from 5-10 weeks of gestation up to term, though the rate of fatty acid synthesis slows down slightly after 25-30 weeks.

3.5 *Correlation coefficients between the developmental profiles of FABPs, ATP citrate lyase and fatty acid synthesis*

The results of table 3 show that the developmental patterns of FABP levels, ATP

Table 1. Ontogenic profile of different fractions of FABP in human placenta.

Group	Gestational ages (weeks)	Amount of FABP* in different fractions			
		DE-I	DE-II	DE-III	Total
I	5–10	3·1 ± 0·6	9·8 ± 1·6	1·4 ± 0·3	14·3 ± 0·9
II	10–15	4·8 ± 0·9	11·9 ± 1·0	2·2 ± 0·5	18·9 ± 1·2
III	15–20	6·6 ± 1·1	16·6 ± 1·4	3·0 ± 0·7	26·2 ± 1·4
IV	20 25	8·4 ± 1·6	21·1 ± 1·9	3·8 ± 0·8	33·3 ± 1·7
V	25–30	10·0 ± 1·2	24·7 ± 2·1	4·6 ± 1·3	39·3 ± 2·1
VI	30–35	10·6 ± 1·0	25·7 ± 2·6	5·2 ± 1·0	41·5 ± 3·0
VII	35–40	10·8 ± 1·5	26·3 ± 2·0	5·6 ± 1·1	42·7 ± 2·4

Values are mean ± SEM of 3 sets of experiments in each case.

Group II *vs* I	—	—	—	$P < 0·05$
Group III *vs* II	—	$P < 0·05$	—	$P < 0·025$
Group IV *vs* III	—	—	—	$P < 0·05$
Group V *vs* IV	—	$P < 0·05$	—	—
Group VI *vs* V	—	—	—	—
Group VII *vs* VI	—	—	—	—

*mg FABP/mg protein × 10^{-3}

Table 2. Development of ATP citrate lyase activity and fatty acid synthesis in human placenta. Values.

Group	Gestational ages (weeks)	ATP citrate lyase (nM NADP produced/ min/mg protein)	Fatty acid synthesis (% [1–^{14}C] acetate incorporated)
I	5–10	2·01 ± 0·27	3·95 ± 0·35
II	10–15	2·97 ± 0·43	7·10 ± 0·54
III	15–20	4·39 ± 0·60	9·18 ± 0·74
IV	20–25	7·21 ± 0·82	12·11 ± 0·59
V	25–30	12·80 ± 1·12	15·78 ± 0·86
VI	30–35	11·74 ± 1·03	15·48 ± 1·25
VII	35–40	10·26 ± 0·96	14·56 ± 0·98
Values are mean ± SEM of 3 sets of experiments in each case.			
Group II *vs* I		—	$P < 0·005$
Group III *vs* II		—	$P < 0·05$
Group IV *vs* III		$P < 0·05$	$P < 0·025$
Group V *vs* IV		$P < 0·01$	$P < 0·02$
Group VI *vs* V		—	—
Group VII *vs* VI		—	—

Table 3. Correlation co-efficients between the developmental profiles of FABPs, ATP citrate lyase activity and fatty acid synthesis in human placenta.

Correlation between	Correlation co-efficient (r)	Significant values (P)
FABPs *vs* ATP citrate lyase	0·950	$P < 0·001$
ATP citrate lyase *vs* fatty acid synthesis	0·973	$P \ll 0·001$
Fatty acid synthesis *vs* FABPs	0·983	$P \ll 0·001$

citrate lyase activity and fatty acid synthesis are highly correlated ($P < 0·001$) in human placenta.

4. Discussion

Placenta is considered to be the sole purveyor of all fetal needs and regulator of the development and maintenance of the fetus. It also serves as an important organ for lipid metabolism for the fetus until the fetal liver becomes more competent. Thus any regulation of placental lipid metabolism must be important for the fetal health. In view of their remarkable abundance in the placental cytosol (3–4%) FABPs may play a major role in regulating G6PD-fatty acid interaction (Das *et al* 1988). The present study indicates that FABPs regulate ATP citrate lyase which supplies acetyl-CoA, and also influence [1–^{14}C] acetate incorporation into fatty acids. Human placental FABPs may thus take part in overall metabolic regulation of fatty acid synthesis by affecting substrate and cofactor production as well as fatty acid synthesis itself. Although albumin and other proteins have been reported to cause similar effect (Goodridge 1972; Sa *et al* 1989), the demonstration that FABPs are

the major cytosolic binder of the inhibitor (Lunzer *et al* 1977) suggests that the effect may be specific in the intact cell. Such efect of FABPs on the inhibition of fatty acid synthesis is attributable to binding of long chain fatty acids and their CoA thioesters rather than a direct efect of the proteins on the lipogenic enzymes. In the absence of externally added inhibitors FABPs stimulate ATP citrate lyase and fatty acid synthesis may be by binding endogenous inhibitors. This conjecture is supported by the finding that almost similar enhanced activity of controls are obtained when dialyzed preparations were used to remove the ligand inhibitors. The actions of fatty acids and fatty acyl-CoAs on the native enzyme structure of ATP citrate lyase are not clear yet. It may be due to the change of active to inactive oligomeric conformation of the enzyme by binding of the inhibitors. Protective effects of DE-II and DE-III have been found to be almost similar when the inhibitor used was PAL-CoA, however, in the case of oleate DE-II was more effective. Such differences may be due to different affinities of these proteins for palmitic acid and oleic acid (Das *et al* 1988).

Correlation coefficients of developmental profiles of FABPs, ATP citrate lyase activity as well as fatty acid synthesis indicate that these parameters are highly correlated in human placenta. Thus with advancement of pregnancy, demand of lipids increases with a parallel increase in FABP content to supply more fatty acids for lipid synthesis. It is known that fetal fatty acids can be derived from two sources: from maternal plasma, in the form of free fatty acids or from placental breakdown of triglycerides and phospholipids, or from direct placental fatty acid synthesis (Beaconsfield and Ginsburg 1979). Thus placental FABPs not only serve the placental need but also indirectly help the fetus to meet its demand of fatty acids for lipogenesis. Near term, when amount of fetal liver FABPs becomes sufficient and this organ can synthesize ample fatty acids, need of placental FABPs subsides. The rate of increase in FABP content therefore slows down in placenta near term whereas the same remains constant up to birth in fetal liver (Das *et al* 1989).

Acknowledgements

We thank Prof. A K Ghosh, National Medical College and Hospital, Calcutta, for clinical materials, Dr K D Mukherjee, Federal Centre of Lipid Research, Munster, Germany, for the gift of [1–^{14}C] acetate and Dr N Sarkar, Indian Statistical Institute, Calcutta, for statistical analysis. This work was supported by a grant from the Council for Scientific and Industrial Research and Indian Council of Medical Research, New Delhi.

References

Bass N M 1985 Function and regulation of hepatic and intestinal fatty acid binding protein; *Chem. Phys. Lipids* **38** 95-114

Beaconsfield P and Ginsburg J 1979 Carbohydrate, fat and protein metabolism in the placenta: a clinicians view; in *Placenta: a neglected experimental animal* (eds) P Beaconsfield and C Villie (Oxford: Pergamon Press) pp 34–62

Chaudhuri D, Kushari J and Mukherjea M 1982 Occurrence of phosphodiesterase IV in the developing human brain, liver and placenta; *Eur. J. Obstet. Gynecol. Reprod. Biol.* **13** 309–316

Das T, Sa G and Mukherjea M 1988 Purification and characterization of fatty acid binding protein from developing human placenta; *Lipids* **23** 528-533

Das T, Sa G and Mukherjea M 1989 Human fetal liver fatty acid binding proteins: Role on glucose 6 phosphate dehydrogenase activity; *Biochim. Biophys. Acta* **1002** 164–172

Das T, Sa G, Ghosh P K, Mukhopadhyay D and Mukherjea M 1989 Isolation of fatty acid binding proteins from human fetal intestine and heart: Comparison with corresponding hepatic proteins; *Med. Sci. Res.* **17** 985-986

Glatz J F C and Veerkamp J H 1985 Intracellular fatty acid binding proteins; *Int. J. Biochem.* **17** 13-22

Goodridge A G 1968 Citrate-cleavage enzyme, malic enzyme and certain dehydrogenases in embryonic and growing chicks; *Biochem. J.* **108** 663-666

Goodridge A G 1972 Regulation of the activity of acetyl coenzyme A carboxylase by palmitoyl coenzyme A and citrate; *J. Biol. Chem.* **247** 6946-6952

Goodridge A G 1973 On the relationship between fatty acid synthesis and the total activities of acetyl coenzyme A carboxylase and fatty acid synthetase in the liver of prenatal and early postnatal chick; *J. Biol. Chem.* **248** 1932-1938

Kawaguchi A and Bloch K 1974 Inhibition of glucose 6 phosphate dehydrogenase by palmitoyl coenzyme A; *J. Biol. Chem.* **249** 5793-5800

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the folin phenol reagent; *J. Biol. Chem.* **193** 265-275

Lunzer M A, Manning J A and Ockner R K 1977 Inhibition of rat liver acetyl coenzyme A carboxylase by long chain acyl coenzyme A and fatty acid; *J. Biol. Chem.* **252** 5483-5487

Mogensen I B, Schulenberg-Schell H, Hansen H O, Spener F and Knudsen J 1988 A novel acyl-CoA binding protein from ovine liver. Efect on fatty acid synthesis; *Biochem. J.* **241** 189-192

Paulussen R J A and Veerkamp J H 1990 Intracellular fatty acid binding proteins, characteristics and function; *Sub-Cell. Biochem.* **16** 175-226

Sa G, Das T and Mukherjea M 1989 Purification and characterization of fatty acid binding proteins from human fetal lung; *Exp. Lung Res.* **15** 619-634

Silpananta P and Goodridge AG 1971 Synthesis and degradation of malic enzyme in chick liver; *J. Biol. Chem.* **246** 5754-5761

Spener F, Borcher T and Mukherjea M 1989 On the role of fatty acid binding proteins in fatty acid transport and metabolism; *FEBS Lett.* **244** 1–5

Spener F and Mukherjea M 1990 Nonenzymatic proteins mediating intracellular lipid transport and metabolism: current status and emerging trends; *Sub-Cell. Biochem.* **16** 1-19

Sweetser D A, Heuckeroth R O and Gordon J I 1987 The metabolic significance of mammalian fatty acid binding proteins; *Annu. Rev. Nutr.* **7** 377-359

Takeda Y, Suzuki F and Iwoue H 1969 ATP citrate lyase (citrate-cleavage enzyme); *Methods Enzymol* **13** 153–160