

Plasma membrane fatty acid-binding protein (FABP_{pm}) is exclusively located in the maternal facing membranes of the human placenta

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Abstract We reported earlier the presence of a 40 kDa plasma membrane fatty acid-binding protein (FABP_{pm}) in human placenta. This protein is thought to be involved in the sequestration of unesterified free fatty acids bound to albumin from the maternal plasma for delivery to the fetus. However, its location in human placental syncytiotrophoblasts is not known. These cells are bipolar; one side facing maternal circulation (microvillous membranes), and the other side facing fetal circulation (basal membranes). Therefore, it is important to resolve the location of this protein in trophoblast membranes in order to understand fatty acid transport and metabolism in human placenta. Isolated plasma membranes vesicles were prepared respectively from the maternal facing microvillous and fetal facing surface of the human full-term placental syncytiotrophoblast. Using these membrane preparations, fatty acid binding activity, the polyacrylamide gel electrophoresis radiobinding assay for FABP_{pm}, and Western blot analysis of FABP_{pm} were carried out to determine the location of this protein in these membranes. Based on the above studies we conclude that the FABP_{pm} is located exclusively in the microvillous membranes. Since FABP_{pm} may be responsible for FFA uptake, its location in the microvillous membranes favours the unidirectional flow of maternal FFA to the fetus.

Key words: Plasma membrane fatty acid-binding protein (FABP_{pm}); Syncytiotrophoblast; Microvillous membrane; Basal membrane; Fatty acid uptake; Human placenta

1. Introduction

The developing fetus and the placenta require essential fatty acids (EFA) and their desaturated and elongated derivatives (LCPUFA) for the formation of structural components of cell membranes, and for participation in intracellular signalling [1–3]. Unesterified free fatty acids (FFA) in the maternal circulation are the major source of fatty acids across the placenta, as triglycerides are not transported intact [6]. The placental lipoprotein lipase [LPL] present in microvillous membranes is thought to be mainly responsible for hydrolysis to facilitate placental uptake of FFA from circulating triglycerides [1,5,7]. In different tissues, particularly in the heart, FFA uptake has been shown to be mediated via the FABP_{pm} [8–11]. We recently purified and characterized a FABP_{pm} both in human [12] and sheep placental membranes [13]. We suggested that this protein may play an important role in the preferential accumulation of EFA/LCPUFA in the fetal circulation [5,12]. However, the exact location of this protein in placental membranes has to be determined in order to understand its role in fatty acid trans-

port and metabolism in the feto-placental unit. Clarification will involve a direct functional comparison of the fatty acid binding sites in the two membranes. The human placental syncytiotrophoblast is a continuous epithelial barrier separating maternal blood from the fetal placental villous vasculature. The apical surface of the syncytium is in direct contact with maternal blood in the placental intervillous space. All substances crossing the placenta in either direction must therefore diffuse or be transported across both this epithelial layer and the endothelial lining of the fetal vessels. In our previous studies crude plasma membranes were used to identify a FABP_{pm} in human placenta [12]. In the present paper, we investigated the presence of FABP_{pm} in the purified maternal or fetal facing plasma membranes from the human placental syncytiotrophoblast. The studies outlined in this paper provide evidence for the presence of FABP_{pm} in microvillous membranes of human syncytiotrophoblast.

2. Materials and methods

2.1. Materials and reagents

[1-¹⁴C]Oleic acid (53 mCi/mmol), [1-¹⁴C]linoleic acid (58 mCi/mmol) were obtained from Amersham, UK. [³H]Dihydroalprenolol (55 Ci/mmol) was obtained from New England Nuclear, UK. Bovine serum albumin (fat free), Triton X-100, and DL-propanolol were obtained from Sigma, Poole, UK. All reagents used were of analytical grade quality.

2.2. Human placental membrane preparations

Human placentas were obtained within 1 h of delivery from Aberdeen Maternity Hospital, Aberdeen. Highly purified microvillous and basal membranes were prepared from the placenta following a method described earlier [14]. After removing chorionic and basal plates, villous tissue was then excised in small chunks and washed in ice cold phosphate buffered saline (PBS) to remove excess blood. The villous tissue was then resuspended in 5 volumes of ice cold PBS and gently stirred for 30 min. The suspension was then centrifuged at 300 × g for 15 min and the supernatant decanted through a 250 μm pore size nylon mesh. The supernatant was then used as the source of the microvillous membrane fraction whereas the sedimented tissue was further treated to isolate basal membrane fraction as described [14].

Both the microvillous and basal membrane preparations were suspended in 50 mM Tris-HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA, 1 mM PMSF and 0.02% sodium azide. The membranes were stored at -80°C for further use. Protein was determined by the Bradford method using bovine serum albumin as a standard [15].

2.3. Alkaline phosphatase assay

Alkaline phosphatase activity of the purified membranes was determined by the release of *p*-nitrophenol from *p*-nitrophenol phosphate in a carbonate/bicarbonate buffer, pH 10.5 at 37°C as described [16].

2.4. [³H] Dihydroalprenol binding assay

Binding of [³H]dihydroalprenol to the purified membrane fractions was carried out as described before [17]. Membrane suspensions (125 μg protein) were incubated in 150 μl incubation medium containing 50 mM Tris-HCl, 10 mM MgCl₂, pH 7.4 for 15 min at 37°C with

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1×10^{-8} M [^3H]dihydroalprenolol. Incubations were terminated by diluting a 150 μl incubation mixture with 2 ml of ice-cold incubation medium, followed by rapid vacuum filtration of the suspension using Whatman glass fibre filter paper GF/C. Filters were dried and radioactivity bound to the filter paper was determined in a scintillation counter. In parallel, non-specific binding was determined by incubating the radio ligand in the presence of excess DL-propanolol.

2.5. Fatty acid binding studies

Binding of [^{14}C]oleate or [^{14}C]linoleate to the purified microvillous and basal membranes was determined as described previously [12,13]. Non-specific binding of each radiolabelled fatty acid was determined by adding a 20-fold excess of the corresponding unlabelled fatty acid to the assay tube, and specific binding was calculated by subtracting the non-specific binding from the total binding (in the absence of excess unlabelled fatty acid).

2.6. Polyacrylamide gel electrophoresis radiobinding assay of FABP_{pm}

Polyacrylamide gel electrophoresis (PAGE) radiobinding assay was used as previously described for the detection of FABP_{pm} in solubilized membrane proteins from both human and sheep placenta [12,13]. Briefly, both microvillous and basal membranes were solubilised with Triton X-100, and the solubilised membranes were then incubated overnight at 4°C with a tracer amount of [^{14}C]oleate in the absence or in the presence of excess unlabelled oleic acid. The incubation mixture was then subjected to non-denaturing PAGE with 20% acrylamide. At the end of the electrophoresis, the gel was transferred onto a nitro-cellulose sheet, dried and autoradiographed with Hyper film- β Max (Amersham International, UK). The film took around 3 to 5 days for development. Bands were visible on the film depending on the [^{14}C]oleate binding activity of the protein.

2.7. Electrophoretic separation of membrane proteins and Western blot analysis of membrane proteins

Polyclonal antisera against the purified human placental FABP_{pm} was used [12]. Western blot analysis of membrane proteins from microvillous and basal membranes was carried out as described before [12]. Polyacrylamide gel electrophoresis of these membrane vesicles in the presence of sodium dodecyl sulphate (SDS) was carried out under reducing conditions on SDS-PAGE gel homogeneous 20 (Phast System, Pharmacia, UK), as described [12]. After electrophoresis, proteins were transferred onto a nitro-cellulose membrane by diffusion blotting at 70°C for 1 h. The membrane was probed for the presence of FABP_{pm} by incubating with rabbit polyclonal antiserum to human placental FABP_{pm}. Antibody antigen complexes were then detected with HRP-anti-rabbit IgG fraction of donkey polyclonal antiserum (Scottish Antibody Production Unit).

3. Results

3.1. Purification of placental membranes

Table 1 shows the distribution of the alkaline phosphatase activity, and dihydroalprenolol binding activity in the purified microvillous and basal membrane fractions obtained from the full term human placentas. The microvillous membranes were enriched around 20-fold in alkaline phosphatase activity, the marker for the microvillous membranes, whereas the basal membranes was enriched around 38-fold in dihydroalprenolol binding, the marker for basal membranes. This indicates that the membrane preparations were free of cross-contamination.

3.2. Binding of radiolabelled fatty acids to membranes

Binding of [^{14}C]oleate or [^{14}C]linoleate to placental membranes attained equilibrium within 20 min of incubation at 37°C at 1:1 molar ratio of fatty acid and albumin. Specific binding for [^{14}C]linoleate and [^{14}C]oleate to the purified microvillous membranes was 1.92 ± 0.19 nmol/mg of protein ($n = 3$) and 1.55 ± 0.03 nmol/mg of protein ($n = 3$), respectively. Whereas the specific binding for these fatty acids to the purified

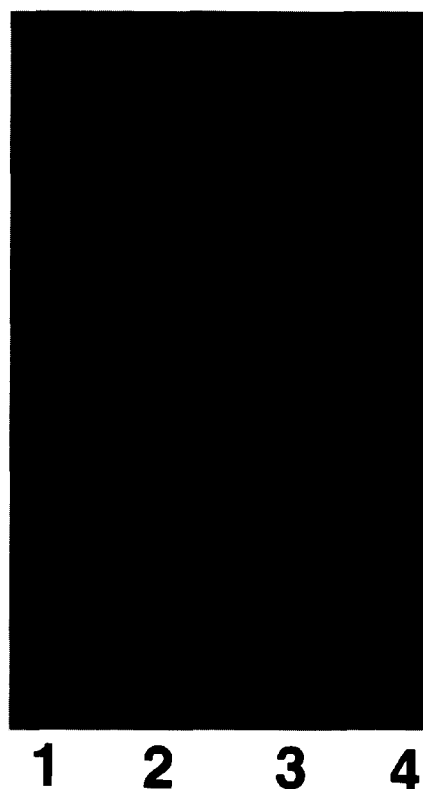


Fig. 1. Autoradiography of FABP_{pm} of solubilised microvillous and basal membranes. Autoblots of 20% homogeneous native PAGE gel. Lanes 1 and 2: Solubilised proteins from the microvillous membranes; Lanes 3 and 4: Solubilised proteins from basal membranes. Approximately, 15 μg membrane proteins were used. All samples were incubated with [^{14}C]oleate whereas the samples in lanes 2 and 4 were incubated in the presence of excess unlabelled oleic acid.

basal membranes was insignificant; [^{14}C]oleate binding (0.13 ± 0.02 nmol/mg of protein), [^{14}C]linoleate binding (0.05 ± 0.02 nmol/mg of protein). Binding of [^{14}C]linoleate to microvillous membranes was around 38-fold higher compared with that of the basal membranes ($P < 0.001$), whereas [^{14}C]oleate binding to the microvillous membranes was around 12-fold higher ($P < 0.001$).

3.3. Polyacrylamide gel electrophoresis radiobinding assay

Fig. 1 shows the [^{14}C]fatty acid binding activity of the solubilised proteins from the purified microvillous and basal membranes in an auto blot. Solubilised proteins from these membranes were incubated with radiolabelled oleate and subsequently examined by autoradiography, only one radioactive band was visible in the solubilised proteins from microvillous membranes which was abolished in the presence of excess oleic acid. Whereas no radioactive band was visible in the case of solubilised proteins from basal membranes. The radioactive band corresponded to that of the purified human placental FABP_{pm} (data not shown).

3.4. Detection of FABP_{pm} in the membrane fractions by Western blot analysis

The presence of the FABP_{pm} in the microvillous and basal membrane fractions, was examined by Western blot analysis using anti-FABP_{pm} polyclonal antiserum. Fig. 2 shows a single

band in the microvillous membranes but not in the basal membranes. This provides further evidence for the presence of the FABP_{pm} in the microvillous membranes.

4. Discussion

The studies presented in the paper attempt to determine the location of FABP_{pm} in the purified syncytiotrophoblast membranes: microvillous and basal membranes. The mechanism by which the placenta takes up fatty acid from the maternal circulation and subsequently transports them to the fetus is still not well understood. Recently, we identified and characterised a FABP_{pm} in human placental membranes, and suggested that this protein may be responsible for fatty acid uptake from the maternal circulation [5,12]. The syncytiotrophoblast of the placenta is the site of exchange of nutrients and minerals between the mother and fetus [18]. Therefore, it is of paramount importance to characterize the transport of fatty acids using purified bipolar syncytiotrophoblast membranes. Studies have shown a symmetrical distribution of various receptors and enzymes in the membranes of this cell [14,17,18]. Asymmetric distribution of the transporting proteins may allow the accumulation of important molecules in the syncytiotrophoblast and their subsequent extrusion to the fetal circulation. In our previous studies, the membranes were prepared from placental syncytiotrophoblasts which might have both the bipolar membranes; microvillous (maternal facing side) and the basal membranes



Fig. 2. Representative Western blot of the microvillous and the basal membranes showing immunoreactivity with mono specific anti-FABP_{pm} antisera. Western blots of the proteins from the microvillous and basal membranes were carried out as described in section 2. Lane 1, Microvillous membrane proteins; Lane 2, Basal membrane proteins. Approximately 15 μ g membrane proteins were used.

Table 1
Parameters of membrane purity in microvillous membranes and basal membranes of human placenta

Markers	Microvillous membranes	Basal membranes
Alkaline phosphatase (μ g phenol/min/mg protein)	2216 \pm 159	347 \pm 67
[³ H]dihydroalprenolol binding activity (pmol/mg protein)	0.06 \pm 0.004	4.4 \pm 0.69

Values are expressed as the mean \pm S.E.M. of three different experiments.

(fetal facing side) [12]. Now, using a different method [14] the sequential production of highly purified microvillous and basal membranes in significant quantities from placentas was possible and allowed us to perform comparative studies using either side of syncytiotrophoblast membranes from the same placenta.

Direct comparison of the binding of radiolabelled fatty acids to these membranes indicate that the maximal binding was in the microvillous membranes compared with that in the basal membranes. Our fatty acid binding data on the purified membranes were similar to those reported earlier by Lafond et al. [19]. This was further confirmed by using PAGE/autoradioblotting of these membranes. We showed earlier that the PAGE/autoradioblotting technique could be used to identify FABP_{pm} in solubilised membranes [12,13]. Auto blot of the membrane proteins clearly shows the presence of a FABP_{pm} in the microvillous membranes but not in the basal membrane fractions. Similarly, monospecific antiserum against the FABP_{pm} which was used previously to identify the FABP_{pm} in the placental membranes and was found to inhibit significantly the binding of [¹⁴C]fatty acids to the membranes [12], produced a single band with solubilised proteins from the microvillous membranes but not with the basal membranes. All the above data clearly demonstrate that the microvillous membranes bind significant amounts of FFA and that the FABP_{pm} is located exclusively in these membranes. Thus, it may represent a unidirectional transport of FFA from the mother to fetus.

The physiological significance of our observation is apparent. Like LPL, FABP_{pm} is also located in the maternal facing membranes of the placenta. Both the membrane proteins are thought to be involved in the sequestration of FFA by the placenta and therefore they may play a critical role in fetal growth and development [4,5]. Increasing knowledge of the fatty acid transporter protein in the placenta, and other environmental modifiers of this protein is required to understand feto-placental fatty acid metabolism.

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