Molecular and Cellular Biochemistry **98**: 57–68, 1990.

Characteristics of fatty acid-binding proteins and their relation to mammary-derived growth inhibitor

Friedrich Spener[1], Christian Unterberg[1], Torsten Börchers[1] and Richard Grosse[2]
[1] *Department of Biochemistry, University of Münster, Wilhelm-Klemm-Str. 2, 4400 Münster, FRG;*
[2] *Central Institute of Molecular Biology, Academy of Sciences of the German Democratic Republic, 1115 Berlin, GDR*

Key words: fatty acid-binding proteins, mammary-derived growth inhibitor, types, isoforms, subcellular distribution

Summary

Based on sequence relationships the cytoplasmic fatty acid-binding proteins (FABPs) of mammalian origin are divided into at least three distinct types, namely the hepatic-, intestinal- and cardiac-type. Highly conserved sequences of FABPs within the same type correlate with immunological crossreactivities. Isoforms of hepatic-type FABP are found in several mammalian species and for bovine liver FABP specific shifts in isoelectric points upon lipidation with fatty acids are observed. Isoforms of intestinal-type FABP are not known and the occurrence of cardiac-type isoforms so far is confined to bovine heart tissue. A bovine mammary-derived growth inhibitor (MDGI) is 95% homologous to the cardiac-type FABP from bovine heart. Dissociation constants of FABP/fatty acid complexes are in the range of 1 μM and 1 : 1 stoichiometries are usually found, but the neutral isoform of hepatic FABP from bovine liver binds 2 fatty acids. On subcellular levels hepatic- and cardiac-type FABPs are differently distributed. Though mainly cytosolic in either case, immunoelectron microscopy as well as a gelchromatographic immunofluorescence assay demonstrate the association of hepatic FABP in liver cells with microsomal and outer mitochondrial membranes and with nuclei, whereas in heart cells cardiac FABP is confined to mitochondrial matrix and nuclei. In mammary epithelial cells MDGI is associated with neither mitochondria nor endoplasmic reticulum, and is expressed in a strictly developmental-dependent spatial and temporal pattern. The specific role proposed for MDGI is to arrest growth of mammary epithelial cells when they become committed to differentiation in the mammary gland.

Introduction

Physiological significances of fatty acid-binding proteins (FABPs) in intracellular lipid metabolism are discussed since their first description [1]. Nevertheless, functions beyond a mere carrying of fatty acids remain to be substantiated. Sequence analyses reveal fatty acid-binding proteins as members of a family of low molecular weight proteins comprising gastrotropin [2], cellular retinoid binding proteins, myelin P2 protein, aP2 protein from adipose tissue [3] and the mammary-derived growth inhibitor [4]. All these proteins act intracellularly except gastrotropin and mammary-derived growth inhibitor (MDGI), which act extracellularly *in vitro*.

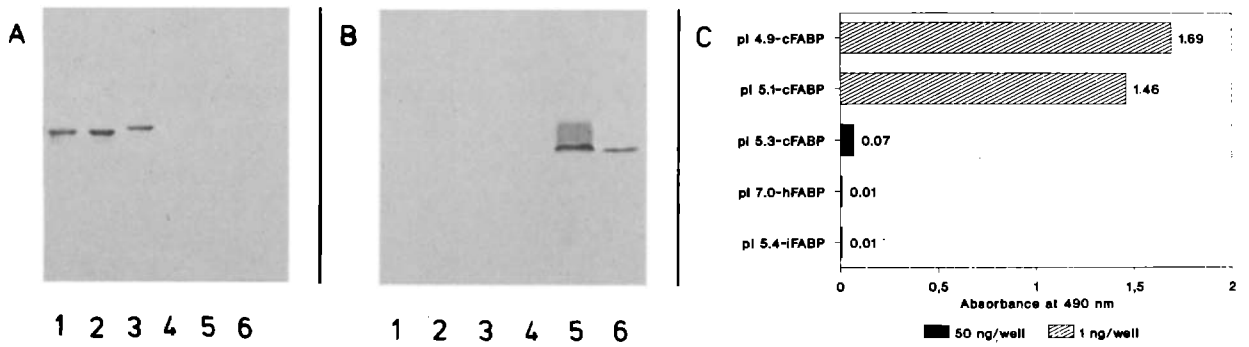

Fig 1. Crossreactivity of antisera with different types of fatty acid-binding proteins. Proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane and immunostained with affinity purified antibodies against bovine cFABP (panel A) and bovine hFABP (panel B). 1, Bovine pI 4.9-cFABP; 2, bovine pI 5.1-cFABP; 3, human pI 5.3-cFABP; 4, bovine pI 5.4-iFABP; 5, bovine intestinal mucosa cell cytosol; 6, bovine pI 7.0-hFABP. Panel C, intensity of peroxidase staining in ELISA specific for bovine cFABP.

Typology of fatty acid-binding proteins and mammary-derived growth inhibitor

Within the family of fatty acid-binding proteins three different types are unequivocally established up to now and are named by the source of their first isolation. The classification into hepatic-, intestinal- and cardiac-type fatty acid binding protein, abbreviated hFABP, iFABP and cFABP respectively, is based on immunological criteria and reflects a homology of usually ~30% between the different types and 80 to 90% within proteins of the same type. In order to demonstrate this a collection of FABPs was immunostained after SDS-polyacrylamide gelelectrophoresis and subsequent Western blotting (Fig. 1). The use of anti-bovine hFABP-antibodies [5] leads to an immunoreactive band in cytosolic proteins of bovine intestinal mucosa and demonstrates the occurrence of hFABP apart from iFABP in that tissue (Fig. 1B), as previously shown for rat tissue [6]. Pure bovine iFABP does not crossreact with antibodies against hFABP and cFABP. With antiserum against this protein the occurrence of iFABP in enterocytes of bovine small intestine and absence from heart and liver tissue was established [7]. The polyclonal antibodies raised against bovine cFABP additionally crossreact with human cFABP [8], whereas the other types remain unstained (Fig. 1A).

Yet immunological methods with higher stringency, like a sandwich ELISA employing affinity purified antibody for antigen capture and a second one for detection [9], can discriminate between the same FABP-type from different species (Fig. 1C). Even the two isoforms of the cardiac-type fatty acid-binding protein reported for bovine heart cytosol (pI 4.9- and pI 5.1-cFABP) [10] show a graduated response under these conditions. In Fig. 1C the intensity of the peroxidase staining reaction used in the ELISA is shown, i.e. upon application of 1 ng of each isoform, bovine pI 5.1-cFABP exhibits somewhat less than 90% of the immunoreactivity of bovine pI 4.9-cFABP,due to the use of a polyclonal antibody raised against the latter. With this antibody and as much as 50 ng antigen only a weak response is found for human cFABP, whereas FABPs of other types, namely bovine hFABP and iFABP, virtually show no staining.

Whether other types of fatty acid-binding proteins exist, e.g. renal [11] and skeletal muscle FABP [12], is an unsettled issue at present that has to await sequencing of these proteins. Recently the renal-type FABP, found together with cardiac FABP in rat kidney, was identified as α_{2u}-globulin by Kimura *et al.* [13], whereas gastrotropin appears to be a further type of FABP expressed in mucosal cells of the gastrointestinal tract [2]. In this regard the classification into types seems somewhat questionable as proteins that originally have not been discovered as fatty acid binding proteins, e.g. aP2 and myelin P2 protein, are highly homologous to cFABP and also bind fatty acids.

From terminally differentiated bovine mammary gland a growth regulating factor, termed mammary-derived growth inhibitor (MDGI) was purified by Böhmer *et al.* [14]. Purification is monitored by an *in vitro* proliferation assay using Ehrlich ascites mammary carcinoma cells from the stationary phase of growth *in vivo* and employs $(NH_4)_2SO_4$-precipitation, Sephadex G 50 gelfiltration and DEAE-Sepharose CL 6B anion exchange chromatography. The fraction with inhibitory activity contains a 14.5 kDa polypeptide as shown by SDS-PAGE as well as size exclusion HPLC in the presence of 0.1% SDS [4]. The amino acid sequence of MDGI [4] which is obtained in a N-terminally blocked form, shows virtually no sequence homology to the proliferation inhibitor TGF-beta or any of the structurally known interferons. Instead, a significant homology (95%) is found with the cardiac-type fatty acid-binding protein from bovine heart [15] (Fig. 2) and thus with a family of proteins associated with the differentiated state of cells such as p422, whose cDNA level is increased during 3T3 preadipocyte differentiation [16] and the cellular retinol and retinoic acid-binding proteins. The sequences of bovine cFABP and MDGI differ in only 6 positions, the latter, however, lacks amino acid 132.

In order to obtain MDGI-cDNA for *in situ* hybridisation experiments (see below), a MDGI-specific probe from the cDNA synthesized from RNA of lactating mammary gland was amplified by the polymerase chain reaction (Kurtz *et al.*, in preparation). The amplification with primers complementary to amino acids 16 to 21 and 97 to 101 generated a 258 bp fragment, which then was used to probe the bovine mammary gland cDNA library under conditons of low stringency. The 9 cDNA clones isolated have a length of 680 bp and an identical sequence. The open reading frame codes for most of the amino acids reported for MDGI, but differences are found at 6 positions together with a Met- and an Ala-residue at positions 1 and 133 of the cDNA deduced sequence, respectively. These differences at positions 13, 15, 41, 44, 94 and 128 of the cDNA coincide with those on protein level between MDGI and cFABP, the latter besides having a C-terminal Ala. The differences at position 13 (Asp for Ser), 15 (Lys for Glu), 94 (His for Gln) and 128 (Thr for Val) are designated as less abundant in the reported MDGI-sequence [4]. Thus, the cDNA deduced MDGI-sequence is identical with that reported for the bovine heart cFABP [15] with the exception that 9 terminal nucleotides of the 5′-untranslated region and 4 nucleotides adjacent to the poly-dA tail are deleted in the MDGI clones.

Presence of isoforms

Multiple forms of hepatic-type fatty acid-binding protein are described in the literature [17, 18, 19]. In order to address a certain FABP out of the variety of isoforms and types, the pI of their apoform is used. Particularly in delipidated bovine liver cytosol, two isoforms exist, namely pI 7.0- and pI 6.0-hFABP [17]. Upon lipidation of these apoproteins with fatty acids or their fluorescent derivatives, e.g. 16-(9′-anthroyloxy)palmitic acid (A16 : 0), a shift in isoelectric points is observed. As shown in Fig. 3 the complex of pI 7.0-hFABP with oleic acid (lane 1) focuses at pH 5, whereas the one with A16 : 0 focuses at pH 6 (lane 2). The corresponding complexes of pI 6.0-hFABP both migrate to pH 5 (data not shown). Thus the heterogeneity of hepatic FABPs is a result of different isoforms as well as differences in lipidation. Apart from hFABP, the cytosol of bovine small intestine contains a single form of intestinal-type FABP with an isoelectric point of 5.4 [7]. This latter pI is not affected by ligand binding (Fig. 3, lanes 4, 5).

As mentioned above two isoforms of cardiac-type FABP are present in the cytosol of bovine heart cells [10]. Hitherto no isoforms have been detected in the myocard of other mammals. Cation exchange chromatography of partially purified 15 kDa fractions from bovine and human heart cytosols [8, 10] resolves two peaks of fatty acid-binding activity in the first, but only one single peak in the latter case (Fig. 4). The insert of Fig. 4 shows the isoelectric points of purified proteins as determined by focusing. When charged with radiolabeled fatty acids prior to isoelectric focusing, cardiac FABPs do not change their pI. The comigrat-

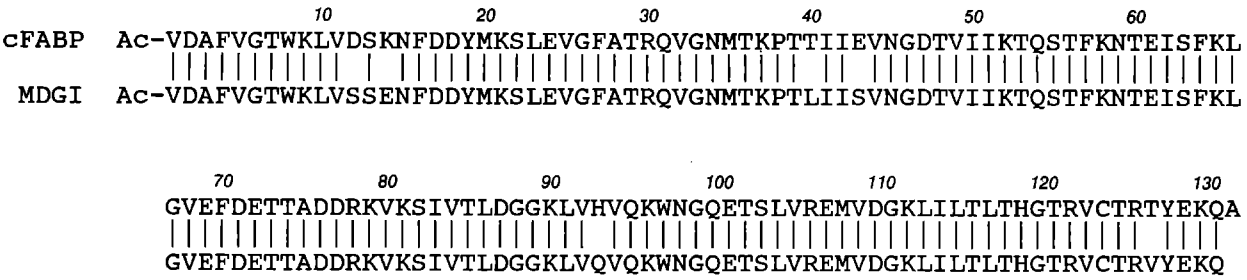

Fig. 2. Sequence relationship between cardiac-type fatty acid-binding protein from bovine heart [15] and bovine mammary-derived growth inhibitor [4].

ing ligands are easily detected by autoradiography indicating their strong affinities to this type of FABP. Recently, Jones *et al.* [20] reported the isolation of two FABP isoforms with pI 4.8 and 4.9, respectively, from rat mammary gland, presumably of the cardiac type. Whether one of these isoforms represents mammary-derived growth inhibitor in this tissue has to be considered. Like for cardiac-type FABP from bovine heart at least two MDGI isoforms differing in their isoelectric points are detected in bovine mammary gland [4]. Whether these forms are coded by different genes is not known.

The results of the sandwich ELISA (Fig. 1C) already indicate that the bovine cFABP isoforms share much more common epitopes than bovine and human cFABP, presumably due to an even higher homology [15, 21, 22]. In fact Unterberg *et al.* [23] recently attributed the molecular origin of bovine cFABP isoforms to a single difference in position 98 of the amino acid sequence, where Asp is found in pI 4.9-cFABP and Asn in pI 5.1-cFABP. A similar difference may at least partly be involved in bovine hFABP diversity (Korf and Spener, unpublished results).

Binding of ligands

In addition of characteristic shifts in isoelectric points upon loading with fatty acids there are yet other peculiarities of the hepatic-type FABPs. While intestinal and cardiac FABPs exhibit a high specificity for fatty acids, hFABP was even discovered as a binding protein for anionic dyes, carcinogens and xenobiotics [6, 18]. Binding of acyl-CoAs, however, seems to be confined to the recently discovered acyl-CoA binding protein [24, 25].

Though the stoichiometry of fatty acid binding to FABPs is still a matter of debate, most investigations,using either the Lipidex- or a liposome-binding assay, found a 2 : 1 ratio for hepatic FABP and a 1 : 1 molar ratio for intestinal and cardiac FABP, respectively. Scatchard analysis allows to separate the isotherms for the binding of the two fatty acids to bovine pI 7.0-hFABP as the affinity of the second is in the order of one magnitude lower than the first [26]. A compilation of binding data is given in Table 1. Furthermore, the circular dichroism induced upon binding of two molecules *trans*-parinaric acid to bovine pI 7.0-hFABP indicates that these fatty acids are located within the same binding site [30]. This concept is supported by a quantitative evaluation of the focusing data presented above. For a complex of pI 7.0-hFABP and radioactively-labeled oleic acid focusing at pH 5, Haunerland *et al.* [17] determined a ligand to protein ratio of 2 : 1. However, from the corresponding complex with 16-(9′-anthroyloxy)-palmitic acid focusing at pH 6 only one mol ligand per mol FABP can be extracted. Obviously the bulky fluorophor of the fatty acid precludes binding of another fatty acid molecule.

Ionic interactions between fatty acid and hFABP can be shown by application of the electrophoretic titration technique [17]. During electrophoresis in a prefocused gel complexes of radioactive fatty acid and pI 7.0-hFABP dissociate below pH 5, most probably due to protonation of the fatty acid. By modification with phenylglyoxal Schulenberg-

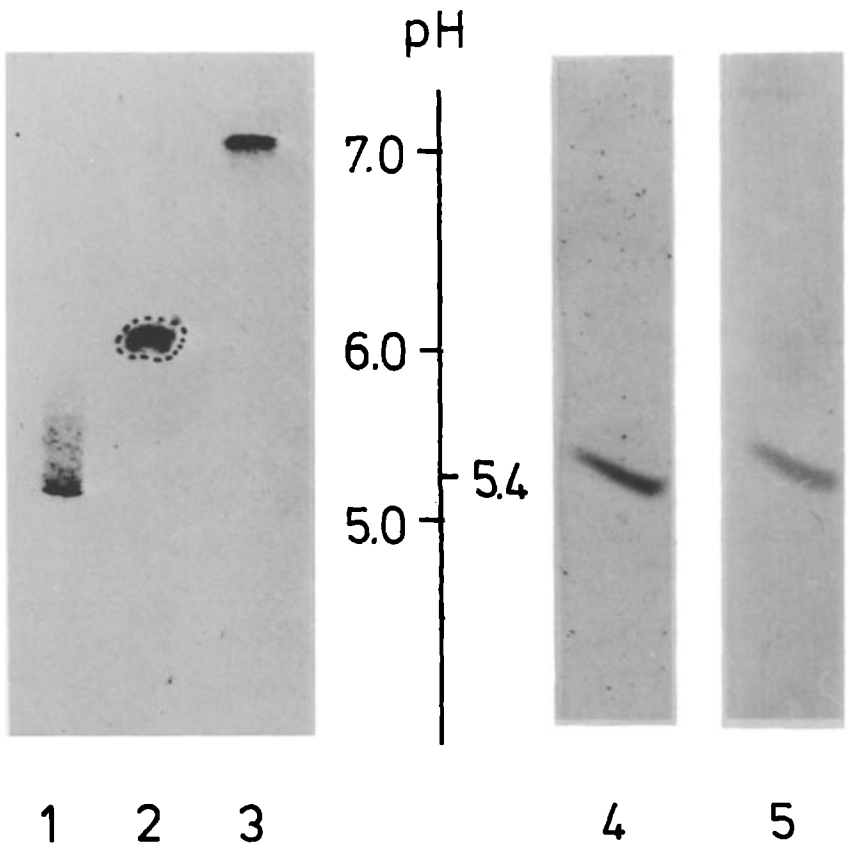

Fig. 3. Isoelectric focusing of fatty acid-binding proteins. 1–3, Bovine pI 7.0-hFABP; 1, loaded with oleic acid; 2, loaded with 16-(9'-anthroyloxy)palmitic acid; 3, delipidated ; 4, 5, bovine pI 5.4-iFABP loaded with [1-^{14}C]oleic acid. 4, Protein stain (Coomassie); 5, autoradiographic track of ligand.

Schell *et al.* [26] then identified arginine as the cationic counterpart of the fatty acyl carboxylate as the binding activity of the modified protein is reduced to 35%. Hence, a similar mechanism may be envisaged for other hepatic- and most probably intestinal-type FABPs, as for the latter X-ray crystallography reveals a close proximity of the fatty acid's carboxylic group to the guanidinium group of Arg^{127} [31].

Fluorescence measurements of complexes of bovine pI 7.0-hFABP and fatty acids, which are substituted with the anthroyloxy group at different positions indicate the contribution of hydrophobic interactions to binding. Blue shifts in emission maxima and increased lifetimes are observed [30], later Storch *et al.* [32] applied this technique with similar results to rat hFABP.

An analysis of putative endogenous MDGI ligands reveals the presence of long chain fatty acids, associated to MDGI prior or during preparation, as well as the capability of MDGI to bind long-chain fatty acids in respective binding assays [33]. The binding constants for the fatty acids were found too low (1 μM) to assume depletion of some essential fatty acids from the medium by MDGI as the reason for growth inhibiton *in vitro*. A synthetic peptide corresponding to the residues 121–131 of the MDGI-sequence exhibits very similar effects as MDGI in a cell proliferation assay (Langen *et al.*, in preparation). This peptide, however, does only bind negligible amounts of fatty acid. Thus, fatty acid binding may not be related to the biological activities described here but may stabilize the MDGI structure and activity because delipidated MDGI undergoes structural changes interpretable as a partial unfolding [34].

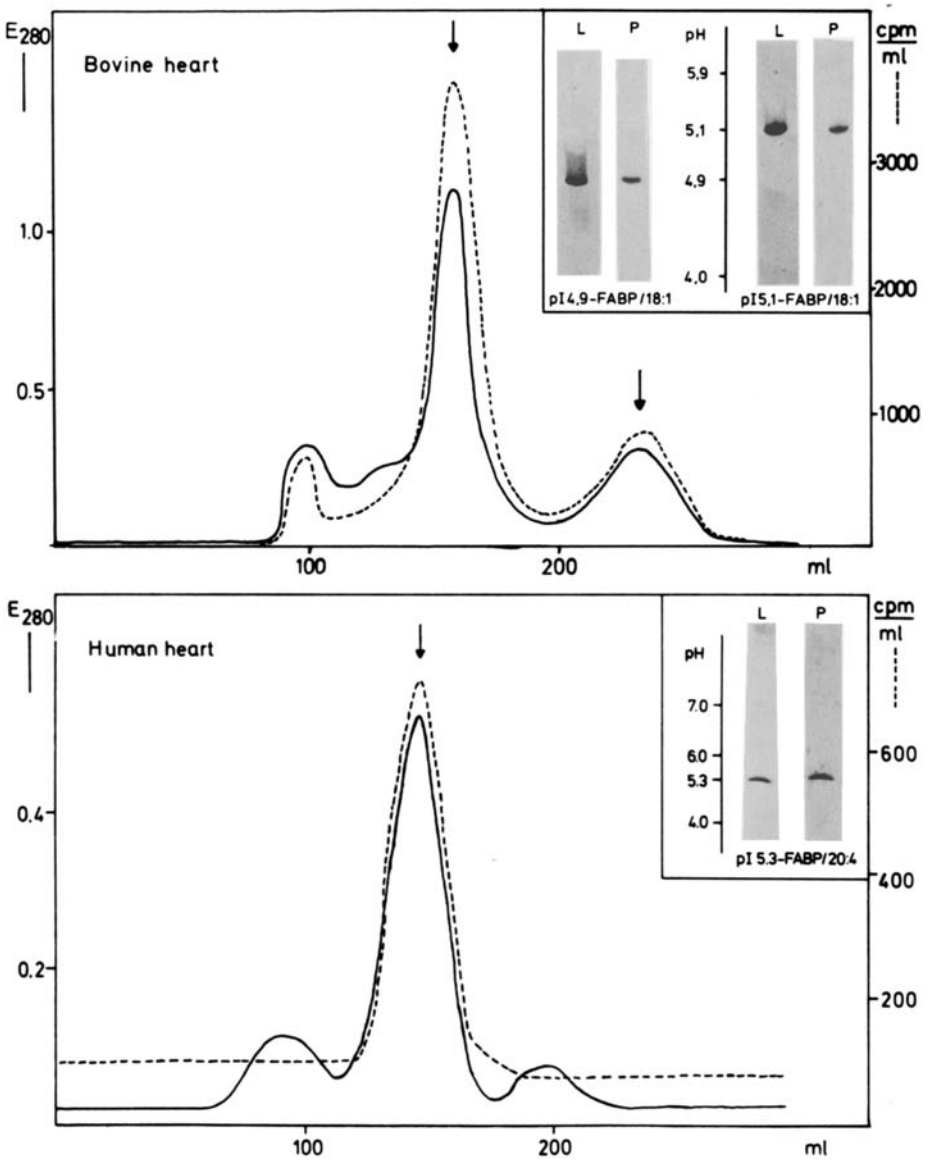

Fig. 4. Cation exchange chromatography of cardiac fatty acid-binding proteins. Partially purified 15 kDa fractions from bovine (upper panel) and human (lower panel) heart cytosols were incubated with [1-^{14}C]oleic acid, applied to a CM-Sephadex C 50 column equilibrated in 20 mM phosphate buffer (pH 6.0) and eluted with the same buffer (12 ml/h). Arrows indicate cFABP-containing peaks. Inserts show isoelectric focusing of these proteins after loading with [1-^{14}C]oleic acid or [1-^{14}C]arachidonic acid. L, Autoradiographic track of ligand; P, protein stain (Coomassie).

Subcellular distribution of fatty acid-binding proteins and mammary-derived growth inhibitor

In order to elucidate the contribution of FABPs to specific metabolic paths of lipids, we and others studied the subcellular distribution of hepatic- and cardiac-type FABP by means of immunomicroscopic [5, 9, 35] and biochemical [5, 9, 36] methods. The elegant protein A-gold method with affinity purified polyclonal antibodies unravels a completely different distribution pattern of FABP-types in liver parenchymal cells and heart myocytes (Fig.

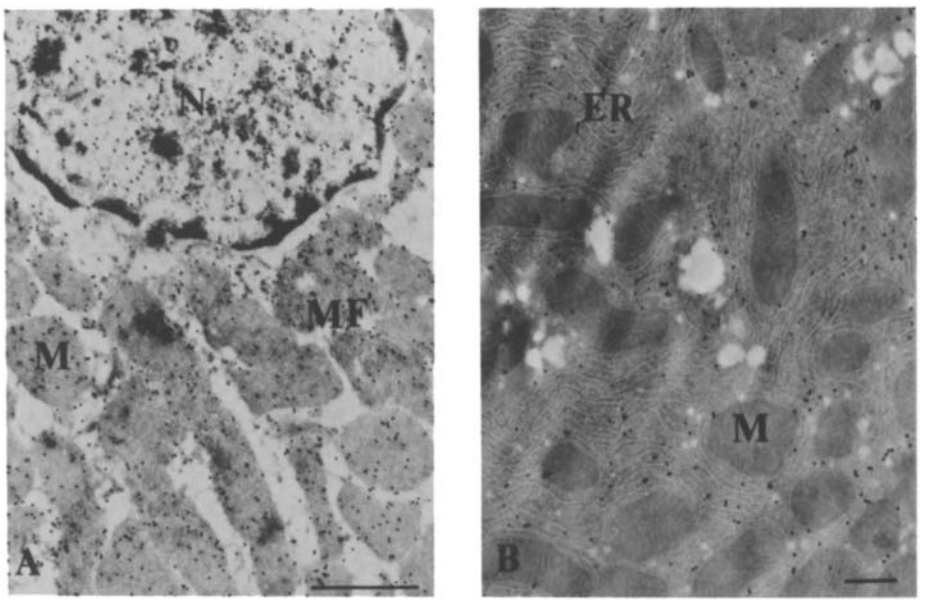

Fig. 5. Immunocytochemical detection of FABP (protein A-gold method) in rat heart and liver cells. N, nucleus; MF, myofibrils; M, mitochondria, ER, endoplasmic reticulum. Panel A, rat heart cell incubated with anti-cFABP-antibodies, × 25500; bar 1 μm. Panel B, rat liver cell incubated with anti-hFABP-antibodies, × 35500; bar 0.5 μm.

5). The electron-micrographs of the liver cell reveal gold-decorated hFABP only in the cytosolic space, preferentially along membranes of endoplasmic reticulum and mitochondria. In contrast to myocytes no staining is observed inside mitochondria and even the Kupffer cells are completely free from label, thus serving as internal controls in those micrographs. Gold-decorated cFABP, however, can be seen within mitochondria and on myofibrils of the heart cell. Moreover, nuclei of both tissues are labeled [5, 9].

Table 1. Binding of oleic acid to fatty acid-binding proteins

Protein	B_{max} [mol/mol]	K_d [μM]	Ref
bovine cFABP[a]	0.93	0.27	
human cFABP	0.45	0.20	[27]
rat cFABP	0.43	0.38	[27]
bovine hFABP	2.1	0.24/2.15[b]	[26]
rat hFABP[c]	1.90	1.77	[28]
rat hFABP	1.2	0.4	[6]
rat hFABP	1.34	1.03	[29]
rat iFABP[c]	0.84	2.87	[28]

[a] pI 4.9-cFABP (Jagschies and Spener, unpublished data).
[b] K_d values for binding of 1st and 2nd ligand molecule to pI 7.0-hFABP from Scatchard plot.
[c] expressed in *E. coli.*

These findings are supported by fractionation of intracellular membranes employing sucrose density gradient centrifugation and subsequent subfractionation of mitochondria after either digitonin treatment or hypotonic swelling. Whole mitochondria and submitochondrial fractions are then analyzed with the aid of a newly developed gelchromatographic immunofluorescence assay, based on the double antibody technique [5]. The membranes are incubated with FABP-specific, affinity purified antibody and, after repeated washings, with an FITC-antibody against the first. Membrane associated fluorescent antibodies are subsequently separated from unbound by gelfiltration on Sephacryl S 1000 (Fig. 6). Control runs without first specific antibody yield a weak fluorescence in the void volume, due to scattering effects of the membranes. In case of intact liver mitochondria an increase in fluorescence is recognized, that is even more pronounced when outer membrane fractions are probed. Liver microsomes react in a similar way in this assay [5]. From our results we conclude that hFABP is associated with these intracellular membranes of liver

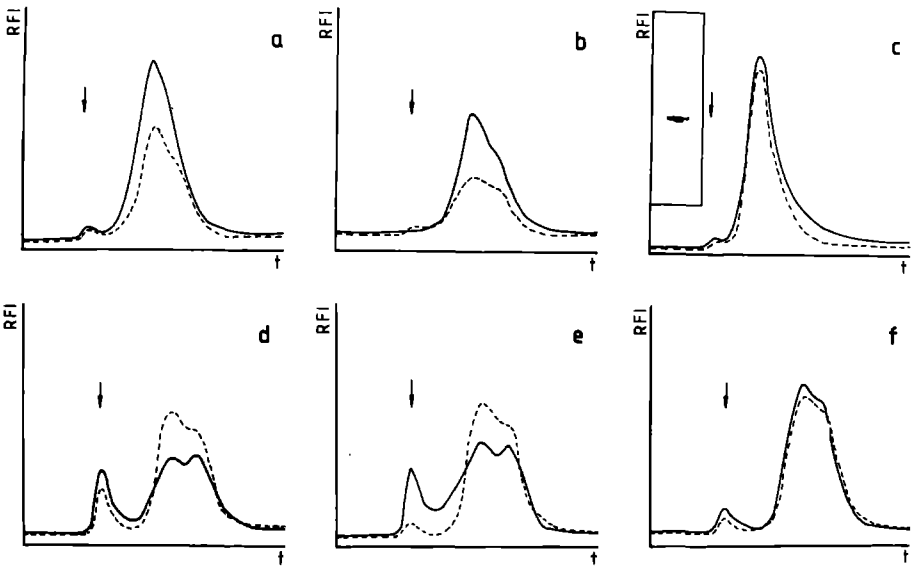

Fig. 6. Gelchromatographic immunofluorescence assay for FABPs in mitochondrial membranes. (——), membranes incubated with FABP specific- and second FITC-labeled antibody; (– – –), control (FABP-specific antibody omitted); t, elution time from Sephacryl S 1000 column (1 × 14 cm); arrow, exclusion volume of the column. a–c, Mitochondria from bovine heart; a, intact mitochondria (1 mg); b, outer membrane (0.5 mg); c, inner membrane (1 mg), insert, western-blot analysis of matrix proteins released after sonication of heart mitoplasts. d–f, Mitochondria from bovine liver; d, intact mitochondria (1.4 mg); e, outer membrane (0.5 mg); f, mitoplasts (0.4 mg).

cells. Heart mitochondrial subfractions, however, exhibit no increase of fluorescence in the void volume, indicating absence of cFABP in these membranes.

Upon sonication of bovine heart mitoplasts, which are essentially free from cytosolic contaminations as ascertained by absence of the cytosolic marker enzyme lactate dehydrogenase [9], a soluble cardiac-type fatty acid-binding protein is released. In the insert of Fig. 6 panel C, the Western blot analysis of matrix proteins clearly demonstrates the presence of this protein. The removal of membrane proteins, which constitute around 90% of mitochondrial proteins is illustrated by the 10-fold increase of cFABP in the mitochondrial matrix (0.18 μg/mg) as compared to whole mitochondria (0.018 μg/mg) [9].

Immunolabeling of mammary epithelial cells with affinity purified antibodies against MDGI is associated with basal invagination, the cytosol and the transcriptionally active euchromatic regions of nuclei, however, at 70 kDa after Western blotting in the latter case [37]. Other cell compartments such as mitochondria and rough endoplasmic reticulum are not labeled.

Biological action of mammary-derived growth inhibitor

Based on findings in Northern blot analysis that transcription of MDGI is related to the proliferative state of mammary gland, cryosections taken from virgin and pregnant cows as well as from different areas of lactating mammary gland are analyzed for MDGI-mRNA using the *in situ* hybridisation technique (Kurtz *et al.*, in preparation). In a developing lobulus MDGI transcription is enhanced in the alveolar cells when compared to ductal epithelial cells (Fig. 7A). Lobuloalveolar structures in glands of a pregnant animal have an increased MDGI-mRNA level in the alveolar epithelial cells bordering on the connective tissue (not shown). In contrast to the pregnant stage both the alveolar and ductal cells of terminally differentiated mammary gland transcribe the MDGI gene

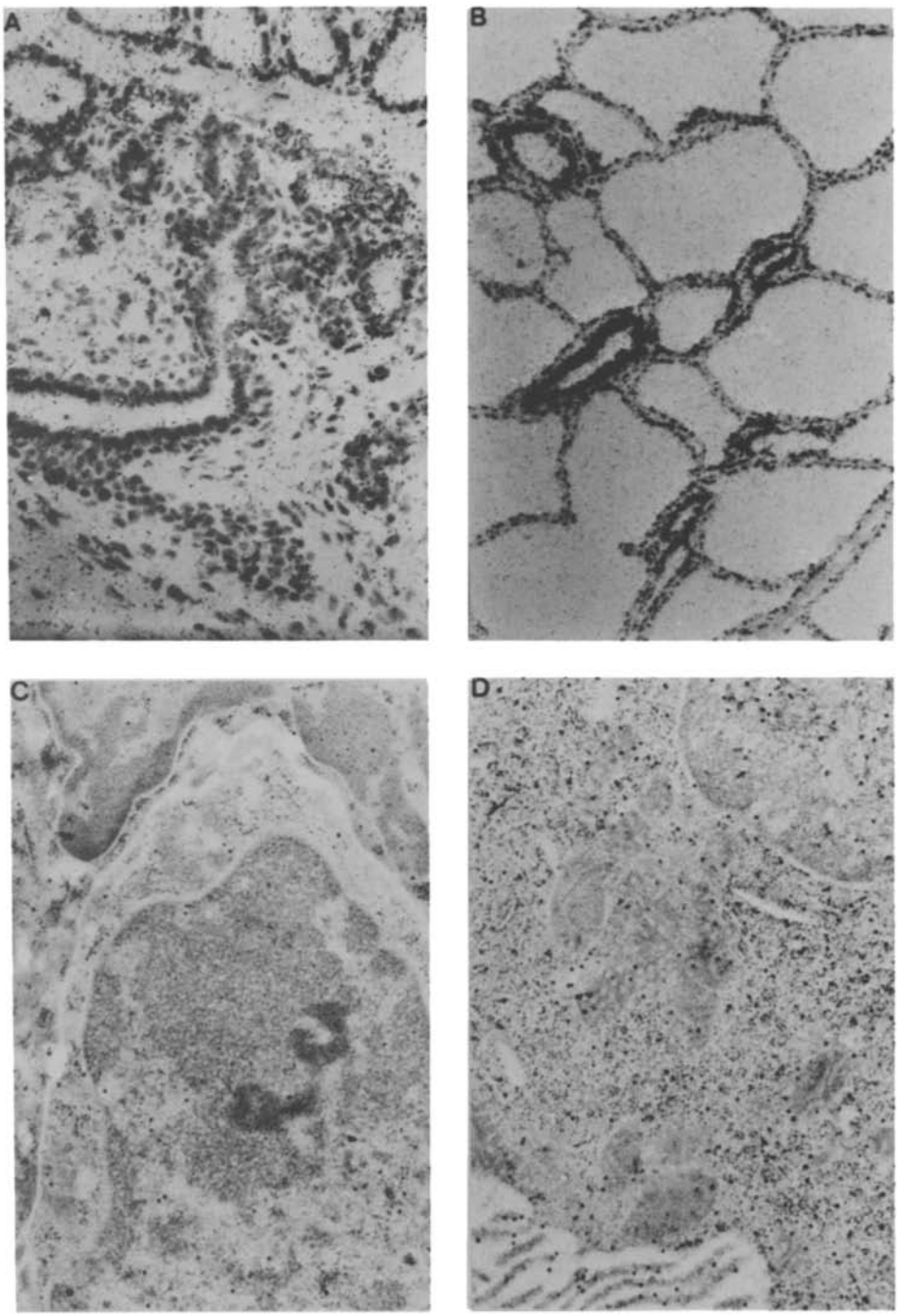

Fig. 7. Distribution of MDGI-mRNA and protein in pregnant and lactating bovine mammary tissue. MDGI-transcripts were detected by *in situ* hybridisation, using cryostat sections hybridized with ^{35}S-labeled antisense RNA probe. A, Longitudinal section of midpregnant mammary gland: B, cross sections of proximal part of lactating mammary tissue; C, Lowicryl K4M embedded sections of pregnant, and D, of lactating mammary gland incubated with anti-MDGI-IgG.

(Fig. 7B). By means of immunohistochemical analysis using affinity purified antibodies MDGI is not espressed in virgin tissue (not shown), whereas a moderate intensity of immunolabeling is found during pregnancy (Fig. 7C). The maximal level is reached during functional differentiation of the mammary gland (Fig. 7D).

Cellular activities are exerted on Ehrlich ascites carcinoma cells as well as on mammary epithelial cell lines. Dose-response curves for inhibition of proliferation of a hyperdiploid line of 'stationary Ehrlich cells' by purified MDGI show a half maximal inhibition at 1 ng/ml. Furthermore, cells become insensitive to inhibition by preincubation for 4 h with serum. Likewise, inhibition is abolished by simultaneous addition of MDGI with EGF or insulin. The inhibitory activity of MDGI is also antagonized by 10 μM 2'-deoxycytidine. Ehrlich cells are shown to possess PDGF receptors (Böhmer, unpublished results). An antagonistic PDGF effect is only observed if the cells are pretreated with 3–10 ng/ml PDGF 4 h before addition of MDGI. The PDGF effect proceeds even if PDGF is washed out before addition of MDGI. Insulin or EGF do not prevent growth inhibition if added to Ehrlich cells during the preincubation period. In summary, the data indicate that MDGI is acting by reducing the rate the cells are passing through some restriction point in G1/S.

The response of permanent mammary carcinoma cell lines (MATU, MCF-7) and normal human mammary epithelial cells to MDGI is based on a serum starvation to trigger cells into quiescence [38] followed by a restimulation with fresh medium. MDGI is present during the restimulation period for 16–20 h. Flow cytophotometric measurements with MATU, MCF-7 and normal human mammary epithelial cells proved them to be arrested in G1/G0. Obviously, synchronization of the cells in G1/G0 is a prerequesite to measure growth inhibition in presence of MDGI as cells not arrested by serum starvation are not responsive. DNA-synthesis in all but MCF-7 cells can be partially blocked by MDGI. For MATU cells the antgonistic effect of insulin can be confirmed (see above). Antisera raised against MDGI have a neutralizing effect on growth inhibition by MDGI in both, the Ehrlich ascites carcinoma cells [14] and in the mammary carcinoma cell line MATU [4]. In summary, MDGI is a growth regulating peptide with different functions including growth arrest during normal development of tissues and organs. The presented data may suggest that MDGI transcription is related to some functional role of MDGI for the onset of early differentiation which is coupled to inhibition of cell proliferation. At present work is in progress to further disclose the functional relationship between cardiac-type fatty acid-binding protein and mammary-derived growth inhibitor.

Acknowledgement

Part of the work presented here was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 310) and by the Fonds der Chemischen Industrie.

References

1. Ockner RK, Manning JA, Poppenhausen RB, Ho WKL: A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium and other tissues. Science 177: 56–58, 1972
2. Walz DA, Wider MD, Snow GW, Dass C, Desiderio DM: The complete amino acid sequence of porcine gastrotropin, an ileal protein which stimulates gastric acid and pepsinogen secretion. J Biol Chem 263: 14189–14195, 1988
3. Jones TA, Bergfors T, Sedzik J, Unge T: The three dimensional structure of P2 myelin protein. EMBO J 7: 1597–1604, 1988
4. Böhmer F-D, Kraft R, Otto A, Wernstedt C, Hellman U, Kurtz A, Müller T, Rohde K, Etzold G, Lehmann W, Langen P, Heldin C-H, Grosse R: Identification of a polypeptide growth inhibitor from bovine mammary gland. Sequence homology to fatty acid- and retinoid-binding proteins. J Biol Chem 262: 15137–15143, 1987
5. Bordewick U, Heese M, Börchers T, Robenek H, Spener F: Compartmentation of hepatic fatty-acid-binding protein in liver cells and its effect on microsomal phosphatidic acid biosynthesis. Biol Chem Hoppe-Seyler 370: 229–238, 1989
6. Bass NM: Function and regulation of hepatic and intestinal fatty acid binding proteins. Chem Phys Lipids 38: 95–114, 1985
7. Meyjohann A, Spener F: Isolation and characterization of the fatty acid binding proteins from bovine small intestine.

Abstract P 4, 1st International Workshop on Fatty Acid-Binding Proteins, Maastricht, 1989
8. Unterberg C, Heidl G, von Bassewitz D-B, Spener F: Isolation and characterization of the fatty acid binding protein from human heart. J Lipid Res 27: 1287–1293, 1986
9. Börchers T, Unterberg C, Rüdel H, Robenek H, Spener F: Subcellular distribution of cardiac fatty acid-binding protein in bovine heart muscle and quantitation with an enzyme-linked immunosorbent assay. Biochim Biophys Acta 1002: 54–61, 1989
10. Jagschies G, Reers M, Unterberg C, Spener F: Bovine fatty acid binding proteins. Isolation and characterization of two cardiac fatty acid binding proteins that are distinct from corresponding hepatic proteins. Eur J Biochem 152: 537–545, 1985
11. Lam KT, Borkan S, Claffey KP, Schwartz JH, Chobanian AV, Brecher P: Properties and differential regulation of two fatty acid binding proteins in the rat kidney. J Biol Chem 263: 15762–15768, 1988
12. Paulussen RJA, Veerkamp JH: Fatty acid transport in muscle: the role of fatty acid-binding proteins. Biochem Soc Trans 15: 331–336, 1987
13. Kimura H, Odani S, Suzuki J, Arakawa M, Ono T: Kidney fatty-acid binding protein: identification as α_{2u}-globulin. FEBS Lett 246: 101–104, 1989
14. Böhmer F-D, Lehmann W, Noll F, Samtleben R, Langen P, Grosse R: Specific neutralizing antiserum against a polypeptide growth inhibitor for mammary cells purified from bovine mammary gland. Biochim Biophys Acta 846: 145–154, 1985
15. Billich S, Wissel T, Kratzin H, Hahn U, Hagenhoff B, Lezius AG, Spener F: Cloning of a full-length complementary DNA for fatty-acid-binding protein from bovine heart. Eur J Biochem 175: 549–556, 1988
16. Bernlohr DA, Angus CM, Lane MD, Bolanowski MK, Kelly TK: Expression of specific mRNAs during adipose differentiation: Identification of a mRNA encoding a homologue of myelin P2 protein. Proc Natl Acad Sci USA 81: 5468–5472, 1984
17. Haunerland N, Jagschies G, Schulenberg H, Spener F: Fatty-acid-binding proteins. Occurrence of two fatty-acid-binding proteins in bovine liver cytosol and their binding of fatty acids, cholesterol, and other lipophilic ligands. Hoppe-Seyler's Z Physiol Chem 365: 365–376, 1984
18. Trulzsch D, Arias IM: Z-protein: Isolation and characterization of multiple forms in rat liver cytosol. Arch Biochem Biophys 209: 433–440, 1981
19. Takahashi K, Odani S, Ono T: Isolation and characterization of the three fractions (DE-I, DE-II and DE-III) of rat-liver Z-protein and the complete primary structure of DE-II. Eur J Biochem 136: 589–601, 1983
20. Jones PD, Carne A, Bass NM, Grigor MR: Isolation and characterization of fatty acid binding proteins from mammary tissue of lactating rats. Biochem J 251: 919–925, 1988
21. Börchers T, Hojrup P, Nielsen S, Roepstorff P, Spener F, Knudsen J: Revision of the amino acid sequence of human heart fatty acid binding protein. Mol Cell Biochem, this issue
22. Offner GD, Brecher P, Sawlivich WB, Costello CE, Troxler RF: Characterization and amino acid sequence of a fatty acid-binding protein from human heart. Biochem J 252: 191–198, 1988
23. Unterberg C, Börchers T, Hojrup P, Roepstorff P, Knudsen J, Spener F: Cardiac fatty acid binding proteins. Isolation of a mitochondrial fatty acid binding protein and its structural relationship with the cytosolic isoforms. J Biol Chem, in press
24. Mogensen IB, Schulenberg H, Hansen HO, Spener F, Knudsen J: A novel acyl-CoA binding protein from bovine liver. Effect on fatty acid synthesis. Biochem J 241: 189–192, 1987
25. Rasmussen JT, Börchers T, Knudsen J: Comparison of the binding affinities of acyl-CoA-binding protein (ACBP) and fatty acid binding protein (FABP) for long-chain acyl-CoA esters. Biochem J 265: 849–855, 1990
26. Schulenberg-Schell H, Schäfer P, Keuper HJK, Stanislawski B, Hoffmann E, Rüterjans H, Spener F: Interactions of fatty acids with neutral fatty-acid-binding protein from bovine liver. Eur J Biochem 170: 565–574, 1988
27. Paulussen RJA, van der Logt CPE, Veerkamp JH: Characterization and binding properties of fatty acid-binding proteins from human, pig, and rat heart. Arch Biochem Biophys 264: 533–545, 1988
28. Lowe JB, Sacchettini JC, Laposata M, McQuillan JJ, Gordon JI: Expression of rat intestinal fatty acid-binding protein in *Escherichia coli.* Purification and comparison of ligand binding characteristics with that of *Escherichia coli*-derived rat liver fatty acid-binding protein. J Biol Chem 262: 5931–5937, 1987
29. Glatz JFC, Janssen AM, Baerwaldt CCF, Veerkamp JH: Purification and characterization of fatty acid binding proteins from rat heart and liver. Biochim Biophys Acta 837: 57–66, 1985
30. Keuper HJK, Klein RA, Spener F: Spectroscopic investigations on the binding site of bovine hepatic fatty acid binding protein. Evidence for the existence of a single binding site for two fatty acid molecules. Chem Phys Lipids 38: 159–173, 1985
31. Sacchettini JC, Gordon JI, Banaszak LJ: The structure of crystalline *Escherichia coli*-derived rat intestinal fatty acid-binding protein at 2.5 Å resolution. J Biol Chem 263: 5815–5819, 1988
32. Storch J, Bass NM, Kleinfeld AM: Studies of the fatty acid-binding site of rat liver fatty acid-binding protein using fluorescent fatty acids. J Biol Chem 264: 8708–8713, 1989
33. Böhmer F-D, Mieth M, Reichmann G, Taube C, Grosse R, Hollenberg MD: A polypeptide growth inhibitor isolated from bovine mammary gland (MDGI) is a lipid carrying protein. J Cell Biochem 38: 199–204, 1988
34. Behlke J, Mieth M, Böhmer F-D, Grosse R: Hydrodynamic and circular dichroid analysis of mammary derived-

growth inhibitor (MDGI). Biochem Biophys Res Commun 161: 363–370, 1989
35. Fournier NC, Rahim M: Control of energy production in the heart: A new function for fatty acid binding protein. Biochemistry 24: 2387–2396, 1985
36. Paulussen RJA, Geelen MJH, Beynen AC, Veerkamp JH: Immunochemical quantitation of fatty acid-binding proteins. I. Tissue and intracellular distribution, postnatal development and influence of physiological conditions on rat heart and liver FABP. Biochim Biophys Acta 1001: 201–209, 1989
37. Müller T, Kurtz A, Vogel F, Breter H, Schneider F, Engström U, Mieth M, Böhmer F-D, Grosse R: A mammary derived growth inhibitor (MDGI) related to 70 kDa antigen identified in nuclei of mammary epithelial cells. J Cell Pysiol 138: 415–423, 1989
38. Lehmann W, Widmaier R, Langen P: Response of different epithelial cell lines to a mammary derived growth inhibitor (MDGI). Biomed Biochim Acta 48: 143–151, 1989

Address for offprints: F. Spener, Department of Biochemistry, University of Münster, Wilhelm-Klemm-Str. 2, D-4400 Münster, FRG