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Review Letter

On the role of fatty acid binding proteins in fatty acid transport and metabolism

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1. INTRODUCTION

Although movement of fatty acids between bilayers can occur spontaneously, the existence of an intracellular carrier protein was postulated in the early 1970's. It has been gradually established that the cytosol of various mammalian cells contains a low molecular mass protein that exhibits a high affinity for fatty acids and their CoA esters and hence may participate in their intracellular transport and/or storage, much like albumin does extracellularly. These fatty acid binding proteins (FABPs), as they are called, are a class of 14–15 kDa proteins that have been isolated from different tissues of rat, bovine and human species. Three structurally distinct FABP types from liver, heart and intestine have been thoroughly characterized thus far. The fatty acid binding proteins belong to a new superfamily of nonenzymic proteins which are characterized by structural homologies indicating a common ancestral gene. This protein family includes, in addition to the three mammalian FABP types, the various cellular retinoid-binding proteins, the P2 protein of peripheral nerve myelin and the p422 adipocyte protein.

The heterogeneity of the FABPs and their endogenous ligands have been defined and numerous physiological functions have been proposed in a recent review [1]. In the present paper, the different types of FABPs, their structural prerequisites to act as fatty acid carriers and their tissue-specific expression have been analysed and their functions in fatty acid transport and metabolism have been discussed in relation to their biochemical characteristics.

2. DIFFERENT TYPES OF FABPs AND THEIR STRUCTURAL ANALYSIS

Of all the FABPs reported to date, perhaps the most thoroughly studied is the hepatic type from rat liver (hFABP) [2]. The protein has an isoelectric point around neutrality, yet a variety of more acidic 'isoforms' have been found, which are believed to result from binding different ligands. However, two distinct isoproteins of hFABP in the apoform isolated from delipidated bovine liver cytosol are known [3]. The cardiac-type protein (cFABP) was obtained from heart muscle of

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human, rat, pig and bovine sources [4]. From the latter, two isoforms of cFABP with acidic isoelectric points have been isolated and characterized [5]. Cardiac and hepatic FABPs were clearly distinct with regard to their immunological characteristics and protein chemistry. Moreover, the 2 types are also different from intestinal-type FABP (iFABP), which is abundantly present in small intestinal lining cells (enterocytes) [2].

The primary structures of the three mammalian FABP types have been fully defined. Human and rat hFABPs contain 127 amino acids (14.3 kDa), whereas corresponding iFABPs as well as cFABPs from rat, bovine and human heart contain 132 amino acids (14.7–15.1 kDa) [1]. Sequence homologies between different types of FABP are around 40% even when expressed in the same cell, but within one FABP type interspecies homologies up to 90% are observed.

CD-spectroscopic data predicted an overall secondary structure of 12% α-helix, 46% β-structure, 15% β-turn and 27% remainder for neutral hFABP from bovine liver [6]. Overwhelming proportions of β-structure have also been predicted for cardiac- [7] and intestinal-type proteins. Confirmation for the latter came from a recent study, where rat iFABPs were expressed in *E. coli* and subsequent X-ray crystallographic data revealed a β-barrel core structure that appears to be a common motif for hydrophobic molecule transporters [8].

3. BINDING OF LIGANDS

Long-chain fatty acids were the only endogenous ligands that copurified with hFABP and cFABP when delipidation was omitted during isolation from mammalian organs. Up to 60% of endogenous long-chain fatty acids in liver and heart cytosols were non-covalently bound to this protein, of which more than 50% were polyunsaturated, while palmitate and stearate were the principal saturated fatty acids [1]. In the case of *E. coli*-expressed FABPs fatty acids bound to recombinant iFABP were only saturated and its affinity for unsaturated fatty acids was indeed significantly lower than that of recombinant hFABP [9]. In vitro experiments showed, however, that FABPs also bind the CoA esters of long-chain fatty acids and modulate acyl-CoA-dependent enzymic reactions. Moreover, hFABP from liver cytosols has been formerly addressed as Z-protein, amino-azodye-binding protein, sterol carrier protein which reflects its properties of binding a variety of amphiphiles [1,2,4].

K_d values of the fatty acid-FABP complexes are in the range of 10^{-7}–10^{-6} M [2,4,8]. A systematic comparison of affinities of all 3 FABP types for saturated and unsaturated fatty acids is not available to date. Lower affinities have been reported for acyl-CoAs and recently, it has been questioned whether cFABP binds acyl-CoAs at all.

There is some controversy concerning the precise stoichiometry of ligand binding. Until recently it has been accepted that all FABPs bind one mol of fatty acids. However, Haunerland et al. [3] were the first to report that bovine hFABP binds 2 fatty acids, a stoichiometry supported by CD measurements and binding studies and now generally found for hepatic-type FABPs. After proper loading of hFABP a minimum binding of 2 mol oleate per mol protein was reported [10]. A clear 1:1 stoichiometry was obtained for iFABP by binding studies and X-ray crystallography [8,9], whereas 1:1 as well as 2:1 ratios have been found for fatty acid/cFABP complexes [4,11].

The molecular basis of ligand-protein interaction is only being gradually understood. The binding site of neutral hFABP, when probed with conjugated as well as fluorescently labeled fatty acids, revealed that two molecules of fatty acids are held in close proximity in one large hydrophobic binding site. A role for Arg residue(s), located near the C-terminus of hFABP, was established for ionic binding of fatty acids in protein modification studies [6], a view challenged by NMR data that did not reveal electrostatic interactions with cationic residues of the protein [10]. A deeply submersed fatty acid in the crevice of recombinant iFABP was deduced from crystallographic data; aromatic amino acid residues appeared to be in the vicinity of the fatty acid's hydrocarbon chain and an Arg residue near its carboxylate group [8].

4. TISSUE-SPECIFIC EXPRESSION AND REGULATION OF THE FABPs

hFABP and iFABP are expressed in considerable abundance in liver and small intestine,

respectively and in the latter both FABPs are expressed. hFABP comprised 3–5% of the cytosolic protein mass in adult male rat hepatocytes, whereas 0.2% of the translatable liver mRNA specified this protein. In intestinal enterocytes, hFABP represented 2% of jejunal and 1% of ileal cytosolic proteins and 3.3% of jejunal epithelial translatable mRNA specified this protein [2]. iFABP was found only in the gastrointestinal tract, mainly in small intestine and in detectable amounts in large intestine and stomach. The pattern of distribution of iFABP corresponds to the anatomical localization of dietary fat absorption and esterification [1,2].

The modulation of hFABP concentration by sex-related factors and by hypolipidemic drugs such as clofibrate was caused by alterations in the synthesis of this protein mediated at the pretranslational level, probably through regulation at the level of gene transcription, although hFABP-mRNA stabilization is also possible in liver. In enterocytes hFABP was hormonally and pharmacologically more responsive than iFABP [2].

Recent data disclosed that cFABP and its mRNA are far more widely distributed than hFABP or iFABP. cFABP is most abundant in adult heart and relatively high levels are also found in skeletal muscle. Distinct pattern of developmental changes of cFABP-mRNA accumulation were found in heart, placenta, brain, kidney and testes, which insinuates a function in oxidative fatty acid metabolism. However, the presence of cFABP in brain suggests a wider role in lipid homeostasis [1].

5. FUNCTIONAL PROPERTIES

Although considerable evidence indicates that the three types of mammalian FABPs serve distinct biological functions, their precise physiological role is yet to be elucidated. Insight into the physiological functions of FABPs has been gained from a vast array of studies on different approaches which may be grouped as follows:

5.1. *Promoting cellular uptake of fatty acids and facilitating their utilization*

Significant correlations have been observed between the tissue content of FABP and the rate of uptake or utilization of fatty acids particularly in response to dietary, hormonal and pharmacological manipulations. Following a long-term high-fat diet the cytosolic FABP content rises in rat liver, heart, intestine and adipose tissue. During diabetes and starvation, however, the markedly increased hepatic fatty acid oxidation is accompanied by a decrease of hFABP [1,2,4].

The utilization of oleate and its incorporation into triacylglycerols were greater in hepatocyte suspensions from adult female rats than from males and corresponded to a higher hFABP concentration in liver cytosol of female rats. Administration of sex steroids to castrated rats could reproduce these sex differences in both fatty acid utilization and hFABP content [2]. Estrogen treatment has been shown to cause marked changes in hepatic lipid metabolism including increased synthesis of VLDL apoproteins, hFABP and augmented lipogenesis. Clofibrate increases hepatic content of hFABP as well as the uptake rate of fatty acids.

FABP titers in rat liver and heart cytosols as determined by fatty acid binding showed a diurnal variation with the highest levels during the dark period, which may be related to the rat's nocturnal feeding habits [4]. This would indicate a rapid turnover of FABPs and, in one instance, a half-life of hFABP in rat liver as low as 2 h was reported. In contrast, Bass and colleages determined a value of 3.1 days, which agrees with all experimental evidence that lipid and effectors of lipid metabolism have no immediate impact on the regulation of FABPs [2].

5.2. *Protection of enzymes and cellular structures from detergent effects*

FABP concentrations up to 300 μM were found in liver and heart cytosols which always provide excess binding sites for fatty acids and acyl-CoAs in the non-diseased state of the organs [4]. FABP may thus serve as an intracellular buffer from which the substrates are rapidly mobilized in response to the cell's metabolic needs. The solubilizing effect of FABPs protects cellular membranes against toxic effects of fatty acids and acyl-CoAs and their critical micellar concentration is not surpassed.

5.3. *Modulation of enzyme activities*

Fatty acids and their CoA esters in their monomeric forms are inhibitory to a variety of en-

zymes and transport systems. FABPs may modulate some aspects of cellular metabolism by binding these effectors, thus attenuating their inhibitory actions. The ability of FABPs to prevent or reverse the feedback inhibition of fatty acid or acyl-CoA on enzymes has been demonstrated, e.g. in the case of acetyl-CoA carboxylase and mitochondrial ATP/ADP translocase from rat liver and human placental and fetal liver glucose-6-dehydrogenase [2,4,12].

A direct modulating effect of FABPs or FABP-substrate complexes on enzyme activities is difficult to assess experimentally in vitro. Usually, conclusions were based on enhanced stimulatory effects observed for FABP vis-à-vis albumin. Enzymes catalyzing acylation reactions in the course of phosphatidic acid and triglyceride biosynthesis in liver and lung microsomes have been reported to be stimulated by the respective FABPs. A general effect on long-chain transacylases, however, cannot be deduced as for example the acylation of lysophosphatidic acid in lung microsomes was not affected by FABP [2,4,13]. Both stimulatory and inhibitory effects of hFABP and cFABP were observed for microsomal fatty acid activation [2]. Whereas FABP interacting with the enzyme-containing membrane could produce the stimulatory effect, inhibition was explained by competitive retainment of fatty acid on FABP in the soluble phase.

5.4. *Targeting fatty acids to specific metabolic pathways*

In model systems FABPs enhance the transfer of fatty acids between microsomes, mitochondria and liposomes and may also remove fatty acids from multilamellar liposomes [14].

Self-aggregates of cFABP in heart cell cytosol have been proposed to transfer fatty acids to mitochondria for β-oxidation [7]. Alternatively, when polyunsaturated fatty acids are retained in the cytosol due to high-affinity binding to cFABP, other fatty acids partition preferentially into mitochondria for activation and subsequent energy production.

A further cue for targeted transport comes from recent findings on the subcellular distribution of FABPs [15]. Since cFABP in heart cells was also found in mitochondrial matrix, one may speculate that it helps to establish an intracellular gradient of binding sites for fatty acids and acyl-CoAs. On the other hand, hFABP in liver cells was detected in intracellular membranes. This membrane-associated hFABP may be a consequence of direct hFABP-membrane interaction that helped to target substrates for specific metabolic paths. Interestingly, cFABP and hFABP have been detected in nuclei of corresponding cells; whether they carry a message related to lipid metabolism remains to be established.

6. CONCLUSION

Long-term in vivo studies demonstrated a clear correlation between cytosolic FABP contents and cellular utilizations of fatty acids. Although many plausible data are available, evidence in vitro is only circumstantial for a direct involvement of FABP in cellular transport and metabolism of fatty acids and their CoA esters. Recent knowledge on the structural details of FABPs indicates a common design for their binding of fatty acids. The expression of different types of FABPs and the occurrence of isoforms in various cells suggest an adaptation to specific metabolic needs of the cells. As the genomic organisation of FABP genes becomes more and more elucidated, it will be interesting to note whether a deficiency or structural defect of this class of proteins has any significance for fatty acid transport and metabolism.

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