Members of the fatty acid-binding protein family inhibit cell-free protein synthesis

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Abstract Fatty acid-binding proteins (FABPs) are 15-kDa cytosolic proteins which are involved in the intracellular binding and targeting of fatty acids. Some members have been implicated in the regulation of cell growth and differentiation. In this study we investigated the effect of a series of FABPs and heart FABP (H-FABP) mutants on cell-free protein synthesis. Human myelin, intestinal, heart and brain FABP showed a dose-dependent inhibition of in vitro mRNA translation. Adipocyte, liver and epidermal types had no effect. The inhibition was not influenced by delipidation and for H-FABP mutants not related to their affinity for fatty acids. Our results indicate that some FABPs may modulate cell growth and/or differentiation by inhibition of protein synthesis.

Key words: Heart fatty acid-binding protein; Myelin fatty acid-binding protein; Brain fatty acid-binding protein; Heart fatty acid-binding protein mutant; RNase A; In vitro translation

1. Introduction

Fatty acid binding proteins (FABPs) are members of a family of conserved intracellular lipid-binding proteins and comprise at least eight types: liver (L-FABP), intestinal (I-FABP), heart or muscle (H-FABP), adipocyte (A-FABP), myelin (M-FABP), ileal (I-LBP), epidermal (E-FABP) and brain (B-FABP) [1,2]. All FABPs are 14–16-kDa cytosolic proteins and display sequence similarity of between 38 and 70%. The main function of FABP is thought to be intracellular binding and targeting of fatty acids. They may also modulate the effect of fatty acids on various metabolic enzymes and receptors and on cellular processes as signal transduction and gene expression.

Some members of the FABP family have been implicated in the regulation of growth and differentiation. Bovine mammary-derived growth inhibitor (MDGI) which was identified as a mixture of H-FABP and A-FABP [3], caused specific growth inhibition and terminal differentiation of mammary epithelial cells [4], and H-FABP cDNA transfection caused a modest antiproliferative activity in human breast cancer cells. Expression of bovine H-FABP in yeast inhibited cell growth [5]. Changes in FABP content have been observed in tumor cells, in an FABP type – and cell type – dependent way [6–11]. Oka et al. reported the isolation and cloning of a 14-kDa perchloric acid-soluble protein (PSP) from rat liver [12]. This protein exhibited a dose-dependent inhibition of mRNA translation. Another PSP isolated from rat heart, identical to H-FABP, also inhibited protein synthesis in vitro [13]. On the basis of these observations we studied the effect of a series of FABPs (different in type, species, preparation, lipidation), mutant FABPs and other proteins on in vitro mRNA translation.

2. Materials and methods

2.1. Materials

Human H-FABP, I-FABP, M-FABP and A-FABP were obtained by recombinant expression and isolation, as described earlier by Veerkamp et al. [14]. Human B-FABP cDNA in pBluescript vector (obtained from Dr. F. Shimizu, Otsuka GEN Research Institute, Kagasuno, Tokushima, Japan) was amplified and modified with PCR to obtain *Nco*I and *Bam*HI sites, checked on sequence in pCR 2.1 vector and ligated in the pET-3d expression vector. B-FABP was expressed in transformed BL21(DE3) cells and isolated as described for H-FABP [14]. Human L-FABP was obtained by recombinant expression, according to the method described by Maatman et al. [15]. Recombinant human E-FABP and bovine H-FABP were obtained from Dr. F. Spener (Dept. Biochemistry, University of Münster, Germany) and Dr. C. Lücke (Institute Biophysical Chemistry, Frankfurt, Germany), respectively. Rat H-FABP and A-FABP were obtained by recombinant expression and isolation as described by Prinsen and Veerkamp [16]. Rat and porcine H-FABP were isolated from heart tissue of these species as described previously [17]. Human H-FABP mutants were obtained by oligonucleotide-directed in vitro mutagenesis and recombinant expression [18]. If indicated, FABPs were delipidated by the Lipidex procedure [19]. All FABP preparations were $>95\%$ pure on the basis of SDS-PAGE and Coomassie brilliant blue staining.

Bovine serum albumin (BSA), ovalbumin and horse heart myoglobin were from Sigma, St. Louis, MO, USA; cytochrome *c* from Aldrich, Milwaukee, MI, USA; RNasin from Promega, Madison, WI, USA; rabbit reticulocyte lysate system, tobacco mosaic virus mRNA, *Xenopus* β-globin mRNA and RNase A from Boehringer, Mannheim, Germany; GF/C filters from Whatman International, Maidstone, UK; pCR2.1 vector from Invitrogen, Leek, the Netherlands. All other reagents were of analytical grade.

2.2. Cell-free mRNA translation

Cell-free protein synthesis was carried out in the presence or absence of different FABP preparations, H-FABP mutants or other proteins with a rabbit reticulocyte lysate assay system, containing tobacco mosaic virus (TMV) mRNA. The kit instructions were basically followed, except that the incubation time was 45 min at 30°C and the TMV mRNA concentration was reduced to 24 μg/ml. After incubation, 4 μl of the mixture were incubated with 0.5 ml 1 M NaOH containing 5% (v/v) H_2O_2 for 10 min at 37°C. Proteins were precipitated by addition of 0.4 ml 50% trichloric acid (TCA)/2% (w/v) casein and put on GF/C filters. Filters were washed with 0.5% (w/v) TCA/10 mM $Na_4P_2O_7$ and radioactivity was measured in a liquid scintillation counter.

2.3. Assay for RNase activity

Various concentrations of RNase A or FABP were added to 250 μl of a solution containing 50 mM Tris-HCl (pH 7.5), 750 μg total yeast RNA, and the reaction mixture was incubated at 37°C for 30 min. The reaction was stopped by precipitation with 0.1 vol. of 3 M NaAc (pH 5.2) and 2.5 vol. of 96% EtOH. RNA pellets were washed with

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70% EtOH, resolved in Milli Q and applied on 1.0% agarose gel containing 0.003% ethidium bromide. After electrophoresis the amount of RNA present in every lane was measured by densitometry.

3. Results

3.1. Effect of FABPs on cell-free protein synthesis

The effect of FABPs on cell-free protein synthesis was examined in a rabbit reticulocyte lysate. Stimulation of translation by TMV mRNA amounted to 350-fold in control incubations. At 150 μg/ml, protein synthesis was inhibited to about 30% and 40% by recombinant human B-FABP and H-FABP, respectively (Table 1). Recombinant human M-FABP and I-FABP inhibited protein synthesis almost completely, whereas human L-FABP, E-FABP and A-FABP did not affect protein synthesis. At 15 μg/ml, the inhibitory effect of FABPs was less. Only human M-FABP inhibited protein synthesis by 70% (Table 1). The same extent of inhibition was caused by human M-FABP, I-FABP and H-FABP with *Xenopus* β-globin mRNA instead of TMV mRNA, indicating that inhibition by FABPs is not dependent on the mRNA type.

To examine the effect of bound ligands on protein synthesis, the effect of delipidation was studied. Delipidation of recombinant M-FABP and I-FABP did not alter their inhibitory effect (Table 1). Several human H-FABP mutants with different binding affinities for oleic acid were also tested. Mutants F57S, F4E, G67S and F4S have a high binding affinity for oleic acid (K_d values are 0.38, 0.39, 0.35 and 0.47 μM, respectively), whereas mutants F16E and R106T do not bind fatty acids or with very low affinity. Mutants F57S, F16E and R106T inhibited protein synthesis, whereas mutants F4E, G67S and F4S did not affect protein synthesis. Together, these results indicate that there is no relation between protein synthesis inhibition and bound ligands as fatty acids or binding activity.

Besides human recombinant FABP types, recombinant and tissue-derived FABPs from other species were tested on their ability to inhibit protein synthesis. Recombinant rat H-FABP did not show any inhibition at 150 μg/ml, whereas recombinant bovine and human H-FABP inhibited by 75% and 62%, respectively. In contrast to recombinant rat H-FABP, tissue-derived rat H-FABP inhibited protein synthesis. Tissue-derived porcine H-FABP did not show any inhibitory effect. Recombinant rat A-FABP had no effect like human A-FABP. Protein synthesis was not, or to a smaller extent, affected by proteins like albumin, ovalbumin, myoglobin and cytochrome *c*, indicating that inhibition of protein synthesis is specific for some FABPs.

Inhibition of protein synthesis by FABPs appeared to be dose-dependent (Fig. 1). Recombinant human M-FABP exhibited 50% inhibition (IC_{50}) at 9.5 μg/ml, whereas IC_{50} values were 36 μg/ml and 88 μg/ml for recombinant human I-FABP and H-FABP, respectively.

3.2. Test on RNase activity

Since contaminating RNase activity might cause the inhibition, we determined the effect of different RNase A concentrations on the translation assay (Fig. 1). Its IC_{50} value amounted to 4.9 μg/ml. This value is the same order of the IC_{50} value for M-FABP. Subsequently we tested all FABP

Table 1
Effect of different FABPs and other proteins on protein synthesis in a rabbit reticulocyte lysate system

Tested protein	Protein concentration	
	150 μg/ml	15 μg/ml
M-FABP	0 (3)	31 ± 5 (3)
I-FABP	8 ± 2 (5)	92 ± 16 (4)
B-FABP	31 ± 11 (3)	–
H-FABP	38 ± 9 (8)	98 ± 7 (4)
A-FABP	89 ± 20 (4)	–
L-FABP	91 ± 24 (5)	95 ± 9 (3)
E-FABP	109 ± 4 (2)	–
M-FABP (delipidated)	0 (2)	–
I-FABP (delipidated)	16 ± 3 (2)	–
F57S H-FABP	28 ± 13 (3)	–
F16E H-FABP	31 ± 3 (2)	–
R106T H-FABP	57 ± 1 (2)	–
F4E H-FABP	82 ± 5 (3)	–
G67S H-FABP	106 ± 19 (3)	–
F4S H-FABP	114 ± 19 (3)	–
Bovine H-FABP	23 ± 1 (2)	–
Rat H-FABP	101 ± 3 (2)	–
Rat A-FABP	91 ± 10 (2)	–
Rat H-FABP (tissue)	12 ± 3 (3)	–
Porcine H-FABP (tissue)	103 ± 1 (2)	–
Cytochrome *c*	71 ± 7 (2)	–
Albumin	106 ± 3 (2)	–
Ovalbumin	111 ± 2 (2)	–
Myoglobin	133 ± 20 (3)	–

Translation was measured at 24 μg/ml TMV mRNA in the absence or presence of 15 or 150 μg/ml protein as described in Section 2, and expressed as percentage of [^{35}S]methionine incorporation of control without protein. Values are means ± S.D. (range) for the number of independent experiments indicated. All FABP preparations are human, recombinant and undelipidated proteins, except otherwise indicated.

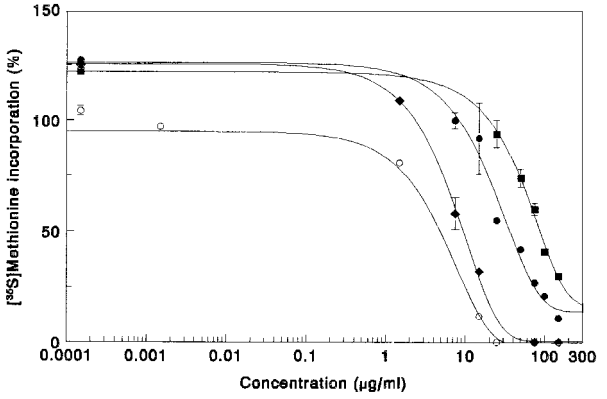

Fig. 1. Effect on cell-free protein synthesis by FABPs and RNase A. Translation was measured in the presence of different concentrations of human recombinant M-FABP (♦), H-FABP (■) or I-FABP (●), or RNase A (○). Each point represents the mean value ± S.D. (range) for at least two independent experiments, expressed as percentage of [^{35}S]methionine incorporation of control.

solutions on the presence of RNase activity, by incubation with yeast RNA and subsequent agarose gel electrophoresis. RNase A clearly showed activity at a concentration of 1.5 μg/ml or higher, whereas all FABPs did not show any RNase activity at a concentration of 150 μg/ml protein. Densitometry revealed that all FABP preparations showed about the same density as the positive control which contained RNA only. Even 1.5 mg/ml of human recombinant H-FABP did not show any RNase activity. To confirm these data, the effect of RNasin in combination with RNase A or FABP on protein synthesis was investigated. However, 2 U/μl RNasin stimulated the protein synthesis of the control by about 300% and could therefore not be used to examine the presence of RNase activity in FABP preparations.

4. Discussion

Several members of the FABP family are possibly involved in the regulation of cell growth and differentiation. In this study we show that M-FABP, I-FABP, B-FABP and H-FABP inhibit in vitro protein synthesis at physiological concentrations of about 1–10 μM. This inhibition appeared to be dose-dependent and is not, or to a smaller extent, exhibited by other proteins. Both agarose gel electrophoresis and the translation assay indicate that the FABPs do not contain RNase activity. Rnase A and M-FABP have about the same IC_{50} value and the IC_{50} values of I-FABP and H-FABP are only 10-fold lower (Fig. 1), indicating that the inhibitory effect of the FABPs cannot be due to RNase contamination. These findings show that FABPs inhibit protein synthesis in the rabbit reticulocyte cell-free lysate system in a different manner from RNase A. The same findings were reported for rat liver- and heart-derived perchloric acid-soluble protein (PSP) [12,13]. It was also shown that these PSPs stimulated the disaggregation of polyribosomes in the lysate, indicating that they inhibit the initiation stage of the protein synthesis [12]. It remains to be investigated whether this is the same for FABPs.

There seems to be no clear relation between protein synthesis inhibition and FABP structure, isoelectric point, fatty acid-binding activity or the presence of a ligand in the FABP molecule. M-FABP and A-FABP with similar isoelectric points and structure show a different action. Delipidation has no effect. H-FABP mutants F16E and R106T which have low affinity for oleic acid show the same inhibitory effect on protein synthesis as wild-type human H-FABP. However, mutants with high affinity for oleic acid have either no effect (F4S, F4E and G67S), or the same effect as wild-type H-FABP (F57S). Furthermore, delipidation of FABPs does not alter their inhibitory effect. Thus, it appears that the inhibitory effect is established by the FABP itself and not by its bound ligand.

Interestingly, H-FABPs derived from different species did not show the same effect on protein synthesis. Both human and bovine recombinant H-FABP inhibited protein synthesis whereas rat recombinant H-FABP had no effect. Surprisingly, tissue-derived rat H-FABP had an inhibitory effect, whereas tissue-derived porcine H-FABP had not. We have no explanation for these contrasting results.

Some members of the FABP family have been implicated in the regulation of growth and differentiation. Yeast, mouse mammary epithelial cells and human breast cancer cell lines showed a specific inhibition of growth and exhibited a differentiated morphology upon H-FABP cDNA transfection [5,6]. In agreement with these data, recombinant human and bovine H-FABP and tissue-derived rat H-FABP inhibited cell-free protein synthesis at physiological concentrations. Studies on rat L6 muscle cells showed that A-FABP cDNA transfection resulted in an increased growth but a decrease in differentiation [16], which may be in accordance with our observation that A-FABP does not influence cell-free protein synthesis. Although L-FABP plays an important role in the modulation of mitogenesis of liver and hepatoma cells [20], addition of L-FABP did not affect cell-free protein synthesis in the system we used. In conclusion, some members of the FABP family possibly regulate cell proliferation and differentiation by interaction with the translation complex, although the mechanism of this interaction remains to be investigated.

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