Isolation and characterization of the fatty acid binding protein from human heart[1]

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Abstract We have isolated in pure form a fatty acid binding protein (FABP) from human cardiac muscle. After preparation of a 100,000 *g* supernatant fraction, the procedure required only one gel chromatographic (Sephacryl S 200) and two cation exchange (CM-Sephadex C 50) steps. The recovery of FABP was 55%. Pure FABP (12.5 mg) was obtained from a 1-g of dry powder equivalent of the high-speed supernatant. The protein had an M_r of 15,500 ± 1,000 Da and an isoelectric point of 5.3. The properties of human cardiac FABP, i.e., molecular mass, isoelectric point, amino acid composition, ultraviolet spectrum, and affinities for hydrophobic ligands, were close to those found for FABPs from bovine heart (Jagschies et al. 1985. *Eur. J. Biochem.* **152:** 537–545). In addition, immunological cross-reactivities showed a relationship between FABPs from several mammalian heart tissues. The data elaborated by us and others support the existence of a cardiac-type FABP that is distinct from the well-defined hepatic-type and gut-type FABPs.—**Unterberg, C., G. Heidl, D-B. von Bassewitz, and F. Spener.** Isolation and characterization of the fatty acid binding protein from human heart. *J. Lipid Res.* 1986. **27:** 1287–1293.

Supplementary key words cardiac muscle • acyl-CoA

The existence of low molecular mass proteins with high affinity for fatty acids in the cytosol of mammalian cells is well established (1–4). Such fatty acid binding proteins (FABPs) were characterized in many instances (5–8) and, based on their primary structures (9–12), immunochemistry, and regulation (4, 13, 14), a hepatic-type and a gut-type protein were defined. The type of FABP does not necessarely preclude its occurrence in different organs; for example, in rat intestinal cells both types of FABP are present (14).

FABPs from heart tissue were isolated from rat (15–19), pig (20, 21), and cattle (22). In a recent review, Glatz, Paulussen, and Veerkamp (17) presented first data on such a binding protein from human heart. The reported M_r of 12 to 15 kDa is within the range found for these proteins, whereas the pI of 7.0 appears to be much higher than their known acidic pIs reported up to now. According to Fournier and collaborators (15, 19–21), cardiac FABP is involved in the transport and supply of fatty acids to the mitochondrial β-oxidation system; a reversible self-association of FABP from pig and rat heart possibly modulates this supply. However, this self-association phenomenon was not observed with bovine FABP (22). The high concentrations of FABP, up to 8% of soluble proteins, in rat heart cytosol may be subject to diurnal variation (23). In this process, cardiac FABP may protect the cell from detergent action of fatty acids and their CoA esters and may buffer the modulation of enzymic activities by these metabolites (23).

In the course of our studies on intracellular transport and recognition of fatty acids (22, 24–26), we present here an expeditious four-step procedure for the isolation of FABP from human heart that affords mg amounts of pure protein. We have characterized the protein and its properties are discussed in context with previous data on cardiac proteins (17, 18, 22) that may justify the definition of a cardiac-type FABP.

MATERIALS AND METHODS

CM-Sephadex C 50, Sephacryl S 200 superfine, and Sephadex G 75 were supplied by Pharmacia; materials for electrophoretic experiments, polyester sheets and ampholines for IEF in ultrathin gels as well as for staining of proteins were from Serva (Heidelberg). Amino acids for electrode solutions, DABITC, and fatty acids were obtained from Sigma. All other reagents were from Merck and were of analytical grade.

Abbreviations: FABP(s), fatty acid binding protein(s); PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing; DABITC 4-N,N-dimethylaminoazobenzene-4′-isothiocyanate; PMSF, phenylmethylsulfonyl fluoride; TLC, thin-layer chromatography.

[1] This paper is part VII of a series Fatty Acid Binding Proteins; part VI appeared elsewhere (25). This work was presented in part at the Vortragstagung der Deutschen Gesellschaft für Fettwissenschaft, Göttingen, 1985 (26).

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Long-chain [1-^{14}C]-labeled fatty acids and their CoA esters (sp act 51–60 Ci/mol), di-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (95 Ci/mol), 1-[1-^{14}C]-palmitoyl-*sn*-glycero-3-phosphocholine (48 Ci/mol), and [4-^{14}C]cholesterol (52.5 Ci/mol) were purchased from New England Nuclear. Medium-chain [1-^{14}C]-labeled fatty acids and their CoA esters (1–5 Ci/mol) were a gift from Dr. J. Knudsen, Institute of Biochemistry, University of Odense, Denmark. Radioactive purity was greater than 96% as checked by TLC and autoradiography.

Anodic disk-PAGE was carried out at pH 8.3 in 1-mm slab gels (T = 11.2%, C = 2.6%) as described by Maurer (27). SDS-PAGE was performed in 1-mm slab gels (T = 15.0%, C = 2.6%) containing urea according to the procedure of Burr and Burr (28); molecular masses were determined with the aid of electrophoresis calibration kits for low molecular mass proteins as well as for polypeptides (Pharmacia).

FABP solutions were added to radioactive ligands placed in 2-ml glass tubes and incubated for 5 min at room temperature with constant shaking. Isoelectric focusing of proteins and protein/ligand complexes was performed in 50-μm polyacrylamide gels and pH gradients from 3 to 10, proteins were stained with Serva Violett, and radioactive ligands were detected by autoradiography (22).

Protein was determined according to Bradford (29). The amino acid composition of cardiac FABP was derived from automatic analysis and individual determinations as described by Jagschies et al. (22). Immunological cross-reactivities were studied by the double diffusion method (30) using the antiserum against bovine pI 4.9-FABP (22).

Human heart tissue was excised from female subjects 12 hr post mortem in accordance with the regulations of the University of Münster Medical School and frozen in liquid nitrogen until work-up. The buffer for homogenization was 0.01 M potassium phosphate, 0.154 M potassium chloride, 1 mM 2-mercaptoethanol, pH 7.4 (buffer A); buffers used in chromatography were 0.02 M potassium phosphate, 1 mM 2-mercaptoethanol, pH 6.3 (buffer B) or pH 6.1 (buffer C). Buffers B and C contained 0.02% sodium azide to prevent microbial growth. The progress of purification (Table 1) was monitored with the aid of [1-^{14}C]oleic acid bound to FABP as described by Jagschies et al. (22). In brief, 1-ml aliquots of fractions were chromatographed on a Sephacryl S 200 column (80 × 1 cm) and assayed for radioactivity in the M_r 15 kDa region. The content of FABP was then calculated from the fractions' total radioactivity relative to that of the high-speed supernatant.

RESULTS

Detection of cardiac fatty acid binding protein

A high-speed supernatant of human cardiac muscle, obtained as described below, was incubated with [1-^{14}C]oleic acid and fractionated on Sephacryl S 200 with buffer C. Radioactivity coeluted with proteins of the 60 kDa and 15 kDa range. The former contained albumin from contaminating blood proteins; the latter was rechromatographed on Sephadex G 75 (buffer B) for removal of residual albumin. The 15 kDa proteins were recharged with [1-^{14}C]oleic acid and focused; the autoradiogram and the stain for protein (**Fig. 1**) showed strong

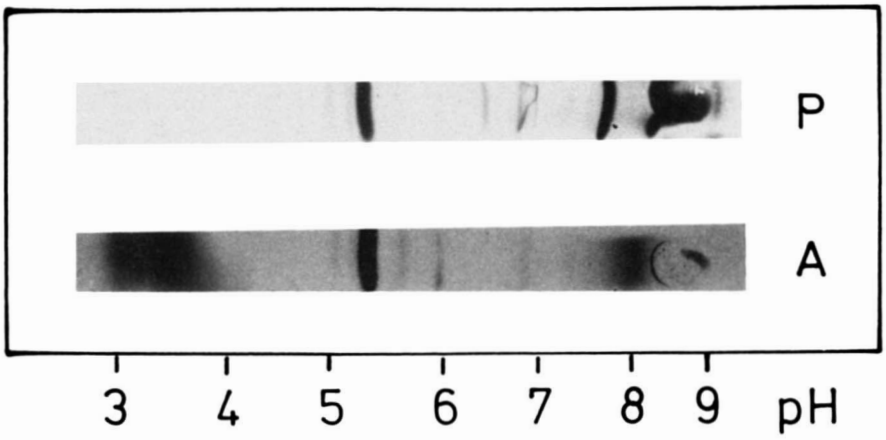

Fig. 1. Detection of human cardiac pI 5.3-FABP in the 15 kDa-fraction obtained after gel chromatography of the high-speed supernatant on Sephacryl S 200 and Sephadex G 75. The fraction was loaded with 40 nCi of [1-^{14}C]oleic acid and separated by IEF (100 μm gels, Servalyte AG 3–10). Lane A, autoradiogram after 24 hr-exposure of the gel; lane P, the gel stained for protein (Serva Violett). Comigration of FABP and ligand is indicated by the dark zones at pH 5.3.

bands for ligand and protein at pH 5.3. Obviously, the bands' superposition reveals the presence of a fatty acid binding protein, whose affinity for oleic acid was strong enough not to dissociate under the electric field applied. Lane A of Fig. 1 shows, furthermore, that excess of ligand had migrated towards the anode, whereas very minor proportions were retained by proteins focusing around pH 5.0, 5.6, 5.9, 6.8, and 7.9. The protein stain (lane P) reveals bands at pH 7.6 and 8.1 as major proteins of the M_r 15 kDa fraction, presumably isoforms of myoglobin as judged by their red color prior to staining.

When the FABP/[1-^{14}C]oleic acid complex was isolated and delipidated with 20% *n*-butanol (31), the resulting apoprotein focused again at pH 5.3. In contrast to observations made with hepatic proteins (7), the state of lipidation of human cardiac FABP, referred to as 'pI 5.3-FABP', did not influence its pI.

Purification

Unless otherwise noted, all steps were performed at 4°C. Thawed heart tissue (150 g wet weight) was cut into small pieces and rinsed three times with buffer A to separate partly from adhering blood proteins. The pieces were added to 250 ml of buffer A containing 1 mM PMSF, homogenized in a Waring Blendor for 1 min followed by a 3-min treatment with an Ultraturrax. The homogenate was centrifuged at 20,000 *g* for 60 min; the supernatant was filtered through glass wool and spun at 100,000 *g* for 120 min. The resulting high-speed supernatant was diafiltrated against distilled water (Sartorius SM 14 138 membrane, cut-off 10,000) and lyophilized to give 3 g of dry powder. The powder was stable for months when kept at −20°C.

Dry powder (0.4 g) was dissolved in 20 ml of buffer B, incubated with 100 nCi of [1-^{14}C]oleic acid, and applied to a CM-Sephadex column (15 × 5 cm, buffer B) after removal of undissolved residues by centrifugation. Elution with buffer B (80 ml/hr) yielded two radioactive peaks (----), the first containing albumin and some overlapping FABP, the second the FABP (**Fig. 2A**). To avoid loss of FABP, the tailing portion of the first peak was thus pooled with the second peak, i.e., fractions between 120 and 220 ml elution volume, and concentrated in an Amicon apparatus (SM 14 138 membrane) to 5 ml (CM-protein).

This solution was fractionated on a Sephacryl S 200 column (100 × 2.5 cm, buffer C) by elution with buffer C (20 ml/hr). The proteins of the 15 kDa range were collected (elution volume 450–550 ml) and ultrafiltrated (Sartorius SM 14 129 membrane, cut off 5,000) to a volume of 5 ml (S 200-protein).

Final purification was achieved when this solution was applied to the second CM-Sephadex column (40 × 2.5 cm, buffer C). Upon elution with buffer C (12 ml/hr) FABP appeared in the fractions between 120 and 160 ml elution volume (Fig. 2B). The pooled fractions were diafiltrated against distilled water (SM 14 129 membrane) and lyophilized to give a stable powder of pI 5.3-FABP in the lipidated form.

The progress of purification was followed by disk-PAGE displayed in **Fig. 3;** quantitative data from two identical runs are summarized in **Table 1.** Taking these data, FABP comprised 2.2% of soluble proteins of the high-speed supernatant. As blood proteins were present, the actual concentration of FABP in human heart cell cytosol was certainly higher. Glatz et al. (23) reported a content of 4–8% FABP in the soluble proteins of rat heart cytosol.

Characterization

The pI 5.3-FABP isolated gave a single band in disk-PAGE as seen in Fig. 3, as well as in IEF and SDS-PAGE. By comparison with standards, a molecular mass of 15,500

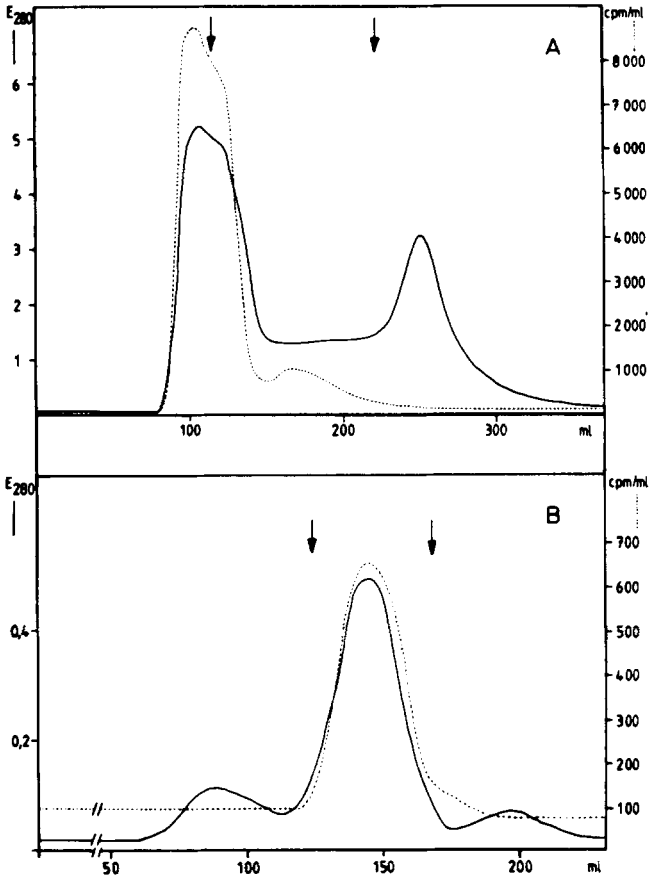

Fig. 2. Purification of FABP on CM-Sephadex C 50. A, first cation-exchange step, the column (15 × 5 cm) was eluted with buffer B. B, final purification in the second cation-exchange step, 40 × 2.5 cm column, buffer C. Fractions between arrows were pooled; (——), protein; (-----), [1-^{14}C]oleic acid bound.

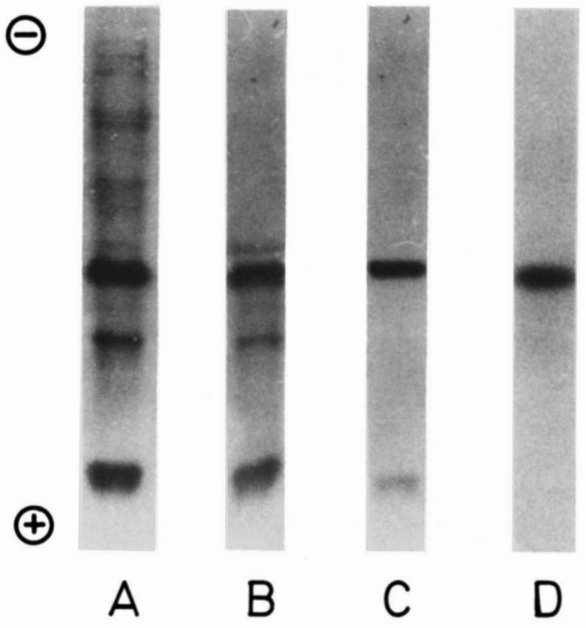

Fig. 3. Disk-PAGE under nondenaturating conditions (pH 8.3, 1-mm slab gels). Lane A, high-speed supernatant (390 μg of protein); lane B, CM-protein (78 μg of protein); lane C, S 200-protein (56 μg of protein); lane D, pI 5.3-FABP (40 μg of protein). Proteins were stained with Serva Violett.

± 1,000 Da was determined by SDS-PAGE and of 15,200 ± 1,000 Da by gel chromatography on Sephadex S 200.

The amino acid composition of human cardiac FABP is shown in **Table 2.** For a comparison with analyses of cardiac FABPs from other species, independent of molecular masses, amino acids are presented in residue numbers/1,000 residues. A calculation of moles of amino acids/mole of FABP is given in parentheses. The content of Phe, Tyr, and Trp appears to be identical in all cardiac proteins so far investigated, a conclusion supported by identical UV-spectra of human and bovine FABPs (see Fig. 4 in ref. 22). The most prominent feature of heart FABPs was their enormous Thr content, up to 140/1,000 residues. Also, their total number of hydrophobic residues remained nearly constant. The number of Lys residues in the human protein was high in comparison to bovine FABPs. This and a decreased Glx content may explain the former's higher isoelectric point. This interpretation of amino acid composition is at variance with the pI 7.0 reported earlier for the cardiac proteins from rat (17, 18) and man (17). Analysis of the N-terminal amino acid with DABITC (32) revealed a blocked N-terminus for human cardiac FABP; the same was found by us for the N-termini of FABPs from bovine heart (22).

Binding of ligands

The current isolation scheme afforded an FABP lipidated with marker oleic acid in addition to endogenous fatty acids. A threefold extraction with 20% *n*-butanol completely delipidated the protein as monitored by the removal of [1-^{14}C]oleic acid. The apoprotein as stated before had a pI of 5.3, however, this treatment produced variably small proportions of denatured protein focusing at pH 5.8. Denaturation could be reversed by relipidation with high affinity fatty acids.

It has been calculated that dissociation constants of about 1 μM and less were necessary to form protein/ligand complexes under conditions of isoelectric focusing (33, 34). We thus incubated 10 μg of delipidated FABP with 40 μCi of radiolabeled ligand and focused the mixture in ultrathin gels. The gels were exposed for 24 hr to a Röntgenfilm and then stained for protein; superposition of the ligand's autoradiographic track and of the protein stain indicated binding. The results qualitatively showed that C_{16} to C_{20} fatty acids with zero to four double bonds served as the foremost substrates for the cardiac FABP, whereas erucic acid and cholesterol were not bound under the conditions applied. The high affinity between this protein and fatty acids commonly found in human tissue accords with the K_d values of 0.8, 0.6, and 1.4 μM reported by Glatz et al. (17) for the binding of palmitic acid, oleic acid, and arachidonic acid, respectively, to human cardiac FABP. Palmitoyl-, stearoyl-, and oleoyl-CoA were also complexed by the protein, but not those with chain-lengths below C_{16}. Interestingly, lysophosphatidylcholine was bound and phosphatidylcholine was not, most probably for steric reasons as these substrates did not differ in charge.

TABLE 1. Progress of purification

Purification Step	Fraction Obtained	Total Protein	Yield FABP	Purification Factor
		mg	%	
1. Homogenization 20,000 *g* 100,000 *g*	High-speed supernatant	758.2	100	1.0
2. CM-Sephadex C 50	CM-protein	243.6	97.6	3.1
3. Sephacryl S 200	S 200-protein	35.7	89.6	19.0
4. CM-Sephadex C 50	pI 5.3-FABP	9.3	54.8	44.7

TABLE 2. Amino acid composition of fatty acid binding proteins from cardiac muscle

Amino Acid	Human Heart	Bovine Heart (22)		Rat Heart (18)
	pI 5.3-FABP (M_r 15,500)	pI 4.9-FABP (M_r 15,300)	pI 5.1-FABP (M_r 15,300)	pI 7.0 (M_r 13,500)
	residues/1,000 residues (total number of residues)			
Ala	47 (6)	42 (6)	42 (6)	58
Arg	32 (4)	31 (4)	31 (4)	25
Asx	108 (15)	113 (16)	110 (15)	120
Cys	13 (2)	18 (3)	30 (4)	0
Glx	79 (11)	98 (14)	92 (13)	106
Gly	69 (10)	82 (12)	81 (11)	100
His	31 (4)	14 (2)	14 (2)	30
Ile	45 (6)	47 (7)	46 (6)	38
Leu	97 (13)	71 (10)	69 (10)	73
Lys	138 (19)	94 (13)	95 (13)	91
Met	15 (2)	15 (2)	14 (2)	8
Phe	41 (6)	44 (6)	46 (6)	42
Pro	13 (2)	13 (2)	11 (2)	11
Ser	48 (7)	52 (7)	49 (7)	71
Thr	126 (17)	133 (19)	139 (20)	130
Trp	15 (2)	16 (2)	15 (2)	n.d.[a]
Tyr	14 (2)	16 (2)	15 (2)	12
Val	70 (10)	101 (14)	102 (14)	83

[a] n.d., Not determined.

All complexes formed focused at pH 5.3. It must be stressed that these data do not necessarily apply to situations in vivo. Under physiological conditions, a weak binding of a ligand not seen here may occur.

Immunological properties

Antiserum against bovine pI 4.9-FABP precipitated human pI 5.3-FABP as well. In the double immunodiffusion gel (**Fig. 4A**), the strong spur formed by precipitins of FABPs from bovine and human heart revealed the presence of similar epitopes. In Fig. 4B, the antigenicity of several heart cell cytosols was tested. The cross-reactivities observed indicated common protein characteristics of the FABPs contained therein.

DISCUSSION

To avoid initial losses of FABP during bulk fractionation with ammonium sulfate (22), we started with a supernatant prepared by high-speed ultracentrifugation. In the present scheme we also deleted anion-exchange chromatography (15–18, 20, 22) that has been reported to cause loss of binding capacity of cardiac FABPs (17) and to cause denaturation that could be overcome only by the use of glycerol-containing elution buffers (17, 22). We preferred, therefore, a twofold application of cation-exchange chromatography; the CM-Sephadex used was operated in the moderate acidic range to allow for weak interactions with FABP only. Basic myoglobin, the major protein of the 15 kDa fraction was 95% removed in the first step at pH 6.3; of the acidic proteins, albumin eluted ahead of FABP and could be 50% separated. The remaining albumin as well as other higher molecular mass proteins was removed by subsequent gel filtration on Sephadex S 200. Final separation from myoglobin and other residual acidic proteins was achieved in the second cation-exchange step. The pH of the column at this step was kept at pH 6.1; a pre-equilibration of the protein solution from pH 6.3 to 6.1 was accomplished by the intermediate chromatography over Sephacryl S 200. The 55% yield of FABP is considerably improved over the 11% reported thus far for the human protein employing anion-exchange chromatography (17). The pI of 5.3 for the protein is well in accord with the range of 4.8 to 5.1 found for cardiac proteins from rat (15, 19), pig (20), and cattle (22). Glatz et al. reported a pI of 7.0 for the cardiac proteins from man (17) and rat (17, 18); however, these authors used gels containing 6 M urea. The denaturating conditions may give rise to higher pIs. Although in our procedure PMSF was immediately added in the work-up, postmortem degradations by proteases cannot be excluded with certainty, especially in the case of the human protein. We can exclude any alteration of the protein's properties by delipidating agents, as the present procedure avoided such treatment.

Molecular masses between 12 and 15 kDa were found for cardiac FABPs (17–20, 22). In the present work we determined 15,500 ± 1,000 by PAGE under denaturating conditions and 15,200 ± 1,000 for the native protein by gel chromatography. Unfortunately, primary structures of cardiac proteins are not available as yet for derivations of protein relationships. However, a comparison of the amino acid data presented in Table 2 does indicate a high Thr content and the co-occurrence of Pro and Trp as

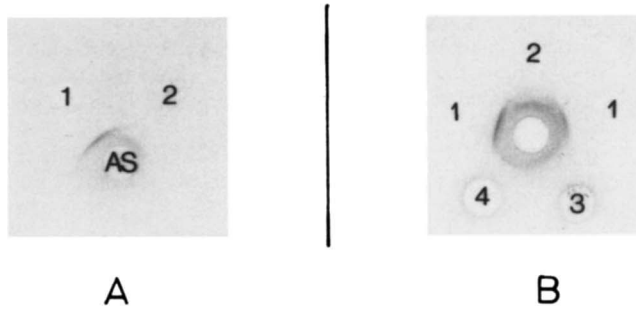

Fig. 4. Cross-reactivity of cardiac FABPs. Double immunodiffusion assay according to ref. 30. The center well (AS) contained 20 μl of anti-(pI 4.9-FABP)-serum. A, 1, bovine pI 4.9-FABP (1 μg); 2, human pI 5.3-FABP (1 μg). B, High-speed supernatants from: 1, bovine heart; 2, human heart; 3, rat heart; 4, pig heart. Precipitins were stained with Serva Violett.

characteristic features of the cardiac proteins. In human (12), bovine (7), and rat (13) hepatic FABPs, Trp is lacking whereas Pro is absent in rat intestinal FABP (11). Our immunological data also support the view that cardiac FABPs constitute their own class of FABP. Antiserum against bovine cardiac FABP cross-reacted with the human FABP and with that from other mammalian heart tissues, whereas we (22) and others (16) have shown that cross-reactions are not obtained between cardiac and hepatic proteins. A blocked N-terminus, as found here and for bovine cardiac FABPs (22), appears quite common among the different classes of FABP, however. The acetyl group was identified as such a blocking group in hepatic (10) and gut (11) proteins and may be responsible for the cytosolic targeting during biosynthesis of these proteins. It remains to be seen whether the different types of FABPs are adapted to the fatty acid metabolism specific for the host organ, or whether they present variations to a mere solubilizer role for fatty acids.

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REFERENCES

1. Ockner, R. K., J. A. Manning, R. B. Poppenhausen, and W. K. L. Ho. 1972. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. *Science.* **177:** 56–58.
2. Dempsey, M. E. 1984. Regulation of lipid metabolism by a lipid-carrying protein. *Curr. Top. Cell. Regul.* **24:** 63–86.
3. Glatz, J. F. C., and J. H. Veerkamp. 1985. Intracellular fatty-acid-binding proteins. *Int. J. Biochem.* **17:** 13–22.
4. Bass, N. M. 1985. Function and regulation of hepatic and intestinal fatty acid binding proteins. *Chem. Phys. Lipids.* **38:** 95–114.
5. Billheimer, J. T., and J. L. Gaylor. 1980. Cytosolic modulators of activities of microsomal enzymes of cholesterol biosynthesis. Role of a cytosolic protein with properties similar to Z-protein (fatty acid binding protein). *J. Biol. Chem.* **255:** 8128–8135.
6. Trulzsch, D., and I. M. Arias. 1981. Z-Protein: isolation and characterization of multiple forms in rat liver cytosol. *Arch. Biochem. Biophys.* **209:** 433–440.
7. Haunerland, N., G. Jagschies, H. Schulenberg, and F. Spener. 1984. 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.
8. Haq, R-U., L. Christodoulides, B. Ketterer, and E. Shrago. 1982. Characterization and purification of fatty acid binding protein in rat and human adipose tissue. *Biochim. Biophys. Acta.* **713:** 193–198.
9. Takahashi, K., S. Odani, and T. Ono. 1982. Primary structure of rat liver Z-protein. A low-M_r cytosol protein that binds sterols, fatty acids and other small molecules. *FEBS Lett.* **140:** 63–66.
10. Gordon, J. I., D. H. Alpers, R. K. Ockner, and A. W. Strauss. 1983. The nucleotide sequence of rat liver fatty acid binding protein mRNA. *J. Biol. Chem.* **258:** 3356–3363.
11. Alpers, D. H., A. W. Strauss, R. K. Ockner, N. M. Bass, and J. I. Gordon. 1984. Cloning of a cDNA encoding rat intestinal fatty acid binding protein. *Proc. Natl. Acad. Sci. USA.* **81:** 313–317.
12. Lowe, J. B., M. S. Boguski, D. A. Sweetser, N. A. Elshourbagy, I. M. Taylor, and J. I. Gordon. 1985. Human liver fatty acid binding protein. Isolation of a full length cDNA and comparative sequence analyses of orthologous and paralogous proteins. *J. Biol. Chem.* **260:** 3413–3417.
13. Ockner, R. K., J. A. Manning, and J. P. Kane. 1982. Fatty acid binding protein. Isolation from rat liver, characterization and immunochemical quantitation. *J. Biol. Chem.* **257:** 7872–7878.
14. Bass, N. M., J. A. Manning, R. K. Ockner, J. I. Gordon, S. Seetharam, and D. H. Alpers. 1985. Regulation of the biosynthesis of two distinct fatty acid binding proteins in rat liver and intestine. Influences of sex difference and of clofibrate. *J. Biol. Chem.* **260:** 1432–1436.
15. Fournier, N., M. Geoffroy, and J. Deshusses. 1978. Purification and characterization of a long chain fatty-acid-binding protein supplying the mitochondrial β-oxidative system in the heart. *Biochim. Biophys. Acta.* **533:** 457–464.
16. Said, B., and H. Schulz. 1984. Fatty acid binding protein from rat heart. The fatty acid binding proteins from rat heart and liver are different proteins. *J. Biol. Chem.* **259:** 1155–1159.
17. Glatz, J. F. C., R. J. A. Paulussen, and J. H. Veerkamp. 1985. Fatty acid binding proteins from heart. *Chem. Phys. Lipids.* **38:** 115–129.
18. Glatz, J. F. C., A. M. Janssen, C. C. F. Baerwaldt, and J. H. Veerkamp. 1985. Purification and characterization of fatty acid-binding proteins from rat heart and liver. *Biochim. Biophys. Acta.* **837:** 57–66.
19. Fournier, N. C., and M. Rahim. 1985. Control of energy production in the heart. A new function for fatty acid binding protein. *Biochemistry.* **24:** 2387–2396.
20. Fournier, N. C., M. Zuker, R. E. Williams, and I. C. P. Smith. 1983. Self-association of the cardiac fatty acid binding protein. Influence on membrane-bound, fatty acid dependent enzymes. *Biochemistry.* **22:** 1863–1872.
21. Fournier, N. C., and M. H. Rahim. 1983. Self-aggregation, a new property of cardiac fatty acid-binding protein. Predictable influence on energy production in the heart. *J. Biol. Chem.* **258:** 2929–2933.
22. Jagschies, G., M. Reers, C. Unterberg, and F. Spener. 1985. 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.
23. Glatz, J. F. C., C. C. F. Baerwaldt, J. H. Veerkamp, and H. J. M. Kempen. 1984. Diurnal variation of cytosolic fatty acid-binding protein content and of palmitate oxidation in rat liver and heart. *J. Biol. Chem.* **259:** 4295–4300.
24. Reers, M., R. Elbracht, H. Rüdel, and F. Spener. 1984. Rapid methods for the characterization of unilamellar phospholipid vesicles. Application to studies on fatty acid donor and acceptor properties of membranes and fatty acid binding proteins. *Chem. Phys. Lipids.* **36:** 15–28.
25. Keuper, H. J. K., R. A. Klein, and F. Spener. 1985. 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.
26. Rüdel, H., C. Unterberg, and F. Spener. 1985. Wechselwirkungen kardialer Fettsäurebindungsproteine mit Fettsäuren und intrazellulären Membranen. *Fette. Seifen. Anstrichm.* **87:** 561–567.
27. Maurer, H. R. 1971. Disk Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis. 2nd ed. Walter deGruyter, Berlin.
28. Burr, F. A., and B. Burr. 1983. Slab gel system for the resolution of oligopeptides below molecular weight of 10,000. *Methods Enzymol.* **96:** 239–244.
29. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
30. Ouchterlony, O., and L. A. Nilsson. 1978. Immunodiffusion and immunelectrophoresis. *In* Handbook of Experimental Immunology. Vol. 1. D. M. Weir, editor. Blackwell Scientific Publications, Oxford. 19. 1–19.39.
31. Morton, R. K. 1955. Methods of extraction of enzymes from animal tissue. *Methods Enzymol.* **1:** 24–51.
32. Chang, J-Y. 1983. Amino-terminal analysis with dimethylaminoazobenzene isothiocyanate. *Methods Enzymol.* **91:** 79–84.
33. Cann, J. R., and K. J. Gardiner. 1979. Isoelectric focusing of interacting systems. IV. Interactions of macromolecules with each other and with ligands. *Biophys. Chem.* **10:** 203–210.
34. Cann, J. R., and K. J. Gardiner. 1979. Isoelectric focusing of interacting systems. V. Determination of ligand binding constants. *Biophys. Chem.* **10:** 211–216.