The purification and characterization of a fatty acid binding protein specific to pig (*Sus domesticus*) adipose tissue

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Western-blot analysis using antiserum to 3T3-L1-cell fatty acid binding protein (FABP) revealed that pig adipose tissue contains a 15 kDa protein immunologically similar to the murine protein. This 15 kDa protein was purified from pig adipose tissue by sequential application of Sephadex G-50 gel filtration, cation exchange and covalent chromatography on Thiol-Sepharose-4B. The purity of the pig protein was established by two-dimensional polyacrylamide-gel electrophoresis. Isoelectric focusing indicated that the pig adipose FABP (a-FABP) exists with two charge isoforms (pI 5.1 and 5.2), both of which persist after delipidation. The N-terminus of the purified pig a-FABP was blocked; however, cleavage with CNBr allowed recovery of a 12-amino-acid peptide which was identical with the murine a-FABP sequence (residues 36–48) at 10 of 12 positions. The pig a-FABP bound 12-(9-anthroyloxy)oleic acid saturably and stoichiometrically, with an apparent dissociation constant of 1.0 μ M. Northern-blot analysis using the cDNA for the murine 3T3-L1 FABP revealed that the pig a-FABP was expressed exclusively in adipose tissue.

INTRODUCTION

The intracellular movement of lipids is thought to be aided by a family of low-molecular-mass (14–15 kDa) cytosolic proteins that bind hydrophobic ligands. FABPs have been purified from liver, small intestine, heart, mammary gland, peripheral nerve tissue and 3T3-L1 cells. The abundance of hepatic and intestinal FABPs is subject to hormonal and dietary control. The abundance of hepatic FABP increased after administration *in vivo* of oestrogen, cholestyramine, or diets with increased fat. Similarly, the abundance of intestinal FABPs was somewhat increased by peroxisome proliferators and increased dietary fat (reviewed by Bass, 1985). Relative to the regulation and adaptivity of a-FABP, feeding with a high-fat diet increased the storage of dietary fatty acids in pig adipocytes and reportedly also increased the palmitoyl-CoA binding activity of the low-molecular-mass protein fraction (St. John *et al.*, 1987). Feeding β -agonists to cattle and sheep decreased the palmitoyl-CoA binding activity of the 14–16 kDa protein fraction in bovine adipose tissue, but increased the palmitoyl-CoA binding activity of the 14–16 kDa protein fraction of sheep adipose tissue (Miller *et al.*, 1988; Coleman *et al.*, 1988). However, conclusions regarding the tissue abundance of FABPs which are based on binding activity should be viewed cautiously, because: (1) a multiplicity of proteins in crude tissue extracts may bind the radiolabelled fatty acid, or its CoA ester, and cause FABP abundance to be overestimated (the non-specific binding component of the assay is often as high as 35–65% of the response); (2) the radiolabelled fatty acid, or its CoA ester, may not displace the ligand already bound to the FABP (thus causing FABP abundance to be underestimated); and (3) in micelle or liposome assays, the monomeric non-esterified fatty acid concentration cannot be accurately quantified. In addition, recent data (Bernier *et al.*, 1988) suggest that 3T3-L1-cell a-FABP is regulated by covalent modification. Such modification may potentially alter the binding affinity of a-FABP for non-esterified fatty acids. These limitations make estimates of FABP concentration, based on binding assays,

equivocal, and probably account for conflicting data in the literature regarding the effects of hormones and dietary fats on FABP concentration (Haq & Shrago, 1985; St. John *et al.*, 1987; Miller *et al.*, 1988; Coleman *et al.*, 1988).

Accurate quantification of a-FABP concentration as affected by hormonal or dietary treatments requires the development of a specific immunological assay. A prerequisite for such an immunological approach is the purification of a-FABP. Pig a-FABP was selected for this study because: (1) the regulation of pig a-FABP may be important for understanding pig adipose metabolism, and hence changes in body composition; (2) pig a-FABP plays a role in the growth-hormone-mediated decrease in the triacylglycerol content of pig adipose tissue (Evock *et al.*, 1988); and (3) pig a-FABP could be used to study the regulation of fatty acid binding by such potential mechanisms as insulin-dependent tyrosine phosphorylation (Bernier *et al.*, 1988).

MATERIALS AND METHODS

Purification

Pig back-fat (100 g) was obtained after slaughter and homogenized in 3 vol. of cold buffer (10 mM-sodium phosphate, 20 mM-NaCl, 1 mM-EDTA and 0.1 mM-phenylmethanesulphonyl fluoride, pH 7.4) with a Polytron homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.) for 30 s at setting 10. The homogenate was centrifuged at 20000 g (4 °C) for 30 min, and the non-fat infranant solution was removed, adjusted to pH 5.0 with 12 M-H₃PO₄ and incubated for 3–4 h at 4 °C before ultracentrifugation at 100000 g (4 °C) for 60 min. The supernatant was concentrated 4-fold by ultrafiltration through a YM-5 membrane (Amicon, Danvers, MA, U.S.A.) and delipidated at 4 °C on a 1 cm $\times$ 30 cm column (Bio-Rad, Richmond, CA, U.S.A.) packed with Lipidex-1000 (Packard Instrument Co., Downers Grove, IL, U.S.A.). Proteins eluted from the Lipidex column were diluted with 5 column vol. of buffer A (50 mM-

Abbreviations used: FABP(s), fatty acid binding protein(s); a-FABP, adipose-tissue FABP; PAGE, polyacrylamide-gel electrophoresis; 12AO, 12-(9-anthroyloxy)oleic acid.

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sodium acetate, pH 5.0) and concentrated by ultrafiltration (YM-5 membrane) to approx. 3 mg/ml.

Approx. 20 mg of protein was applied to a pre-calibrated column (2.5 cm $\times$ 100 cm) of Sephadex G-50 (Pharmacia, Piscataway, NJ, U.S.A.) and eluted (20 ml/h) in buffer A. The protein absorbance in the column eluate was monitored at 280 nm, and the low-molecular-mass proteins were pooled and exhaustively delipidated by Lipidex 1000 chromatography at 37 °C. The delipidated eluate was applied to a 1 cm $\times$ 20 cm column of CM-Sephadex (Sigma Chemical Co., St. Louis, MO, U.S.A.), washed with 5 column vol. of buffer A, and the bound proteins were eluted with buffer A containing 500 mM-NaCl. The fractions containing pig FABP were identified by SDS/PAGE and Western-blot analysis. The FABP-containing fractions were pooled, dialysed against buffer B (50 mM-Tris/100 mM-NaCl, pH 8.0), and added to 500 mg of Thiol-Sepharose-4B (Sigma) which had been equilibrated in buffer B. The slurry was gently agitated at 4 °C for 60–65 h, then poured into a 1 cm $\times$ 5 cm column, washed with 10 column vol. of buffer B, and the protein fraction containing pig FABP was eluted with buffer B containing 25 mM-dithiothreitol. The eluted protein fraction was dialysed against buffer B with 1 mM-dithiothreitol, followed by buffer B alone. Purity and identity were determined by SDS/PAGE, Western-blot analysis, two-dimensional PAGE, isoelectric focusing, partial amino acid sequence and fatty acid binding activity.

Electrophoresis

Slab SDS/PAGE was performed in 1.5 mm-thick 9 cm $\times$ 6 cm gels with a 3.5%-acrylamide stacking gel and a 10–20% linear gradient separating gel (Laemmli, 1970). Voltage (175 V) and temperature (18 °C) were held constant, and the separated proteins were made visible by silver staining (Morrissey, 1981). Molecular-mass markers, prestained with Coomassie Blue, were purchased from Diversified Biotech, Newton Centre, MA, U.S.A.

Western-blot analysis

For this, protein samples were separated by electrophoresis as described above and then transferred to nitrocellulose (Towbin *et al.*, 1979) as modified by Burnette (1981). Transfer of low-molecular-mass proteins was performed at 40 V for 16 h at 4 °C. Immunochemical detection was accomplished at room temperature by incubating the nitrocellulose in blocking buffer [3% (w/v) gelatin in 20 mM-Tris/500 mM-NaCl, pH 7.5] for 30 min, followed by a 2–4 h incubation in rabbit anti-(murine FABP) serum diluted 1:1000 which had previously been prepared and shown to be monospecific (Bernlohr *et al.*, 1984). Detection was accomplished by using goat anti-(rabbit IgG)-alkaline phosphatase conjugate (Immun-Blot Kit from Bio-Rad). The pre-stained molecular-mass markers were visible immediately on transfer of the proteins to the nitrocellulose.

Isoelectric focusing and two-dimensional PAGE

The pI values for the FABP isoforms were determined by flat-bed electrofocusing at 10 °C in a polyacrylamide gel (5% gel concentration, 3% cross-linkage) containing a pH range of 4.5–6.0 (Multiphor System and Ampholine PAGplate; LKB, Bromma, Sweden). Gels were silver-stained (Morrissey, 1981), and the apparent pI was determined with a surface pH electrode. Two-dimensional electrophoresis was performed by using non-equilibrium isoelectric focusing followed by SDS/PAGE (Bernier *et al.*, 1987).

Protein hydrolysis and sequence analysis

After purification from pig adipose tissue, 65 nmol of freeze-dried pig FABP (estimated from the A_{280} by assuming ϵ 1.55 $\times$

10^4 M⁻¹·cm⁻¹) were redissolved in 70% (w/v) formic acid, and peptides were generated by cleavage with CNBr (Aldrich Chemical Co., Milwaukee, WI, U.S.A.). Based on the number of methionine residues predicted from the murine FABP sequence (Bernlohr *et al.*, 1984), a 100-fold molar excess of CNBr over protein was employed. Cleavage was carried out at room temperature for 24 h. After chemical degradation, the sample was freeze-dried, redissolved in 0.15% (v/v) trifluoroacetic acid, and the peptide fragments were separated on a Perkin-Elmer Series 4 chromatograph fitted with a 250 mm $\times$ 4.6 mm-internal-diam. Vydac TP-RPC4 column (The Separation Group, Hesperia, CA, U.S.A.). The eluents were: buffer C, 0.15% trifluoroacetic acid; buffer D, 0.15% trifluoroacetic acid in propan-2-ol/acetonitrile (7:3, v/v). The programmed gradient was 0–100% D in 45 min. The flow rate was 1.0 ml/min and the column eluate was monitored at 220 nm.

N-Terminal amino acid sequence analysis was performed by automated Edman degradation with an Applied Biosystems model 470A gas-phase protein sequencer on-line to a model 120A amino acid phenylthiohydantoin analyser.

Fluorescence measurements and fatty acid binding

Fluorescence spectra were obtained at 24 °C with an SLM 8000C fluorescence spectrophotometer. Excitation wavelengths were 280 and 383 nm for tryptophan and 12AO respectively. Emission intensities were estimated by integrating the area under the emission peak $\pm$ 10 nm.

Binding was determined by the quenching of protein tryptophan fluorescence and the enhancement of 12AO fluorescence on addition of fluorophore as previously described (Storch *et al.*, 1989). The 12AO was added from concentrated ethanolic stock, and the final ethanol concentration was always less than 1% (v/v). The 12AO fluorescence intensities were measured 3 min after probe addition, and binding affinities were established by fluorescence titration (Cogan *et al.*, 1976) and tryptophan quenching (Vincent & Muller-Eberhard, 1985).

Tissue distribution

To examine the tissue distribution of FABP by Western-blot analysis, samples of diaphragm, heart, intestine, kidney, liver, lung and adipose tissue were obtained from a barrow immediately after slaughter and homogenized as described above for purification. The crude homogenate protein (40 μ g of protein/lane) was separated by SDS/PAGE and the presence of an immuno-reactive protein identified as previously described.

The tissue specificity of a-FABP expression was also examined by Northern-blot analysis by using 3T3-L1 FABP cDNA (Bernlohr *et al.*, 1984). Samples of brain, heart, diaphragm, intestine, liver, lung and spleen were removed from a barrow immediately after slaughter and frozen in liquid N₂. Each tissue was homogenized in RNazol (Cinna/Biotechx, Friendwood, TX, U.S.A.) and the RNA was extracted as recommended by the manufacturer. The aqueous fraction containing RNA was extracted once with an equal volume of chloroform/butanol (4:1, v/v). RNA was precipitated from the aqueous fraction by adding an equal volume of propan-2-ol and incubating the samples at –20 °C for 45 min. RNA (10 μ g) from each tissue was denatured with 2 M-glyoxal and size-fractionated by electrophoresis in a 1.5% agarose gel. After electrophoresis, the gel was equilibrated in 40 mM-Tris/acetate/1 mM-EDTA, pH 8.0, for 15 min before electroblotting the RNA on to Zeta Probe (Bio-Rad, Rockville Centre, NY, U.S.A.). The transfer conditions were 60 V for 90 min at 4 °C in 40 mM-Tris/acetate/1 mM-EDTA, pH 8.0. The nucleic acids were cross-linked to the membrane by u.v. irradiation (3 min at 1200 μ W/cm²) and hybridized to ³²P-labelled 3T3-L1 FABP

Fig. 1. A positive Western blot for FABP in 100 000 *g* supernatant from pig adipose tissue (20 μ g of protein) and purified FABP (1 μ g) from 3T3-L1 cells

The blot was incubated for 4 h with rabbit anti-(3T3-L1 FABP) antiserum and developed as described in the Materials and methods section.

Fig. 2. Chromatography of protein (20 mg) from the high-speed-supernatant fraction of pig adipose tissue

The Sephadex G-50 column (2.5 cm $\times$ 100 cm) was eluted at room temperature with 50 mM-sodium acetate buffer (pH 5.0; 20 ml/h). Fractions (6.67 ml) were assayed by SDS/PAGE to determine which contained the relatively greatest amount of 15 kDa proteins. Inset: SDS/PAGE (10–20%-acrylamide linear gradient) performed on fractions 11–14 and molecular-mass markers (MW: carbonic anhydrase, 29 kDa; cytochrome *c*, 12.4 kDa). Fractions 12–14 were pooled before further purification.

Fig. 3. CM-Sephadex ion-exchange chromatography of partially purified 15 kDa proteins

Proteins eluted from the column (1 cm $\times$ 20 cm) after addition of 0.5 M-NaCl to the buffer (50 mM-sodium acetate, pH 5.0) were collected as one fraction (10 ml). Inset: SDS/PAGE (10–20% linear gradient) shows molecular-mass (MW) markers (left) and the putative a-FABP (centre); the positive Western blot (far right) shows that the protein in greatest abundance immunoreacts with antisera developed against 3T3-L1 FABP. Prestained molecular-mass markers are visible on the Western blot; however, they were not immunoreactive.

cDNA. The murine FABP cDNA was radiolabelled by the random-primer method of Feinberg & Vogelstein (1983).

RESULTS

Western-blot analysis of the 100 000 *g* supernatant demonstrated that pig adipose tissue contains a 15 kDa protein which immunoreacts with murine a-FABP antiserum (Fig. 1). Consequently, this antiserum was used to identify the putative pig a-FABP during purification.

Chromatography of the partially delipidated supernatant on a pre-calibrated Sephadex G-50 column resolved the low-molecular-mass proteins from the bulk of other protein in the sample. Fig. 2 (inset) shows that fractions 12–14 in fact contained an abundant 15 kDa protein. Western-blot analysis of these fractions revealed the presence of a 15 kDa protein which immunologically reacted with antiserum to 3T3-L1 FABP (results not shown). These fractions were pooled for further purification by CM-Sephadex ion-exchange chromatography. In order to retain the putative a-FABP on CM-Sephadex, the pooled protein fraction had to be further delipidated. This was accomplished by Lipidex 1000 chromatography at 37 $^{\circ}$ C as described in the Materials and methods section.

CM-Sephadex chromatography was a key step in the purification of the putative pig a-FABP. This ion-exchange procedure removed nearly 75% of the protein without loss of FABP.

Fig. 4. (a) Titration of pig a-FABP (12 μmol) with 12AO and (b) tryptophan fluorescence when pig a-FABP binds 12AO

(a) Increasing concentrations of 12AO were added to 12 μM-a-FABP in 50 mM- Na_2HPO_4 /100 mM-NaCl, pH 8.0. Background for probe in buffer alone was subtracted. In (b) intensity values, I , are reported as a fraction of the initial intensity, I_0 .

Western-blot analysis of the proteins eluted from CM-Sephadex with buffer A indicated that FABP was absent from these fractions (results not shown). Upon addition of 0.5 M-NaCl to buffer A, a single 10 ml fraction, highly enriched with a 15 kDa protein, was eluted from the CM-Sephadex. Western analysis revealed that the 15 kDa protein was immunologically similar to 3T3-L1 FABP (Fig. 3).

The final step of the purification made use of the observation that 3T3-L1 FABP contained free thiol groups, i.e. Cys-2 and Cys-118 (Bernlohr *et al.*, 1984). We speculated that the pig a-FABP would also contain these reactive thiol groups, and capitalized on this characteristic, by subjecting the CM-Sephadex eluate containing the putative a-FABP to covalent chromatography with Thiol-Sepharose-4B. The pig a-FABP did in fact bind to the Thiol-Sepharose-4B, and was subsequently eluted from it with buffer B containing 25 mM-dithiothreitol. A single 15 kDa protein was identified by SDS/PAGE, and this protein was immunoreactive with antiserum developed against 3T3-L1 FABP (results not shown).

The putative pig a-FABP bound to the fluorescent oleic acid

	37	40	45	48
Pig	Ala-Lys-Pro-Asn-Leu-Ile-Ile-Thr-Val-Asn-Gly-Asp			
Mouse*	Ala-Lys-Pro-Asn- <u>Met</u> -Ile-Ile- <u>Ser</u> -Val-Asn-Gly-Asp			

Fig. 5. Partial amino acid sequence of the pig FABP aligned with the similar partial sequence for the mouse adipose lipid-binding protein

Amino acid sequence differences are underlined. * Sequence from Bernlohr *et al.* (1984).

Fig. 6. (a) Silver-stained two-dimensional SDS/PAGE [non-equilibrium isoelectric focusing (NEPHGE) in the first dimension and SDS/PAGE in the second dimension] of purified putative pig a-FABP, and (b) two-dimensional Western blot of purified pig a-FABP, showing two charge isoforms that were positive to rabbit anti-(3T3-L1 FABP) antisera

In (a), the respective molecular mass and pI for each standard are: A, β -lactoglobulin, 20.1 kDa and 4.6; B, carbonic anhydrase, 29.0 kDa and 6.6; C, trypsinogen, 24.0 kDa and 9.3. D indicates the two putative isoforms of pig a-FABP.

analogue 12AO also indicated a ligand/protein molar ratio of 0.7:1 (Fig. 4a). Quenching of protein tryptophan emission by 12AO also indicated a ligand/protein ratio of approx. 1 (Fig. 4b). The reason for the emission quenching is likely to reflect energy transfer from the tryptophan to the anthroxyloxy fluorophore (Storch *et al.*, 1989). The apparent K_d for fatty acid binding obtained by fluorescence titration or tryptophan quenching was 1 μM. When 12AO binding was assessed at pH 6.5 (results not shown), the relative quantum yield was about 3-fold less than that observed at pH 8.0, but the apparent K_d was still 1 μM. Tryptophan quenching at pH 6.5 for the a-FABP was comparable with that at pH 8.0 (results not shown).

Results from the amino acid sequence further support a conclusion that the purified 15 kDa protein was a-FABP. CNBr cleavage of the purified porcine a-FABP yielded four peptide fragments as separated by h.p.l.c. Sequence analysis of the best-resolved fragment allowed placement of 12 amino acid residues corresponding to residues 37–48 of the 3T3-L1 FABP sequence (Fig. 5). Ten of the 12 amino acids in the pig peptide were identical in sequence with the 3T3-L1 FABP.

Further characterization of the pig a-FABP by two-dimensional electrophoresis (non-equilibrium isoelectric focusing in the first dimension and SDS/PAGE in the second dimension)

Fig. 7. Flat-bed isoelectrically focused gel (5% acrylamide, 3% cross-linkage, pH range 4.5–6.0) showing the two charge isoforms of putative pig FABP before delipidation by solvent extraction (a) and after solvent extraction (b)

pI was determined by using a pH-meter with a surface electrode, and the gel was silver-stained.

Fig. 8. Northern blot showing the pig tissue distribution of hybridizable mRNA (10 µg per lane) to 3T3-L1 FABP cDNA

followed by Western-blot analysis revealed that the pig a-FABP existed as two isoforms (Fig. 6). Incomplete delipidation of hepatic FABP has previously led to the identification of multiple isoforms (Ketterer *et al.*, 1976; Ockner *et al.*, 1976; Trulzsch & Arias, 1981; Takahashi *et al.*, 1983; Glatz *et al.*, 1985; Bass, 1985). In order to verify that the two a-FABP isoforms were not the result of incomplete delipidation, the a-FABP was extracted with butanol/di-isopropyl ether (4:1, v/v) (Cham & Knowles, 1976) and subjected to flat-bed isoelectric focusing. Fig. 7 demonstrates that organic-solvent delipidation of the pig a-FABP did not result in a single predominant isoform.

The previous data indicated that an a-FABP had been purified

from pig adipose tissue. To determine if this FABP was unique to adipose tissue, other pig tissues (diaphragm, heart, kidney, liver, lung and intestine) were analysed for the presence of an immunoreactive 15 kDa protein by Western-blot analysis. Immunoreactivity was found in diaphragm, kidney and heart as well as adipose tissue (results not shown). This could imply that the pig a-FABP was expressed in other tissues, or that an immunologically similar protein is expressed in these tissues. To evaluate these two possibilities, Northern-blot analyses of these pig tissues were conducted. Hybridization analysis revealed the pig a-FABP mRNA was specific to adipose tissue (Fig. 8). Hence, in pigs, the presence of immunopositive a-FABP in non-adipose tissues probably reflects cross-reactivity among the FABPs of heart, diaphragm, kidney and adipose tissue.

DISCUSSION

Our data demonstrate that pig adipose tissue contains a 15 kDa FABP which binds fatty acids with saturation kinetics, with an apparent K_d of approx. $1 \mu\text{M}$ and a stoichiometry of 1:1 FABP:fatty acid. The affinity of a-FABP for fatty acids is similar to that reported for the heart FABP (Sacchetti *et al.*, 1986; Paulussen *et al.*, 1988), liver FABP (Bass, 1985; Storch *et al.*, 1989) and 3T3-L1 FABP (Matarese & Bernlohr, 1988). The pig a-FABP appears to be similar to the 3T3-L1 FABP, as judged by its cross-reactivity with antisera prepared against the 3T3-L1 FABP (Fig. 1). More specifically, we found that the sequence of 10 amino acids in the 12-amino-acid peptide fragment from the pig a-FABP was identical with the sequence of residues 37–48 of the 3T3-L1 FABP (Bernlohr *et al.*, 1984).

The pig a-FABP appears to be unique to adipose tissue, as judged by Northern-blot analysis of RNA from pig adipose tissue, brain, heart, diaphragm, intestine, liver, lung and spleen. Interestingly, Western-blot analysis with antisera raised against 3T3-L1 FABP revealed the presence of immunopositive FABP-like proteins in diaphragm, kidney and heart. Given the high abundance of a-FABP mRNA in pig adipose tissue, and yet the complete absence of cDNA hybridization in heart, kidney and diaphragm (Fig. 8), it seems unlikely that adipose contamination of these tissues would explain the degree of immunochemical cross-hybridization. Dempsey *et al.* (1986) have interpreted similar results for the tissue distribution of liver FABP to indicate that liver FABP is a secreted protein which is removed from the blood by various tissues. However, neither the adipose nor the liver FABP has a secretory leader sequence, i.e. a pre-form of FABP does not exist. Thus a more plausible explanation is the presence of an FABP within these tissues, which possesses a primary structure similar to that of pig a-FABP. This is particularly likely for the 20–30 amino acid residues of the N-terminal domain of the lipid-binding-protein multigene family (Sweetser *et al.*, 1987). In support of this explanation, rat heart FABP has 62% sequence similarity with murine 3T3-L1 FABP (Sacchetti *et al.*, 1986). Moreover, the 12-amino-acid sequence obtained from our pig a-FABP had a high similarity to residues 37–48 of rat heart FABP, and within this sequence 7 of 12 residues were identical. Finally, heart-like FABP has been found in both kidney and diaphragm.

Unlike the murine 3T3-L1 FABP, the pig a-FABP existed as two isoforms, as judged by isoelectric focusing and two-dimensional electrophoresis. Two-dimensional electrophoresis followed by Western blotting indicated that the two charge isoforms possessed pI values of 5.1 and 5.2. Multiple pI values for FABP within a tissue are commonplace (Ockner *et al.*, 1976; Ketterer *et al.*, 1976; Trulzsch & Arias, 1981; Takahashi *et al.*, 1983; Glatz *et al.*, 1985; Bass, 1985). One suggestion has been that fatty acid binding to the FABP alters the protein's pI. In this respect, the

isoforms of liver are interconvertible and become more basic after delipidation (Ockner *et al.*, 1976; Ketterer *et al.*, 1976; Trulzsch & Arias, 1981; Takahashi *et al.*, 1983; Glatz *et al.*, 1985; Bass, 1985). Lipidex chromatography and subsequent solvent delipidation of pig a-FABP had no effect on the isoelectric-focusing pattern (Fig. 7). An intriguing possibility exists that the two pI values represent differences in phosphorylation status. This speculation is based on reports that murine 3T3-L1 FABP is phosphorylated by insulin-dependent tyrosine kinase (Bernier *et al.*, 1988). The phosphorylation of the 3T3-L1 FABP results in the appearance of two phosphorylated spots, one at pI 5.0 and one at pI 5.2. It will be of future interest to determine if the pig a-FABP is a substrate for tyrosine kinase and if the phosphorylation of a-FABP affects its affinity for non-esterified fatty acid.

In conclusion, our data indicate that pig adipose tissue contains a highly abundant FABP and its mRNA, and that the expression of this FABP is specific for adipose tissue. Furthermore, the pig a-FABP appears to have a single fatty-acid-binding site, with an apparent K_d of approx. $1 \mu\text{M}$. Although our data support the conclusion that the pig a-FABP is a member of the family of low-molecular-mass FABPs located in the brain, liver, heart, intestine and kidney, the regulation and physiological function of these proteins remain enigmatic.

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