Characterization and amino acid sequence of a fatty acid-binding protein from human heart

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The complete amino acid sequence of a fatty acid-binding protein from human heart was determined by automated Edman degradation of CNBr, BNPS-skatole [3'-bromo-3-methyl-2-(2-nitrobenzenesulphenyl)indolenine], hydroxylamine, *Staphylococcus aureus* V8 proteinase, tryptic and chymotryptic peptides, and by digestion of the protein with carboxypeptidase A. The sequence of the blocked *N*-terminal tryptic peptide from citraconylated protein was determined by collisionally induced decomposition mass spectrometry. The protein contains 132 amino acid residues, is enriched with respect to threonine and lysine, lacks cysteine, has an acetylated valine residue at the *N*-terminus, and has an M_r of 14768 and an isoelectric point of 5.25. This protein contains two short internal repeated sequences from residues 48–54 and from residues 114–119 located within regions of predicted β -structure and decreasing hydrophobicity. These short repeats are contained within two longer repeated regions from residues 48–60 and residues 114–125, which display 62% sequence similarity. These regions could accommodate the charged and uncharged moieties of long-chain fatty acids and may represent fatty acid-binding domains consistent with the finding that human heart fatty acid-binding protein binds 2 mol of oleate or palmitate/mol of protein. Detailed evidence for the amino acid sequences of the peptides has been deposited as Supplementary Publication SUP 50143 (23 pages) at the British Library Lending Division, Boston Spa, Yorkshire LS23 7BQ, U.K., from whom copies may be obtained as indicated in Biochem. J. (1988) 249, 5.

INTRODUCTION

Fatty acid-binding proteins (FABPs) belong to a class of abundant low- M_r cytosolic proteins that have been shown to bind fatty acids and other organic anions (Ockner *et al.*, 1972). FABPs are thought to function in the intracellular transport and metabolism of long-chain fatty acids. Such proteins would be expected to have a particularly important role in heart tissue, where up to 80% of the energy requirement is derived from oxidation of long-chain fatty acids (Neely & Morgan, 1974). The concentration of long-chain fatty acids and their metabolites rises dramatically during ischaemia, and the elevated concentration of these amphipathic molecules has been correlated with both reversible and irreversible damage to cell and organellar membranes (Corr *et al.*, 1984). Since heart FABP constitutes 4–8% of the protein in myocyte cytosol (Bass & Manning, 1986; Chrisman *et al.*, 1987), this abundant protein may serve as an intracellular storage reservoir for these potentially toxic metabolites, and thus may play a protective role in addition to its normal function in cardiac energy metabolism.

In the present paper we describe the isolation, characterization and complete amino acid sequence of FABP from human heart.

EXPERIMENTAL

Isolation and characterization

Human left ventricular heart tissue (50 g), obtained at autopsy within 12 h *post mortem*, was minced, fat strips

were removed and the tissue was homogenized in 80 ml of 0.1 M-potassium phosphate buffer, pH 7.2, in a Polytron homogenizer. This and subsequent purification steps were performed at 4 °C. The homogenate was centrifuged at 12000 *g* for 30 min and the resulting supernatant was centrifuged at 120000 *g* for 60 min to yield heart high-speed supernatant. [14 C]Oleic acid (1.5 μ Ci; 56 mCi/mmol; New England Nuclear) was added to the high-speed supernatant, and the sample was applied to a Sephadex G-75 (Pharmacia) column equilibrated and developed with 0.02 M-Tris/HCl buffer, pH 8.0. FABP-containing fractions were applied to a DEAE-Bio-Gel A (Bio-Rad Laboratories) column equilibrated with the same buffer and developed with a linear gradient of 0.02–0.2 M-Tris/HCl buffer, pH 8.0. Fractions containing FABP were dialysed against 0.01 M-sodium acetate buffer, pH 4.9, and applied to a CM-Bio-Gel A (Bio-Rad Laboratories) column equilibrated with the same buffer and developed with a linear gradient of 0.01–0.2 M-sodium acetate buffer, pH 4.9. Fractions from CM-Bio-Gel A containing purified FABP were dialysed against 0.05 M-NH₄HCO₃ and stored at –70 °C until used.

SDS/polyacrylamide-gel electrophoresis was performed in 12.5% slab gels (Laemmli, 1970). Isoelectric focusing was performed in an LKB Multiphor apparatus with Servalyt Precotes (pH 3–10 and pH 4–7) according to the manufacturer's instructions. Amino acid analysis was performed on a Beckman System 6300 amino acid analyser on samples hydrolysed *in vacuo* in 0.5 ml of 6 M-HCl or 4 M-methanesulphonic acid (Simpson *et al.*, 1976).

Abbreviations used: FABP, fatty acid-binding protein; BNPS-skatole, 3'-bromo-3-methyl-2-(2-nitrobenzenesulphenyl)indolenine.

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for 22 h at 110 °C. The c.d. spectrum of FABP in 0.01 M-sodium phosphate buffer, pH 6.5, at protein concentrations from 2.5 to 0.125 mg/ml was determined at 25 °C in a Cary model 61 spectropolarimeter as described previously (Offner *et al.*, 1986). Computer analysis of the c.d. spectrum to predict secondary structure was performed by a linear least-squares method (Chen *et al.*, 1974).

Sequence determination

FABP (1 mg, 67 nmol) was subjected to several chemical and enzymic cleavages, and peptides from the resulting digests were separated by reverse-phase liquid chromatography. Cleavage of FABP with CNBr was performed in 70% (v/v) formic acid at a 500-fold molar excess of reagent to methionine *in vacuo* for 18 h at room temperature (Gross, 1967). BNPS-skatole cleavage was carried out in 50% (v/v) acetic acid at a 200-fold molar excess of reagent to protein for 18 h at room temperature (Fontana, 1972). FABP was cleaved with hydroxylamine as described by Bornstein & Balian (1977) for 3 h at 45 °C. FABP was digested with trypsin [phenylalanyl-chloromethane ('TPCK')-treated; Cooper Biomedical Co.] with or without prior modification with citraconic anhydride (Aldrich Chemical Co.) (Offner *et al.*, 1981), and with *Staphylococcus aureus* V8 proteinase (ICN) at an enzyme to substrate ratio of 1:50 in 0.1 M-NH₄HCO₃ at 37 °C for 18 h. The N-terminal tryptic peptide, CT-1, derived from citraconylated FABP was subdigested first with trypsin to yield peptides CT-T1-CT-T4, and peptide CT-T1 was digested with chymotrypsin (Sigma Chemical Co.) to yield peptides CT-T-CH-1 and CT-T-CH-2. The C-terminal sequence of FABP was determined by using carboxypeptidase A (Sigma Chemical Co.) as described previously (Offner & Troxler, 1983).

Amino acid sequences were determined on an Applied Biosystems 470A gas-phase sequencer equipped with a 120 phenylthiohydantoin analyser. The amino acid sequence of the blocked N-terminal peptide, CT-T1, was determined by collisionally induced decomposition mass spectrometry (m.s./m.s.) on a JEOL HX110/HX110 four-sector tandem mass spectrometer in the laboratory of Professor K. Biemann in the Department of Chemistry at Massachusetts Institute of Technology. The 6 keV xenon atom beam was produced by a JEOL ion/neutral gun. Accelerating voltage was +10 kV and -18 kV post-acceleration voltage was applied at the detectors. He was used for the collision gas in a cell located between MS-1 and MS-2, at a pressure sufficient to diminish the precursor beam to 20% of its original value.

Sequence data were analysed for predicted secondary structure by the Chou & Fasman (1979*a,b*) method, for hydropathicity by the method of Kyte & Doolittle (1982) by using the PEP program and for internal homology by using the programs ALIGN and SEARCH, obtained from Intelligenetics (Palo Alto, CA, U.S.A.).

Fatty acid-binding studies

Binding of oleate and palmitate to purified FABP was measured in the multilamellar liposome assay (Brecher *et al.*, 1984). Liposomes were prepared by mixing chloroform/methanol (2:1, v/v) solutions of egg-yolk phosphatidylcholine ('lecithin') (Lipid Products, Surrey, U.K.) and oleic acid (Sigma Chemical Co.) or palmitic acid (Sigma Chemical Co.) so that the final concentrations of

Fig. 1. Purification of human heart FABP

(a) Elution profile of proteins from heart high-speed supernatant from a Sephadex G-75 column (5.0 cm × 85 cm) equilibrated and developed with 0.02 M-Tris/HCl buffer, pH 8.0, at a flow rate of 30 ml/h. The column eluate was monitored at 280 nm (●) and each fraction was assayed for radioactivity (□). (b) Pooled fractions (bar) from (a) were chromatographed on a DEAE-Bio-Gel A column (1.5 cm × 10 cm) equilibrated in 0.02 M-Tris/HCl buffer, pH 8.0, and developed with a linear buffer gradient (0.02–0.2 M) at a flow rate of 16 ml/h. (c) Pooled fractions (bar) from (b) were dialysed against 0.01 M-sodium acetate buffer, pH 4.9, and chromatographed on a CM-Bio-Gel A column (1.5 cm × 10 cm) equilibrated in the same buffer and developed with a linear buffer gradient (0.01–0.2 M) at flow rate of 16 ml/h.

phosphatidylcholine and fatty acid were 40 mM and 2 mM respectively. [1-¹⁴C]Oleic acid or [1-¹⁴C]palmitic acid and dipalmitoyl phosphatidyl[Me-³H]choline (all from New England Nuclear) were added to the liposomes to a

Table 1. Amino acid composition of human heart FABP and derived peptides

Values for FABP are based on the average of three 24 h hydrolysates assuming an M_r of 15000. Values in the columns headed 'Sequence' are derived from the final deduced sequence (see Fig. 2). Abbreviation: N.D., not determined.

Amino acid	Amino acid composition (mol of residue/mol)					
	FABP		Peptide CT-1		Peptide CT-T1	
	Calc.	Sequence	Calc.	Sequence	Calc.	Sequence
Asx	14.6	14	4.8	5	1.2	1
Thr	19.2	19	1.9	2	0.9	1
Ser	6.6	7	2.2	2	0	0
Glx	11.5	11	0	0	0	0
Pro	1.1	1	0	0	0	0
Gly	10.5	10	3.5	3	1.4	1
Ala	7.0	6	2.1	2	1.1	1
Val	9.1	10	3.4	3	0.9	1
Cys	0	0	0	0	0	0
Met	2.3	2	1.2	1	0	0
Ile	5.8	7	0	0	0	0
Leu	13.7	13	2.9	3	0.9	1
Tyr	2.2	2	1.0	1	0	0
Phe	6.0	6	2.7	3	0.8	1
Lys	14.7	15	3.0	3	0.9	1
His	3.0	3	0	0	0	0
Arg	4.2	4	1.1	1	0	0
Trp	2.4	2	N.D.	1	N.D.	1
Total...	133.9	132	30.2	30	8.6	9

specific radioactivity of 1 $\mu\text{Ci}/\text{ml}$ and the mixture was evaporated to dryness under a stream of N_2 . Liposomes were obtained by suspending the lipid residue in 0.01 M-Tris/HCl buffer, pH 7.4, containing 0.1 M-NaCl (assay buffer). Binding assays were performed in 150 μl incubation mixtures containing assay buffer, 1.25–10 μM -FABP in assay buffer, and the appropriate volume of liposomes to give a final fatty acid concentration of 5–200 μM . After incubation at 25 $^\circ\text{C}$ for 0–60 min, the liposomes were separated from FABP by centrifugation in a Beckman Airfuge for 5 min. ^{14}C -labelled fatty acid and ^3H -labelled dipalmitoyl phosphatidylcholine remaining in the supernatant were measured by double-label counting in a Packard liquid-scintillation spectrometer. The quantity of [^{14}C]-oleate or -palmitate in the supernatant represents the amount of fatty acid bound to FABP. The quantity of ^3H -labelled phospholipid in the supernatant represents the liposomes not pelleted, and this amount was always less than 3 %.

RESULTS

Isolation and characterization

Heart high-speed supernatant, to which [$1\text{-}^{14}\text{C}$]oleate had been added, was chromatographed on Sephadex G-75. This procedure resolved proteins in the high-speed supernatant into two broad A_{280} peaks containing high- M_r and low- M_r proteins (Fig. 1a). Radiochemical assay of column fractions revealed peaks of radioactivity

Table 2. Average values of mean residue ellipticity calculated from the c.d. spectrum of human heart FABP

Values represent average mean residue ellipticity at FABP concentrations of 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 and 0.125 mg/ml.

Wavelength (nm)	Mean residue ellipticity (θ) (degrees $\cdot \text{cm}^2 \cdot \text{dmol}^{-1}$)	Deviation from mean (%)
240	0	—
238	0	—
236	512 ± 5	1.0
234	675 ± 36	5.3
232	1299 ± 20	1.5
230	2114 ± 86	4.1
228	3252 ± 8	0.3
226	5495 ± 80	1.5
224	7017 ± 79	1.1
222	8978 ± 178	2.0
220	10611 ± 34	0.3
218	11346 ± 9	0.1
216	1194 ± 591	0.5
214	9977 ± 346	3.5
212	9327 ± 21	0.2
210	9182 ± 27	0.3
208	5903 ± 180	3.0

associated with the high- M_r peak, due to the presence of albumin, and with the low- M_r peak, due to the presence of FABP. Chromatography of the pooled fractions containing FABP on DEAE-Bio-Gel A (Fig. 1b) partially resolved FABP from myoglobin, the other major protein in the low- M_r fraction from Sephadex G-75. Complete separation of FABP from myoglobin and other minor proteins was achieved by chromatography on CM-Bio-Gel A (Fig. 1c).

Analysis of purified FABP by SDS/polyacrylamide-gel electrophoresis and isoelectric focusing revealed a single protein band with an M_r of 15000 and an apparent isoelectric point (pI) of 5.25. The u.v.-absorption spectrum of FABP displayed a prominent peak at 280 nm and a shoulder at 292 nm. The molar absorption coefficient (ϵ) of FABP at 280 nm in 0.01 M-potassium phosphate buffer, pH 7.5, was $1.57 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (protein concentration determined by quantitative amino acid analysis). The amino acid composition of FABP (Table 1) shows that this protein is enriched with respect to threonine (14 mol%) and lysine (11 mol%), contains two residues of tryptophan and methionine and one residue of proline, and does not contain cysteine. The c.d. spectrum of FABP displayed a large negative ellipticity peak from 216 nm to 218 nm, characteristic of proteins with a high degree of β -structure (results not shown). The mean residue ellipticity from 240 nm to 208 nm did not vary significantly as a function of protein concentration (Table 2). Analysis of the c.d. spectrum to predict secondary structure indicated that FABP contained 25 % α -helix, 45 % β -structure and 30 % reverse turns.

Amino acid sequence and FABP

The complete amino acid sequence of FABP is given in Fig. 2. The protein contains 132 amino acid residues, has

Fig. 2. Amino acid sequence of human heart FABP

Continuous lines indicate regions of peptides sequenced, dashed lines represent peptides sequenced by m.s., arrows indicate residues identified by using carboxypeptidase A, and dotted lines indicate peptides identified on the basis of amino acid composition. Abbreviations: CNBr-cleavage peptides, CNBr; BNPS-skatole-cleavage peptides, SK; hydroxylamine-cleavage peptides, HA; *S. aureus* V8 proteinase peptides, V8; tryptic peptides, T; tryptic peptides from citraconylated protein, CT; chymotryptic peptides, CH.

an M_r of 14768 and has an acetylated valine as the *N*-terminal amino acid residue. Approx. 10% of the sequence consisted of amino acid doublets, with -Thr-Thr- occurring three times, -Asp-Asp- occurring twice and -Ile-Ile- and -Gly-Gly- each occurring once.

When FABP (500 pmol) was subjected to five cycles of automated Edman degradation no phenylthiohydantoin derivatives could be identified, indicating that the *N*-terminal amino acid was blocked. The amino acid sequence of the first nine residues of FABP was determined by m.s. of peptide CT-T1. Fast-atom-bombardment m.s. (results not shown) showed a molecular ion $(M+H)^+$ at m/z 1078.6 that would be predicted from the amino acid composition assuming an

acetylated *N*-terminal residue (Table 1). M.s./m.s. of peptide CT-T1 enabled the sequence to be deduced from the fragmentation pattern (Fig. 3) and from the m/z values of the product ions (Table 3).

The sequence of peptide SK-1 (residues 9-97, sequenced to residue 35) provided a one-residue overlap with the sequence of peptide CT-T1 (residues 1-9) and peptide CT-T-CH-2 (residues 5-9). The sequence of peptide CT-2 (residues 31-78, sequenced to residue 47) provided a five-residue overlap with peptide SK-1 and aligned peptides CNBr-2 (residues 21-35) and CNBr-3 (residues 36-132, sequenced to residue 53). The sequence of peptide HA-1 (residues 46-132, sequenced to residue 64) provided an eight-residue overlap with the sequence

Fig. 3. Collisionally induced decomposition mass spectrum of peptide CT-T1

All ions in the spectrum have been assigned, but only the more prominent sequence ion series are marked. The b fragment ions arise by cleavage of the polypeptide chain from the *N*-terminus, and these ions were used to deduce the sequence. The sequence of peptide CT-T1 $[(M+H)^+ m/z 1078.6]$ is given in the one-letter code at the top of the spectrum. The b, y, and z structures are a slight variation (Johnson & Biemann, 1987) on the nomenclature proposed by Roepstorff & Fohlman (1984).

Table 3. Amino acid sequence of the *N*-terminal tryptic peptide CT-T1 determined from the collisionally induced decomposition mass spectrum

The b fragments arise from cleavage of the polypeptide chain from the *N*-terminus and have the structure:

Fragment	<i>m/z</i>	Amino acid sequence
b ₁	142.06	Ac-Val-
b ₂	257.07	Ac-Val-Asp-
b ₃	328.12	Ac-Val-Asp-Ala-
b ₄	475.03	Ac-Val-Asp-Ala-Phe-
b ₅	588.20	Ac-Val-Asp-Ala-Phe-Leu-
b ₆	645.27	Ac-Val-Asp-Ala-Phe-Leu-Gly-
b ₇	746.35	Ac-Val-Asp-Ala-Phe-Leu-Gly-Thr-
b ₈	1060.25	Ac-Val-Asp-Ala-Phe-Leu-Gly-Thr-Trp-Lys-

of peptide CNBr-3 and aligned peptides T-3 (residues 53–58) and T-4 (residues 59–65). The sequence of peptide V8-1 (residues 62–101, sequenced to residue 81) aligned peptides T-4 and T-5 (residues 66–78) and provided a three-residue overlap with the sequence of peptide CT-3 (residues 79–106). Peptides CT-3 and CT-4 (residues 107–126) were aligned by the sequence of peptide SK-2 (residues 98–182, sequenced to residue 118), and peptides CT-4 and CT-5 (residues 127–132) were aligned by the sequence of peptide V8-2 (residues 108–129). The sequence -Lys-Glu-Ala, determined by carboxypeptidase A digestion of FABP, was identical with the last three residues of peptide CT-5. This showed that peptide CT-5 was the C-terminal tryptic peptide from citraconylated protein and established the complete amino acid sequence of heart FABP.

Evaluation of the secondary structure of FABP by the Chou-Fasman method predicted that the protein contained three regions of α -helix (residues 17–36, 62–76 and 126–132), six regions of β -structure (residues 1–10, 37–43, 49–56, 81–85, 90–97 and 102–121) and seven reverse turns (Fig. 4). By summation, this analysis predicted

Fig. 4. Predicted secondary structure of human heart FABP

Each dot represents an amino acid residue. α -Helix, ; β -structure, ; reverse turn, . The internal repeated sequences (residues 48–60 and 114–125) are indicated by dashed lines.

Fig. 5. Hydropathicity plot of human heart FABP

The plot was generated with the use of a window size of six residues. The two half-molecules contained in FABP (residue 48–86 and 90–132) are represented by a line. Dashed portions of the line indicate the internally repeated sequences (residues 48–60 and 114–125) where the sequence homology is 62%.

the protein was composed of 31.8% α -helix, 44.0% β -structure and 24.2% reverse turns. These results are in excellent agreement with the secondary structure predicted from analysis of the c.d. spectrum. The hydropathicity plot of FABP (Fig. 5) shows that the protein contains alternating hydrophobic and hydrophilic regions characteristic of other globular proteins (Kyte & Doolittle, 1982).

Fatty acid binding

Fatty acid binding to FABP was measured by gel-filtration chromatography and in the multilamellar liposome assay. Gel filtration of FABP that had been preincubated with [14 C]oleic acid on a calibrated Sephadex G-75 column showed that the protein and radiolabelled fatty acid were co-eluted, providing direct evidence that human heart FABP binds fatty acid (Fig. 6a).

In the multilamellar liposome assay, binding of either radiolabelled oleate or radiolabelled palmitate to FABP (5 μ M) reached equilibrium within 30 min (Fig. 6b). When binding was measured in the presence of 100 μ M-oleate or -palmitate and 1.25–10 μ M-FABP, binding was

Fig. 6. Fatty acid binding to human heart FABP

(a) Measurement of fatty acid binding to FABP by gel filtration on a calibrated Sephadex G-75 column. The protein was preincubated with 1.5 μ Ci of [14 C]oleate before application to the column. The column eluate was assayed for radioactivity (■) and for protein (A_{595} , ●) by the method of Bradford (1976). Abbreviations: BSA, bovine serum albumin; CA, carbonic anhydrase; M, myoglobin; In, insulin; F, human heart FABP. (b)–(d) Characterization of fatty acid binding to FABP in the multilamellar liposome assay: ●, palmitate; ■, oleate. (b) Kinetics of fatty acid binding to FABP; (c) fatty acid (100 μ M) binding to FABP at various protein concentrations; (d) fatty acid binding to FABP (5 μ M) at various fatty acid concentrations.

48	60
- I le- L eu- T hr- L eu- L ys- T hr- H is- S er- T hr- P he- L ys- A sn- T hr-	
- I le- L eu- T hr- L eu- - - T hr- H is- G ly- T hr- A la- V al- S er- T hr-	
114	125

Fig. 7. Internal repeated sequence in human heart FABP

Identical residues are shown in **bold** type.

saturated at an FABP concentration of 10 μ M, and at 1–2.5 μ M-FABP approx. 2 mol of fatty acid was bound per mol of protein (Fig. 6c). Binding stoichiometries of 1.8–2.0 mol of fatty acid per mol of protein were also observed when binding was measured in the presence of 5–200 μ M-oleate or -palmitate and 5 μ M-FABP (Fig. 6d).

DISCUSSION

The amino acid sequence of human heart FABP described in this paper provides the complete structural characterization of a fatty acid-binding protein from human heart. Two properties of this protein complicated elucidation of the amino acid sequence. First, the absence of cysteine precluded reduction and alkylation of the protein before fragmentation procedures, and in many cases complex mixtures of peptides were obtained, indicating incomplete cleavage at susceptible residues. Secondly, the protein contained a blocked *N*-terminal amino acid and m.s./m.s. was used to determine the *N*-terminal amino acid sequence. By the use of this methodology the *N*-terminal residue was identified as an acetylated valine. This is unusual, since of 250 acetylated proteins for which the sequence is known only one, the β -subunit of tadpole haemoglobin, had valine as the *N*-terminal amino acid (Persson *et al.*, 1985).

Evaluation of the secondary structure of human heart FABP by the Chou–Fasman method predicted that the protein has a high degree of ordered structure. It can be seen from the predicted secondary structure that the reverse turn with a midpoint at residue 88 divides the polypeptide chain between residues 48 and 132 into two half-molecules (Fig. 4). The hydropathicity plot shows a pattern of alternating hydrophobic and hydrophilic regions for residues 48–86 and a similar pattern for residues 90–132, with a midpoint at residue 88, which also divides the protein into two half-molecules (Fig. 5). By using the programs ALIGN and SEARCH a region of internal sequence homology was found in each of the half-molecules defined by the predicted secondary structure and hydropathicity plot of human heart FABP. The sequence of residues 48–54 is identical with the sequence of residues 114–119, with one gap. These repeats are contained within two longer repeated regions, residues 48–60 and residues 114–125, which are themselves 62% homologous (Fig. 7). The short repeats are located in regions of predicted β -structure (Fig. 4) and decreasing hydrophobicity (Fig. 5). Such regions could accommodate both the uncharged and the charged moieties of long-chain fatty acids and could represent fatty acid-binding domains, consistent with the finding that human heart FABP binds 2 mol of fatty acid/mol of protein

(Fig. 6). Although the amino acid sequence of human heart FABP displays approximately 90% sequence identity (with a single one-residue gap) with that of rat heart FABP (Sacchettini *et al.*, 1986), the ALIGN and SEARCH programs revealed that the internal repeated sequences in human heart FABP were not present in rat heart FABP.

FABPs have been isolated from pig (Fournier *et al.*, 1978), rat (Offner *et al.*, 1986), bovine (Jagschies *et al.*, 1985) and human (Glatz *et al.*, 1985; Unterberg *et al.*, 1986) heart. These proteins have M_r values of approx. 15000, have somewhat different amino acid compositions and have been shown to bind fatty acids *in vitro*. Glatz *et al.* (1985) isolated an FABP from human heart high-speed supernatant by gel filtration on Sephacryl S-200 and anion-exchange chromatography on DEAE-Sephacel. This protein had an apparent M_r of 15000, a pI of 7.5 and the amino acid composition was not reported. Unterberg *et al.* (1986) isolated an FABP from human heart by gel filtration on Sephacryl S-200 and cation-exchange chromatography on CM-Sephadex C-50. This protein had an apparent M_r of 15500 and a pI of 5.3, and amino acid analysis indicated that the protein contained two residues of cysteine. The human heart FABP described in the present study is an anionic protein with an apparent pI of 5.25, which contains no cysteine, and is clearly a different protein from the human heart FABPs described previously (Glatz *et al.*, 1985; Unterberg *et al.*, 1986). These results suggest that isoforms of FABP may occur in human heart.

It has been reported that the c.d. spectrum of pig heart FABP (Fournier *et al.*, 1983; Fournier & Rahim, 1983) displayed changes in the mean residue ellipticity at 225 nm at protein concentrations ranging from approx. 3.0 to 0.5 mg/ml, and it was suggested that these changes were due to concentration-dependent aggregation of the protein. Furthermore, physiological significance was attributed to this postulated aggregation based on experiments performed *in vitro* where the rate of mitochondrial β -oxidation of stearate bound to rat heart FABP was reported to vary at different donor FABP concentrations (Fournier & Rahim, 1985). At similar protein concentrations we found no concentration-dependent changes in the mean residue ellipticity of rat heart FABP from 240 nm to 208 nm (Offner *et al.*, 1986) and in the present investigation it was found that the c.d. spectrum of human heart FABP did not vary significantly at protein concentrations ranging from 2.5 to 0.125 mg/ml (Table 2). The latter results are supported by those of Jagschies *et al.* (1985), who found no evidence for concentration-dependent changes in the c.d. spectrum of FABP from bovine heart and also showed that the sedimentation coefficient ($s_{20,w}$) was unchanged at protein concentrations ranging from 4.0 to 0.1 mg/ml. In view of the foregoing, the results reported by Fournier and co-workers (Fournier *et al.*, 1983; Fournier & Rahim, 1983, 1985) describing aggregation of pig and rat heart FABPs are difficult to explain.

The isolation of FABPs from human heart with different amino acid compositions and isoelectric points (Glatz *et al.*, 1985; Unterberg *et al.*, 1986; the present study) suggests that multiple isoforms of this protein occur in human heart. This is supported by the finding that the nucleotide sequences of two rat heart FABP cDNAs are different in the 5' and 3' untranslated regions (Heuckeroth *et al.*, 1987; Claffey *et al.*, 1987). In addition,

the amino acid sequences deduced from the coding regions of these cDNAs differ from the directly determined amino acid sequence of an FABP from rat heart (Sacchettini *et al.*, 1986). It is possible that different isoforms of heart FABP have evolved to perform distinct physiological functions. Different isoforms of FABP may have unique binding specificities and stoichiometries and may transport different fatty acids to mitochondria or to the endoplasmic reticulum, where acylation and subsequent metabolism occurs. In addition, one or more isoforms may serve as specific intracellular binding reservoirs for fatty acids, acyl-CoAs and other amphipathic molecules whose concentrations become dramatically elevated in ischaemia (Corr *et al.*, 1984). The molecular details of the structural and functional relationships between FABP isoforms in heart tissue can be understood only when all of these proteins are isolated and fully characterized and their precise role in cardiac energy metabolism is established.

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