Cloning and Tissue Distribution of Rat Heart Fatty Acid Binding Protein mRNA: Identical Forms in Heart and Skeletal Muscle†

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ABSTRACT: A fatty acid binding protein (FABP) has been identified and characterized in rat heart, but the function and regulation of this protein are unclear. In this study the cDNA for rat heart FABP was cloned from a λ gt11 library. Sequencing of the cDNA showed an open reading frame coding for a protein with 133 amino acids and a calculated size of 14 776 daltons. Several differences were found between the sequence determined from the cDNA and that reported previously by protein sequencing techniques. Northern blot analysis using rat heart FABP cDNA as a probe established the presence of an abundant mRNA in rat heart about 0.85 kilobases in length. This mRNA was detected, but was not abundant, in fetal heart tissue. Tissue distribution studies showed a similar mRNA species in red, but not white, skeletal muscle. In general, the mRNA tissue distribution was similar to that of the protein detected by Western immunoblot analysis, suggesting that heart FABP expression may be regulated at the transcriptional level. S1 nuclease mapping studies confirmed that the mRNA hybridized to rat heart FABP cDNA was identical in heart and red skeletal muscle throughout the entire open reading frame. The structural differences between heart FABP and other members of this multigene family may be related to the functional requirements of oxidative muscle for fatty acids as a fuel source.

The regulation of fatty acid metabolism is dependent upon a controlled flux of fatty acids between intracellular sites for activation, esterification, and oxidation. Fatty acid binding proteins (FABP)[1] are thought to be the intracellular transport carriers of fatty acids (Bass, 1985; Burnett et al., 1979; Glatz & Veerkamp, 1985; Gordon & Lowe, 1985; Ockner et al., 1977). They are a group of different but homologous proteins with several common characteristics: a relatively low molecular mass (14–16 kDa), localization within the cell cytosol, high concentration (2–8% of total cytosolic protein), and a distribution in tissues actively involved in fatty acid transport and/or dependent on fatty acid metabolism. FABP from intestine, liver, and heart have been purified and characterized (Ockner & Manning, 1974; Ockner et al., 1982; Takahashi et al., 1983; Said & Schulz, 1984). Two forms have been identified in intestinal tissue, one of which is identical with liver FABP (Bass & Manning, 1986). The cDNAs for each of these FABP's have been obtained (Alpers et al., 1984; Gordon et al., 1983), and Northern blot analysis showed that both intestinal mRNA isoforms were abundant: the intestine-specific isoform represented almost 5% of the total mRNA, and the intestine/liver isoform represented 3%, in contrast to much lower levels detected in other tissues (Gordon et al., 1985).

Rat heart FABP also has been purified and characterized (Said & Schulz, 1984; Offner et al., 1986) and the complete amino acid sequence determined directly (Sacchettini et al., 1986). Although some homology with the gastrointestinal FABP's was shown, rat heart FABP showed greatest homology to the murine adipocyte "FABP" and to the myelin P2 protein with 62% and 59% homology, respectively (Sacchettini et al., 1986). Immunological data using anti-rat heart FABP polyclonal antibodies showed this protein to be abundant in heart (1.5 mg/g wet weight), localized in the cytosol, and expressed early in development (Crisman et al., 1987). Crisman et al. (1987) also detected an immunologically similar protein in oxidative muscle fibers and kidney. Whether those proteins were identical with rat heart FABP or represented immunologically similar isoforms could not be resolved.

To further understand the function and regulation of rat heart FABP, the work described in the present study was initiated. We have obtained a full-length cDNA for the rat heart FABP and used it to establish the amino acid sequence and tissue distribution of heart FABP mRNA.

Materials and Methods

Materials. Restriction endonucleases and DNA modifying enzymes were purchased from New England Biolabs, Inc., Bethesda Research Laboratories, Inc., and New England Nuclear Research Products. [γ-^{32}P]ATP (3000 Ci/mmol), [α-^{32}P]ddATP (3000 Ci/mmol), [^{35}S]dATPαS (650 Ci/mmol), and [α-^{32}P]dCTP (3000 Ci/mmol) were from New England Nuclear or from Amersham Corp. Double-stranded M13 sequencing vectors M13mp18 RF and M13mp19 RF were obtained from New England Biolabs, Inc., and M13 sequencing kits were purchased from New England Biolabs or from Pharmacia. All other chemical reagents were of molecular biology grade.

Oligonucleotide Probe Specific for Rat Heart FABP. A 24-base oligodeoxynucleotide probe with 32-fold redundancy

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[1] Abbreviations: FABP, fatty acid binding protein; aa, amino acid; bp, base pair(s); ELISA, enzyme-linked immunosorbent assay; kb, kilobase(s); kDa, kilodalton(s); SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SSC, standard saline–citrate buffer; SSPE, standard saline–sodium phosphate–EDTA buffer; EDTA, ethylenediaminetetraacetic acid.

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(5′-CTT-CAT-$\frac{A}{G}$TA-$\frac{A}{G}$TC-$\frac{A}{G}$AA-$\frac{A}{G}$TT-CTT-3′) was synthesized on the basis of amino acids 15–22 of the sequence reported by Sacchettini et al. (1986). The two terminal lysine codons were specified as TTC on the basis of codon usage frequencies from isolated eucaryotic cDNAs (Grantham et al., 1981). The oligonucleotide probe was purified and labeled with [γ-^{32}P]-ATP by T4 polynucleotide kinase, specific activity 1×10^6 cpm/μg of DNA. Specificity of this oligonucleotide probe was determined by analysis of rat heart total cellular and poly(A+) RNA. Northern blot hybridization was performed at 42 °C with 6× SSPE for at least 18 h. The nitrocellulose filters were washed with 2× SSC–0.1% SDS, at 55 °C for 1–2 h with multiple buffer changes.

Synthesis and Screening of the Rat Heart λ gt11 Library. A rat heart cDNA λ gt11 library was constructed according to the modified Okayama–Berg procedure (Gubler & Hoffman, 1983; Young & Davis, 1983). The cDNA library contained 1.5×10^6 independent clones. Approximately 80 000 plaques were screened with the oligonucleotide probe under the conditions optimized in the Northern blot analysis. three cDNA clones were isolated and plaque-purified.

DNA Sequence Analysis. DNA from one isolated clone was digested with *Eco*RI restriction endonuclease, and an 845-bp cDNA insert was subcloned into pUC18 and propagated in *Escherichia coli* (Maniatis et al., 1982). Restriction fragments from the insert isolated were generated by *Eco*RI and *Rsa*I restriction endonucleases. These restriction fragments and the full-length 845-bp cDNA were subcloned into M13mp18 and M13mp19 vectors for DNA sequencing by the Sanger dideoxy chain termination method (Sanger et al., 1977). Nucleotide sequences were obtained in both directions.

Northern Blot Analysis. Equal amounts of fetal (18-day gestation) and adult rat tissue total cellular RNAs, isolated from Sprague-Dawley rats by the method of Chirgwin et al. (1979), were subjected to Northern blot analysis (Lehrach et al., 1977). Rat heart FABP cDNA probes were isolated from the 845-bp cDNA as either a 355-bp *Nco*I–*Nco*I fragment containing the open reading frame coding for amino acids 1–118 or a 676-bp *Ssp*I–*Eco*RI fragment consisting of the 5′ untranslated region, the entire open reading frame, and the complete 3′ untranslated region. The DNA probes were labeled by the random hexanucleotide primed synthesis method (Feinberg & Vogelstein, 1984), with resulting specific activities of 1×10^9 cpm/μg of DNA. Hybridization was performed at 42 °C in 50% formamide–5× SSPE for 18 h and washed at high stringency conditions (0.1× SSC, 0.1% SDS, at 65 °C for 1–2 h, with three buffer changes).

Rat Tissue Extract Preparation and Western Immunoblot Analysis. Cell cytosol and low-speed supernatant extracts were prepared from respective tissues from adult Sprague-Dawley rats (250–300 g) as previously described (Crisman et al., 1987). Protein concentration was determined with the method of Lowry et al. (1951). SDS–polyacrylamide electrophoreses of protein extracts were analyzed as described by Laemmli (1970). Rat heart FABP was purified as described previously (Offner et al., 1986). Western blot transfer was performed in a Bio-Rad Trans-blot cell. Immunological detection was performed with a rabbit monospecific polyclonal anti-rat heart FABP antibody described previously (Crisman et al., 1987) with a goat anti-rabbit alkaline phosphatase conjugate second antibody reacted with 0.33 mg/mL nitro blue tetrazolium and 0.16 mg/mL 5-bromo-4-chloro-3-indolyl phosphate substrates.

S1 Nuclease Mapping of Rat Tissue RNA. S1 nuclease mapping experiments were performed essentially according to the method of Berk and Sharp (1977). Two S1 nuclease

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CACGCCTCCTCTCTCATTGCACC ATG GCG GAC GCC TTT GTC GGT ACC TGG     50
                        Met Ala Asp Ala Phe Val Gly Thr Trp      9

AAG CTA GTG GAC AGC AAG AAT TTT GAT GAC TAC ATG AAG TCA CTC     95
Lys Leu Val Asp Ser Lys Asn Phe Asp Asp Tyr Met Lys Ser Leu     24

GGT GTG GGC TTT GCC ACC AGA CAG GTC GCT AGC ATG ACC AAG CCG    140
Gly Val Gly Phe Ala Thr Arg Gln Val Ala Ser Met Thr Lys Pro     39

ACC ACA ATC ATT GAG AAG AAT GGG GAT ACC ATC ACC ATA AAG ACA    185
Thr Thr Ile Ile Glu Lys Asn Gly Asp Thr Ile Thr Ile Lys Thr     54

CAC AGT ACC TTC AAG AAC ACA GAG ATC AGC TTT CAG CTG GGA GTA    230
His Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Gln Leu Gly Val     69

GAG TTT GAC GAG GTC ACA GCA GAT GAC AGG AAG GTC AAG TCG GTC    275
Glu Phe Asp Glu Val Thr Ala Asp Asp Arg Lys Val Lys Ser Val     84

GTG ACA CTG GAC GGA GGC AAA CTG GTC CAT GTG CAG AAG TGG GAC    320
Val Thr Leu Asp Gly Gly Lys Leu Val His Val Gln Lys Trp Asp     99

GGG CAG GAG ACT ACG CTT ACA CGG GAA CTA AGT GAT GGG AAA CTC    365
Gly Gln Glu Thr Thr Leu Thr Arg Glu Leu Ser Asp Gly Lys Leu    114

ATC CTG ACT CTC ACC CAT GGC AAT GTG GTG AGC ACT CGG ACT TAC    410
Ile Leu Thr Leu Thr His Gly Asn Val Val Ser Thr Arg Thr Tyr    129

GAG AAG GAG GCG TGA CCTGGCTGCCCCGTCACTGACTGCTCCTCTGCCAATGGC    464
Glu Lys Glu Ala  •                                             133

TACCCCTAACTCAGCACCACGTTGCCTCATGTTTCTCCCCTCTGACGTTTTATATAAAT    523

ACTCTTTGGTTGGGCTTTTCCTGGAGATATGGGACACCAGCCTGGACCCAGGTCCCATT    582

GTGTATGTGGTTTATTTTTTAAAACTGTATCCAAAGGGTGCTCCAAGGTCAATAAAGCA    641

GAGCCAAGGCCAAAAAAAAAAAAAAA                                     667
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FIGURE 1: Nucleotide sequence and deduced amino acid sequence of rat heart FABP cDNA. The 5′ untranslated segment depicted (bases 1–23) was protected in an S1 nuclease mapping assay of rat heart RNA (data shown in Figure 3B). The additional 178 bases upstream from base 1 present in the cDNA but not protected from S1 nuclease are not included. The asterisk denotes the translation termination codon.

mapping probes were isolated from the 845-bp rat heart FABP cDNA. The probe to assay the 5′ end was a 403-bp *Eco*RI–*Pvu*II fragment that contained aa codons 1–65 and the 5′ untranslated region. This *Eco*RI–*Pvu*II 403-bp fragment was end labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. The 3′ specific probe consisted of a 442-bp *Pvu*II–*Eco*RI fragment radiolabeled at the 3′ termini with [α-^{32}P]ddATP by terminal deoxynucleotide transferase. Total cellular rat tissue RNA (10 μg) was hybridized to 20 000–40 000 cpm of denatured, end-labeled, double-stranded DNA probes under RNA loop conditions (Casey et al., 1977) and then digested with 100 units of S1 nuclease at 25 °C for 1 h. The digestion products were then size separated in a 5% polyacrylamide–8.5 M urea denaturing gel, subsequently dried, and exposed to X-ray film (Kodak X-Omat) overnight.

Results and Discussion

Northern blot analysis of rat heart total RNA (20 μg) and poly(A+) RNA (1 μg) with the end-labeled oligonucleotide probe [24-mer, aa 15–22 (Sacchettini et al., 1986)] detected a significant level of an RNA of about 0.85 kb (data not shown), indicating that rat heart contained a relatively abundant putative FABP mRNA. A rat heart λ gt11 cDNA library was then screened with the oligonucleotide probe, and three clones having inserts that ranged in size between 0.65 and 0.85 kb were isolated.

The nucleotide and deduced amino acid sequences determined for rat heart FABP cDNA are shown in Figure 1. An initiator ATG codon (position 24) is followed by a single open reading frame of 399 nucleotides, coding for a protein containing 133 amino acids with a calculated molecular weight of 14 776. The ATG initiation site is contained in the sequence GCACCATGG, which is homologous to the optimal sequence for initiation by eukaryotic ribosomes described by Kozak (1986). The open reading frame ended with a TGA terminator codon at position 423 followed by 242 nucleotides in the 3′ untranslated region. Twenty nucleotides upstream of the poly(dA) tail there was a consensus AATAAA polyadenylation signal. The open reading frame codes for most of the amino

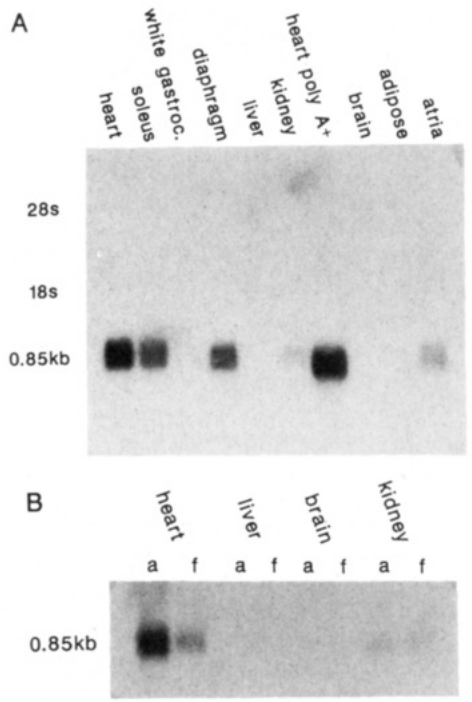

FIGURE 2: Northern blot analysis of rat tissue RNAs. Hybridization was performed with the 355-bp *Nco*I–*Nco*I fragment of rat heart cDNA. (A) Blots were prepared with total cellular RNA (20 μg) from the indicated tissues and heart poly(A+)-selected RNA (1 μg). Exposure time, 18 h. (B) Blots contained total cellular RNA (10 μg) from either adult (a) or fetal (f) tissue. Exposure time, 30 h.

acids reported in the published sequence for rat heart FABP (Sacchettini et al., 1986), although several differences were found. The most striking is at the amino-terminal end, where the first four amino acids predicted by the cDNA sequence (Met-Ala-Asp-Ala) differed completely from the first four amino acids reported by protein sequencing techniques (Thr-Glu-Lys-Asn). An Asn residue between aa 64 and aa 66 in the protein-derived sequence (Sacchettini et al., 1986) is not present in the cDNA-derived protein sequence (Figure 1, codons 64–65). These differences account for the cDNA-derived sequence to be shorter by one amino acid (133 vs 134) than that determined by Sacchettini et al. (1986). Other differences are at amino acids 51 and 52 (Figure 1), where the cDNA-derived sequence codes for Thr-Ile instead of Tyr-Gly, and at amino acid 71, Phe instead of Asp. These differences may indicate heterogeneity in the population of heart FABP molecules rather than sequencing errors.

The general structural relationships reported by Sacchettini et al. (1986) for rat heart FABP versus other members of the multigene family were not changed appreciably by the differences noted above. The homology between the rat heart FABP sequence, myelin P2 protein, and murine adipocyte 422 protein remains high, and the hydropathy plot data were not altered significantly. No additional insight can be deduced as to the location or nature of the fatty acid binding sites. The rat heart FABP is most homologous to the lipid binding protein of mouse 3T3-L1 adipocytes, murine adipocyte 422 protein, with 62% homology at the amino acid level and 47% at the nucleotide level (Bernlohr et al., 1984; Cook et al., 1985). The first 100 amino acids are most conserved showing 69% homology at the nucleotide level. This sequence analysis confirms that rat heart FABP is a unique gene product within the growing number of proteins in this multigene family.

Analysis for tissue-specific expression of rat heart FABP by Northern blot (Figure 2A) using a probe spanning aa 1–118 detected a mRNA of about 0.85 kb in heart [total RNA and poly(A+) RNA], diaphragm, soleus, red gasterocnemius muscle, and atria. There was no hybridizing RNA detected in liver, adipose tissue, and brain. Small, but detectable, amounts were found in white gastrocnemius muscle and kidney in contrast to the relatively abundant RNA detected in heart and red muscle. Northern blot analysis of RNA from adult and fetal tissues (Figure 2B) detected less FABP mRNA in fetal heart as compared to adult heart. Fetal and adult kidney RNA showed equivalent amounts, although much less than that in heart. No rat heart FABP RNA was detected in adult or fetal tissue RNAs from rat adipose and brain.

The Northern blot analyses were done at high stringency conditions; therefore, it is unlikely that RNAs detected were homologous FABP mRNA isoforms in tissues other than the heart. Nevertheless, in order to establish nucleotide sequence identity among the different heart FABP mRNAs observed in the Northern blot experiments, we performed S1 nuclease mapping analysis of RNAs isolated from various rat tissues. The 443-bp *Pvu*II–*Eco*RI 3′ labeled probe utilized in the S1 mapping studies spanned aa 65 to the poly(A) tract. As shown in Figure 3A, full-length protection from S1 nuclease digestion was observed in RNAs from heart, soleus, red gastrocnemius, and diaphragm muscle and a small amount in kidney, whereas virtually no protection against S1 nuclease digestion was found with RNAs from liver, white gastrocnemius, or adipose tissues. Figure 3B shows S1 nuclease protection of rat tissue RNAs utilizing the 403-bp *Eco*RI–*Pvu*II 5′ labeled S1 nuclease probe. Interestingly, only a partially protected fragment (about 210 nucleotides) was noted in heart, soleus, and diaphragm. On the basis of the nucleotide sequence, this corresponds to a region spanning 20–30 nucleotides 5′ to the ATG codon. Analysis of the nonprotected 5′ region of the cDNA clone indicated that it is not part of the heart FABP mRNA. Most likely it represents a cloning artifact as it gave a different expression pattern on Northern blot analysis and showed 91% homology to a 12S mitochondrial ribosomal RNA sequence (data not shown). Again, liver and white gastrocnemius demonstrated no protection of the S1 nuclease probe. Figure 3C depicts a partial restriction map of the rat heart FABP 845-bp cDNA clone, including a schematic of the S1 nuclease mapping experiments.

S1 nuclease mapping experiments confirm the relative abundance and tissue distribution observed in the Northern blot experiments. In addition, the S1 nuclease mapping results strongly support the idea that the FABP in oxidative tissues (soleus, red gastrocnemius, diaphragm, and kidney) is identical with rat heart FABP. This is based on observations that members of multigene families are most likely to have sequence differences in the 3′ untranslated region, whereas our studies show complete protection for the S1 nuclease mapping probe that contained the entire 3′ untranslated region. It is possible that single base differences not detected by S1 nuclease mapping could be found by more sensitive techniques such as RNase protection assays or direct sequencing.

The tissue distribution of mRNA for rat heart FABP was similar to the distribution of immunoreactive protein previously found in cytosol fractions with ELISA or radial immunodiffusion analysis (Crisman et al., 1987; Bass & Manning, 1986). Figure 4 shows a Western immunoblot of rat tissue extracts using polyclonal antibodies developed against the purified heart protein (Offner et al., 1986). To include the possibility that membrane-associated protein may be present in some tissues lacking an immunoreactive heart FABP in the cytosol fraction, low-speed supernatant fractions from all tissues were used, and the immunoblot was overdeveloped to determine if small

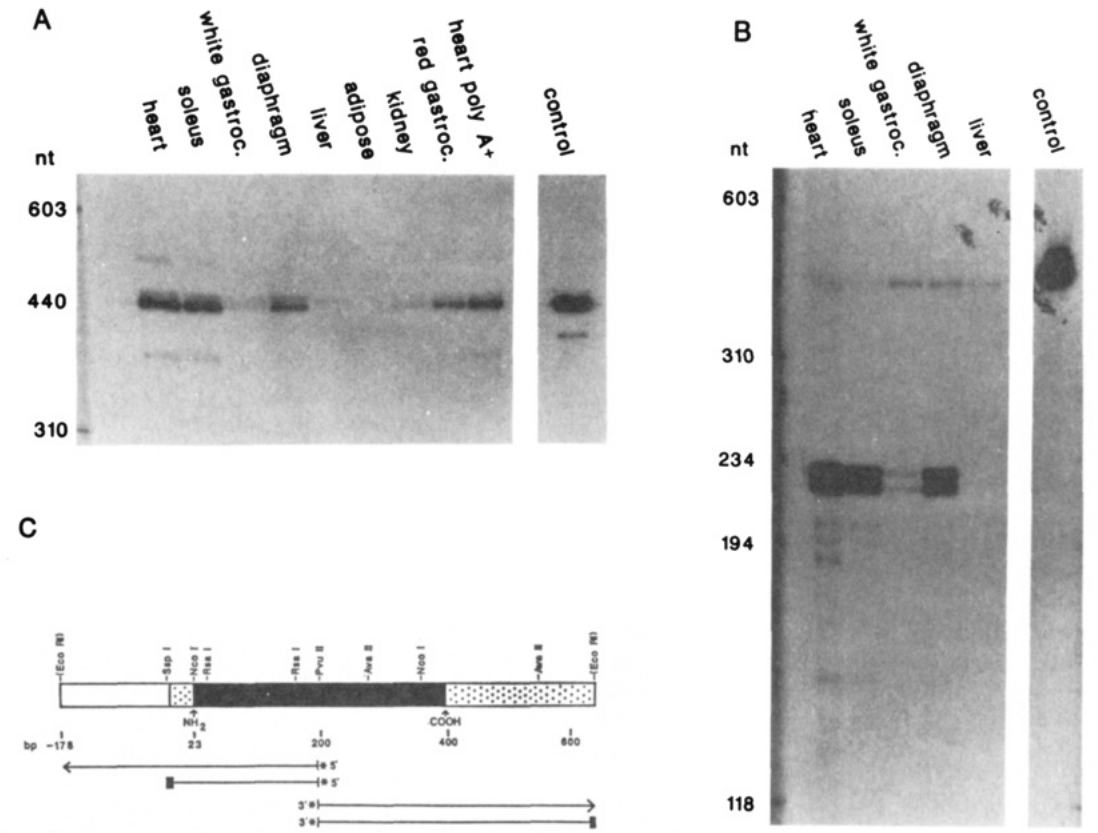

FIGURE 3: S1 nuclease mapping of rat tissue RNAs for rat heart FABP mRNA. (A) Total cellular RNA (10 μg) or rat heart poly(A+) RNA (0.2 μg) was hybridized to the 442-bp *Pvu*II–*Eco*RI fragment labeled at the 3′ end. Control lane contains the untreated 442-bp probe. Number of nucleotides denoted as nt. (B) Total RNA (10 μg) was hybridized to the 403-bp *Eco*RI–*Pvu*II fragment labeled at the 5′ end. Control lane contains the untreated 403-bp probe. (C) Partial restriction map of rat heart FABP cDNA. Asterisks denote the position end labeled for the fragments used for S1 nuclease mapping. Also indicated are the protected sequences.

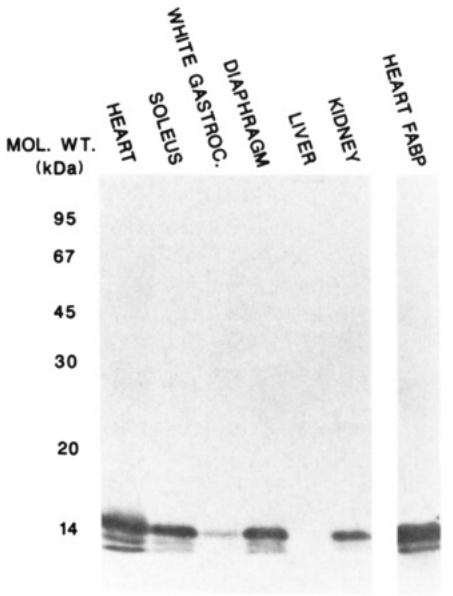

FIGURE 4: Western immunoblot analysis of rat tissue extracts. Aliquots (50 μg of protein) of low-speed supernatant fractions were subjected to SDS–PAGE. Purified rat heart FABP (0.3 μg) was utilized as a positive control. The immunoblot was overdeveloped to emphasize the absence of immunoreactive rat heart FABP in liver.

amounts might be present in certain tissues. Heart and red muscle contain the most immunoreactive heart FABP, lesser amounts were present in kidney and white muscle, and no detectable protein was found in liver. Additional experiments (not shown) indicated no detectable protein in adipose tissue or brain. Clearly the protein levels parallel the RNA tissue distribution, suggesting that heart FABP expression may be regulated at the transcriptional level although other post-transcriptional mechanisms cannot be ruled out.

An important finding of this study was that heart and metabolically distinct skeletal muscle (both slow twitch oxidative and fast twitch oxidative glycolytic fibers) express identical heart FABP mRNA. This was suggested by similarity in immunoreactive proteins and the Northern hybridization data and was confirmed by the S1 mapping experiments suggesting identity of the mRNA for heart and several oxidative muscle types. In fast twitch glycolytic muscle (white gastrocnemius), however, no evidence for an abundant species of mRNA or immunoreactive protein was found. These findings are important with regard to establishing functional differences between the different FABP's. Heart and red muscle are the major mammalian tissues involved in utilization of circulating fatty acids as a fuel source, whereas tissues such as intestine, adipose, and liver tend to esterify fatty acids for transport or storage. Although speculative, it is possible that heart (i.e., muscle) FABP has structural features that facilitate transfer from plasma membrane to mitochondria for subsequent activation and oxidation, whereas other FABP's are capable of directing bound ligand to sites on the endoplasmic reticulum.

It is likely that the same gene is responsible for FABP expression in heart and the different red muscle cell types, but we cannot rule out the possibility that skeletal muscle also produces a different gene product that might be classified as FABP. There have been anecdotal reports in the literature of a skeletal muscle FABP that is similar but distinct from heart FABP (Said & Schulz, 1985), but to date, only intestinal tissue seems to have two abundant gene products that are

members of this multigene family. Interestingly, kidney contained an immunologically similar FABP that appears to be relatively abundant, in contrast to the low level of RNA detected with the rat heart FABP cDNA. This suggests that the major kidney FABP may not be identical with heart FABP. The kidney also utilizes fatty acids as an important fuel source, and comparative studies of such proteins will be informative in eventually correlating structure and function.

An interesting analogy between the characteristics of myoglobin and heart FABP is suggested from the data. Both proteins have a relatively low molecular weight, are found almost exclusively in the cytosol, and appear similar with respect to tissue distribution, being present in muscle tissue considered oxidative but absent in those muscle fibers commonly classified as fast twitch glycolytic. In addition, both proteins presumably function to provide fuel for mitochondrial oxidation and are among the most abundant of the soluble proteins in myocytes. The availability of cDNA probes for both proteins should make it feasible to determine if these genes are expressed constitutively in adult myocytes and if common mechanisms are involved in regulating their coordinate gene expression. More importantly, the availability of the rat heart FABP cDNA will make it possible to definitively determine the biological function of heart FABP by expression studies coupled with site-directed mutagenesis.

Added in Proof

A manuscript reporting a cDNA sequence for rodent heart FABP was published recently while this paper was in press (Heuckeroth et al., 1987).

Registry No. DNA (rat heart fatty acid binding protein messenger RNA complementary), 111025-61-7; protein (rat heart fatty acid binding), 111025-62-8.

References

Alpers, D. H., Strauss, A. W., Ockner, R. K., Bass, N. M., & Gordon, J. I. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 313–317.

Bass, N. M. (1985) *Chem. Phys. Lipids 38*, 95–114.

Bass, N. M., & Manning, J. A. (1986) *Biochem. Biophys. Res. Commun. 137*, 929–935.

Berk, A. J., & Sharp, P. A. (1977) *Cell (Cambridge, Mass.) 12*, 721–732.

Bernlohr, D. A., Angus, C. W., Lane, M. D., Bolanowski, M. A., & Kelly, T. J., Jr. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5468–5472.

Burnett, D. A., Lysenko, N., Manning, J. A., & Ockner, R. K. (1979) *Gastroenterology 77*, 241–249.

Casey, J., & Davidson, N. (1977) *Nucleic Acids Res. 4*, 1539–1552.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Cook, K. S., Hunt, C. R., & Spiegelman, B. M. (1985) *J. Cell Biol. 100*, 514–520.

Crisman, T. S., Claffey, K. P., Saouaf, R., Hanspal, J., & Brecher, P. (1987) *J. Mol. Cell. Cardiol. 19*, 423–431.

Feinberg, A. P., & Vogelstein, B. (1984) *Anal. Biochem. 137*, 266–267.

Glatz, J. F. C., & Veerkamp, J. H. (1985) *Int. J. Biochem. 17*, 13–22.

Gordon, J. I., & Lowe, J. B. (1985) *Chem. Phys. Lipids 38*, 137–158.

Gordon, J. I., Alpers, D. H., Ockner, R. K., & Strauss, A. W. (1983) *J. Biol. Chem. 258*, 3356–3363.

Gordon, J. I., Elshourbagy, N., Lowe, J. B., Liao, W. S., Alpers, D. H., & Taylor, J. M. (1985) *J. Biol. Chem. 260*, 1995–1998.

Grantham, R., Gautier, C., Gouy, M., Jacobzone, M., & Mercier, R. (1981) *Nucleic Acids Res. 9*, r43–r74.

Gubler, U., & Hoffman, B. J. (1983) *Gene 25*, 263–269.

Heuckeroth, R. O., et al. (1987) *J. Biol. Chem. 262*, 9709–9717.

Kozak, M. (1986) *Cell (Cambridge, Mass.) 44*, 2813–292.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–686.

Lehrach, H., Diamond, D., Wosney, J. M., & Boedtker, H. (1977) *Biochemistry 16*, 4743–4751.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Ockner, R. K., & Manning, J. A. (1974) *J. Clin. Invest. 54*, 326–338.

Ockner, R. K., Manning, J. A., Poppenhausen, R. B., & Ho, W. K. L. (1977) *Science (Washington, D.C.) 177*, 56–58.

Ockner, R. K., Manning, J. A., & Kane, J. P. (1982) *J. Biol. Chem. 257*, 7872–7878.

Offner, G. D., Troxler, R. F., & Brecher, P. (1986) *J. Biol. Chem. 261*, 5584–5589.

Sacchettini, J. C., Said, B., Schulz, H., & Gordon, J. I. (1986) *J. Biol. Chem. 261*, 8218–8223.

Said, B., & Shultz, H. (1984) *J. Biol. Chem. 259*, 1155–1159.

Said, B., & Schulz, H. (1985) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 44*, 1415 (Abstr. 5871).

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.

Takahashi, K., Odani, S., & Ono, T. (1983) *Eur. J. Biochem. 136*, 589–601.

Young, R. A., & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1194.