PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 189, 183–188 (1988)

Fatty Acid Binding Proteins in the Three Types of Rat Skeletal Muscle (42795)

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Abstract. By means of Sephadex G-50 column chromatography, a M_r 12,000 fatty acid binding protein (FABP) was found to be present in all three types of skeletal muscle. FABP concentrations in muscle cytosols (105,000*g* supernatant) were fiber type specific with binding levels (expressed as pmole [^{14}C]oleate bound/mg protein) of 70 ± 7 in fast-twitch white (FTW) (heart FABP = 469 ± 33). Cytosols from all three fiber types cross-reacted with antibody to pure heart FABP on Ouchterlony immunodiffusion analysis. FABP content, determined by radial immunodiffusion, followed the same pattern in the muscle types as that in the binding assay. The values (in μg/mg protein) were 3.3 ± 0.1 in FTW, 17.0 ± 0.4 in FTR, and 31.7 ± 1.4 in STR fibers (heart = 55). Disc gel electrophoresis revealed a protein band in each fiber type that had migration identical to that of pure heart FABP and was proportional to the amounts determined by Sephadex G-50 chromatography and immunoassay. In addition, Western blots of tissue cytosols, using antibody to heart FABP, detected single protein bands identical in size to pure heart FABP in all three types of skeletal muscle. These results show the presence of a FABP in all skeletal muscle types with an immunologic and electrophoretic characterization identical to that of heart FABP.

Fatty acid binding proteins (FABPs) have been demonstrated to be present in a number of tissues including skeletal muscle (1–6). Skeletal muscles, however, are composed of cell populations with a wide variation in metabolic and functional capacity. They have been classified into three general fiber-type categories that are based on their physiological and biochemical characteristics (7–9). Fast-twitch white (FTW) fibers contain high glycogenolytic capacity, low respiratory capacity, and high myosin ATPase activity; fast-twitch red (FTR) fibers contain high glycogenolytic capacity, high respiratory capacity, and high myosin ATPase activity; and slow-twitch red (STR) fibers contain low glycogenolytic capacity, high respiratory capacity, and low myosin ATPase activity (10). As the expression of FABP in the three types of skeletal muscle has not received any systematic attention, the present experiments were undertaken to quantify FABP in each type. Furthermore, on the basis of electrophoretic, immunologic, and functional criteria, heart FABP appears structurally different from liver FABP (2, 11–14). Thus, a second aim was to characterize the relationship of the skeletal muscle FABPs with purified heart FABP. A preliminary communication of this work has been reported previously (15).

Methods. *Preparation of tissue.* Male Sprague–Dawley rats (250 g) were anesthetized with ether. The abdominal aorta and ascending vena cava were then ligated and catheters (20 and 16 gauge, respectively) were inserted into the vessels. Both hindlimbs of the rat were perfused with nonrecirculating, ice-cold saline/heparin (5 units heparin/ml) for 2 min at a rate of 25 ml/min in order to wash the plasma proteins from the muscles. Next, the heart was removed and perfused with the same solution for 1 min. The muscles selected for study were removed, trimmed of fat and connective tissue, and kept on ice with the ventricles until homogenization. The specific muscles studied were as follows: the superficial portion of the quadriceps (white vastus lateralis), a muscle region containing mostly fast-twitch white fibers; the deep quadriceps (red vastus lateralis), a muscle region containing predominantly fast-twitch red fibers; and the soleus, a muscle composed primarily of slow-twitch red fibers (10). All muscles were thoroughly minced with scissors and homogenized in an ice water bath using a Polytron (Brinkman instruments) at setting six for three 10-sec

0037-9727/88 $1.50

passes (with the samples cooled on ice for 30 sec between passes) in 3 vol of KCl–PO_4 buffer (150 m*M* KCl in 10 m*M* KH_2PO_4, 0.02% NaN_3, pH 7.4). Tissue samples from three to six animals were pooled for each assay. The homogenates were initially centrifuged at 10,000*g* for 20 min. The supernatant fractions were removed and centrifuged again at 105,000*g* for 60 min. These supernatant fractions (cytosol) were assayed for protein content (16) and were stored at −20°C for further analysis.

G-50 Column chromatography binding assay. The total concentration of available FABP-associated fatty acid receptors in the cytosol was estimated by the binding of [^{14}C]oleate to the 12,000 M_r fraction of cytosol as determined by Sephadex G-50 chromatography (17). In this assay, 20 nmole of [^{14}C]oleate was added in 5 μl propylene glycol to 4.0 mg cytosolic protein brought to 0.5 ml with KCl–PO_4 buffer. All of this mixture was subjected to gel filtration on Sephadex G-50 Columns (1 × 48 cm, 20°C). Fractions (0.6 ml) were analyzed for [^{14}C]oleate and compared to an [^{14}C]oleate standard.

Immunological studies. Rat heart FABP was purified from the cytosol according to the procedures published by Said and Schulz (11). Heart FABP antiserum, raised in New Zealand rabbits as previously described (13), was used in these studies. This antiserum shows no cross-reaction with FABPs purified from rat liver or intestine (13). Cytosol from all four muscle types was tested for cross-reaction with heart FABP antibody on Ouchterlony discs purchased from Miles Laboratories (Elkhart, IN).

Quantitative analysis of FABP content in all muscles was determined by radial immunodiffusion as described by Mancini *et al.* (18). The gel consisted of 4.0 ml antiheart FABP antiserum, 1.0 ml barbital buffer (4.5 g Na barbital, 0.25 g NaN_3 + 3.3 ml 1.0 *N* HCl, pH 8.6, brought to 500 ml), and 5.0 ml of 2.0% agarose in barbital buffer. Aliquots of 10–20 μg of cytosolic protein in 10 μl buffer were added to the gel and incubated for 48 hr. Comparisons were made to standards of purified heart FABP treated in the same manner as the samples.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) was performed as previously described (19). Separating gels contained 12% (w/v) acrylamide. Proteins separated by gel electrophoresis in SDS were transferred to nitrocellulose paper using equipment and reagents obtained from Bio-Rad Laboratories, Richmond, California, according to the manufacturer's instruction manual. After electrophoretic transfer at 250 mA for 3 hr, the nitrocellulose was incubated overnight in 3% gelatin in 500 m*M* NaCl, 20 m*M* Tris, pH 7.5 (TBS). The paper was washed with 0.05% Tween 20 in TBS (T-TBS) and incubated with antiserum to heart FABP diluted 1:500 in T-TBS containing 1% gelatin for 1 hr. After further washing, the nitrocellulose paper was incubated with a 1:3000 dilution of goat anti-rabbit IgG–horseradish peroxidase conjugate for 1 hr. The paper was washed again and immune complexes were detected using color development with 60 mg of 4-chloro-1-naphthol in 20 ml cold methanol added to 100 ml of TBS containing 60 μl of 30% H_2O_2.

Analysis of data. Comparisons among groups for FABP in the different muscle

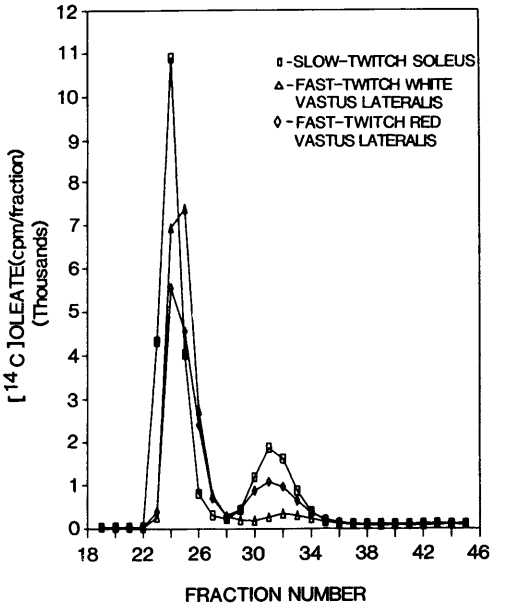

FIG. 1. Gel filtration on Sephadex G-50 of 4.0 mg cytosolic protein from soleus, red vastus lateralis, and white vastus lateralis muscles. Eluting buffer was 150 m*M* KCl, 10 m*M* KH_2PO_4, 0.02% NaN_3, pH 7.4. Volume per fraction = 0.6 ml.

TABLE I. FABP CONTENT IN THE THREE SKELETAL MUSCLE TYPES AND HEART

Tissue	[^{14}C]Oleate binding (pmole/mg cytosolic protein)	Radial immunodiffusion (μg/mg protein)
Soleus	353 ± 22*	31.7 ± 1.4*
Red vastus	263 ± 11*	17.0 ± 0.4*
White vastus	70 ± 7*	3.3 ± 0.1*
Heart	469 ± 33*	55

Note. Cytosol samples from 3 to 6 rats were pooled for each assay. Values are means ± SE. There were four or five observations per muscle with the exception of the immunodiffusion assay in heart, in which one determination was made.

* Significantly different from all other values ($P < 0.01$).

types were made using an analysis of variance followed by a Fisher's LSD test for multiple comparisons. Statistical significance was accepted at $P < 0.01$.

Results. As shown in Fig. 1, Sephadex G-50 chromatography profiles revealed two distinct peaks eluting from the columns for each of the three skeletal muscle fiber types. The first, corresponding to the excluded or void volume, contains residual albumin and other large macromolecules in the muscle cytosols. The second peak, corresponding to the elution volume of the 12,000 M_r proteins, represents FABP bound [^{14}C]oleate. The magnitude of the elution profiles was significantly different among all muscle types with soleus > red vastus > white vastus muscles (Table I, Fig. 1). Heart FABP eluted from the same fractions as the skeletal muscle types (Fig. 1) and displayed the highest concentration of all muscle types studied (Table I). A major protein species in fractions 25–35 comigrated with heart FABP on SDS–polyacrylamide gels (data not shown).

Cytosol from each of the three skeletal muscle types showed a single precipitant line with antibody to pure heart FABP on Ouchterlony immunodiffusion (data not shown), and FABP in skeletal muscle was quantitated by a radial immunodiffusion assay (18). These results demonstrated the same pattern of concentration differences among fiber types as that observed in the binding assay (Table I). Figure 2 shows that there was a strong correlation ($r = 0.98$) between the two methods for determining FABP content in muscles, which was highly significant ($P < 0.001$).

Polyacrylamide gel electrophoresis revealed a protein band in each skeletal muscle fiber type with migration identical to that of heart cytosol and pure heart FABP (Fig. 3). Furthermore, the Western blotting showed that pure heart muscle FABP, heart muscle cytosol, and soleus and red vastus muscle cytosols each showed a strong band of immunostaining of identical molecular size (Lanes A–D, Fig. 4). Lane E of Fig. 4 contained cytosol from white vastus and, although in other experiments we were able to see a faint band corresponding in size to the others on Western blotting when 100 μg cytosol protein was added, it was not visible in this experiment. Figure 5 shows Western blots from three separate white vastus muscle cytosols (Lanes B–D) that are also identical to heart (Lane A) cytosol FABP when 250 μg cyto-

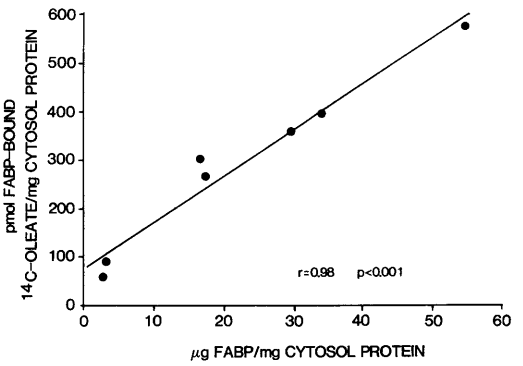

FIG. 2. Correlation of muscle FABP from the same samples that were analyzed using the G-50 column chromatography [^{14}C]oleate binding assay and the radial immunodiffusion assay.

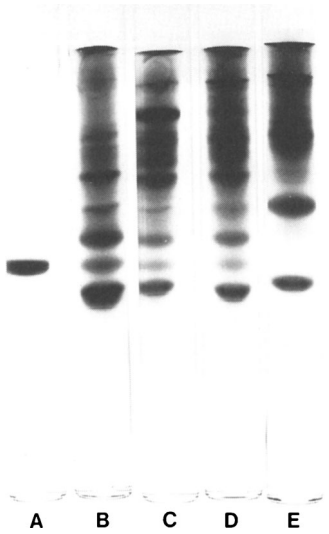

FIG. 3. Polyacrylamide disc gel electrophoresis pattern of (A) purified heart FABP, (B) heart cytosol, (C) soleus cytosol, (D) red vastus lateralis cytosol, and (E) white vastus lateralis cytosol.

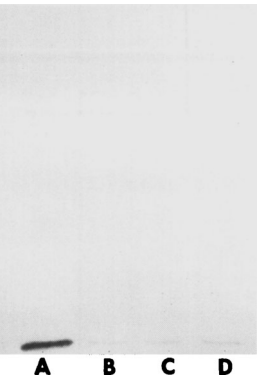

FIG. 5. Immunoblot detection of white muscle proteins transferred from SDS–polyacrylamide gel to nitrocellulose paper. Protein bands were detected using the immunoperoxidase method after incubation of the nitrocellulose paper with antibody to rat heart FABP (under Methods). The lanes contained (A) 100 μg of cytosolic protein from heart muscle and (B–D) 250 μg of cytosolic protein from separate white vastus muscles of three rats.

solic protein is added to these three lanes. It is unlikely that contamination of red fibers is responsible for FABP activity in white muscle region, but this alternative cannot be completely ruled out. Resolution of this possibility will require FABP analysis in single fibers. No reaction with liver cytosol proteins was seen in any experiment, nor was any reaction with pure liver or intestinal type FABP seen (data not shown).

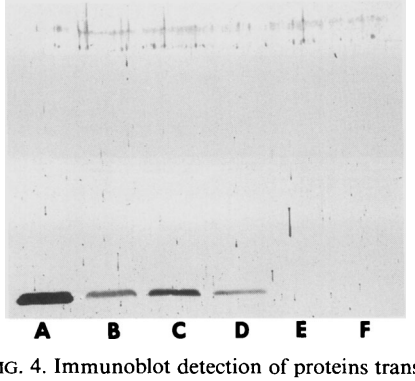

FIG. 4. Immunoblot detection of proteins transferred from SDS–polyacrylamide gel to nitrocellulose paper. Protein bands were detected using the immunoperoxidase method after incubation of the nitrocellulose paper with antibody to rat heart FABP (under Methods). The lanes contained (A) pure heart muscle FABP, 15 μg, and 100 μg of cytosolic protein from (B) soleus, (C) heart muscle, (D) red vastus, (E) white vastus, and (F) liver.

Discussion. Two distinct assay procedures (Sephadex G-50 chromatography and radial immunodiffusion) demonstrated that there is a clear distinction in FABP expression between slow-twitch red and fast-twitch red muscle fibers. A previous report had failed to observe any FABP activity in white muscle (3). However, using Sephadex G-50, a small FABP peak was detected in all cytosols (n = 5) of white vastus lateralis muscles assayed. In further experiments, using anti-heart FABP antiserum, we found that white vastus muscles (and all other fiber types) cross-reacted qualitatively using Ouchterlony and Western blotting procedures and quantitatively by radial immunodiffusion. Thus, white muscle which has high glycogenolytic capacity and a very low capacity for aerobic metabolism, still contains measurable levels of FABP.

In this present study, muscle cytosols were not delipidated prior to the binding assay. In more recent preliminary studies (W. C. Miller, unpublished), the binding of fatty acids with delipidated cytosol was found to

be higher than that of nondelipidated cytosol but the proportional binding of fatty acids to the cytosolic extracts from the different muscle types was the same as that seen with nondelipidated extracts.

Fournier and co-workers (20) have indicated that the FABP in the heart (which appears to contain an optimal amount of enzymatic machinery for aerobic metabolism) is a key factor in the utilization of fatty acids for energy production. They have also shown that FABP can transfer fatty acids to the mitochondria *in vitro* (20). But in rat skeletal muscles, the capacity for fatty acid oxidation is higher in fast-twitch red fibers than in slow-twitch red fibers (7, 21). Thus, the higher FABP levels in slow-twitch fibers than in fast-twitch fibers are surprising. These results do not initially suggest a direct relationship of FABP concentration with fatty acid oxidation capacity in skeletal muscle. Nevertheless, an intracellular lipase, which has been proposed to regulate triacylglycerol hydrolysis, follows the same quantitative pattern in the three muscle types as that of FABP (22, 23). Additional work is needed to establish the specific functioning of FABP in each of the fiber types as FABP may regulate other cellular processes such as triacylglycerol synthesis (1).

Another purpose of this work was to compare skeletal muscle FABP characteristics with those found for heart FABP. The results from the [^{14}C]oleate binding assay, gel electrophoresis, immunodiffusion, and Western blots show that FABP in all fiber types has the same M_r and immunoreactivity as heart FABP. Consequently, the FABP in these skeletal muscle fiber types appears immunologically and electrophoretically identical to heart FABP.

This work was supported by NIH Grants AM-32926, AM-13328, K04-AM-01100, and 1F32 HL07100-01 B1-2. Secretarial assistance is greatly appreciated.

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Received March 21, 1988. P.S.E.B.M. 1988, Vol. 189.
Accepted July 7, 1988.