FATTY ACID BINDING TO CYTOPLASMIC PROTEINS OF MYOCARDIUM AND RED AND WHITE SKELETAL MUSCLE IN THE RAT. A POSSIBLE NEW ROLE FOR MYOGLOBIN

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SUMMARY: The presence of a cytoplasmic binding protein with an affinity for [1 - ^{14}C] oleic acid was demonstrated in the 105,000 x g supernatant of rat heart. The molecular weight was estimated to be approximately 16,000 which is higher than that of the Z protein in liver. The presence of myoglobin was demonstrated in this peak. Myoglobin itself was found to bind labelled oleic acid in vitro. The amount of binding observed in myocardial and skeletal muscle cytosol seemed to depend on the amount of myoglobin present.

INTRODUCTION: Two cytoplasmic proteins, Y and Z, which bind various organic ions have been shown to be present in the cytosol of liver (1, 2, 3). The smaller of these two proteins, the Z protein, has also been shown to bind long-chain unsaturated fatty acids (4) and their CoA thioesters (5). The Z protein is probably identical with the fatty acid binding protein occurring in intestinal mucosa which was first reported by Ockner et al. (6). It has been suggested that these fatty acid binding proteins have a role in fatty acid transport and metabolism within the cell (4, 7, 8, 9). Fatty acid binding proteins have been demonstrated in a variety of tissues (4, 5, 6) but most attention has been given to the liver and intestinal mucosa.

Less attention has been given to the fatty acid binding characteristics of muscle cytosol. The utilisation of fatty acids varies greatly according to muscle type. Myocardium and red skeletal muscle have a high requirement for fatty acids, while white skeletal muscle has a low requirement. The present communication describes the binding of [1 - ^{14}C] oleic acid to the cytoplasm of the myocardium and of red and white skeletal

muscle. The results are compared with the well-characterised binding occurring in hepatic cytoplasm.

MATERIALS: [1 - ^{14}C] oleic acid (specific activity 55 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, England. Bovine serum albumin, fatty acid free, (Pentex) was purchased from Miles Laboratory. Pepsin, chymotrypsinogen, myoglobin and cytochrome c were all obtained from Sigma Ltd. Blue Dextran and Sephadex G-75 were from Pharmacia.

METHODS: Male Sprague Dawley rats, guinea-pigs of the Dunkin-Hartley strain and New Zealand white rabbits were killed by cervical dislocation. The organs were quickly removed and rinsed in ice-cold Krebs bicarbonate buffer pH 7.4. The liver and heart were perfused from a syringe with cold Krebs bicarbonate buffer to remove blood. In some experiments rat and guinea-pig hearts were perfused by the Langendorff technique for 1 hour at 4°C with Krebs bicarbonate buffer gassed with 95% O_2 : 5%CO_2. Sartorius muscle was taken as an example of white muscle. A mixed sample of red muscle was taken from the hind legs.

A 20% homogenate was prepared from each tissue in 0.25 M sucrose-0.01 M Tris-HCl buffer (pH 7.4). A cytoplasmic fraction was then obtained by centrifugation at 105,000 x g for 90 minutes at 4°C. Aliquots of 3 ml cytoplasmic fraction were mixed with 2 nmoles [1 - ^{14}C] oleic acid dissolved in 50 µl dioxane : propylene glycol (2:1 v/v).

After standing in ice for 30 minutes 2.0 ml of the sample was applied to a column of Sephadex G-75 (2.5 x 52 cm) and separation achieved by upward flow elution using 0.01 M Tris-HCl buffer (pH 7.4) with a flow-rate of 18 ml/hr. 3.0 ml fractions were collected. The protein concentration in the eluted fractions was determined by the method of Lowry et al. (10) using bovine serum albumin as standard. The concentration of myoglobin present was determined by absorbance at 410 mµ. Radioactivity in each fraction was determined by liquid scintillation counting of 1.0 ml aliquots in 10 ml of Bray's solution (11). The results are expressed as cpm per fraction.

An estimation of the molecular weight of the binding proteins was made according to the method of Andrews (12). Bovine serum albumin, pepsin, chymotrypsinogen, myoglobin and cytochrome c were used as standards to calibrate the column.

RESULTS AND DISCUSSION: In rat liver the two cytoplasmic proteins Y and Z accounted for most of the bound radioactivity derived from [1 - ^{14}C] oleic acid (Figure 1). In four experiments 22.9 $\pm$ 3.2% of the added radioactivity was associated with the Z protein and 6.6 $\pm$ 2.3% with the Y protein. In agreement with Levi et al. (1) and Reyes et al. (2) it was found that, when sulphobromophthalein was used in place of [1 - ^{14}C] oleic acid, more of the bound dye was associated with the Y than the Z protein. The Y protein was estimated to have a molecular weight of about 38,000 and the Z protein of

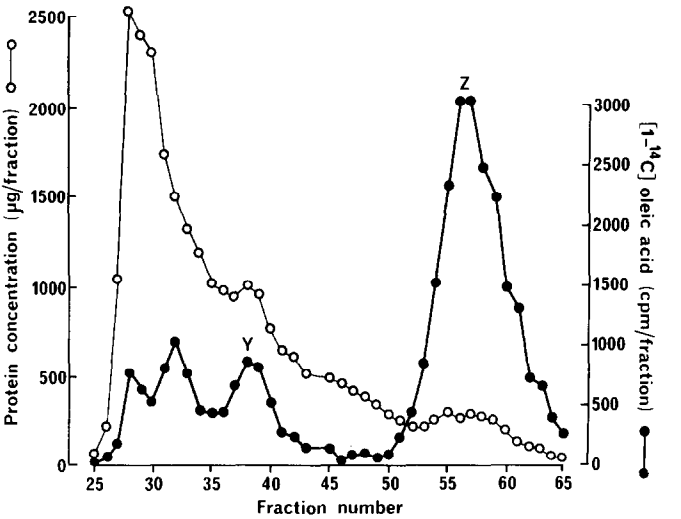

Figure 1: Gel chromatography of rat liver cytoplasmic proteins in the presence of [1-^{14}C] oleic acid. [1-^{14}C] oleic acid (2 nmol in 50 µl dioxane: propylene glycol 2:1 v/v) was added to rat liver cytoplasm (26 mg protein). Elution was from Sephadex G-75 (2.5 x 52 cm) using 0.01 M Tris-HCl buffer (pH 7.4). Flow rate was 18 ml/hr and 3 ml fractions were collected. (o—o protein concentration µg per fraction : ●—● [1-^{14}C] oleic acid cpm per fraction).

between 12,000–13,000, figures comparable with those previously published (5, 6, 13). The radioactivity occurring in the first two small peaks was due to binding to high molecular weight proteins and to albumin. The binding in guinea-pig and rabbit liver supernatant was similar to that occurring in the rat.

The binding of [1-^{14}C]oleic acid to the 105,000xg supernatant protein of rat heart is shown in Figure 2. Only two peaks were evident. In five experiments 9.3 ± 2.9% of the added radioactivity was associated with the smaller second peak. This is a little lower than the 13.8% reported by Mishkin et al. (4). When rat hearts were perfused for 1 hour with Krebs bicarbonate buffer before homogenisation, it was found that binding to the high molecular weight protein was markedly reduced. The perfusate was concentrated by ultra-filtration and was shown to have a high binding affinity for fatty acids. Polyacrylamide gel electrophoresis demonstrated that the binding protein in the perfusate migrated in a

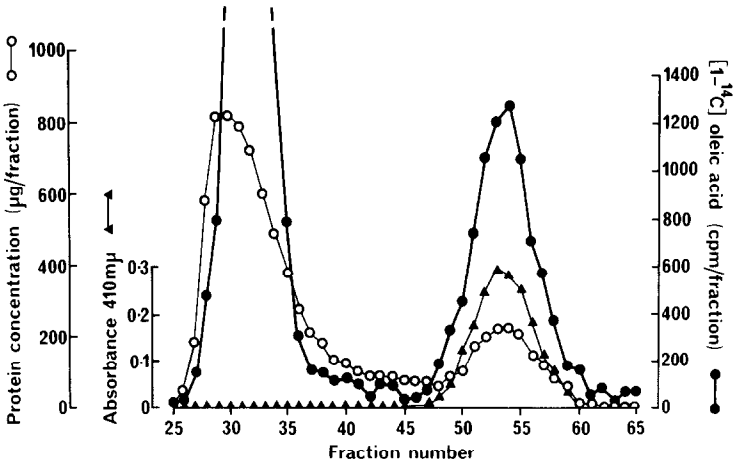

Figure 2: Binding of [1 - ^{14}C] oleic acid to the proteins in rat heart cytoplasm (8.7 mg protein). Elution conditions as given in Figure 1. (o—o protein concentration µg per fraction : ●—● [1 - ^{14}C] oleic acid cpm per fraction : ▲—▲ absorbance at 410 mµ).

similar manner to standard serum bovine albumin. In liver, most intracellular albumin occurs in the microsomal fraction (14) which may explain why the amount of unspecific binding is lower than in heart.

The peak of radioactivity associated with the low molecular weight proteins of rat heart supernatant was eluted earlier than it was with the liver supernatant. The protein had an apparent molecular weight of about 16,000. There was a noticeable brown colour in this fraction which had an absorption maximum at 410 mµ, similar to myoglobin. This led us to consider the possibility that myoglobin itself might be binding fatty acid. The binding property of myoglobin was, therefore, determined by adding 2 nmoles [1 - ^{14}C] oleic acid to 3 ml solution containing 9 mg horse heart myoglobin. After equilibration at 4°C for 30 min 2 ml of the sample was eluted from a column of Sephadex G-75. A mean of 18.5% of the added radioactivity was associated with the myoglobin peak (Fig. 3). Myoglobin and the fatty acid binding protein of heart had identical elution volumes. When a solution containing 9 mg cytochrome c was treated with [1 - ^{14}C] oleic acid there was no radioactivity associated with the eluted protein peak.

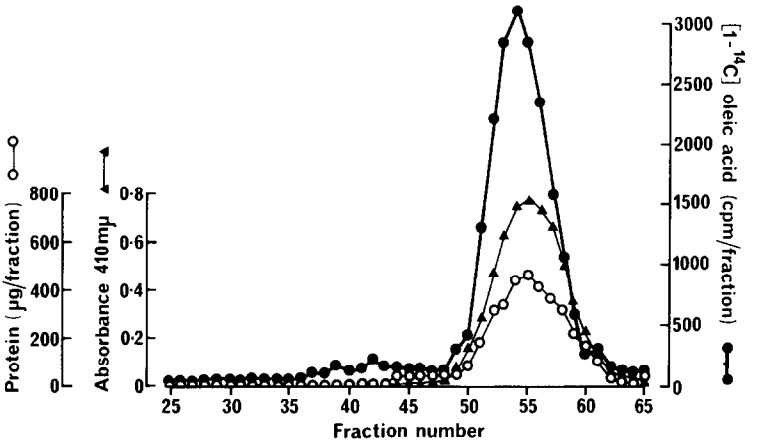

Figure 3: Binding of [1 - ^{14}C] oleic acid to horse heart myoglobin (9 mg protein). Elution conditions as given in Figure 1. (o—o protein concentration µg per fraction : ●—● [1 - ^{14}C] oleic acid cpm per fraction : ▲—▲ absorbance at 410 mµ).

The binding pattern in the 105,000 x g supernatant of rat heart was consistent, but there was some variation in the case of guinea-pig and rabbit hearts. In these two species the amount of binding to the low molecular weight protein was less and did not exceed 5% of the added radioactivity. There was also variable binding to a higher molecular weight protein eluted in a position corresponding to the Y protein of liver and mucosa.

The amount of binding of labelled oleic acid to the low molecular weight protein seemed to depend on the amount of myoglobin present. Rat heart contained more myoglobin than the other two species as judged by absorbance at 410 mµ and the percentage binding of the [1 - ^{14}C] oleic acid added was higher. When the radioactivity in this low molecular weight protein was expressed in terms of cpm/mg protein present, rat heart contained three times more radioactivity than did rabbit heart.

To pursue the possible relation between the myoglobin content and the binding of fatty acid by low-molecular weight proteins further, we compared the binding of [1 - ^{14}C] oleic acid to the 105,000 x g supernatant from red and white skeletal muscle in the rat

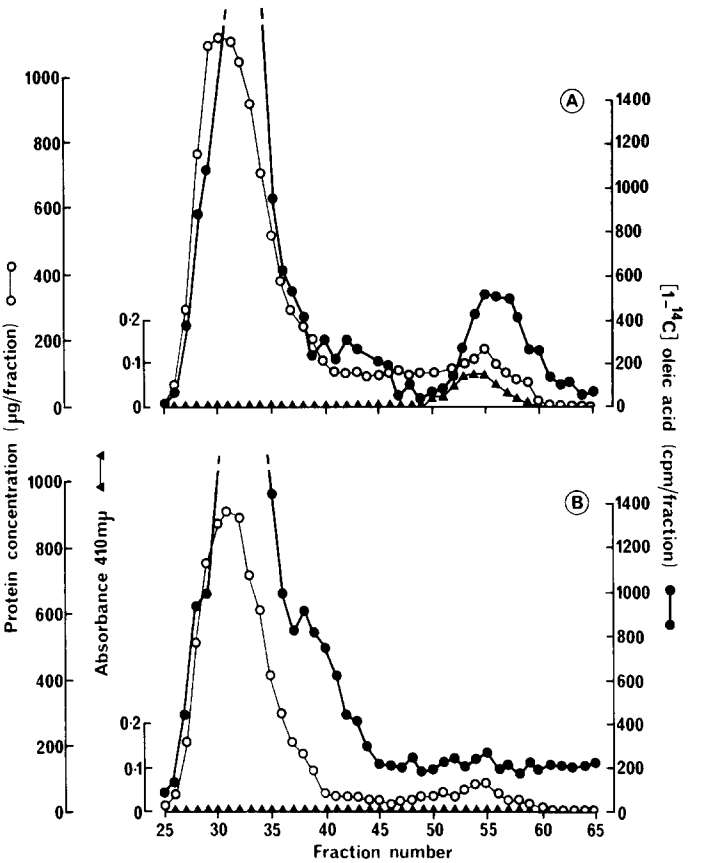

Figure 4 : Binding of [1 - ^{14}C] oleic acid to the proteins in (A) rat white skeletal muscle cytoplasm (7.9 mg protein) and (B) to the cytoplasmic proteins in rat red skeletal muscle (11.0 mg protein). Elution conditions were the same as given in Figure 1. (o—o protein concentration µg per fraction : ●—● [1 - ^{14}C] oleic acid cpm per fraction : ▲—▲ absorbance at 410 mµ).

(Fig. 4). Non-specific binding to high molecular weight proteins accounted for most of the recovered activity in both red and white muscle. In red skeletal muscle there was, in addition, a small peak of radioactivity corresponding to the low molecular weight peak which had an absorbance at 410 mµ (Fig. 4a). In white skeletal muscle (Fig. 4b) there was no low molecular weight peak of protein, no peak at 410 mµ and no second peak of radioactivity.

The above results do not entirely exclude the possibility that the binding of fatty

acid is due solely to a specific protein, distinct from myoglobin. If this is the case, however, the specific binding protein must have a molecular weight similar to that of myoglobin, must fail to be separated during the commercial purification of myoglobin, and must be distributed in different types of muscle in proportion to the distribution of myoglobin.

Preliminary experiments, using separation by polyacrylamide gel electrophoresis of the proteins from the low molecular weight peak of rat heart, have demonstrated the presence of three protein bands. When the gel was sliced and the distribution of radioactivity determined, it was found that the band containing the most radioactivity corresponded to the position occupied by the myoglobin standard. Caution, however, is necessary in the interpretation of these results as electrophoresis can dissociate the anion-protein bond and lead to movement of the identifying label away from its original binding site.

Taking the above evidence together, there seems reason to believe that myoglobin may function as a transport protein for fatty acids in the cytoplasm. If this were the case, it is of interest to note that myoglobin occurs in those muscles which preferentially utilise free fatty acids. White skeletal muscle fibres store glycogen and depend on glycolysis for their energy needs (15). Red skeletal muscle fibres and especially the myocardium (16) have a high capacity for oxidation and the utilisation of free fatty acids.

Recent work (17, 18, 19) has shown that the hepatic Z protein plays an important role in fatty acid metabolism. Before such a role can be demonstrated in cardiac muscle it will be necessary to prove that the Z protein has a separate and distinct entity.

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