The binding of \([^{3}H]\)oleic acid and \([^{3}H]\)palmitic acid to the low molecular weight fatty acid binding proteins present in the cytosols of rat liver and heart was studied. Both fatty acids were bound by Z protein of liver, whereas only oleic acid was bound by the fraction of heart that contains the fatty acid binding protein. However, after delipidation of heart cytosolic proteins with butanol, the binding of palmitic acid to the fatty acid binding protein was detected. In contrast to a published report (Gloster, J., and Harris, P. (1977) Biochem. Biophys. Res. Commun. 74, 506-513), oleic acid was not bound by rat heart or bovine heart myoglobin. Both rat heart fatty acid binding protein and rat liver Z protein were purified to homogeneity or near homogeneity. On polyacrylamide gel electrophoresis under non-denaturing conditions, liver Z protein gave rise to three bands, none of which was identical with the single band due to heart fatty acid binding protein. Rabbit antibodies to rat liver Z protein were used to demonstrate that the purified fatty acid binding protein from rat liver (Z protein) and rat heart are immunologically unrelated and that no Z protein is present in rat heart cytosol.

Taken together, these observations lead to the conclusion that the low molecular weight fatty acid binding proteins from rat liver and heart are different proteins.

The existence of low molecular weight fatty acid binding proteins in the cytosols of various animal tissues was first reported by Ockner et al. (1). The best characterized of these binding proteins is that from rat liver which appears to be identical with the anion-binding protein or Z protein described by Levi et al. (2). The rat liver fatty acid binding protein or Z protein has been purified to homogeneity and characterized (3-5). Since its identification, the physiological function of this protein has been discussed. Several reports have provided indirect evidence for its involvement in fatty acid metabolism in liver (6, 7) because it is capable of binding fatty acids and possibly even transporting them through the cytosol. Since this protein binds fatty acyl-CoA beside fatty acids, it may also protect enzymes like the adenine nucleotide transporter of mitochondria (8) and enzymes of cholesterol biosynthesis in the endoplasmic reticulum (9) against inhibition by long chain acyl-CoAs. However, a definite proof for the physiological role of this protein is still lacking. Fatty acid binding proteins similar to or identical with Z protein from liver are present in the small intestines (10) and in adipose tissue (11). However, with respect to heart, two conflicting reports have appeared. Fournier et al. (12) have identified in heart a fatty acid binding protein with properties similar to that of liver Z protein, whereas Gloster and Harris (13) have suggested that myoglobin may be the myocardial fatty acid binding protein.

Because of our interest in fatty acid metabolism in heart, we set out to determine the identity of the myocardial fatty acid binding protein and to evaluate its possible function in cytosolic fatty acid transport and thus in β-oxidation.

**EXPERIMENTAL PROCEDURES**

**Materials**—\([^{3}H]\)Palmitic acid (500 mCi/mmol) was purchased from Amersham. \([^{3}H]\)Oleic acid (9.5 Ci/mmol) was bought from New England Nuclear. Sephadex G-50 and Sephadex G-75 were purchased from Pharmacia (Piscataway, NJ). Thioglycolate and thioglycollate medium (2) were purchased from Miles Laboratories, Inc. Sephadex (2-50 and 50-75) was obtained from Pharmacia Fine Chemicals, Inc. Miles Laboratories, Inc. was the source of immunodiffusion plates. Antibodies to Z protein from rat liver were generously provided by Dr. I. Arias, Liver Research Center, Albert Einstein College of Medicine, Bronx, NY.

**Purification of Z Protein from Rat Liver**—All operations were performed at 4 °C. Z protein from rat liver was purified by a modified version of the procedure published by Trulzsch and Arias (3). Livers from 17 adult male Sprague Dawley rats were cut into small segments and carefully rinsed with 5 mm Tris-HCl (pH 7.4) containing 210 mM mannitol, 70 mM sucrose, and 1 mM EGTA.1 The liver segments were suspended in eight volumes of the same solution and homogenized by three passes of a glass-Teflon homogenizer. The homogenate was centrifuged for 90 min at 110,000 g. The clear solution of cytosolic proteins was concentrated in an Amicon concentrator (PM-10 membrane). To the resulting solution of cytosolic proteins (1.4 g) were added 4 nmol of \([^{3}H]\)oleic acid (9.5 Ci/mmol) dissolved in 4 μl of ethanol. This sample was chromatographed on a Sephadex G-75 column (4 x 42 cm) equilibrated with 10 mM sodium acetate (pH 5). Fractions of 5 ml each were collected and assayed for protein and radioactivity. Most of the radioactivity appears in two peaks. Fractions corresponding to the second peak were pooled and concentrated in an Amicon concentrator (PM-10 membrane). The concentrate was chromatographed on a Sephadex G-50 column (1.5 x 90 cm). Fractions of 4 ml each were collected. Those fractions containing the highest amount of radioactivity were pooled and concentrated in an Amicon concentrator (PM-10 membrane). The resulting concentrate (21.3 mg) was chromatographed on a CM-cellulose column (2.5 x 30 cm) equilibrated with 10 mM sodium acetate (pH 5). The column was developed with a linear gradient made up of 50 ml each of 10 mM sodium acetate (pH 5) and 0.2 M sodium acetate (pH 5). The fractions containing most of the radioactivity were pooled and concentrated in an Amicon concentrator (PM-10 membrane) to yield 7.5 mg of Z protein.

**Purification of the Fatty Acid Binding Protein from Rat Heart**—All operations were performed at 4 °C. Purification of the fatty acid binding protein from rat heart was achieved by a modified version of the procedure published by Fournier et al. (12). Hearts from nine adult male Sprague Dawley rats were perfused with Krebs' bicarbonate buffer (pH 7.4) for 10 min. The perfusate was removed and washed with 5 mm Thioglycollate medium (2). Hearts were cut into small sections, suspended in eight volumes of the same solution and homogenized by three passes of a glass-Teflon homogenizer. The homogenate was centrifuged at 110,000 g for 90 min at 0°C. The clear solution of cytosolic proteins (1.4 g) were added 4 nmol of \([^{3}H]\)oleic acid (9.5 Ci/mmol) dissolved in 4 μl of ethanol. This sample was chromatographed on a Sephadex G-75 column (4 x 42 cm) equilibrated with 10 mM sodium acetate (pH 5). The column was developed with a linear gradient made up of 50 ml each of 10 mM sodium acetate (pH 5) and 0.2 M sodium acetate (pH 5). The fractions containing most of the radioactivity were pooled and concentrated in an Amicon concentrator (PM-10 membrane) to yield 7.5 mg of Z protein.

1 The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetracetic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; SDS, sodium dodecyl sulfate.
of the rinsing solution, and homogenized by six passes of a glass-Teflon homogenizer. The homogenate was centrifuged for 20 min at 28,000 × g and the resulting supernatant was centrifuged for 90 min at 110,000 × g. The final preparation of cytosolic proteins was concentrated in an Amicon concentrator (PM-10 membrane). To 30 mg of cytosolic proteins were added 4 nmol of [9,10-3H]oleic acid (817 mCi/mmol) dissolved in 20 liters of ethanol. The resulting mixture was passed through a Sephadex G-75 column (2.5 × 42 cm) equilibrated with 5 mM sodium acetate (pH 5). Fractions of 4 ml were collected and those corresponding to the second radioactive peak were pooled. After concentrating these fractions in an Amicon concentrator (PM-10 membrane), 6 mg of protein were obtained. This material was rechromatographed on a Sephadex G-75 column (1.2 × 47 cm). The fractions containing most of the radioactive material were pooled and concentrated in an Amicon concentrator (UM-2 membrane). The 2.7 mg of protein were applied to a DEAE-cellulose column (0.9 × 7 cm) equilibrated with 10 mM Tris phosphate (pH 8.3). The column was washed with 200 ml of the same buffer to yield a foreuron that contained mostly myoglobin. The column was then developed with a linear gradient made up of 20 ml each of 10 mM Tris phosphate (pH 6.3) and 20 ml of 10 mM Tris phosphate (pH 6.3) containing 0.1 M KCl. Since no radioactivity was detected in the eluate, all fractions were combined and concentrated in an Amicon concentrator (UM-2 membrane). The resulting protein sample (1.3 mg) was recharged with [9,10-3H]oleic acid as described above and chromatographed on a CM-cellulose column (0.9 × 7 cm) equilibrated with 10 mM sodium acetate (pH 5). The column was developed with a linear gradient made up of 40 ml of 10 mM sodium acetate (pH 5) and 40 ml of 0.5 M sodium acetate (pH 5). Radioactive material emerged as a symmetrical peak. The fractions containing the radioactive material were pooled and concentrated in an Amicon concentrator (UM-2 membrane) to yield 0.11 mg of pure fatty acid binding protein.

Fractionation of Cytosolic Proteins from Rat Liver and Heart Charged with Either [3H]Oleic Acid or [3H]Palmitic Acid—Cytosolic rat liver or rat heart proteins (2 mg) prepared as described above were dissolved in 1 ml of 10 mM MOPS (pH 7.4) containing 150 mM KCl and mixed with either 2 nmol of [9,10-3H]oleic acid (0.85 Ci/nmol) or 2 nmol of [9,10-3H]palmitic acid (0.5 Ci/nmol). After incubation for 25 min at 37°C, the samples were filtered through a Sephadex G-75 column (1.2 × 22 cm) equilibrated with 10 mM MOPS (pH 7.4) containing 150 mM KCl. Fractions of 1 ml were collected and assayed for protein and radioactivity. After each experiment, bovine serum albumin (1 mg/ml) was passed through the column to remove traces of radioactive fatty acids bound to the column or column material.

Polyacrylamide Gel Electrophoresis—Disc gel electrophoresis was performed on 6.2% polyacrylamide gels at 13°C and pH 8.6 as described principally by Davis (14). Gels were stained overnight with 0.4% Coomassie brilliant blue in 7% acetic acid and destained in 7% acetic acid. Polyacrylamide gel electrophoresis in the presence of SDS was carried out at pH 8.3 as described by Laemmli (15).

Protein Determinations—Concentrations of protein solutions were determined by the method of Lowry et al. (16).

RESULTS

Identification of Low Molecular Weight Fatty Acid Binding Proteins in the Cytosols of Rat Liver and Heart—When the cytosolic proteins from rat liver were charged with [3H]oleic acid and separated by chromatography on Sephadex G-75, the radioactive material emerged from the column in two peaks (see Fig. 1A). The first peak according to its position corresponds to serum albumin, whereas the second peak was eluted where Z protein with a molecular weight of 14,272 (17) would be expected to emerge. An experiment in which the cytosolic rat liver proteins were charged with [3H]palmitic acid instead of [3H]oleic acid gave a similar result (see Fig. 1A), especially when the higher specific radioactivity of the latter fatty acid is considered. When the cytosolic fraction of rat heart was charged with [3H]oleic acid and analyzed by fractionation on Sephadex G-75, a similar picture was observed (see Fig. 1B). However, compared to liver, more radioactivity was associated with the first peak, especially when the hearts were homogenized without being first perfused. This observation, together with the fact that the position of the first peak corresponds to that of serum albumin, leads to the conclusion that the first radioactive peak to emerge from the column reflects the binding of fatty acids to residual serum albumin. Most interesting was the absence of a second radioactive peak, when the rat heart cytosolic proteins had been charged with [3H]oleic acid (see Fig. 1B). This observation raises doubts about the proposed function of the low molecular weight fatty acid binding protein as a general carrier of fatty acids necessary for their oxidation. Since Fournier et al. (12) have reported the binding of stearic acid to delipidated (18) rat heart fatty acid binding protein, we have repeated their experiment and observed binding of palmitic acid to the fatty acid binding protein after delipidation with butanol (data not shown). Unfortunately, we do not know what, if any, material was removed from the protein by extraction with butanol and what structural changes the protein may have undergone as a result of this procedure. Finally, we tested bovine heart myoglobin for its ability to bind oleic acid. As demonstrated in Fig. 1C, bovine heart myoglobin has no measurable affinity for this fatty acid. This result raises doubts about the reported binding of fatty acids to myoglobin (13).

Purification and Comparison of the Low Molecular Weight Fatty Acid Binding Proteins from Rat Heart and Liver—Fatty acid binding protein or Z protein from rat liver was purified to near homogeneity by the procedure of Trulzsch and Arias.
(3) with some modifications. In the last purification step on CM-cellulose, all radioactivity was co-eluted with the major protein fraction, presumably Z protein, which was well resolved from a minor protein contaminant (see Fig. 2). When subjected to polyacrylamide gel electrophoresis in the presence of SDS, this preparation of Z protein gave rise to one major band which accounted for more than 90% of the applied protein (see Fig. 3). On polyacrylamide gel electrophoresis under non-denaturing conditions, Z protein was resolved into two major and one minor fraction (see Fig. 4, lane 1). This result is in good agreement with the reported separation of purified Z protein into three to four immunologically identical fractions on isoelectric focusing (3).

The purification of the fatty acid binding protein from rat heart was achieved by a modified version of the procedure of Fournier et al. (12). Passing the cytosolic heart proteins twice through a Sephadex column yielded a fraction of low molecular weight proteins which had oleic acid bound to it (see Fig. 5A). The absorbance of this fraction was indicative of the presence of hemoproteins, most likely myoglobin. On polyacrylamide gel electrophoresis under non-denaturing conditions, this fraction was separated into more than seven proteins (see Fig. 4, lane 3). This partially purified preparation of fatty acid binding protein was further purified by chromatography on DEAE-cellulose on which myoglobin was removed and finally on CM-cellulose from which the fatty acid binding protein emerged as a symmetrical peak (data not shown). The final preparation of the heart fatty acid binding protein behaved as a homogeneous protein on polyacrylamide gel electrophoresis in the absence (see Fig. 4, lane 2) and in the presence of SDS (see Fig. 3). On polyacrylamide gel electrophoresis in the absence of SDS, the myoglobin fraction gave rise to three bands (see Fig. 4, lane 4) all of which were visible before staining for protein. Both myoglobin and the purified fatty acid binding protein from rat heart were recharged with [3H]oleic acid and rechromatographed on a small Sephadex G-75 column. Since little protein was available, only the radioactivity eluted from the column was measured. As can be seen in Fig. 5B, curve 1, the first peak of radioactivity was centered around fraction 20 where the fatty acid binding protein is expected to emerge (compare Fig. 5, A and B). The second radioactive peak was most likely due to unbound fatty acids which were always observed to emerge in later fractions. A similar experiment with rat heart myoglobin proved that no radioactivity was associated with this protein (see Fig. 5B, curve 2) which was eluted in fractions 15-25 (for
**Fatty Acid Binding Protein from Rat Heart**

**DISCUSSION**

Although fatty acid binding proteins of low molecular weight have been identified in various tissues of the same animal, mostly in rat, their structural relationships have not yet been studied in detail. The results of this study clearly demonstrate the nonidentity of the fatty acid binding proteins from rat liver (Z protein) and rat heart. This conclusion is based on three lines of evidence. (a) The two proteins apparently differ in their abilities to bind fatty acids. Z protein binds palmitic acid, whereas the native fatty acid binding protein from heart does not. (b) The two proteins behave differently on polyacrylamide gel electrophoresis under non-denaturing conditions. (c) The two proteins are immunologically unrelated. Ockner and Manning (10), who have looked for proteins in heart that cross-react with antibodies to purified fatty acid binding proteins from small intestine, reported their detection in some, but not in all, experiments. In contrast, they (10) and others (11) have presented evidence for the immunological identity of fatty acid binding proteins from rat liver, adipose tissues, and small intestines, the latter of which contains additionally another fatty acid binding protein (20). Thus, it is possible that tissues like liver, intestines, and adipose tissues which have a high capacity to synthesize lipids may contain one type of fatty acid binding protein, namely Z protein, whereas tissues like heart and possibly others, that have a high capacity to oxidize fatty acids, contain another type of fatty acid binding protein. If so, each type of fatty acid binding protein may have a different physiological function. A detailed study of the binding characteristics of the two types of fatty acid binding proteins so far identified may provide a clue to the intracellular functions of these proteins. In a recent report, evidence for the identity of the fatty acid binding protein from rat liver (Z protein) and sterol carrier protein has been presented (21). Hence, this protein binds and possibly plays a role in the metabolism of cholesterol, precursors of cholesterol, fatty acids, fatty acyl-CoAs, and other organic anions. However, whether this protein is a biological carrier or has only a storage function for one, several, or all of these compounds remains to be established.

The physiological function of the fatty acid binding protein from heart has also not been established. Fournier et al. (12) have suggested that this protein may function as a carrier of fatty acids for their delivery to mitochondria. The ability of this protein to bind fatty acids and the high capacity of heart to oxidize fatty acids would agree with this suggested role of the heart fatty acid binding protein. However, the observed inability of the native fatty acid protein from heart to bind palmitic acid, which is a good substrate of \(\beta\)-oxidation, does not agree with a function of this protein as a general carrier. Before a definite function can be assigned to this protein, it needs to be determined (a) which ligands are bound to the protein intracellularly, (b) whether the protein can bind palmitic acid after fatty acids are removed from the protein without denaturing it, and (c) if the protein is required for or accelerates the transfer of fatty acids from the cell membrane to mitochondria.

**Fig. 5. Gel filtration on Sephadex G-75.** A, partially purified rat heart fatty acid binding protein (6 mg) corresponding to the second radioactive peak shown in Fig. 1B. Buffer, 5 mM sodium acetate (pH 5). Column size, 1.2 X 47 cm. Fraction size, 2 ml. B, curve 1, purified rat heart fatty acid binding protein (90 \(\mu\)g), curve 2, rat heart myoglobin (93 \(\mu\)g). Buffer, 10 mM MOPS (pH 7.4) containing 150 mM KCl. Column size, 1.2 X 22 cm. Fraction size, 1 ml. The samples were charged with [9,10-\(^3\)H]oleic acid as described under "Experimental Procedures."

**Fig. 6. Ouchterlony immunodiffusion analysis of the low molecular weight fatty acid binding proteins from rat heart and liver.** The center well contained antibodies (0.34 mg) to rat liver Z protein raised in rabbit. Antigen, well 1, cytosolic rat liver proteins (40 \(\mu\)g); well 2, purified rat liver Z protein (4 \(\mu\)g); well 3, cytosolic rat liver proteins (10 \(\mu\)g); wells 4 and 5, purified rat heart fatty acid binding protein (1.1 and 2.2 \(\mu\)g, respectively); well 6, cytosolic rat heart proteins (40 \(\mu\)g).

(see Fig. 1C). All radioactivity emerged in later fractions characteristic of unbound fatty acids.

The structural relationship between rat liver Z protein and rat heart fatty acid binding protein was also evaluated by an Ouchterlony double immunodiffusion experiment (19). The picture of an Ouchterlony plate shown in Fig. 6 demonstrates the formation of strong precipitin lines between antibodies to rat liver Z protein (center well) and purified rat liver Z protein (well 2) as well as cytosolic rat liver proteins (wells 1 and 3). In contrast, neither purified rat heart fatty acid binding protein (wells 4 and 5) nor cytosolic rat heart proteins (well 6) gave rise to a corresponding precipitin line. A very weak precipitin line close to the antibody well was seen with cytosolic rat heart proteins. This line is not visible in Fig. 6. Since this precipitin line was not observed with pure heart fatty acid binding protein, it must be due to an antigen other than the fatty acid binding protein.
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REFERENCES
1. Ockner, R. K., Manning, J. A., Poppenhausen, R. B., and Ho, W. K. L. (1972) Science 177, 56–58
2. Levi, A. J., Gatmaitan, Z., and Arias, I. M. (1969) J. Clin. Invest. 48, 2156–2167
3. Trulzsch, D., and Arias, I. M. (1981) Arch. Biochem. Biophys. 209, 433–440
4. Ockner, R. K., Manning, J. A., and Kane, J. P. (1982) J. Biol. Chem. 257, 7872–7878
5. Takahashi, K., Odani, S., and Ono, T. (1982) FEBS Lett. 140, 83–86
6. Burnett, D. A., Lysenko, N., Manning, J. A., and Ockner, R. K. (1979) Gastroenterology 77, 241–249
7. Wu-Rideout, M. Y. C., Elson, C., and Shrago, E. (1976) Biochem. Biophys. Res. Commun. 89, 1198–1177
8. Barbour, R. L., and Chan, S. H. P. (1979) Biochem. Biophys. Res. Commun. 89, 1198–1177
9. Grinstead, G. F., Trzaskos, J. M., Billheimer, J. T., and Gaylor, J. L. (1983) Biochim. Biophys. Acta 751, 41–51
10. Ockner, R. K., and Manning, J. A. (1974) J. Clin. Invest. 54, 326–338
11. Hag, R., Christodoulides, L., Ketterer, B., and Shrago, E. (1982) Biochim. Biophys. Acta 713, 193–198
12. Fournier, N., Geoffroy, M., and Deshusses, J. (1978) Biochim. Biophys. Acta 533, 457–464
13. Gloster, J., and Harris, P. (1977) Biochem. Biophys. Res. Commun. 74, 506–513
14. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427
15. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
17. Takahashi, K., Odani, S., and Ono, T. (1982) Biochem. Biophys. Res. Commun. 106, 1099–1105
18. Morton, R. K. (1955) Methods Enzymol. 1, 25–51
19. Ouchterlony, O. (1949) Ark. Kemi 1, 43–48
20. Bass, N. M., Manning, J. A., and Ockner, R. K. (1983) Gastroenterology 84, 1099
21. Dempsey, M. E., McCoy, K. E., Baker, H. N., Dimitriadou-Vafiadou, A., Lorsbach, T., and Howard, J. B. (1981) J. Biol. Chem. 256, 1867–1873
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