THE ISOLATION OF ACYL CARRIER PROTEIN FROM THE PIGEON LIVER FATTY ACID SYNTHETASE COMPLEX[1] II.

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SUMMARY

A low molecular weight protein of less than 10,000 Daltons has been isolated from Subunit I (β-ketoacyl thioester reductase) of the pigeon liver fatty acid synthetase complex and purified to homogeneity. This protein contains all of the [^{14}C]-labeled pantetheine incorporated into the fatty acid synthetase on injection of [^{14}C]-labeled pantetheine into pigeons. It also has one β-alanine and one sulfhydryl group. This protein is an acceptor of an acetyl group from acetyl-CoA and a malonyl group from malonyl-CoA in the presence of Subunit II (transacylase). In these respects it is very similar to E. coli acyl carrier protein.

The properties and functions of acyl carrier proteins associated with fatty acid synthesizing systems in bacteria, yeast and plants have been extensively reviewed by Prescott and Vagelos (1). The presence of a similar acyl carrier protein in the tightly-bound avian and mammalian fatty acid synthetase complexes has been sought, without success, for a considerable period of time (2). Joshi et al. indicated in a preliminary report (3) the isolation of an acyl carrier protein-like substance from pigeon liver fatty acid synthetase. However, they could not substitute this protein for acyl carrier protein in the E. coli fatty acid synthesizing system. Neither did they report that this protein accepts acetyl or malonyl groups in the presence of the pigeon liver fatty acid synthetase complex.

The principal evidence up to now for an acyl carrier protein in the pigeon liver fatty acid synthetase has been the demonstration of one, and only one, 4′-phosphopantetheine prosthetic group in the complex (4). Re-

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cently we have separated the two non-identical, half-molecular weight subunits of 4'-phospho-[^{14}C]pantetheine-labeled fatty acid synthetase from one another by a combination of affinity chromatography and sucrose density gradient centrifugation (5). In this study it was shown that the 4'-phosphopantetheine group is located in the β-ketoacyl thioester reductase subunit (I). The separated half-molecular weight subunits can be further dissociated slowly and partially into smaller components in 0.1 M Tris-phosphate buffer, pH 8.4, at 0-4°. In lower ionic strength buffer the dissociation is more rapid and complete.[3]

In the present report we describe the isolation of a small molecular weight protein from Subunit I (the β-ketoacyl thioester reductase) dissociated under the above conditions. This protein passes through a PM 10 ultrafilter and it has properties similar to those of the E. coli acyl carrier protein. Thus it has a similar molecular weight and it accepts acetyl and malonyl groups from acetyl- and malonyl-CoA in the presence of the transacylase subunit (II).

EXPERIMENTAL

An authentic sample of E. coli acyl carrier protein (6) was obtained from Sigma. When this compound was incubated for one-half hour with 0.02 M β-mercaptoethanol or dithiothreitol at 30°, and then dialyzed in 0.01 M potassium phosphate buffer, pH 7.0, containing 0.1 M KCl or NaCl and 1 mM dithiothreitol for 14-16 hours at 0°, a single peak (1.35S) was obtained on sedimentation velocity centrifugation.

Pigeon liver fatty acid synthetase and 4'-phospho-[^{14}C]pantetheine-labeled pigeon liver fatty acid synthetase were prepared as previously described (4). Subunit I (β-ketoacyl thioester reductase) of the pigeon liver enzyme was also prepared as previously described (5).

Protein was estimated by the methods of Lowry (7) and Murphy and Kies (8), and sedimentation velocity was carried out using an artificial boundary cell with schlieren optics (9). High voltage paper electrophoresis was carried out in a Gilson apparatus in pyridine-acetate buffer. Sulfhydryl groups in the isolated protein were determined with carefully matched cells by the method of Ellman (10).

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[3]Qureshi, A.A., Lornitzo, F.A., and Porter, J.W. Unpublished results.

RESULTS

When 4′-phospho-[^{14}C]pantetheine-labeled Subunit I (10 mg of protein and 30,000 dpm), obtained from an affinity column (5), was allowed to stand overnight in 0.1 M Tris-phosphate buffer, pH 8.5, at 0-4° and then concentrated by means of PM-10 ultrafiltration, 10% of the total radioactivity was found in the filtrate. The filtrate was concentrated further by either lyophilization or by means of a UM-2 ultrafilter to 0.5 ml. The radioactive material was then passed through a 1 × 30 cm Sephadex G-50 column. A single peak of radioactivity and protein was observed (Fig. 1A), at the same volume as found with [1-^{14}C]acetyl-labeled E. coli acyl carrier protein (Fig. 1B). The molecular weight of E. coli acyl carrier protein as determined from its amino acid structure is 8847 (11). Hence, it is concluded from the behavior of the pigeon liver acyl carrier protein on Sephadex G-50 that it has the same, or nearly the same, molecular weight as the E. coli acyl carrier protein.

The [^{14}C]pantetheine-labeled material was then subjected to high voltage electrophoresis on paper at pH 3.7 and 6.5. Under each of these conditions the protein moved as a single sharp band toward the anode (Figs. 2A and B). Similar migrations were obtained with E. coli acyl carrier protein (not shown).

At this point studies were carried out to determine whether acyl carrier protein could be obtained quantitatively from Subunit I of the pigeon liver fatty acid synthetase complex. Subunit I, prepared by affinity column chromatography, was subjected to the conditions effecting reassociation of the fatty acid synthetase complex (5). This protein was subjected to sucrose density gradient centrifugation. The purified Subunit I was collected, concentrated and then dialyzed in Tris:glycine:EDTA:β-mercaptoethanol buffer (5:35:1:10 mM) or in 0.01 M potassium phosphate buffer, pH 7, at 0-4° for 4 hours. The sucrose density gradient step was repeated with the dialyzed protein. Most of the radioactivity was found at the top of the gradient in tubes 29 and 30. This material was also passed through Sephadex G-50 and then subjected to electrophoresis as reported above. It behaved as reported in the previous paragraphs.

The rates of transacylation of acetyl groups from acetyl-CoA and malonyl groups from malonyl-CoA were determined. Five hundred μg of unla-

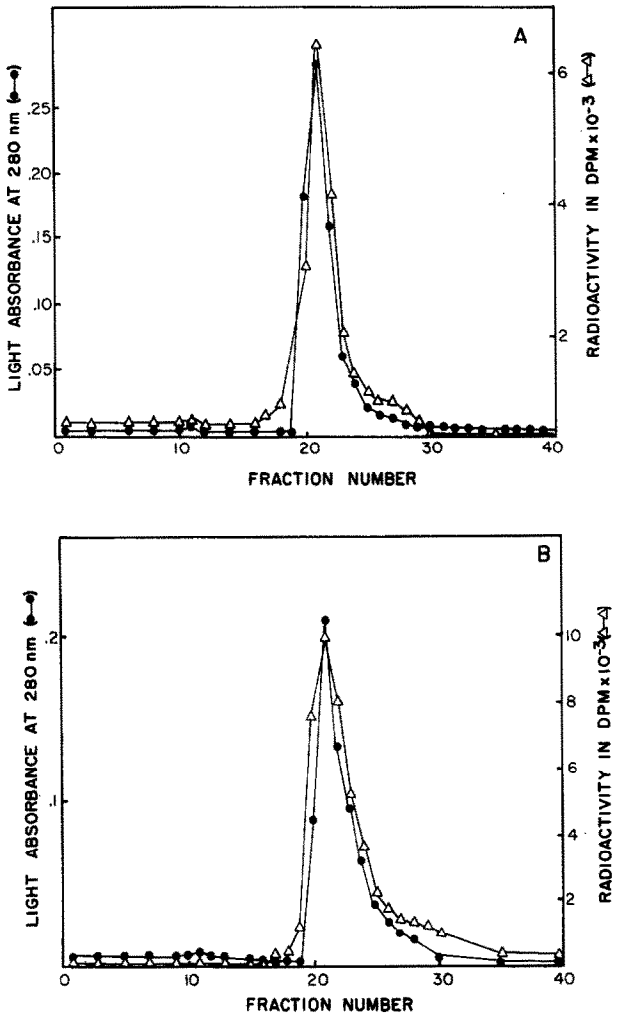

Fig. 1. (A) Molecular filtration on Sephadex G-50 of [^{14}C]pantothenate-labeled acyl carrier protein obtained from the pigeon liver fatty acid synthetase complex. The experimental procedure followed is given in the text. Δ—Δ, radioactivity in dpm; •—•, light absorption at 280 nm. Fractions of 1 ml were collected. (B) Molecular filtration on Sephadex G-50 of [^{14}C]-acetyl-labeled E. coli acyl carrier protein. The [^{14}C]acetyl-labeled acyl carrier protein was prepared by incubation of acyl carrier protein, transacylase (Subunit II) and [1-^{14}C]acetyl-CoA. The [^{14}C]labeled acetyl-acyl carrier protein was then purified by filtration on Sephadex G-50. Δ—Δ, radioactivity in dpm; •—•, light absorption at 280 nm. Fractions of 1 ml were collected.

beled protein (isolated in a manner identical to that of the [^{14}C]pantetheine-labeled protein) were incubated with 12 nmoles of [1-^{14}C]acetyl-CoA (100,000 dpm) or 12 nmoles [1,3-^{14}C]malonyl-CoA (84,000 dpm), and 10 μg of pro-

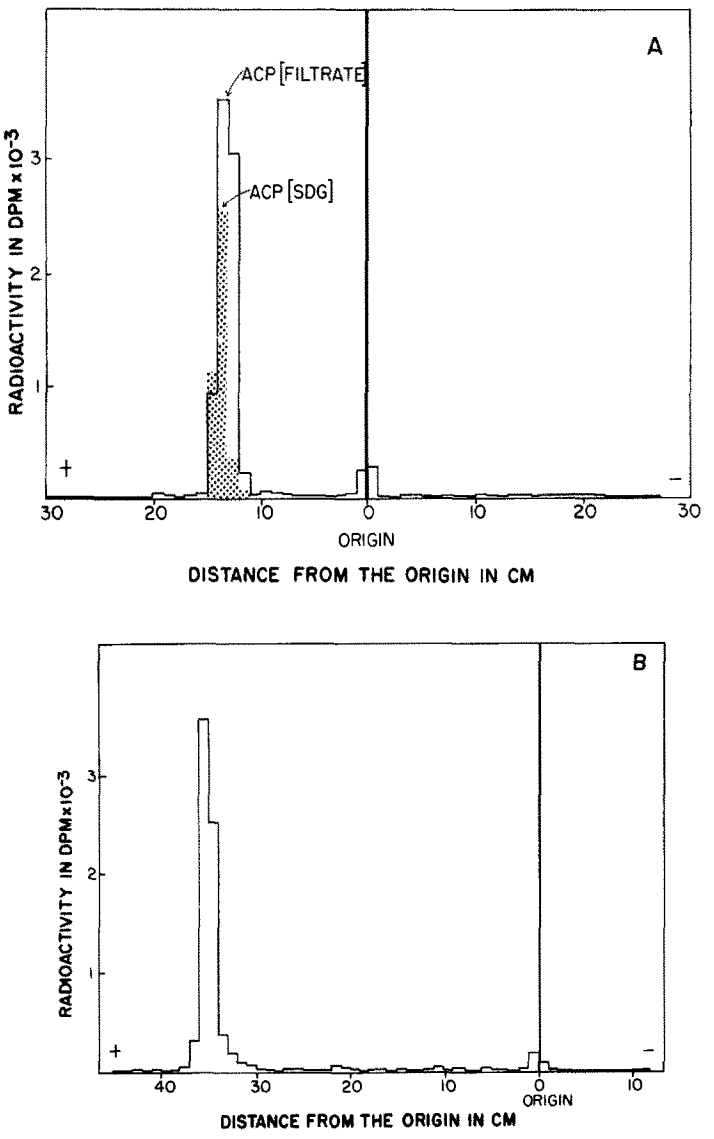

Fig. 2. High voltage electrophoresis of [^{14}C]pantetheine-labeled pigeon liver acyl carrier protein obtained by Sephadex G-50 gel filtration. Electrophoresis was carried out at 2500 V for 90 minutes. Strips of paper of 1 cm x 1" were cut from the electrophorogram and assayed for radioactivity in a Packard liquid scintillation spectrometer. (A) Electrophoresis of a PM-10 ultrafiltration membrane filtrate, —; and a sucrose density gradient centrifugation separated fraction, ---; at pH 3.7. (B) Electrophoresis of a PM-10 ultrafiltration membrane filtrate at pH 6.5.

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tein of pure Subunit II (transacylase) in 1 ml of 0.2 M potassium phosphate buffer at 0°. The reaction was stopped after 5 minutes incubation by adding 30 µl of 60% $HClO_4$. 10 µl of 10% bovine serum albumin was added and the

TABLE I

TRANSFER OF ACETYL AND MALONYL GROUPS FROM ACETYL- AND MALONYL-CoA TO ACYL CARRIER PROTEIN (ACP)

	dpm
1. Acetyl-CoA:	
a. Blank; ACP only, no transacylase	702
b. Pigeon liver ACP (from reductase filtrate)	17,897
c. Pigeon liver ACP (from reductase sucrose density gradient centrifugation)	15,767
d. E. coli ACP	10,989
2. Malonyl-CoA:	
a. Blank; ACP only, no transacylase	205
b. Pigeon liver ACP (from reductase filtrate)	12,084
c. Pigeon liver ACP (from reductase sucrose density gradient centrifugation)	9,176
d. E. coli ACP	7,299

Assays were carried out with ACP, 500 μg protein; acetyl-CoA (12 nmoles and 100,000 dpm); malonyl-CoA (12 nmoles and 84,000 dpm); and transacylase, 10 μg protein, in 1.0 ml of 0.2 M potassium phosphate buffer at 0°C for 5 minutes. The reaction was stopped with 0.05 ml of $HClO_4$, and the precipitated, labeled acyl carrier protein was centrifuged and then washed 3 times with 0.2 M acetic acid. The precipitate was dissolved in phosphate buffer and an aliquot was assayed for radioactivity.

reaction tube was shaken and centrifuged. The precipitate was washed three times with 0.2 M acetic acid and then taken up in 1 ml of 10 mM potassium phosphate buffer, pH 7.0. This material was passed over a Sephadex G-50 column. A single sharp peak of radioactivity was obtained. These fractions were combined and an aliquot was assayed for radioactivity. The remainder of the combined fractions was retained for use in further studies. A similar experiment was carried out with E. coli acyl carrier protein. [It was previously shown by Plate et al. (12) that E. coli acyl carrier protein accepts acetyl and malonyl groups from acetyl- and malonyl-CoA in the presence of pigeon liver fatty acid synthetase complex.] A confirmation of this result with pigeon liver fatty acid synthetase Subunit II (transacylase) is reported in

Table I. Table I also shows that the pigeon liver acyl carrier protein, prepared by two different methods, acts as an acceptor of acetyl and malonyl groups from acetyl-CoA and malonyl-CoA in the presence of the transacylase Subunit II. The activity of the pigeon liver acyl carrier protein as an acceptor of acetyl and malonyl groups appears to be slightly higher than that of E. coli acyl carrier protein. The rate of transacylation with acyl carrier protein is roughly the same as found when pantetheine is used as the acceptor of acetyl and malonyl groups. However, the concentration of acyl carrier protein used is two orders of magnitude lower than that of pantetheine in the standard transacylase assay (5).

Sulfhydryl analyses of the pigeon liver acyl carrier protein showed 1 nmole of sulfhydryl moiety per 8.4 μg of protein, or 1 equivalent per 9000 Daltons. A sample submitted for amino acid analysis showed the presence of 82 amino acids and 1 β-alanine residue.

DISCUSSION

This is the first report[4] of the purification from a source other than E. coli or plants to homogeneity of an acyl carrier protein that accepts acetyl and malonyl groups from acetyl- and malonyl-CoA. This protein is found associated only with Subunit I of the pigeon liver fatty acid synthetase complex and the properties of this protein; i.e., molecular weight, electrophoretic mobility, amino acid composition and ability to accept acetyl and malonyl groups from acetyl- and malonyl-CoA, are the same, or nearly the same, as those reported for E. coli acyl carrier protein.

After Subunit I is separated from Subunit II the conditions required for the isolation of the pigeon liver acyl carrier protein are relatively mild. In contrast, acyl carrier protein was not obtained either by ultrafiltration or by sucrose density gradient centrifugation prior to the separation of the dissociated fatty acid synthetase complex subunits.

[4]Since the completion of this report, acyl carrier proteins have been isolated and purified, in this laboratory, from rat and human liver fatty acid synthetases. The details of this work will be included in a future paper on comparative studies of acyl carrier proteins.

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