Separation of the Half-Molecular Weight Nonidentical Subunits of Pigeon Liver Fatty Acid Synthetase by Affinity Chromatography*

(Received for publication, November 6, 1973)

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SUMMARY

Two half-molecular weight subunits of 4'-phospho[14C]pantetheine-labeled pigeon liver fatty acid synthetase have been separated and shown to be nonidentical. An affinity chromatographic column containing ε-aminocaproypantetheine bound to cyanogen bromide-activated Sepharose via the ε-amino group was used to achieve this separation. The subunit containing 4'-phospho[14C]pantetheine and β-ketoacyl thioester reductase activity is only slightly retarded on this column, whereas the subunit containing acetyl coenzyme A transacylase activity is strongly adsorbed. The latter subunit is eluted from the column with 0.1 M phosphate buffer at pH 10. Each subunit is then purified free of small amounts of the other subunit. This is accomplished by subjecting each to conditions which effect fatty acid synthetase formation. Subsequent sucrose density gradient centrifugation then separates the pure half-molecular weight subunits containing transacylase or β-ketoacyl thioester reductase activity from the small amount of fatty acid synthetase complex. Recombination of the purified subunits under appropriate conditions yields enzymatically active fatty acid synthetase complex.

The pigeon liver fatty acid synthetase complex dissociates reversibly† into two subunits of approximately equal molecular weight (1, 2). However, the separation of these subunits from one another had not been accomplished prior to this communication. Indeed, the similarity of the masses and the mass to charge ratios of the two half-molecular weight subunits is such that the question of their identity or nonidentity was unanswered until it was shown only that one phosphopantetheine group is present per intact fatty acid synthetase molecule (3). This result suggested that the half-molecular weight subunits of 240,000 are of two types which differ by at least the presence or absence of a bound phosphopantetheine group. Therefore, it appeared likely the subunits would have unequal affinity for an external analog of the internal substrate family of acyl 4'-phosphopantetheines. If so, the separation of nonidentical subunits could then be proven by assay of each for the partial reactions of fatty acid synthesis.

Two partial reactions of fatty acid synthesis (Equations 1 and 2) were used in the present investigation to follow the separation of the half-molecular weight subunits of pigeon liver fatty acid synthetase.

β-Ketoacyl thioester reductase:

\[
\begin{align*}
\text{CH}_3\text{C}-\text{CH}_2-\text{C}-\text{S}-\text{CH}_2\text{CH}_2\text{N}-\text{C}-\text{CH}_3 \\
\text{O} & \quad \text{O} & \quad \text{O}
\end{align*}
\]

† TPNI + H⁺ →

\[
\begin{align*}
\text{CH}_3\text{CH}-\text{CH}_2-\text{C}-\text{S}-\text{CH}_2\text{CH}_2\text{N}-\text{C}-\text{CH}_3 + \text{TPN}^+ (1)
\end{align*}
\]

Acyl thioester transacylase:

\[
\begin{align*}
\text{CH}_3\text{C}-\text{C}=\text{O} + \text{HS-pantetheine} \rightarrow \\
\text{CH}_3\text{C}-\text{S-pantetheine} + \text{HSCoA} (2)
\end{align*}
\]

We chose as an affinity ligand in this investigation the substrate analog ε-aminocaproypantetheine bound to Sepharose by means of the amino group. This ligand is analogous to the intermediate caproyl-4'-phosphopantetheine (4). Sepharose ε-amino n caproic acid was prepared according to the method of Larsson and Mosbach (5) from cyanogen bromide-activated Sepharose (6). Crystalline pantetheine was obtained from Sigma Chemical Co., and it was reduced with sodium amalgam to pantetheine prior to use. The pH of the reaction was kept below 7 by the addition of Dowex 50-H⁺. Sepharose ε-aminocaproypantetheine was prepared by the method of Cuztrecuras (7) but with the —SH compound in the solvent and the carbonyl compound in the stationary phase. An amount of ethyldimethylamino-n-propylcarbodiimide sufficient to enable the binding of only 1.5 μmoles of pantetheine to 1 g of gel (wet weight) was used. This is less than the amount given by Cuztrecuras. Larger amounts of the carbodiimide lead to excessive nonspecific (ionic) binding of fatty acid synthetase subunits. Bound pantetheine was assayed by the Ellman method after hydrolyzing the washed Sepharose-ε-aminocaproypantetheine in 0.1 M KOH for 15 min at room temperature.

The Sepharose ε-aminocaproypantetheine was washed with 1 liter of distilled water and then with 150 ml of buffer (pH 8.7) containing 0.1 M potassium phosphate, 0.1 M Tris, and 0.0025 M β-mercaptoethanol or dithiothreitol. The final pH of the solution was 8.1. The Sepharose substrate analog was then packed in an ice-salt-water-jacketed column (3 mm x 15 cm).

*This investigation was supported in part by Research Grant AM01863 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, United States Public Health Service.

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‡ R. A. Meusing, F. A. Lornitzo, S. Kumar, and J. W. Porter, unpublished results.

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and 5 mM β-mercaptoethanol or dithiothreitol (1). The dialyzed, dissociated fatty acid synthetase was diluted with the same buffer to 2 ml and then loaded onto the Sepharose ε-amino-
propyl-pantetheine column at 0–4°C. Elution was carried out with at least 50 ml of a 0.1 M potassium phosphate-0.1 M Tris buffer (pH 8.5) containing 5 mM β-mercaptoethanol at -1.0–0.0°C and then at 25°C with the same buffer adjusted to pH 10 with ammonia. Loading and elution were carried out at a rate of 3 ml per hour and 1-ml fractions were collected. The fractions were monitored for ultraviolet light absorption at 280 nm. 

β-Ketoacetyl thioester reductase in each fraction was assayed spectrophotometrically, with N-acetyl-S-acetoacetyl cysteamine (Sigma) and TPNH as substrates by the method of Kumar et al. (9). Acetyl-CoA-pantetheine transacylase activity was measured radiochemically in a system consisting of 0.03 to 0.05 μg of protein, 3.2 nmoles of [14C]acetyl-CoA (4000 cpm per nmole), and 0.34 μmole of pantetheine in 0.1 ml of 0.2 M potassium phosphate buffer, pH 7.0, at 0°C. The reaction was carried out for 2 to 4 min and then stopped with 20 μl of 2 N acetic acid. The unchanged labeled starting material [14C]acetyl-CoA was removed from the product (acetyl-pantetheine) by passing the reaction mixture through a column (4 mm × 5 cm) of Dowex 1-C2-anion exchange resin in 0.2 N acetic acid. The product, [14C]labeled S-acetylpantetheine was washed from the column with 1.5 ml of 2 N acetic acid. One-half of the combined eluents and washes were added to 15 ml of dioxane-2,5-bis[2-(5-tert-
butylbenzoxazolyl)]thiophene scintillation fluid and then assayed for radioactivity. The course of elution of labeled pantetheine is shown in Fig. 1. About 90% of the β-ketoacetyl thioester reductase is eluted in fractions 20 to 35 with about 10 to 15% of the transacylase. The elution of radioactive pantetheine parallels that of reductase.

Out of 6140 cpm recovered, 5299 cpm appeared in tubes 20 to 35. Fractions 60 to 70 which contained about 70% of the transacylase activity had only 750 cpm. Therefore, it is evident that reductase and transacylase activities are in separate subunits and that 4'-phosphopantetheine is associated with the subunit containing reductase activity.

In order to confirm the enzymatic nonidentity of the subunits and to complete their purification, the following procedure was used. The reductase Fractions 25 to 35 and the transacylase Fractions 62 to 70 were separately concentrated by means of a Diaflo PM-10 membrane filter. The concentrated reductase fraction was then dialyzed 2 hours against 0.2 M potassium phosphate buffer containing 3 mM β-mercaptoethanol at 25°C and the transacylase fraction was dialyzed against 0.2 M potassium phosphate buffer containing 2 mM dithiothreitol. This procedure results in reassociation of unlike subunits to fatty acid synthetase complex. A third dialysis was carried out with a mixture of equal amounts of reductase and transacylase protein. As a control fatty acid synthetase complex was subjected to the same temperature (0–5°C) and time as the above reductase and transacylase fractions. This enzyme complex was then dialyzed under the same conditions as used for the transacylase fraction.

Sucrose density gradient centrifugation was then carried out on each of the above fractions in a Spinco model L-350 ultra-

**Fig. 1.** The separation of the two half-molecular weight subunits of [14C]labeled fatty acid synthetase on a column of Sepharose ε-amino-propyl-pantetheine. Ethyldimethylamino-n-propylcarbodi-

imide, 90 to 100 mg (freshly opened), was reacted with 2.2 g of Sepharose ε-amino-propyl-pantetheine and 0.1 nmole of pantetheine at pH 4.7. The details of the loading and elution of protein and the collection of fractions are as given in the text. Protein (light absorption at 280 nm) in units per ml (□——□); β-keto thioester reductase activity in nanomoles per min per ml (▲——▲); acetyl-CoA-pantetheine transacylase activity in nanomoles per min per ml (●——●); transacylase activity in 0.2 mg of standard fatty acid synthetase complex (14 units (nanomoles per min)). Twice as much enzyme activity was obtained with 0.2 mg of purified reductase (see peak tube, Fraction 25). **, transacylase activity in 0.2 mg of standard fatty acid synthetase complex (35 units (nanomoles per min)). Twice as much enzyme activity was obtained with 0.2 mg of purified transacylase (see peak tube, Fraction 65) after dialysis against 5 mM Dithiothreitol for 3 hours (●——●).

Centrifugations were carried out at 56,000 × g for 44 hours at 4°C in a 5 to 20% w/v sucrose gradient containing 0.1 M potassium phosphate buffer, pH 7.0, 1 mM β-mercaptoethanol or dithiothreitol, and 1 to 2 mg of protein. Fatty acid synthetase, reductase, and transacylase fractions, and the reassociated mixture of each were loaded separately onto 38 ml of gradient. After centrifugation the bottoms of the tubes were punctured and fractions were collected dropwise. Thirty fractions were collected from each tube. The fractions were monitored for protein by ultraviolet light absorption at 280 nm and for fatty acid synthetase, reductase, and transacylase activities. The fractions, 400-μl aliquots, containing 14C-labeled pantetheine and reductase activity were also monitored for radioactivity.

The profiles of fatty acid synthetase complex, reductase, and transacylase fractions, and complex reassociated from a mixture of reductase and transacylase obtained on sucrose density gradient centrifugation are shown in Fig. 2, A to D. The 9 S reductase peak (Fig. 2B) is free from transacylase activity and the 9 S transacylase (Fig. 2C) peak is free from reductase activity. The reassociation of the unlike subunits to complex (Fig. 2D) is virtually complete. A significant amount of fatty acid synthetase activity, determined spectrophotometrically as previously described (9), was also obtained in this fraction on reassociation. This is the first report of the separation of the half-molecular weight subunits of a fatty acid synthetase complex from one another and the recombination of the separated halves with the recovery of fatty acid synthetase activity.

A number of factors are of critical importance in achieving the separations reported in this communication. A low temperature is required to secure the complete dissociation of the fatty acid
Synthetase complex (9) and to prevent reassociation of the subunits. Also, dialysis of the dissociated fatty acid synthetase must not be carried out for more than 12 hours, since some of the enzyme protein is converted to a form which cannot be eluted from the column. Furthermore, the fatty acid synthetase must be frozen at -20° after purification on DEAE-cellulose. If dissociation is effected without freezing, the dissociated subunits are not eluted from the Sepharose substrate analog at 0°. However, they are eluted at room temperature without separation. A slow flow rate on the affinity column is required to ensure binding of the transacylase-containing subunit at the temperature we use. Moderately high salt concentrations, along with a pH of 8.5 or higher, are required to dissociate the bound enzyme protein from the column. At pH 8.5, only one-third of the transacylase activity is eluted from the column, whereas at pH 10 two-thirds or more of this fraction is eluted.

Pantetheine bound to ε-aminocaproic acid is essential for the binding of the transacylase subunit. Neither the binding of this subunit nor the separation of fatty acid synthetase subunits occur on the column when the Sepharose ε-aminocaproic acid is allowed to react with ethyldiaminopropylcarbodiimide in the absence of pantetheine.

β-Ketocarboxyl thioester reductase activity is relatively unstable. Half of the original enzyme activity is lost in the presence of dithiothreitol within 12 hours. Reductase activity can be restored, however, on dialysis for 3 hours in buffer containing freshly prepared dithiothreitol or β-mercaptoethanol. In the presence of β-mercaptoethanol the reductase fraction loses 10% of its activity in 24 hours. The transacylase-containing subunit is stable for 3 days in dithiothreitol, but loses 30% of its activity in 24 hours in β-mercaptoethanol.

Further chemical and physical studies are being carried out on each subunit.

REFERENCES
1. Kumar, S., Dorsey, J. K. & Porter, J. W. (1970) Biochem. Biophys. Res. Commun. 40, 825–832
2. Kumar, S., Muesing, R. A. & Porter, J. W. (1972) J. Biol. Chem. 247, 4749–4762
3. Jacob, E. J., Butterworth, P. H. W. & Porter, J. W. (1968) Arch. Biochem. Biophy. 124, 392–400
4. Nixon, J. E., Phillips, G. J., Abramovitz, A. S. & Porter, J. W. (1970) Arch. Biochem. Biophy. 138, 372–379
5. Larsson, P. & Mostbach, H. (1970) Biotechnol. Bioeng. 14, 394
6. Axen, H., Forath, J. & Eichen, S. (1967) Nature 214, 1302–1304
7. Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059–3065
8. Chesterton, C. J., Butterworth, P. H. W., Abramovitz, A. S., Jacob, E. J. & Porter, J. W. (1968) Arch. Biochem. Biophy. 124, 386–391
9. Kumar, S., Dorsey, J. A., Muesing, R. A. & Porter, J. W. (1970) J. Biol. Chem. 245, 4732–4744
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J. Biol. Chem. 1974, 249:1654-1656.

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