Vol. 84, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 14, 1978 Pages 225-231

THE ISOLATION OF THE TWO SUBUNITS OF YEAST FATTY ACID SYNTHETASE*

James K. Stoops and Salih J. Wakil
Marrs McLean Department of Biochemistry
Baylor College of Medicine, Houston, Texas 77030

Received August 1,1978

SUMMARY: The two subunits that comprise the yeast fatty acid synthetase (designated α and β) have been isolated. The separation was performed using DEAE Biogel A chromatography after first treating yeast fatty acid synthetase with 3,4,5,6 tetrahydrophthalic anhydride. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the fractions eluted from the ion exchange column indicated that the separation of the subunits was essentially complete. It was possible to remove the 3,4,5,6 tetrahydrophthalate derivative from the subunits and regenerate certain of the partial activities. The α subunit was found to have the β-keto reductase activity as well as the acyl carrier protein component associated with it. The β subunit had the acetyl and malonyl transacylases and the palmitoyl transferase activity associated with it. The different extent to which the malonyl and acetyl transacylase activities were regained indicated that these two catalytic sites have separate domains in the β subunit.

INTRODUCTION: It has been shown by genetic (1) and physicochemical studies that the yeast fatty acid synthetase complex (2.4×10^6 molecular weight) consists of two subunits in nearly equal amounts (2). The higher molecular weight subunit (designated α, molecular weight 213,000) is separated from the lower molecular weight subunit (designated β, molecular weight 203,000) by sodium dodecyl sulfate (SDS)[1] polyacrylamide gel electrophoresis (2). However, the small difference between the molecular weights of the two subunits has prevented us and others from obtaining complete separation of the two subunits by this technique (2,3). Furthermore, the use of SDS in the separation of the subunits appears to suffer from the disadvantage of irreversibly inactivating the enzyme since no method is known to obtain active enzyme after treatment with and removal of SDS from the synthetase. Recently, analytical urea polyacrylamide gel electrophoresis has been used to separate the two subunits after first modifying the enzyme with citraconic anhydride and iodoacetate (3). This method suffers from the same drawback as the use of SDS described above since extensive carboxymethylation of the enzyme would be expected to result in irreversible loss of enzyme activity. In this communication, we report the separation of the α and β subunits of the yeast fatty acid synthetase by ion exchange chromatography after first dissociating the complex by

*This investigation was supported in part by the National Science Foundation Grant No. NSF PCM 77-00969, by the United States Public Health Service Grant No. NIH USPH GM 19091-07 and the Welch Foundation Grant No. Q-587.

[1]The abbreviations used are: SDS, sodium dodecyl sulfate; ACP, acyl carrier protein.

0006-291X/78/0841-0225$01.00/0

reaction with 3,4,5,6 tetrahydrophthalic anhydride. The modifying phthalate groups were removed from the α and β subunits and partial enzymic activities were restored to the two separated subunits.

EXPERIMENTAL PROCEDURES: [^{14}C]Pantothenate labeled yeast fatty acid synthetase was prepared and assayed as described previously (2). The enzyme had a specific activity at 25° of 3000 nmol NADPH oxidized min^{-1} mg^{-1} protein.

Treatment of the Enzyme with 3,4,5,6 Tetrahydrophthalic Anhydride: A freshly prepared solution of the anhydride (Aldrich) in p-dioxane was added to the enzyme (5 mg/ml) in 50 mM sodium phosphate, 2 mM 2-mercaptoethanol (pH 8.0) so that the final concentration of the anhydride was nominally 5 mM. The pH was regulated by the addition of 0.5M NaOH during the addition of the anhydride. Lower amounts of the anhydride resulted in poor separation of the subunits by ion exchange chromatography while larger amounts made it impossible to elute the protein from the column.

Column Chromatography: The anhydride treated synthetase (35 mg) was subjected to DEAE Biogel A chromatography. The column (0.8 x 17 cm) was equilibrated with a buffer consisting of 50mM Tris-HCl, 2 mM EDTA, 2 mM 2-mercaptoethanol, and 0.05 M KCl (pH 8.0) and the enzyme was eluted with a linear gradient consisting of 50 ml of this buffer and 50 ml of the same buffer containing 0.5 M KCl. In some cases, DEAE cellulose was substituted for DEAE Biogel A.

Assays for the Partial Enzymic Activities and Pantothenate Content: Malonyl and acetyl transacylases were assayed by measuring the transfer of [1-^{14}C] acetyl or [1,3-^{14}C] malonyl group from CoA to E. coli ACP. The reaction mixture contained 100 μM [1-^{14}C] acetyl-(2 μCi/μmol) or [1,3-^{14}C] malonyl-CoA (2 μCi/μmol), 1-5 μg enzyme, 10 mM dithiothreitol, 0.05 M potassium phosphate, and 150 μg of E. coli ACP in a volume of 0.2 ml (pH, 7.0). The reaction was started by the addition of acetyl- or malonyl-CoA and was terminated after the addition of 5% perchloric acid. The precipated protein was counted with a New England Nuclear Mark II Liquid Scintillation counter as described below in the assay of palmitoyl-CoA transferase. E. coli ACP was prepared as described previously (4).

Protein was determined by the absorbance at 280 nm (2,5) and the radioactivity of the [^{14}C] pantothenate-labeled protein was determined by counting a volume of 1 ml of the protein solution in a Triton-toluene solution described previously (6). SDS-tris glycine polyacrylamide gel electrophoresis was performed as described previously (5).

The palmitoyl-CoA transferase activity was measured as described previously (7) except that 1 mM 2-mercaptoethanol was substituted for glutathione and [^{3}H]CoA (New England Nuclear) 1.3 μCi/μmol was substituted for the [^{14}C]CoA. The precipitated palmitoyl-CoA was collected on a Millipore filter (HAWP02500) which was then placed in a scintillation vial, and 0.1 ml of 0.5M NaOH and 1 ml of methanol was added to the vial, and after 10 min 0.1 ml of 0.75 M acetic acid was added to the vial. Radioactivity was estimated using a Nuclear-Chicago Mark II Scintillation system after adding 10 ml of a solution containing 75% ρ-dioxane, 12.5% 1,2 dimethoxyethane, 12.5% anisole and 0.08% omnifluor to the vial.

The β-keto reductase activity was determined by measuring the oxidation of NADPH at 340 nm (25°). The assay solution contained 0.14 mM NADPH, 60 mM N-acetyl-S-acetyl-cysteamine (Sigma), 0.1 M sodium phosphate, 1 mM EDTA, (pH 7.0).

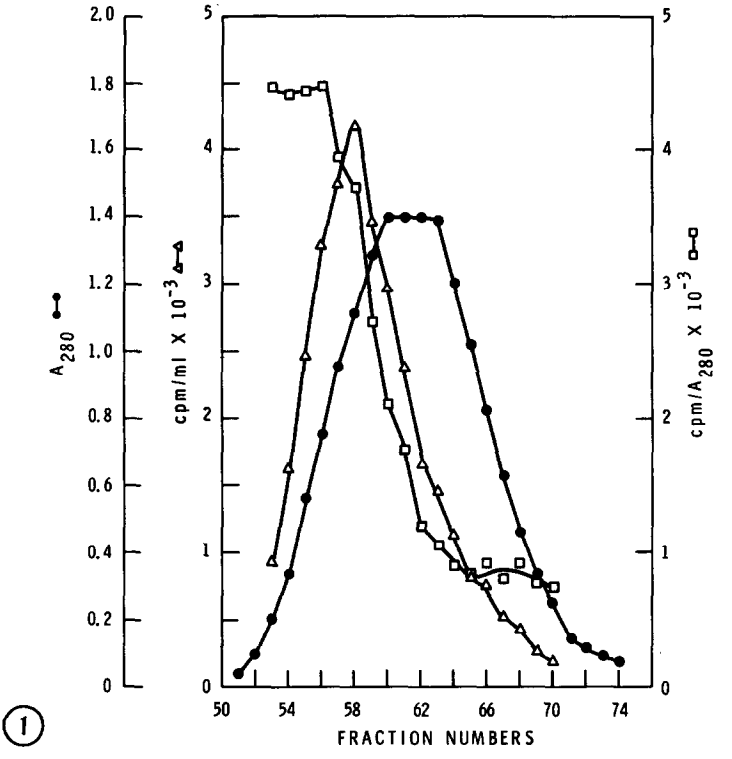
RESULTS AND DISCUSSION: Reaction of the yeast fatty acid synthetase with 3,4,5,6 tetrahydrophalic anhydride as described above results in the immediate (within the 30 minute time period required for measurement) and complete dissociation of the complex into the component subunits as determined by sedimentation velocity studies. The protein sediments as a single peak with a sedimentation coefficient of 4S at 20°C. The dissociation of the complex by reacting it with the anhydride,

then, makes it possible to separate the subunits by ion exchange chromatography. Furthermore, the carboxylic acid derivative of the enzyme has the effect of increasing the charge difference between the two subunits, thus improving the separation of the two subunits by ion exchange chromatography. Since we have previously shown (2) that the α-subunit carries the 4'-phosphopantetheine moiety of the synthetase, [^{14}C]pantetheine labeled fatty acid synthetase was used in order to monitor the separation of the α and β subunits.

As shown in Figure 1, DEAE Biogel A chromatography of the carboxylic acid derivative of the yeast fatty acid synthetase resulted in the separation of the two subunits. The elution profile shows that there is a peak of radioactivity that preceeds somewhat the elution of the protein peak and that the early fractions contain nearly a constant 2.8-fold increase in the specific radioactivity of the protein loaded on the column. This latter result indicates that the α subunit has been separated from the β subunit. Further, the protein eluted near the end of the gradient had lower specific radioactivity indicating that these fractions are enriched in the β subunit. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of these fraction supports these proposals. As can be seen in Figure 2 (gel a) there is nearly equal distribution of the synthetase protein that was subjected to the chromatography between α- and β- subunits. Gel electrophoresis of Fractions 53-54 of Figure 1 show one distinct band corresponding to the mobility of α-subunit (Figure 2, gel b) whereas fractions 64-68 of Figure 1 gives a major band corresponding to the mobility of the β-subunit (Figure 2, gel d) with less than 10% contamination due to the α subunit as determined by a scan of the polyacrylamide gel. Other fractions (58-60) show variable amounts of α and β subunits on these gels (Figure 2, gel c).

The increase in the radiospecific activity of the fractions containing the α subunit by 2.8 fold instead of 2 is unexpected if the yeast complex consist of only the α and β subunits in equal amounts as proposed earlier (2,8). However, we have found that our preparations of the yeast fatty acid synthetase contain 0.7 mols of the prosthetic group pantetheine per two subunits instead of one and we suggested that possibly the lower value may have resulted from partia "nicking" of the α subunit or the presence of an apo-form of this subunit (2)[2]. The present finding of the 2.8 increase in the radiospecific activity of the α subunit then is expected if the α subunit containing the 4'-phosphopantetheine has been separated from the α subunit which contains no pantetheine as well as

[2]The possibility that the carboxylic acid derivative of the protein may alter the A_{280}-mass relationship was ruled out since comparison of the cpm/A_{280} ratio with that obtained by determining protein mass using the procedure of Gornall, et al. (9) gave the ratios that agreed within 10%.



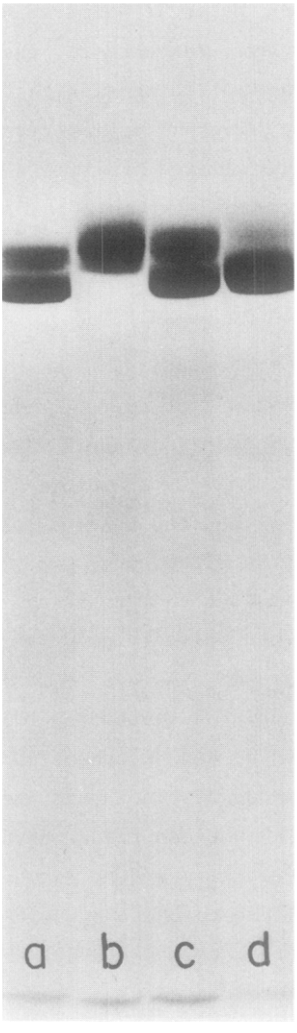


FIGURE 1: DEAE Biogel A chromatography of 3,4,5,6 tetrahydrophthalate derivative of the [^{14}C]pantothenate-labeled yeast fatty acid synthetase. The conditions of the run are described in Experimental Procedures. Fractions (1 ml) were collected. The gradient was initiated at fraction 14 and no protein was eluted until fraction 50. The radiospecific activity of the fatty acid synthetase loaded on the column was 1540 cpm/A_{280}.

FIGURE 2: SDS-tris glycine polyacrylamide gels (5%) of the fractions eluted from the DEAE Biogel A column. The gels were loaded with 10 µg of protein and gels were loaded with (a) the protein subjected to chromatography (b) fractions 53-54 eluted from the column (c) fractions 58-60 eluted from the column and (d) fractions 64-68 eluted from the column. The polyacrylamide gels were stained with coomassie blue (5).

Table 1. Comparison of the Partial Activities Associated with the α and β Subunits with that of the Yeast Fatty Acid Synthetase (nmol min^{-1} mg^{-1}) Specific Activities

Enzymic Activity	α Subunit	β Subunit	Synthetase
Malonyl Transacylase	10	170	200
Acetyl Transacylase	<0.05	0.5	12
Palmitoyl Transferase	2	70	650
β Keto Reductase[a]	560	130	5000

[a]The α and β subunits assayed in this experiment were obtained by DEAE cellulose column chromatography. On SDS polyacrylamide gel electrophoresis the α subunit was free of β, however, the β subunit contained some α subunit.

the β subunit. A number of attempts were made to separate the subunits by substituting DEAE cellulose for DEAE Biogel A. Generally the yield of the α subunit was less than one half that obtained from DEAE Biogel A chromatography and no fractions were obtained which yielded the β subunit essentially free of the α subunit as determined by SDS polyacrylamide gel electrophoresis. However, this substitution was necessary in order to obtain the α subunit with restored β-keto reductase activity (see below).

Restoration of the Partial Activities of the Fatty Acid Synthetase: The subunits isolated from the column do not initially have any of the partial activities associated with the complex. In order to remove the carboxylic acid groups thereby restoring partial activities, fractions 53-54 and 64-68 containing α and β subunits, respectively, were dialysed separately for 24 hr against 0.1 mM sodium phosphate, 2 mM EDTA, and 2 mM 2-mercaptoethanol (pH 7.0). (Even though dialysis against the buffer at pH 6.0-6.5 would be expected to result in more rapid release of the dicarboxylic acid, no restoration of the partial activities was obtained). When the dialysed fractions were assayed for various activities, it was found that the β subunit exhibited a slow increase over a period of a month of the malonyl and acetyl transacylase and palmitoyl transferase activities (Table I). (Polyacrylamide gel electrophoresis showed that the gel patterns in Figure 2 remained unchanged thereby eliminating the possibility that proteolysis had occurred during the storage period). The difference between the extent of the reactivation of the malonyl and acetyl transacylase activities suggest that there are two different active sites catalyzing these reactions. Knobling, et al (1) have made a similar proposal based on genetic studies.

The possibility that these activities which we have assigned to the β sub-

unit result from complexing of the β subunit with the small amount of the α subunit present in these preparations can be ruled out since there was no stimulation of these activities on combining the α and β subunits.

The α subunit has the acyl carrier protein component associated with it as evidenced by increased levels of the radiospecific activity associated with these fractions (Fig. 1). When the α subunit was isolated by DEAE cellulose chromatography and the tetrahydrophthalate was removed, the β-keto reductase activity of this subunit was partially restored (Table I). It is not clear at this time why this activity can be regained after DEAE cellulose chromatography but cannot be similarly regained after DEAE Biogel A chromatography. At any rate, it is apparent that the β-keto reductase activity is associated with the α subunit and the four-fold lower specific activity found associated with the β subunit has probably resulted from the presence of the α subunit in these fractions (Table I). The gels run on fractions containing the β subunit indicated the presence of the α subunit (approximately 30%).

It is apparent from the results summarized in Table I that the α subunit has the acyl carrier protein component and the β keto reductase activity associated with it while the β subunit contains the malonyl and acetyl transacylase and palmitoyl transferase activities. This result is in agreement with the assignment of these activities based on genetic studies (1).

Attempts are being made to restore the other enzymatic activities associated with the α and β subunits as well as the overall fatty acid synthetase activity. If this proves feasible, then it may be possible to gain some insight into what advantage the formation of the large complex imparts to this fatty acid synthesizing system. Furthermore, studies of separated subunits are now possible which may result in a more definitive assignment of how the α and β subunits are arranged in the electron microscope picture of the complex (2).

ACKNOWLEDGMENTS: It is a pleasure to acknowledge the excellent technical assistance of Peggy Ross and Stanley Gunsberg for preparation of the enzyme.

REFERENCES

1. Knobling, A., Schiffman, D., Sickinger, H., and Scweizer, E. (1975) Eur. J. Biochem. 56, 359-367 and references therein.
2. Stoops, J.K., Awad, E.S., Arslanian, M.J., Gunsberg, S., and Wakil, S.J. (1978) J. Biol. Chem., 253, 4464-4475.
3. Engeser, H., Wieland, F., and Lynen, F. (1977) FEBS Letters 82, 139-142.
4. Vanaman, T.C., Wakil, S.J., and Hill, R.J. (1969) J. Biol. Chem. 243, 6420-6431.
5. Stoops, J.K., Arslanian, M.J., Aune, K.C., and Wakil, S.J. (1978) Arch. Biochem. Biophys., 188, 348-359.

6. Stoops, J.K., Arslanian, MlJ., Yang, H.O., Aune, K.C., Vanaman, T.C. and Wakil, S.J. (1975) Proc. Nat. Acad. Sci. USA 72, 1940-1944.

7. Lynen, F. (1969) Methods Enzymol. 14, 17-33.

8. Kresze, G.B., Oesterhelt, D., Lynen, F., Castorph, H., and Schweizer, E. (1976) Biochem. Biophys. Res. Commun. 69, 893-899.

9. Gornall, A.B., Bardawill, C.J., and David, M.M. (1949) J. Biol. Chem. 177, 751-756.