Partial Characterization of Fatty Acid Synthase of *Propionibacterium shermanii*

By PATRICIA M. AHMAD, LYNNE A. STIRLING
AND FAZAL AHMAD*

Papanicolaou Cancer Research Institute, P.O. Box 016188, Miami, Florida 33101, U.S.A.

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Fractionated extracts of *Propionibacterium shermanii* were found to contain the readily dissociable type of fatty acid synthase complex. For optimal activity the partially purified synthase required the presence of acetyl-CoA, malonyl-CoA, NADH and NADPH as well as a cell-free fraction containing the acyl carrier protein (ACP). When ACP-containing fractions obtained from *P. shermanii* grown in the presence of [1-¹⁴C]pantothenate were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis, the radioactivity was located in a peak corresponding to a molecular weight of approx. 8500–9000. The biological activity of the ACP was relatively heat-stable and trypsin-resistant. When the labelled ACP-containing fraction was subjected to gel filtration on a calibrated Sephadex G-75 column, a radioactive peak corresponding to a molecular weight of 22 000 was obtained that could participate in the ¹⁴CO₂–malonyl-CoA exchange reaction in the presence of the other requisite components.

INTRODUCTION

The biosynthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH is catalysed by fatty acid synthases which contain 4'-phosphopantetheine as the prosthetic group (Lynen, 1961; Vagelos, 1974; Bloch & Vance, 1977). The enzymes isolated from a variety of sources catalyse the same type of chemical reactions, but differ in certain molecular properties, especially with regard to the structural organization of the individual enzymic components within the complex (Brindley *et al.*, 1969). The fatty acid synthases isolated from yeast (Lynen, 1961), animal tissues (Stoops *et al.*, 1975; Bloch & Vance, 1977) and certain bacteria (Brindley *et al.*, 1969; Rossi & Corcoran, 1973; Kawaguchi & Okuda, 1977) are of comparatively high molecular weight and individual enzyme activities are present in a tightly associated complex. These synthases are very difficult to dissociate into their constituent enzymes and free acyl carrier protein (ACP). In contrast, the enzyme systems present in *Escherichia coli*, *Clostridium kluyveri* (Vagelos, 1974; Bloch & Vance, 1977) and certain plants (Overath & Stumpf, 1964; Simoni *et al.*, 1967) can be readily fractionated to yield free ACP and the different enzymic components. Therefore, upon fractionation, the synthases belonging to the latter group require ACP for long-chain fatty acid synthesis.

There is a general lack of information on the biosynthesis of long-chain fatty acids from acyl-CoA derivatives in *Propionibacterium shermanii*. Studies concerning fatty acid biosynthesis in this organism are complicated by the presence of methylmalonyl-CoA transcarboxylase (methylmalonyl-CoA:pyruvate carboxyltransferase; EC 2.1.3.1), a biotin-containing enzyme which can catalyse the following exchange reaction (Wood *et al.*, 1969):

In the presence of methylmalonyl-CoA transcarboxylase and/or other enzyme(s) catalysing the above exchange reaction it is difficult to interpret data concerning fatty acid

biosynthesis by following the incorporation of radioactivity from acetyl- and/or malonyl-CoA into the end-product. This problem was circumvented in the present work by conducting fatty acid synthase assays in the presence of avidin, a protein known to inhibit biotin-containing enzymes irreversibly. Our results indicate that the fatty acid synthase of *P. shermanii* is of the readily dissociable type and that there is a small protein that replaces the need for *E. coli* ACP in the fatty acid synthase system of *P. shermanii*.

METHODS

Organism, media and growth conditions. The stock cultures of *Propionibacterium shermanii* were maintained in 'stabs' containing 1.5% (w/v) agar, 1% (w/v) glucose, 2% (w/v) peptone and 1% (w/v) yeast extract and were subcultured periodically and stored at 4 °C. Cells were routinely grown in 4 l conical flasks containing 3.5 l of a medium of the following composition (g l⁻¹): KH₂PO₄, 10.0; Bacto yeast extract, 5.0; Bacto peptone, 5.0; Co(NO₃)₂·6H₂O, 0.016; thiamin·HCl, 0.015; calcium pantothenate, 0.015; D-biotin, 0.0006; Na₂CO₃, 5.5 (autoclaved separately); and glycerol, 30 ml. To this was added 1.3 ml l⁻¹ of a trace element solution of the following composition (mg l⁻¹): H₃BO₃, 750; CuSO₄, 60; NaI, 135; FeSO₄, 662; ZnCl₂, 284; and SeO₂, 166. Growth flasks were inoculated with 80 ml of a vigorously fermenting culture and were incubated at 30 °C. The flasks were shaken manually for a few minutes once daily to ensure adequate suspension of cells. Bacteria were harvested during the late-exponential phase of growth by centrifugation at 10000 g for 10 min and washed twice with 0.2 M-Tris/HCl buffer, pH 7.5. Cell pastes were stored at -20 °C until use.

For studies involving growth of the organism of [1-¹⁴C]pantothenate, cells were grown in a semi-defined medium (Delwiche, 1949) supplemented with [1-¹⁴C]pantothenate [50 µCi l⁻¹; 55 µCi µmol⁻¹ (2.045 MBq µmol⁻¹)].

Preparation of cell-free extracts. Frozen cell pastes (25–35 g) were thawed and suspended in 0.2 M-Tris/HCl buffer, pH 7.5. The cell suspensions were disrupted by sonication (10 × 2 min bursts) in a Heat Systems model W375A sonicator operating at maximum power output while maintaining the temperature below 5 °C. Unbroken cells and cell debris were removed by centrifugation at 37000 g for 30 min at 4 °C.

Fractionation of cell-free extracts. All procedures were carried out at 0–4 °C. Three volumes of 5% (w/v) streptomycin sulphate were added to the crude cell-free extract with stirring, followed by centrifugation at 37000 g for 15 min. The resultant pellet was discarded. Solid ammonium sulphate was added to the supernatant solution and the proteins precipitating at 0–45%, 45–55%, 55–65% and 65–100% saturation were collected by centrifugation. All precipitated protein fractions were retained, dissolved in a minimal amount of 0.2 M-Tris/HCl buffer, pH 7.5 and were quick-frozen in an acetone/dry ice bath prior to storage at -70 °C. Samples were dialysed for 16 h at 4 °C against 0.1 M-triethanolamine/HCl buffer, pH 7.5 containing 1 mM-β-mercaptoethanol to remove ammonium sulphate.

Preparation of ACP-containing fraction from *P. shermanii*. The method used was essentially that of Majerus *et al.* (1969) for the preparation of *E. coli* ACP with the following minor modifications. All procedures were carried out at 0–4 °C. Cells were disrupted by the sonication method already described and then the supernatant solution was diluted to 15 mg protein ml⁻¹ by the addition of 20 mM-triethanolamine/HCl buffer, pH 7.5 containing 10 mM-β-mercaptoethanol. The ACP-containing fractions obtained by this method (Majerus *et al.*, 1969) which had been carried through the purification step involving solubilization of the acid precipitate contained 2–5 mg protein ml⁻¹ and were stored at -20 °C until use.

Fatty acid synthase assay. The assay method used was based on that of Birge & Vagelos (1972). Optimal concentrations of reactants were determined and reaction mixtures contained, in a total volume of 0.25 ml: 10 µmol triethanolamine/HCl buffer, pH 7.5, 0.2 µmol NADP, 1.0 µmol glucose 6-phosphate, 1 unit glucose-6-phosphate dehydrogenase, 0.6 µmol NADH, 70 µg avidin, 0.04 µmol acetyl-CoA, 0.06 µmol [2-¹⁴C]malonyl-CoA (1100 d.p.m. nmol⁻¹), indicated amounts of *P. shermanii* or *E. coli* ACP-fraction protein (reduced prior to use by incubating at 37 °C for 30 min in the presence of 0.25 M-Tris/HCl buffer, pH 8.1 and 50 mM-β-mercaptoethanol), and a suitable amount of protein derived from the 55–65% saturation ammonium sulphate fraction. After incubation at 37 °C for 30 min the reaction was terminated by the addition of 1.0 ml methanolic KOH (15%, w/v) and mixtures were incubated at 85 °C for 45 min. Following the addition of 0.35 ml conc. HCl the free fatty acids were extracted into 2 × 5 ml portions of petroleum spirit (b.p. 20–40 °C). The pooled petroleum fraction was back-extracted with 3 ml 4 M-acetic acid, the aqueous phase was discarded, and the petroleum phase was transferred to scintillation vials and evaporated to dryness in an 80 °C sand bath. The contents of each vial were dissolved in 0.5 ml toluene prior to the addition of 5 ml scintillant (2.0 g Omnifluor l⁻¹ in toluene).

¹⁴CO₂-malonyl-CoA exchange assay. The assay used was based on the method of Majerus *et al.* (1969). Reaction mixtures contained, in a total volume of 0.125 ml: 12.5 µmol imidazole/HCl buffer, pH 6.5, 5.0 µmol β-mercaptoethanol, 0.113 µmol malonyl-CoA, 0.045 µmol hexanoyl-CoA, 35 µg avidin, 0.039 mg *E. coli* Fraction A protein (prepared by the method of Majerus *et al.*, 1969), 3.125 µmol NaH¹⁴CO₃ (3000 c.p.m.

nmol⁻¹) and a suitable amount of the ACP-containing fraction from *P. shermanii*. Reaction mixtures were incubated for 15 min at 37 °C. The reaction was stopped by the addition of 0.035 ml 10% (v/v) perchloric acid. After centrifugation at 2000 g for 5 min, 100 µl samples from the supernatant fraction were transferred to scintillation vials and dried in a forced air oven at 85 °C. Dried samples were dissolved in 0.5 ml water prior to the addition of 5.0 ml Tritosol (Fricke, 1975).

Product identification. The fatty acid products of the synthase reaction (standard reaction mixture scaled up 10-fold) were extracted with petroleum spirit (b.p. 20–40 °C) and converted to their methyl esters by treatment with BF₃/methanol reagent (Supelco). Samples were analysed on a Varian Series 2400 gas-liquid chromatograph equipped with a flame-ionization detector and a coiled copper column (1.83 m × 2 mm internal diameter) packed with 15% diethylene glycol succinate on 10% Chromosorb with a carrier gas flow rate of 45 ml min⁻¹ at 175 °C. Provisional identification of fatty acids was achieved by comparing their retention times with those of standard compounds.

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was performed according to Weber & Osborn (1969) except that, to provide gels that could later be solubilized, *N,N'*-methylenebisacrylamide was replaced by *N,N'*-diallyltartaramide (DATD) as described by Anker (1970). Protein samples in 0.01 M-sodium phosphate buffer, pH 7.0 containing 1.0% (w/v) SDS and 1.0% (v/v) β-mercaptoethanol were denatured by heating at 100 °C for 10 min. The resulting mixtures were then applied to 15% gels and electrophoresis was performed at a constant current of 3 mA per gel in the presence of SDS until the Bromophenol Blue tracking dye had migrated to the opposite end of the gels. Gels were stained overnight in 0.025% (w/v) Coomassie Blue R250 in 10% (v/v) glacial acetic acid/25% (v/v) isopropyl alcohol and were destained in a Bio-Rad 255 destainer containing 30% (v/v) ethanol/10% (v/v) glacial acetic acid. After destaining, the gels were manually sliced into small sections which were placed in scintillation vials containing 0.5 ml 2% (w/v) periodic acid. After allowing the gel slices to solubilize at room temperature for 30 min, 5 ml Aquasol 2 was added to each vial and the radioactivity was determined.

Gel filtration of *P. shermanii* ACP-containing fraction. The ACP-containing fraction prepared from *P. shermanii* grown in the presence of [1-¹⁴C]pantothenate, and dialysed for 2 h against 10 mM-imidazole/HCl buffer, pH 6.5 containing 10 mM-β-mercaptoethanol, was applied (0.4 ml) to a Sephadex G-75 column (0.8 × 43 cm) previously equilibrated with dialysis buffer and calibrated using cytochrome c, chymotrypsinogen and ovalbumin. Fractions of 0.32 ml were collected and monitored for radioactivity. Those fractions that constituted a sharp peak of radioactivity (elution volume approx. 11 ml) were combined, and shown to contain ACP by its requirement in the ¹⁴CO₂-malonyl-CoA exchange reaction. Appropriate controls were also included during the assay.

Determination of radioactivity and protein, and reproducibility of assays. Radioactivity was determined in a Mark III (Searle Analytic) scintillation spectrometer with 75–80% counting efficiency. Assay conditions were selected to ensure that the reaction rate was proportional to the enzyme protein concentration as well as to the time of incubation. Protein was estimated by the biuret method as described by Layne (1957) using bovine serum albumin as standard, or by the method of Bradford (1976) using ovalbumin as standard. The appropriate controls for the assays performed are detailed in the figure and table legends. All assays were done in duplicate with ± 10% variation and variation in activity from batch to batch of cells was negligible.

Chemicals. Acetyl-CoA, malonyl-CoA, NADH and NADPH were obtained from P-L Biochemicals. [1-¹⁴C]Acetyl-CoA [40 µCi µmol⁻¹ (1.482 MBq µmol⁻¹)], [2-¹⁴C]malonyl-CoA [15 mCi mmol⁻¹ (556 MBq mmol⁻¹)], [1-¹⁴C]pantothenate [25 mCi mmol⁻¹ (926 MBq mmol⁻¹)], Omnifluor and Aquasol 2 were purchased from New England Nuclear Corporation. Avidin, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, NADP, trypsin, chymotrypsinogen, ovalbumin, lysozyme and the methyl esters of myristic, palmitic and stearic acids were purchased from Sigma. The methylating agent BF₃/methanol was obtained as a kit from Supelco. All reagents for SDS-polyacrylamide gel electrophoresis, including DATD, were obtained from Bio-Rad. All other reagents were of the highest purity commercially available.

RESULTS

Fractionation of cell-free extracts of P. shermanii and determination of fatty acid synthase activity

Resting cell suspensions of *P. shermanii* readily incorporated radiolabelled acetate into long-chain fatty acids when incubated in the presence of glucose or glycerol under both aerobic and anaerobic conditions. However, when the crude cell-free protein derived from these bacteria was tested for fatty acid synthase activity, only extremely low levels of activity could be detected. Experiments were therefore conducted to determine whether fractionation of *P. shermanii* cell-free extracts with ammonium sulphate would facilitate detection of fatty

Table 1. *Fatty acid synthase activity of ammonium sulphate fractions prepared from cell-free extracts of P. shermanii and the requirement of ACP for activity*

Fatty acid synthase activities were determined with or without ACP as indicated. The *E. coli* and *P. shermanii* ACPs were prepared as described in Methods. The amounts of ACP-fraction protein added were 0.12 mg from *E. coli* and 0.20 mg from *P. shermanii*.

(NH ₄) ₂ SO ₄ fraction (% saturation)	Enzyme activity {nmol [¹⁴ C]malonyl-CoA incorporated (mg protein) ⁻¹ }		
	<i>E. coli</i> ACP	<i>P. shermanii</i> ACP	No ACP
None	0.05	0.05	—
0–45	0.71	0.23	0.06
45–55	1.66	1.60	0.04
55–65	3.12	3.51	0.086
65–100	0.67	0.22	0.77

Fig. 1. Determination of the optimal concentration of *P. shermanii* ACP during fatty acid biosynthesis. The source of fatty acid synthase was the *P. shermanii* cell-free fraction precipitating between 55 and 65% ammonium sulphate saturation.

acid synthase activity. The results given in Table 1 show that fatty acid synthase activity could be readily detected in ammonium sulphate fractions and that the majority of the activity was present in the fraction precipitating between 55 and 65% ammonium sulphate saturation. In order to assay fatty acid synthase activity, an exogenous source of ACP was also required which was initially met by the addition of an ACP fraction prepared from *E. coli*. However, preparation of *P. shermanii* cell-free extracts in a manner analogous to that described for the isolation of *E. coli* ACP yielded a fraction that could effectively replace *E. coli* ACP during fatty acid synthesis catalysed by different *P. shermanii* fractions (Table 1). Subsequent experiments were conducted in the presence of *P. shermanii* ACP.

It is clear from the results of Table 1 that ACP isolated from both *E. coli* and *P. shermanii* was essentially devoid of fatty acid synthase activity.

Conditions for optimal assay

ACP concentration. Assays were conducted in the presence of various amounts of the protein fraction that contained ACP to establish its optimal concentration during fatty acid biosynthesis. Increasing concentrations of the ACP-containing fraction resulted in increased incorporation of [2-¹⁴C]malonyl-CoA into the end-product (Fig. 1). Addition of about 200 μg protein was nearly sufficient to saturate the assay system with respect to ACP and in subsequent experiments this concentration of ACP was used. Reactions were also carried out using the optimal ACP concentration but varying the concentration of protein present in the fraction containing highest fatty acid synthase activity; approximately 400 μg protein was required for optimal activity.

Dual requirement for NADH and NADPH. Fatty acid synthesis was found to proceed in the presence of either NADH or NADPH. At identical concentrations (0.9 mM) the rate of catalysis in the presence of NADPH was about twofold higher than that observed with NADH. For optimal activity, both were added to the reaction mixture, the concentrations being 1.6 mM for NADH and 0.8 mM for NADPH. The effects of these pyridine nucleotides were additive.

Effect of avidin on the incorporation of [1-¹⁴C]acetyl-CoA into fatty acids. As stated in the Introduction the presence of methylmalonyl-CoA transcarboxylase and/or any other biotin-containing enzyme(s) could lead to misinterpretation of data concerning actual fatty acid synthase activity, particularly when measured by the incorporation of radiolabelled acetyl- and/or malonyl-CoA into the end-product. When the assay was conducted in the absence of avidin, radioactivity corresponding to the incorporation of 0.74 nmol [1-¹⁴C]acetyl-CoA was found in the end-product. In the presence of avidin the incorporation decreased to 0.065 nmol which most probably represents the actual rate of fatty acid biosynthesis under these experimental conditions. Since the 55–65% ammonium sulphate fraction contains about 10 units transcarboxylase mg⁻¹, reaction (1) would proceed rapidly. The increased incorporation of radioactivity into the fatty acids in the absence of avidin results from the incorporation of radioactivity not only from acetyl-CoA but also from radiolabelled malonyl-CoA which is formed via reaction (1).

Requirement for acetyl-CoA and malonyl-CoA. The fatty acid synthase of *P. shermanii* showed an absolute requirement for malonyl-CoA. The optimal concentration during assay was estimated at 0.24 mM. Although an absolute requirement for acetyl-CoA could not be demonstrated for this system, optimal reaction proceeded only when the concentration of this acyl-CoA derivative was at least 0.1 mM. In the absence of acetyl-CoA, the rate of fatty acid synthesis dropped to approx. 40% of that observed in its presence. At present it is difficult to offer a plausible explanation for this observation except that the fraction containing fatty acid synthase also catalysed the decarboxylation of malonyl-CoA, albeit at a slow rate [0.17 nmol malonyl-CoA decarboxylated min⁻¹ (mg protein)⁻¹ at 37 °C]. The concentration of acetyl-CoA formed by the decarboxylation of malonyl-CoA probably provides the acetyl units for the acetyl transacetylase partial reaction of fatty acid synthase.

*End-product of *P. shermanii* fatty acid synthase reaction.* The end-product could be extracted into petroleum spirit only after the saponified reaction mixtures had been acidified. This observation indicated that free fatty acid(s) was not the end-product of the *P. shermanii* fatty acid synthase reaction. Further characterization of the end-product [fatty acyl-ACP complexes and/or fatty acyl-CoA derivative(s)] was not attempted. The long-chain fatty acyl moieties of the synthase end-product were identified as myristic acid (19%), palmitic acid (68%) and stearic acid (13%).

Characterization of *P. shermanii* ACP

The overall fatty acid synthase reaction of *P. shermanii* has been shown to proceed in the presence of a fraction which contains ACP-like activity (Table 1). The ¹⁴CO₂-malonyl-CoA exchange reaction is considered to be a sensitive method for the detection of free ACP (Majerus *et al.*, 1969). Therefore, experiments were conducted to obtain additional evidence for the presence of free ACP in fractions derived from cell-free extracts of *P. shermanii*. The optimal assay conditions for the detection of ACP by the *E. coli* system have been described (Majerus *et al.*, 1969). Using this method, the participation of the *P. shermanii* fraction in the ¹⁴CO₂-malonyl-CoA exchange reaction was readily demonstrated (Table 2).

The effect of heat treatment and trypsin on the biological activity of *P. shermanii* ACP were also studied. Approximately 55% of the activity was lost on heating the ACP for 3 min at 100 °C. In contrast, trypsin-treatment had a less deleterious effect on the ability of ACP to promote the ¹⁴CO₂-malonyl-CoA exchange reaction. Exposure to trypsin (1.5 mg per

Table 2. Evidence for the requirement of *P. shermanii* ACP in the $^{14}\text{CO}_2$ -malonyl-CoA exchange reaction

The source of fatty acid synthase activity was Fraction A from *E. coli*, prepared according to the method of Majerus *et al.* (1969). For each of the assays, 0.075 mg protein of *P. shermanii* ACP-containing fraction was used.

Assay mixture	^{14}C Malonyl-CoA formed (nmol)
Complete	0.715
— source of fatty acid synthase	0.051
— malonyl-CoA	0.083
— hexanoyl-CoA	0.060
— malonyl-CoA and hexanoyl-CoA	0.032
— source of ACP	0.040

Fig. 2

Fig. 3

Fig. 2. Determination of the molecular weight of *P. shermanii* ACP. The ^{14}C -labelled ACP-containing fraction was prepared from *P. shermanii* grown in the presence of $[1-^{14}\text{C}]$ pantothenate as described in Methods. Gels of 15% polyacrylamide were used, and 20–40 μg protein was applied to each gel following denaturation in the presence of SDS and β -mercaptoethanol. For the ACP-containing fraction, 7712 c.p.m. were applied and 5375 c.p.m. were recovered (b). Proteins of known molecular weights were used to construct the standard curve shown in (a): 1, ovalbumin; 2, chymotrypsinogen; 3, lysozyme; 4, cytochrome c; 5, bovine trypsin inhibitor; the arrow indicates the relative mobility of the radioactive peak of (b).

Fig. 3. Participation of *P. shermanii* ACP in the $^{14}\text{CO}_2$ -malonyl-CoA exchange reaction. Various amounts of the radiolabelled pooled fraction obtained after Sephadex G-75 chromatography were used in the $^{14}\text{CO}_2$ -malonyl-CoA exchange reaction. Fraction A from *E. coli* (0.039 mg) was used as the source of fatty acid synthase. Appropriate corrections were made for the radioactivity present in the samples containing ACP.

100 mg ACP-fraction protein) for up to 2 h led to approx. 15–17% loss in the biological activity of *P. shermanii* ACP.

An attempt was made to determine the molecular weight of *P. shermanii* ACP. Since the 4'-phosphopantetheine moiety of ACP is derived from pantothenic acid, bacteria were grown in the presence of $[1-^{14}\text{C}]$ pantothenate to facilitate the location of ACP. The ^{14}C -labelled

bacteria were disrupted and the cell-free extract was fractionated. A sample of the protein fraction exhibiting ACP activity (Tables 1 and 2) was treated with SDS in the presence of β -mercaptoethanol and then electrophoresed in polyacrylamide gel columns in the presence of SDS. An example of such an experiment is presented in Fig. 2. More than 70% of the radioactivity applied to the gel was recovered following electrophoresis. The major portion of the label was located in a gel segment where protein(s) of molecular weight 8500–9000 were found to migrate.

In addition, the radiolabelled ACP-containing fraction (0.4 ml = 1.5 mg protein) was chromatographed on a calibrated Sephadex G-75 column. A radioactive peak emerged at an elution volume of 10.88 ml, which corresponded to a molecular weight of approx. 22 000. The fractions comprising this peak were combined and tested for their ability to promote the $^{14}\text{CO}_2$ -malonyl-CoA exchange reaction in the presence of *E. coli* Fraction A. Addition of increasing amounts of the pooled fractions from the Sephadex G-75 column caused a proportional increase in the rate of $^{14}\text{CO}_2$ -malonyl-CoA exchange reaction (Fig. 3). Besides these results, the data given in Tables 1 and 2 provide reasonably reliable evidence for the presence of free ACP in the cell-free extracts of *P. shermanii*. These results also indicate that during fractionation of cell-free extracts with ammonium sulphate, the ACP of *P. shermanii* readily separates from the enzymic components of fatty acid synthase.

A sample of the pooled fractions from the Sephadex column was denatured, reduced and then subjected to polyacrylamide gel electrophoresis in the presence of SDS. The radioactivity was again located in a band of molecular weight 8500–9000. Therefore, its elution behaviour from the Sephadex column (mol. wt 22 000) might indicate an asymmetric nature for *P. shermanii* ACP. Another possibility is that the ACP may undergo dimerization under certain conditions.

DISCUSSION

The fatty acid biosynthetic system of *P. shermanii* has been studied in detail for the first time. Data concerning the apparent fatty acid synthetic ability of this organism can be misleading due to the presence of large amounts of methylmalonyl-CoA transcarboxylase. The inclusion of avidin during the assays allows more reliable measurement of fatty acid synthase activity in the presence of biotin-containing enzyme(s) catalysing reaction (1).

The fatty acid synthase of *P. shermanii* resembles that of *E. coli* in a number of aspects. The majority of the activity in *P. shermanii* was found in the protein fraction precipitating between 55 and 65% ammonium sulphate saturation. Overall activity could only be detected when an exogenous source of free ACP was included in reaction mixtures. Free ACP is encountered in those organisms which employ individual reactions rather than a multienzyme aggregate for fatty acid synthesis. Therefore the fatty acid synthase of *P. shermanii*, like that of *E. coli*, appears to be of the readily dissociable type. The results presented here do not exclude the possible existence of fatty acid synthase as a complex in vivo which dissociates during cell rupture. A protein fraction derived from the crude cell-free extract of *P. shermanii* was found to participate in both the overall reaction and the $^{14}\text{CO}_2$ -malonyl-CoA exchange reaction, thus substantiating the claim that this fraction contains the free ACP necessary for fatty acid biosynthesis. The biological activity of *P. shermanii* ACP was not modified by trypsin. Majerus (1968) and Majerus *et al.* (1969) found that trypsin also did not affect the ability of *E. coli* ACP to function in fatty acid biosynthesis. The activity of *P. shermanii* ACP, however, was not as stable to heat as has been observed for the ACPs purified from *E. coli* and *Mycobacterium phlei* (Majerus, 1967; Majerus *et al.*, 1969; Matsumara *et al.*, 1970). The reason for this difference is not yet known.

The molecular weight of *P. shermanii* ACP, as estimated by monitoring the radioactivity derived from labelled pantothenic acid in fractions applied to SDS-polyacrylamide gels, was found to be in the range 8500–9000. The *E. coli* ACP has been purified to homogeneity and

has a molecular weight of 8847 (Vanaman *et al.*, 1968) and *Arthrobacter* ACP has a molecular weight of 9200 (Simoni *et al.*, 1967), indicating that the ACPs from a number of sources are of comparable size. They also function interchangeably, e.g. *E. coli* ACP can function in the fatty acid biosynthetic system of *P. shermanii* and vice versa.

The requirements and optimal conditions for assaying the fatty acid synthase activity from *P. shermanii* have been established and the end-products of the reaction, primarily C₁₆ and C₁₈ saturated fatty acids, have been provisionally identified. There is an atypical requirement for both NADH and NADPH for optimal fatty acid synthase activity. Dual reduced pyridine nucleotide requirements for fatty acid synthases have been documented in the multienzyme complexes from *Saccharomyces cerevisiae*, *Euglena gracilis* and *Mycobacterium phlei* by White *et al.* (1971) who have examined the *Mycobacterium phlei* system in some detail. The effect of both nucleotides in this system was synergistic. NADPH alone supported long-chain fatty acid synthesis more effectively than did NADH and the nucleotides were assigned to specific reduction steps, NADPH being the specific coenzyme for the β -ketoacyl reductase while NADH was preferentially utilized in α,β -enoyl reduction. We cannot at this time assign a specific nucleotide to a specific reductive step in the *P. shermanii* system.

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