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Purification and characterization of [acyl-carrier-protein] acetyltransferase from *Escherichia coli*

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A multi-step procedure has been developed for the purification of [acyl-carrier-protein] acetyltransferase from *Escherichia coli*, which allows the production of small amounts of homogeneous enzyme. The subunit M_r was estimated to be 29000 and the native M_r was estimated to be 61000, suggesting a homodimeric structure. The catalytic properties of the enzyme are consistent with a Bi Bi Ping Pong mechanism and the existence of an acetyl-enzyme intermediate in the catalytic cycle. The enzyme was inhibited by *N*-ethylmaleimide and more slowly by iodoacetamide in reactions protected by the substrate, acetyl-CoA. However, the enzyme was apparently only weakly inhibited by the thiol-specific reagent methyl methanethiosulphonate. The nature of the acetyl-enzyme intermediate is discussed in relationship to that found in other similar enzymes from *E. coli*, yeast and vertebrates.

INTRODUCTION

The pathway of fatty acid biosynthesis has been well elucidated in both prokaryotes and eukaryotes (Vagelos, 1973; Volpe & Vagelos, 1976; Fulco, 1983; Wakil *et al.*, 1983; Schweizer, 1986; Hardie & McCarthy, 1986). Two distinct classes of molecular organization of enzyme activities catalysing the reactions necessary for synthesis of long-chain fatty acids occur. In many bacteria and plants the Type II fatty acid synthetase system exists, in which each type of chemical reaction involved in fatty acid synthesis is catalysed by a discrete monofunctional polypeptide. Type I systems, which occur in other organisms, are multifunctional enzyme complexes of M_r 400000 to over 2000000. Much of the early work on the elucidation of the pathway was carried out on the Type II system found in *Escherichia coli*. However, in recent years attention has centred on the multifunctional proteins from fungi and vertebrates, where a combination of elegant protein chemistry, molecular biology and genetics have given detailed insight into their structure and function (Wakil *et al.*, 1983; Schweizer, 1986; Hardie & McCarthy, 1986). Except for the *E. coli* acyl-carrier protein (ACP), most of the *E. coli* enzymes no longer attract much attention.

The initial reactions of fatty acid synthesis are the transfers of acetate and malonate from CoA to ACP which, in *E. coli*, are catalysed by two quite specific transacylases, [acyl-carrier-protein] acetyltransferase (EC 2.3.1.38, acetyl-CoA:[acyl-carrier-protein] *S*-acetyltransferase) and [acyl-carrier-protein] malonyltransferase (EC 2.3.1.39, malonyl-CoA:[acyl-carrier-protein] *S*-malonyl transferase). In yeast, these two enzymes activities are catalysed by different domains of the same multifunctional protein, whereas in vertebrates both activities are catalysed at the same active site.

Although the *E. coli* [ACP] malonyltransferase has been purified to homogeneity (Joshi & Wakil, 1971), the *E. coli* [ACP] acetyltransferase has only previously been partially purified (Alberts *et al.*, 1964, 1969; Williamson & Wakil, 1966). In the present paper, we report the complete purification of the latter enzyme and describe some of its properties.

EXPERIMENTAL

Purification of [ACP] acetyltransferase from *E. coli*

All steps were at 0–4 °C, and all buffers contained 1 mM-dithiothreitol.

Step 1: preparation of cell-free extract. Frozen *E. coli* B cells (65 g wet wt.) were stirred with 130 ml of ice-cold 50 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA, until thawed. The cells were broken by sonication in 40 ml batches in a 50 ml beaker for 2 min in 30 s bursts by using a MSE sonicator with a 0.5 cm-diam. probe at full power. Cell breakage was monitored by release of aspartate aminotransferase and NADH oxidase activity. The sonicated material was centrifuged at 124000 ***g*** for 60 min. The supernatant was retained.

Step 2: $(NH_4)_2SO_4$ fractionation. The supernatant (155 ml) was adjusted to 35% saturation with 30.5 g of $(NH_4)_2SO_4$. After stirring for 30 min, the mixture was centrifuged at 10000 ***g*** for 10 min. The pellet was discarded and the supernatant (163 ml) adjusted to 80% saturation by addition of 48 g of $(NH_4)_2SO_4$. After 30 min, the centrifugation was repeated. The supernatant could be retained for purification of ACP from it. The pellet was resuspended in a minimum volume of 50 mM-potassium phosphate buffer (pH 7.4)/1 mM-EDTA, and the suspension transferred to a dialysis bag. After dialysis for 1 h against 1000 ml of the same buffer, the protein had dissolved, yielding 34 ml of a dark-yellow solution.

Step 3: first gel filtration. The solution was applied to a column of Sephacryl S-200 (88 cm × 2.6 cm), eluted

Abbreviation used: ACP, acyl-carrier protein.

with the same buffer at 22 ml/h. Fractions of volume 7.3 ml were collected. The acetyltransferase activity was eluted after the peak of acetyl-CoA hydrolase, but complete separation was not achieved. The bulk of the fractions containing acetyltransferase activity were pooled and concentrated to a volume of 40 ml in an Amicon ultrafiltration cell fitted with a Diaflo PM10 membrane. The solution was then dialysed for 2 h against 1000 ml of 20 mM-Tris/HCl, pH 7.4.

Step 4: chromatography on DEAE-cellulose DE-52. The enzyme solution was applied to a column of DEAE-cellulose DE-52 ion-exchanger (20 cm × 2.6 cm) equilibrated in 20 mM-Tris/HCl, pH 7.4. The column was washed with this buffer for 100 min at a flow rate of 0.69 ml/min, and the enzyme was eluted by application of a gradient consisting of 400 ml of start buffer and 400 ml of the same buffer containing 0.7 M-KCl. The acetyltransferase activity was eluted with approx. 0.27 M-KCl, which was similar to the concentration that eluted acetyl-CoA hydrolase activity. The acetyltransferase-containing fractions were pooled and concentrated, as and concentrated to a volume of 8 ml.

Step 5: second gel filtration. The enzyme solution was re-applied to the column of Sephacryl S-200, as in step 3, and the eluted acetyltransferase, now largely freed from contamination with acetyl-CoA hydrolase, was pooled and concentrated to a volume of 8 ml.

Step 6: chromatography on Mono Q. The enzyme was applied to a column of Mono Q exchanger (10 cm × 1 cm diam.), equilibrated in 20 mM-Tris/HCl, pH 7.4. The column was washed for 10 min with this buffer at a flow rate of 5 ml/min. Then, over 60 min, a gradient of 0–0.4 M-KCl in buffer was applied. Acetyltransferase activity was eluted at approx. 0.26 M-KCl. The enzyme was concentrated to a volume of 2.7 ml and dialysed for 4 h against 1000 ml of 10 mM-potassium phosphate buffer, pH 7.4.

Step 7: chromatography on Blue Sepharose. The acetyltransferase was applied to a column of Blue Sepharose (5.8 cm × 1 cm) equilibrated with the same buffer. The column was eluted at 0.28 ml/min with 14 ml of equilibration buffer, followed by a linear gradient over 140 min of 0–1 M-KCl in the same buffer. Acetyltransferase was eluted as a broad peak with approx. 0.5 M-KCl. The enzyme-containing fractions were pooled and concentrated to a volume of 2.8 ml, and dialysed overnight against 1000 ml of 10 mM-potassium phosphate buffer, pH 7.0.

Step 8: fractionation on hydroxyapatite. The enzyme was then applied to a column of Bio-Gel HPHT hydroxyapatite (100 cm × 0.78 cm, fitted with a 50 cm × 0.4 cm guard column) equilibrated and eluted with 10 mM-potassium phosphate, pH 6.95, containing 0.01 mM-$CaCl_2$. The enzyme was only weakly retained by the column, and most of the protein was eluted by washing the column with 0.5 M-potassium phosphate buffer, containing 0.01 mM-$CaCl_2$ (Fig. 3). The acetyltransferase-containing fractions were concentrated to approx. 1 ml.

Step 9: chromatofocusing. The enzyme was dialysed for 70 min against 600 ml of 25 mM-Bistris/HCl, pH 5.75, and then applied to a column of Mono P exchanger (20 cm × 0.5 cm), equilibrated in the same buffer. The enzyme was eluted at a flow rate of 1 ml/min with a 1:10 dilution of Polybuffer 74, adjusted to pH 3.8 with HCl. The acetyltransferase, which was eluted at approx. pH 4.75, was concentrated to a volume of 0.56 ml.

Step 10: chromatography on TSK G-3000. The enzyme was applied to a column of TSK G-3000SW (600 mm × 7.5 mm) fitted with a guard column (75 mm × 7.5 mm), and eluted with 50 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA at a flow rate of 0.5 ml/min. The acetyltransferase was eluted at 38 min as a symmetrical peak of activity whose profile corresponded with that of a band of M_r approx. 30000 by SDS/polyacrylamide-gel electrophoresis. This was the only protein visible in the peak fractions.

Production of larger amounts of highly purified acetyltransferase for the kinetic studies was achieved by omitting steps 7, 9 and 10. Approx. 5000-fold purified protein could be obtained, with a 10% recovery of activity.

Purification of ACP from *E. coli*

ACP was purified from 200 g batches of *E. coli* B cells by the following modification of the procedure of Rock & Cronan (1981). After step 2, the crude ACP-containing solution was dialysed overnight against 1000 ml of 5 mM-Tris/HCl, pH 6.8, containing 1 mM-dithiothreitol, and freeze-dried. The sample was dissolved in 2–3 ml of 50 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-dithiothreitol and 1 mM-EDTA, and applied to a column of Superose 12 (preparative grade) packed in a Pharmacia HR16/50 column. The column was run at 0.5 ml/min in the same buffer. If more than 60 mg of protein was present, two runs were made. The presence of ACP was detected either by non-denaturing polyacrylamide-gel electrophoresis (Rock & Cronan, 1981) or by its activity as a substrate in the *E. coli* [ACP] acetyltransferase assay. The ACP-containing fractions were pooled and applied to a column of Mono Q exchanger (10 cm × 1 cm diam.) equilibrated in 20 mM-potassium phosphate buffer, pH 6.3, containing 1 mM-dithiothreitol, at a flow rate of 5 ml/min. The ACP was eluted by applying a gradient between 0 and 20 min of 0–0.5 M-KCl, in the start buffer. ACP was eluted at about 0.42 M-KCl. The ACP could be used at this stage, but for the kinetic experiments the ACP-containing fractions were pooled, dialysed against 800 ml of 5 mM-potassium phosphate buffer, pH 7, containing 1 mM-dithiothreitol, and freeze-dried. The sample was dissolved in 0.7 ml of water and applied to the column of Superose 12 as above. The eluted protein was free from contamination with 260 nm-absorbing material. The concentration of pure ACP was determined by using $\epsilon_{280} = 1.8 \times 10^3$ litre·mol^{-1}·cm^{-1}.

Determination of M_r by gel filtration and SDS/polyacrylamide-gel electrophoresis

The native M_r of [ACP] acetyltransferase was estimated by gel filtration on a Superose 12 (preparative grade) column run and eluted as above. The column was calibrated with ribonuclease A (bovine pancreas, M_r 13700), chymotrypsinogen A (bovine pancreas, M_r 25000), ovalbumin (M_r 43000), 3-dehydroquinase (M_r

52750), bovine serum albumin (M_r 67000), aspartate aminotransferase (pig heart, M_r 90000), aldolase (rabbit muscle, M_r 158000) and catalase (M_r 232000).

The subunit M_r was estimated by SDS/polyacrylamide-gel electrophoresis, calibrated with SDS-6H and SDS-VII L M_r marker protein mixtures obtained from Sigma.

Assay of [ACP] acetyltransferase activity

[ACP] acetyltransferase activity was measured as the production of acid-insoluble radiolabelled acetyl-ACP, by using a modification of the method of Alberts *et al.* (1969). The incubation mixture was 30 μl of 0.33 M-potassium phosphate buffer, pH 6.5, containing 13.3 mM-dithiothreitol and 13.3 mM-EDTA, 10 μl of 0.5 mM-ACP, enzyme-containing sample, and water to give a final volume of 90 μl, in a 1.5 ml plastic Eppendorf tube. The reaction was started by addition of 10 μl of 1 mM-[^{3}H]acetyl-CoA (5–30 Ci/mol). After incubation at 30 °C for 12 min, the reaction was stopped by addition of 1 ml of ice-cold 0.015% (w/v) sodium deoxycholate, followed immediately by 0.1 ml of ice-cold 72% (w/v) trichloroacetic acid (Peterson, 1977). The reaction mixture was left at 4 °C for 10 min, and then centrifuged at 10000 ***g*** for 10 min. The supernatant was carefully removed, and the pellet washed by addition of 1 ml of ice-cold 7.2% trichloroacetic acid, followed by centrifugation at 10000 ***g*** for 2 min. The supernatant was again discarded and the washing procedure repeated. The pellet was then dissolved by addition of 1 ml of 2.5% (w/v) SDS dissolved in 0.2 M-NaOH. A sample (0.9 ml) of this was counted for radioactivity by liquid scintillation.

For kinetic measurements, the concentrations of [^{3}H]acetyl-CoA and ACP were varied. In all assays the amount of enzyme used was such that the rate of incorporation of [^{3}H]acetyl groups into ACP was linear with time for at least 12 min under the assay conditions.

With crude enzyme, it was essential to run blanks without ACP, as radioactivity was trapped by the large amounts of protein precipitated. With purified enzyme, blanks without ACP or without enzyme gave negligible radioactivity.

Other enzyme assays

Acetyl-CoA hydrolase was assayed either from the rate of decrease of A_{232} by addition to 100 μM-acetyl-CoA dissolved in 0.1 M-potassium phosphate buffer, pH 6.5, assuming a change of ϵ_{232} of 4250 litre·mol^{-1}·cm^{-1} for the hydrolysis of the thioester, or from the rate of increase of A_{412} by addition to a reaction mixture containing 0.3 mM-5,5′-dithiobis(2-nitrobenzoic acid) and 100 μM-acetyl-CoA dissolved in 0.1 M-potassium phosphate buffer, pH 6.5, assuming ϵ_{412} = 12800 litre·mol^{-1}·cm^{-1}. Both assays were performed at 30 °C and gave identical rates of acetyl-CoA hydrolysis.

Aspartate aminotransferase activity was assayed by the method of Morino *et al.* (1977).

[Pantotheine] malonyltransferase activity was measured spectrophotometrically essentially as described by Caughey & Kekwick (1982), except that the pH of the buffer was 6.5 rather than 7.0. In this method the formation of malonyl-pantotheine and CoA is coupled to the formation of acetyl-CoA from acetyl phosphate by phosphotransacetylase. It is assumed that this activity is a measure of the [ACP] malonyltransferase activity.

Yeast fatty acid synthase activity was measured spectrophotometrically essentially as described by Lynen (1962), by monitoring the reaction at 340 nm, and omitting cysteine and bovine serum albumin.

Inactivation by chemical modification reagents

E. coli [ACP] acetyltransferase (purified by steps 1–6 and 8), crude *E. coli* [pantotheine] malonyltransferase and yeast fatty acid synthase fractions were freed of dithiothreitol by centrifuge gel-filtration on columns of Sephadex G-25 (superfine) equilibrated in 0.1 M-potassium phosphate buffer, pH 6.5, by the method of Penefsky (1977). Samples of enzyme (100 μl) were incubated with the chemical modification reagent in the absence or the presence of 100 μM-acetyl-CoA for 10 min at either 4 °C or 30 °C. The mixtures were then subjected to centrifuge gel-filtration again, and samples of the eluate were assayed for [ACP] acetyltransferase, [pantotheine] malonyltransferase and fatty acid synthase activities as appropriate. When methyl methanethiosulphonate was the reagent used, the assay mediums did not contain dithiothreitol, and the ACP was also centrifuge gel-filtered to ensure that free thiols were not present.

Other methods

Protein was determined by the method of Bradford (1976) with γ-globulin as standard, except after steps 8–10 of the purification, where protein was estimated from the A_{280}, by assuming $A_{1\,\mathrm{cm}}^{0.1\%} = 1$.

Polyacrylamide-gel electrophoresis in the presence of SDS was performed by the method of Anderson *et al.* (1973), with a running gel containing 19.5% acrylamide and 0.087% *NN′*-methylenebisacrylamide. Gels were stained with Coomassie Blue R250 and then with a silver stain (Morrissey, 1981).

Kinetic parameters (K_m and K_i) were obtained by using the computer program and statistical methods described by Lowe & Rowe (1987).

Materials

Mono Q, Mono P, Superose 12 (preparative grade), Sephacryl S-200 and Blue Sepharose were obtained from Pharmacia; Bio-Gel HPHT hydroxyapatite was from Bio-Rad Laboratories, and TSK G-3000 SW from LKB. *E. coli* B cell paste was purchased from Porton Products, Porton Down, Wilts., U.K.

D-Pantotheine was prepared by borohydride reduction of D-pantothine, and was quantified by reaction of its thiol group with 5,5′-dithiobis-(2-nitrobenzoic acid).

Fatty acid synthase fraction was prepared from brewer's yeast, by breakage of cells in a French pressure cell, and two rounds of $(NH_4)_2SO_4$ fractionation and differential high-speed centrifugation to pellet the enzyme. The specific activity of the preparation was 0.26 μmol·min^{-1}·mg^{-1} in the fatty acid synthase assay.

E. coli [pantotheine] malonyltransferase was partially purified from *E. coli* B cells by using steps 1–3 of the purification procedure for [ACP] acetyltransferase.

RESULTS

Purification of [ACP] acetyltransferase from *E. coli*

The purification of [ACP] acetyltransferase from *E. coli* is summarized in Table 1 and Fig. 1. A major problem was the presence of high activities of an acetyl-

Table 1. Purification scheme for *E. coli* [ACP] acetyltransferase

The results presented are for a typical purification starting from 65 g of *E. coli* cells (see the Experimental section). Abbreviation: n.d., not determined.

	Step	Volume (ml)	Protein (mg)	[ACP] acetyltransferase activity (nmol/min)	(nmol/min per mg)	Acetyl-CoA hydrolase activity (μmol/min)	(μm/min per mg)
1.	Cell-free extract	155	3875	n.d.		1674	0.43
2.	35–80%-satd. $(NH_4)_2SO_4$	34	3264	n.d.		862	0.26
3.	Sephacryl S-200	40	1120	~ 40	0.03	13	0.01
4.	DEAE-cellulose DE-52	6.9	343	~ 7	0.02	6	0.02
5.	Sephacryl S-200	10.0	149	28	0.19	0	0
6.	Mono Q	2.7	52	12	0.23		
7.	Blue Sepharose	2.8	25	5	0.17		
8.	Hydroxyapatite	1.0	~ 0.2	4	~ 19		
9.	Mono P	0.56	n.d.	2.3			
10.	TSK G-3000	1.5	~ 0.001	0.8	~ 800		

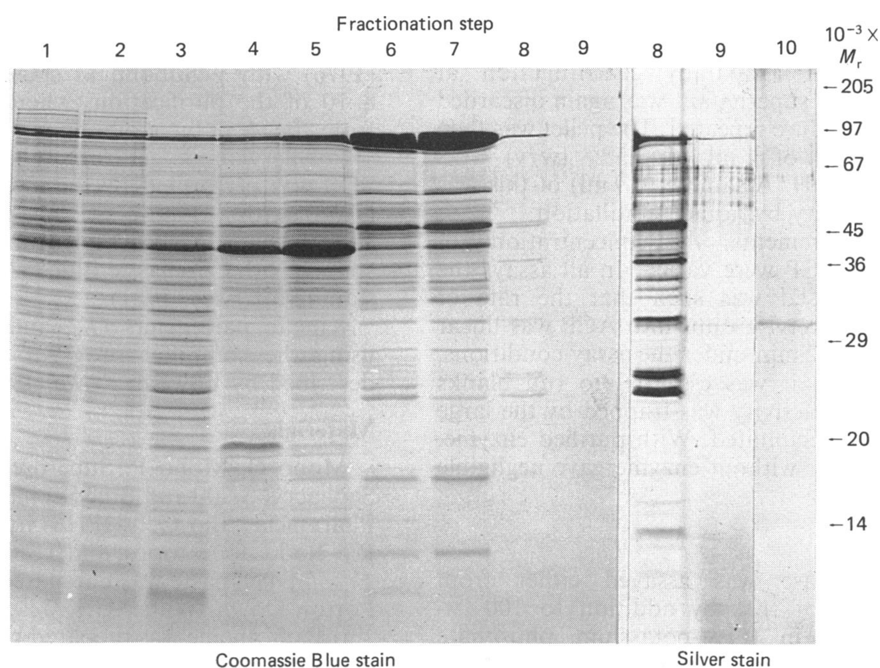

Fig. 1. Purification of [ACP] acetyltransferase from *E. coli*

This SDS/polyacrylamide-gel electrophoresis monitors the purification of [ACP] acetyltransferase. The numbers above the tracks refer to the steps in the purification scheme (see the Experimental section and Table 1). The first nine tracks (labelled 1–9) were stained with Coomassie Blue and photographed. The last three tracks (labelled 8–10) were subsequently stained by the silver method. Positions of M_r standards are indicated at the right.

CoA hydrolase in the early steps of the purification. In the cell-free extract the activity of this enzyme was about 50000 times that of the acetyltransferase, making assays in the first stages of the purification far from quantitative. The effect of addition of cell-free extract, containing this hydrolase, to partially purified enzyme is shown in Fig. 2, demonstrating the marked interference that this caused to the acetyltransferase assay. When acetyltransferase free from contamination with acetyl-CoA hydrolase was used, the rate of production of [^{3}H]acetyl-ACP was linear with time for the duration of the enzyme assay. The hydrolase activity was largely removed by gel filtration (steps 3 and 5), and after each of these steps the total measured acetyltransferase appeared to increase, making an accurate assessment of recoveries and purification impossible. However, we estimate that the acetyltransferase was purified approx. 30000–80000-fold, with a 2% recovery of activity. The purified enzyme could be

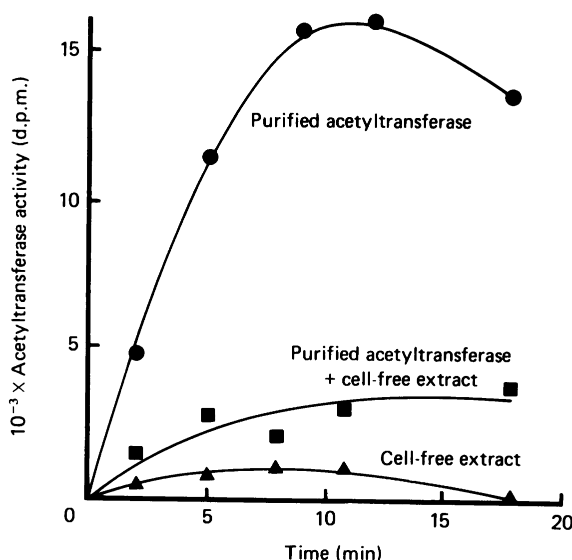

Fig. 2. Effect of *E. coli* cell-free extract on the activity of purified [ACP] acetyltransferase

[ACP] acetyltransferase assays were performed for various incubation times, with 10 μl of [ACP] acetyltransferase purified by steps 1–6 and 8 (see the Experimental section) (●), or 10 μl of *E. coli* cell-free extract (▲), or both 10 μl of cell-free extract and 10 μl of purified [ACP] acetyltransferase [■).

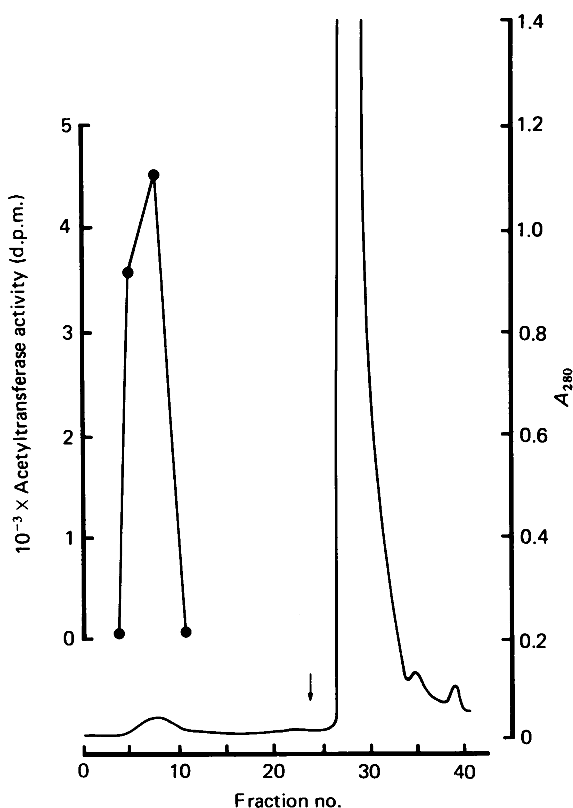

Fig. 3. Fractionation of *E. coli* [ACP] acetyltransferase on hydroxyapatite

E. coli [ACP] acetyltransferase eluted from Blue Sepharose was applied to a column of Bio-Gel HPHT hydroxyapatite and eluted as described in the Experimental section (step 8). ——, A_{280}; ●——●, [ACP] acetyltransferase activity.

stored at −20 °C for several months with little loss of activity.

The production of homogeneous enzyme required a large number of steps, as, except for the chromatography on hydroxyapatite (Fig. 3), no large degree of purification was achieved with any single step.

The purified enzyme showed a single band on polyacrylamide-gel electrophoresis in the presence of SDS (Fig. 1), but unfortunately a yield of only 1 μg of pure enzyme could be obtained. By omission of steps 7, 9 and 10 a high recovery of highly purified, but not homogeneous, enzyme was obtained.

Molecular and catalytic properties

The subunit M_r of the [ACP] acetyltransferase was estimated by polyacrylamide-gel electrophoresis in the presence of SDS to be 29000 ± 2000. The native enzyme had an apparent M_r of 61000 ± 10000 when subjected to gel filtration on Superose 12, which was in agreement with its elution position on a TSK G-3000 SW column (step 10 of purification). These data suggest that the *E. coli* enzyme is a simple dimer.

A steady-state kinetic analysis of the enzyme in which both the concentration of acetyl-CoA and of ACP were varied (Fig. 4) are consistent with a Ping Pong Bi Bi kinetic scheme and showed K_m for acetyl-CoA = 50 ± 10 μM and K_m for ACP = 35 ± 6 μM. At higher concentrations of acetyl-CoA (> 30 μM) and ACP (> 20 μM) some substrate inhibition was seen (Fig. 4).

CoA was, within experimental error, a competitive inhibitor of [ACP] acetyltransferase with respect to varied acetyl-CoA concentration ($K_i = 30 \pm 3$ μM at 20 μM-ACP). Pantotheine was a weak competitive inhibitor ($K_i = 3.2 \pm 0.6$ mM at 20 μM-ACP).

Interactions with potential inhibitors

E. coli [ACP] acetyltransferase activity was inhibited by preincubation with *N*-ethylmaleimide or iodoacetamide (Table 2). The inhibition by *N*-ethylmaleimide was prevented by inclusion of acetyl-CoA at 4 °C, but not at 30 °C. At 30 °C, it is likely that the rate of reaction with *N*-ethylmaleimide was sufficiently rapid that, with the preincubation time used, acetyl-CoA appeared to show little protection. Iodoacetamide was a much less potent inhibitor than *N*-ethylmaleimide, and acetyl-CoA also gave good protection from this inhibitor.

E. coli [ACP] acetyltransferase activity was not inhibited by preincubation with phenylmethanesulphonyl fluoride (Table 2). A low but significant inhibition was seen with high concentrations of methyl methanethiosulphonate, which reacts rapidly and specifically with thiol groups (Roberts *et al.*, 1986) (Table 2).

For comparison, in parallel experiments, *E. coli* [pantotheine] malonyltransferase and yeast fatty acid synthase preparations were incubated with these compounds. Both overall fatty acid synthase and the partial acetyl- and malonyl-transferase activities of the yeast preparation were measured (Table 2). Under these conditions phenylmethanesulphonyl fluoride did not inhibit *E. coli* acetyl- or malonyl-transferases or overall yeast fatty acid synthase activities. Methyl methanethiosulphonate was an extremely potent inhibitor of overall yeast fatty acid synthase activity, but not of its acetyltransferase activity. *N*-Ethylmaleimide and iodo-

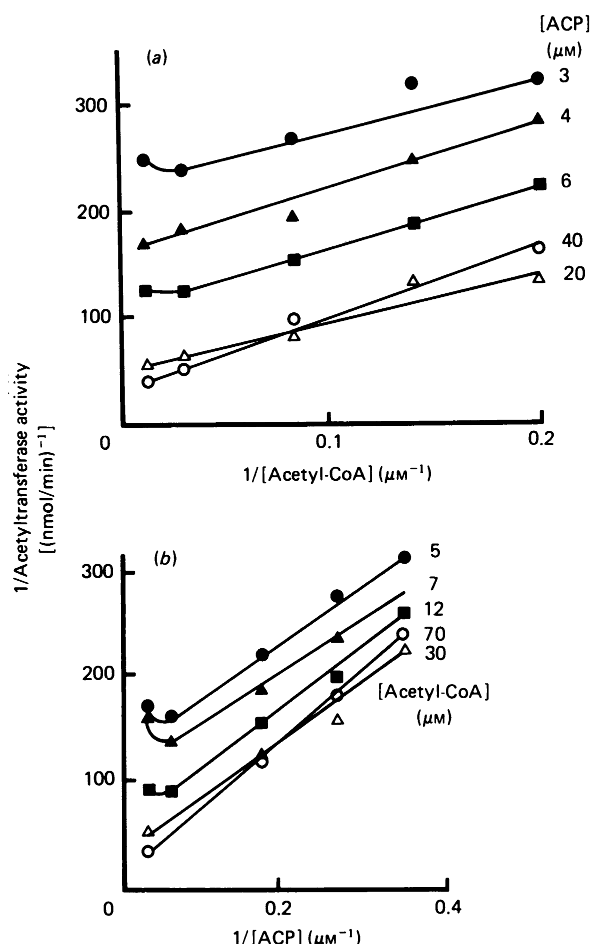

Fig. 4. Kinetics of *E. coli* [ACP] acetyltransferase activity

(*a*) Double-reciprocal plot of [ACP] acetyltransferase velocity versus concentration of acetyl-CoA at the indicated fixed concentrations of ACP. (*b*) As (*a*), but the concentration of ACP was varied at the indicated fixed concentrations of acetyl-CoA.

acetamide inhibited the overall fatty acid synthase activity of the yeast preparation to a high degree at both 30 °C and 4 °C. However, iodoacetamide did not inhibit either the acetyl- or the malonyl-transferase activities of the yeast enzyme and *N*-ethylmaleimide was only weakly active at the higher temperature. Moreover, this latter reaction was not protected by acetyl-CoA. The *E. coli* malonyltransferase was not inhibited by *N*-ethylmaleimide or iodoacetamide, even at 30 °C. Thus the *E. coli* acetyltransferase is sensitive to inhibition by *N*-ethylmaleimide and iodoacetamide, in contrast with the *E. coli* malonyltransferase, but it is less sensitive than is the overall yeast fatty acid synthase activity.

When *E. coli* [ACP] acetyltransferase was incubated with acetyl-CoA and then gel-filtered to remove unbound ligand (see the Experimental section), the eluted enzyme was partially resistant to inhibition by *N*-ethylmaleimide: incubation of gel-filtered, but otherwise untreated, enzyme with 1 mM-*N*-ethylmaleimide at 4 °C for 10 min resulted in 74 % inhibition of enzyme activity, but the enzyme pre-treated with acetyl-CoA was only inhibited by 40 % under these conditions. This experiment demonstrates the existence of a tightly bound or covalently bound acetyl-enzyme derivative.

Thiolactomycin (10 μM–1 mM) did not inhibit *E. coli* [ACP] acetyltransferase, in either crude or purified form, when preincubated for 10 min at 4 °C or 30 °C.

DISCUSSION

The preparation of homogeneous [ACP] acetyltransferase from *E. coli* represents the first report of a purification of this enzyme from a type II fatty acid synthase system. There have been reports of the partial purification of this enzyme from *E. coli* (Alberts *et al.*, 1964, 1969; Williamson & Wakil, 1966) and from spinach (Shimikata & Stumpf, 1983, 1986), but it is likely, judging by the degree of purification required in the work described above (Table 1 and Fig. 1), that those enzyme preparations were a considerable way from homogeneity.

Table 2. Effect of chemical modification reagents on enzyme activities

Inhibitors were incubated with purified *E. coli* [ACP] acetyltransferase, or partially purified *E. coli* [pantotheine] malonyltransferase, or yeast fatty acid synthase fraction for 10 min at the indicated temperatures and concentrations. The excess ligand was separated by centrifuge gel-filtration, and enzyme assays were performed as described in the Experimental section. Abbreviation: n.d., not determined.

Temperature	Treatment	*E. coli* [ACP] acetyltransferase activity		*E. coli* [pantotheine] malonyltransferase activity	Yeast fatty acid synthase activity		Yeast [ACP]acetyltransferase activity		Yeast [pantotheine] malonyltransferase activity	
	Acetyl-CoA...	−	+	−	−	+	−	+	−	+
4 °C	*N*-Ethylmaleimide (1 mM)	94	21	0	98	52	0	0	5	11
	Iodoacetamide (1 mM)	9	0	0	74	16	2	0	9	4
30 °C	*N*-Ethylmaleimide (1 mM)	96	97	0	100	99	44	78	50	73
	Iodoacetamide (1 mM)	64	10	0	99	87	3	0	0	16
	Methyl methanethiosulphonate (1 mM)	40	n.d.	n.d.	100	n.d.	20	n.d.	n.d.	n.d.
	Methyl methanethiosulphonate (0.1 mM)	22	n.d.	n.d.	100	n.d.	23	n.d.	n.d.	n.d.
	Phenylmethanesulphonyl fluoride (1 mM)	3	n.d.	0	14	n.d.	n.d.	n.d.	n.d.	n.d.

The *E. coli* [ACP] acetyltransferase was difficult to purify mainly because of the low abundance of the protein, but also because of the presence of a contaminating acetyl-CoA hydrolase in the cell-free extract. The presence of this enzyme prevented the accurate quantification of [ACP] acetyltransferase, and hence the activity of the acetyltransferase could only be estimated in the early stages of the purification. In order to establish a reliable purification procedure, we therefore found that it was essential to try to remove the hydrolase activity as early as possible during the purification, even at the expense of lower recovery, in order to allow early accurate quantification of the acetyltransferase activity. The chromatography on Blue Sepharose was required finally to achieve homogeneous enzyme, although this step did not give a high degree of purification, and large losses of activity occurred at this step. If highly purified, but not completely homogeneous, enzyme is required, this step could be omitted and much higher recoveries of activity obtained.

After taking into account the low recovery of activity, it would appear that the [ACP] acetyltransferase is not an abundant protein, and even the total activity is low, lending weight to the hypothesis that this enzyme is the rate-determining step in fatty acid biosynthesis in Type II systems (cf. Shimikata & Stumpf, 1983). In some plant fatty acid synthase preparations, the acetyltransferase was undetectable (Caughey & Kekwick, 1982), and it is possible that the explanation for this might be the same unfortunate combination of acetyl-CoA hydrolase and low total acetyltransferase activity.

Alberts *et al.* (1964) demonstrated that the [ACP] acetyltransferase and [ACP] malonyltransferase activities in *E. coli* could be separated by gel filtration, the former being eluted before the latter. Using the M_r of the malonyltransferase (35500) reported by Joshi & Wakil (1971), we can estimate from the data of Alberts *et al.* (1964) that the M_r of the acetyltransferase is about double that value, and is in reasonable agreement with the value of the M_r measured by us.

The properties of the *E. coli* [ACP] acetyltransferase were further investigated by Williamson & Wakil (1966), again using partially purified enzyme. They showed that the enzyme was completely inhibited by millimolar concentrations of either *N*-ethylmaleimide or iodoacetamide when incubated with the enzyme for 5 min at 38 °C. Acetyl-CoA decreased the inhibition caused by iodoacetamide to 50%. They concluded that the acetyltransferase was a thiol enzyme. They further showed that the impure enzyme fraction could be acetylated by [^{14}C]acetyl-CoA, and that these acetyl groups could be removed by CoA or ACP. The nature of the bond between acetyl groups and enzyme was not investigated, but it was presumed to be an acetyl-*S*-enzyme. In the same paper, Williamson & Wakil (1966) presented evidence that the *E. coli* malonyltransferase might also be a thiol enzyme.

Later results from Lynen's and Wakil's groups clearly showed that the acetyl- and malonyl-transferase components of the yeast and vertebrate fatty acid synthases catalysed the reaction via an acetyl-*O*-enzyme intermediate in which the acetyl group was bound to a serine residue (Wakil *et al.*, 1983; Hardie & McCarthy, 1986). Subsequently, Joshi & Wakil (1971) purified the *E. coli* malonyltransferase to homogeneity. They found that this preparation was not inhibited by *N*-ethylmaleimide or iodoacetamide at concentrations up to 10 mM, in agreement with our own results (Table 2), and that the malonyl group was bound at a non-thiol site, as the adduct was stable to performic acid. From these data, it was concluded that the *E. coli* [ACP]malonyltransferase was not a thiol enzyme, and thus resembled its yeast and vertebrate counterparts. To our knowledge no further work has been reported on the mechanism of the *E. coli* [ACP] acetyltransferase.

The effects of chemical modification reagents on the *E. coli* [ACP] acetyltransferase reported here (Table 2) demonstrate some clear differences between the *E. coli* acetyl- and malonyl-transferases, in that only the latter is not sensitive to *N*-ethylmaleimide and iodoacetamide. Under the experimental conditions employed, it is likely, though by no means certain, that these two reagents are modifying thiol groups on the acetyltransferase. The effective protection by acetyl-CoA suggests that the reactive residue is at the active site. We expected that the use of methyl methanethiosulphonate, a highly reactive and selective thiol-modifying reagent, would unequivocally answer whether a thiol group is essential for activity. However, under conditions which gave complete inhibition of yeast fatty acid synthase activity, the *E. coli* [ACP] acetyltransferase was only partially inhibited. We can consider two explanations; either the enzyme does not have an essential thiol group, and the inhibition by *N*-ethylmaleimide and iodoacetamide is caused by reaction at a non-thiol group, or methyl methanethiosulphonate does thiomethylate a thiol group at the active site, but under the acetyltransferase assay conditions this group is removed. The mixed disulphide is known to be labile to thiol groups, and it is possible that the thiol group of ACP used as the substrate in the assay removes the thiomethyl group from the acetyltransferase, thus reactivating the protein. If this latter explanation is correct, the *E. coli* [ACP] acetyltransferase differs in mechanism from its yeast and vertebrate counterparts.

The steady-state kinetics of the *E. coli* [ACP] acetyltransferase (Fig. 4) were consistent with a Bi Bi Ping Pong mechanism with an acetyl-enzyme intermediate. Similar results were obtained for the *E. coli* [ACP] malonyltransferase (Joshi & Wakil, 1971). Further evidence for a stable acetyl-enzyme derivative was obtained from the resistance of enzyme pre-treated with acetyl-CoA to inhibition by *N*-ethylmaleimide.

The question of the nature of the acetyl-enzyme derivative would be finally resolved if sufficient homogeneous *E. coli* [ACP] acetyltransferase was available to acetylate the enzyme, in order to check the lability of this linkage to performic acid. Unfortunately, we were unable to produce sufficient material to do this, but using partially purified enzyme we found that, after incubation with high-specific-radioactivity [^{3}H]acetyl-CoA, all the trichloroacetic acid-precipitable radioactivity was removed by performic acid (results not shown). Controls using yeast fatty acid synthase showed that half of the acetyl groups were bound in linkages stable to performic acid, in accordance with the known acetylation sites (Lynen, 1967). Although these results strongly suggested that the *E. coli* [ACP] acetyltransferase operated by an *S*-acetyl intermediate, we have not presented the results in detail here, since with non-homogeneous protein, we might just possibly be examining the acetylation of some other protein.

Thiolactomycin, an antibiotic produced by *Nocardia*

sp., selectively inhibits type II fatty acid synthases, whereas type I synthases are insensitive to it (Oishi *et al.*, 1982; Hayashi *et al.*, 1984; Nishida *et al.*, 1986). It has been reported that, of the six *E. coli* enzymes of the fatty acid synthase system, both [ACP] acetyltransferase and 3-oxoacyl-ACP synthase were inhibited (Nishida *et al.*, 1986). In our hands, the [ACP] acetyltransferase was not inhibited. We do not know the reasons for this discrepancy, but we note that Nishida *et al.* (1986) assayed the enzyme in crude cell-free extract, under conditions which we find do not reliably measure the acetyltransferase activity (cf. Fig. 2). Therefore it is possible that the site of action of thiolactomycin in *E. coli* is on the 3-oxoacyl-ACP synthase component.

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