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Purification and some properties of thiolase from *Escherichia coli*

The induction of enzymes involved in the β-oxidation of fatty acids has been shown recently in *Escherichia coli* when the cells were grown on long-chain fatty acids as a unique carbon source[1-4]. Thiolase (acetyl-CoA: acetyl-CoA *C*-acetyltransferase, EC 2.3.1.9) played a key role in this process; this enzyme, purified to homogeneity from pig heart, has been extensively studied by GEHRING *et al.*[5-7]. We now wish to report the purification and some general properties of the enzyme extracted from *E. coli* grown on oleate, as previously described[2]. *E. coli* thiolase is a soluble enzyme since essentially all the enzyme activity found in homogenates can be recovered in the 45 000 $\times$ *g* supernatant. The enzyme has been purified 218-fold (Table I) by streptomycine sulfate precipitation, ammonium sulfate fractionation, DEAE-cellulose and Sephadex G-200 chromatography. This last technique indicates a molecular weight of 140 000 $\pm$ 5000. Ultracentrifugation data were obtained by the Yphantis method, using for calculation the partial specific volume of the pig heart enzyme[6]. A molecular weight of 139 900 was found. The polyacrylamide gel electrophoresis revealed a minor band (4%) and two major bands (20 and 76%, respectively). It is possible that one major band derives from the other, since gel electrophoresis in 6 M urea shows a shift in their respective proportions (46 and 50%), the minor one being unchanged.

During the purification, multiple peaks of enzyme activity can be observed if very shallow ionic strength gradients are used. Determination of the molecular weights on Sephadex G-200 of the different peaks gives values of 40 000–45 000, 70 000 and 105 000–110 000. Furthermore the 40 000–45 000 x *g* molecular-weight fraction, after concentration by pressure dialysis, shows a molecular weight of 143 000. These results suggest that thiolase is a tetrameric molecule like the pig heart enzyme[6];

TABLE I

PURIFICATION OF *E. coli* THIOLASE

E. coli cell disruption is accomplished as previously described[2], as well as streptomycin and ammonium sulfate precipitations. All steps are made at 4°. LiCl is used in 0.01 M potassium phosphate buffer, pH 7.3 (containing 0.01 M 2-mercaptoethanol), for the ionic strength gradients (0.01–0.25 M in the first DEAE-cellulose chromatography, 0.03–0.09 M in the second one). Sephadex G-200 is equilibrated with 0.2 M LiCl containing potassium phosphate buffer, pH 7.3, and 2-mercaptoethanol, as above. Assays were performed according to the method of STERN[8] with slight modifications. Acetoacetyl-CoA and acetoacetyl-pantetheine were synthetized as described by DECKER[9]. A molecular activity of 19 800 moles of substrate transformed per min per mole of enzyme can be calculated for the purified enzyme.

Step	*Total protein (mg)*	*Total activity (units)*	*Specific activity (units/mg)*	*Purification (-fold)*	*Yield (%)*
50 000 $\times$ *g* (30 min) supernatant	3680	2390	0.65		
Streptomycin sulfate precipitation	2950	2720	0.92	1.4	114
40–80% ammonium sulfate sediment	915	3180	3.48	5.3	133
1st DEAE-cellulose	126	2440	19.4	30	102
2nd DEAE-cellulose	26	980	37.7	58	41
Sephadex G-200	5.9	840	142	218	35

TABLE II

INVOLVEMENT OF SULFHYDRYL GROUPS IN THIOLASE ACTIVITY

Treatment	*Activity (%)*
Experiment 1	
(a) 1 h in 0.01 M Tris–HCl buffer, pH 7.5, containing 0.01 M 2-mercaptoethanol at 4°	100
(b) 1 h dialysis against 0.01 M Tris–HCl buffer, pH 7.5, at 4°	48
(c) 1 h dialysis (same conditions) followed by reduction in 0.01 M Tris–HCl buffer, pH 8, containing 0.01 M 2-mercaptoethanol (15 min at 30°)	77
Experiment 2	
(a) Enzyme in 0.01 M potassium phosphate buffer, pH 7.3, time, 0,	100
1 month storage,	0
(b) 1 month (same conditions) followed by incubation (30 min, at 30°, pH 8.2)	0
(c) 1 month (same conditions) followed by reduction in 0.01 M Tris–HCl buffer containing 0.01 M 2-mercaptoethanol (30 min at 30°, pH 8.2)	32
Experiment 3	
(a) Enzyme in 0.01 Tris–HCl buffer, pH 7.3, (80 min at 30°)	100
(b) Iodoacetamide $0.1 \cdot 10^{-3}$ M (40 min at 30°, pH 7.3)	30
(c) *p*-Chloromercuribenzoate $0.5 \cdot 10^{-3}$ M (80 min at 30°, pH 7.3)	0

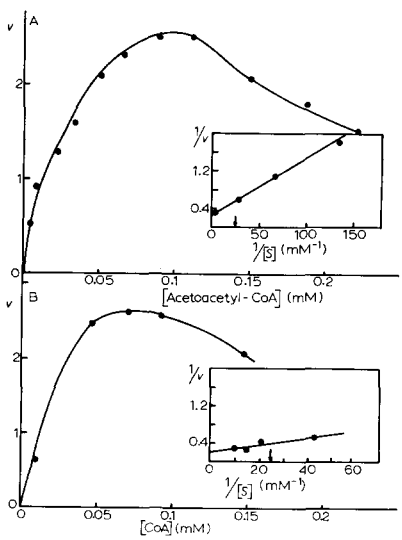

Fig. 1. Dependence on the rate of acetyl-CoA formation on substrate concentration and determination of K_m values. A. The experiments are performed in the presence of $0.5 \cdot 10^{-3}$ M CoA. B. The experiments are performed in the presence of $0.06 \cdot 10^{-3}$ M acetoacetyl-CoA. v is expressed in nmoles/min.

the molecular weight of the subunits should be around 35 000, owing to the fact that determination of molecular weights under 50 000 with Sephadex G-200 lacks accuracy.

Stability as a function of pH shows inactivation of the enzyme below pH 6.8 in the presence of reducing agents (40% inactivation at 0° during 75 h; 48% inactivation at 30° during 3 h).

Table II indicates that *E. coli* thiolase, as expected[5], is a sulfhydryl enzyme. Losses of activity occur either by oxidation or by blocking the –SH groups with specific reagents. Protection of the inhibition by *N*-ethylmaleimide or iodoacetamide was not prevented by preincubation with acetyl-CoA or acetoacetyl-CoA*.

Michaelis–Menten curves described in Fig. 1 allow the determination of K_m for acetoacetyl-CoA ($4.2 \cdot 10^{-5}$ M) and CoA ($4 \cdot 10^{-5}$ M). Surprisingly, as compared to the pig heart enzyme, pantetheine does not behave as a substrate but as a non-competitive inhibitor ($K_i = 5.4 \cdot 10^{-4}$ M). *E. coli* acyl carrier protein is inactive. Acetoacetyl-pantetheine as a substrate is a poor substitute for the CoA derivative, giving at $0.03 \cdot 10^{-3}$ M only 16% of the value obtained with acetoacetyl-CoA. Thiol-reducing agents such as mercaptoethanol and dithiothreitol maintain activity of refrigerated stored enzyme. However, slow but definite inactivation was observed on storage even in the presence of reducing agents. This inactivation could not be prevented by adding the substrates, by changing the pH or the ionic strength, or by increasing the storage temperature. Purified enzyme is completely inactivated by freezing.

The mechanism by which this enzyme is inactivated on storage requires study. It seems reasonable to postulate that the equilibrium is shifted toward inactive trimeric, dimeric and/or monomeric species[6].

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* *Pseudomonas putida* thiolase gives similar results. A molecular weight of 135 000 was found by chromatography on Sephadex G-200.