[4] Acetyl-CoA Carboxylase from Yeast

EC 6.4.1.2 Acetyl-CoA : carbon-dioxide ligase (ADP-forming)

By MANFRED SUMPER

$$CH_3—CO—SCoA + ATP + CO_2 \rightleftharpoons \overset{\overset{COOH}{|}}{CH_2}—CO—SCoA + ADP + P_i$$

Assay Methods

The assay methods for acetyl-CoA carboxylase have been described previously.[1] The optical assay, which measures formation of malonyl-CoA in a combined reaction with fatty acid synthase[2] by the decrease of NADPH,[1,3] was used throughout the purification procedure described here.

Units. One unit of enzyme is defined as that amount of protein which catalyzes the carboxylation of 1 μmol of acetyl-CoA per minute at 25°. Specific activity is expressed as units per milligram of protein.

Purification Procedure

The purification procedure previously described in this series[1] yields an enzyme preparation with a specific activity of about 0.4 unit per milligram of protein. Acetyl-CoA carboxylase purified by the alternative procedure described below has a specific activity of 6.0–9.0 units per milligram of protein and yields a single polypeptide band on sodium dodecyl sulfate gel electrophoresis. All operations were carried out at 4°. Brewer's yeast (4 kg, wet weight), from Löwenbräu (Munich) was washed twice with about 6 liters of 0.1 *M* potassium phosphate, pH 6.5, and cells were collected by centrifugation.

Step. 1. Disruption of the Cells.[4] The washed cells (4 kg) were sus-

[1] M. Matsuhashi, this series, Vol. 14 [1].
[2] F. Lynen, Vol. 14 [3].
[3] M. Matsuhashi, S. Matsuhashi, S. Numa, and F. Lynen, *Biochem. Z.* **340**, 243 (1964).
[4] Disruption of the cells in a French press yields crude extracts with considerably lower enzymatic activity.

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pended in 6 liters of 0.2 *M* potassium phosphate, pH 6.5, containing 1 m*M* EDTA, and each 250 ml suspension was agitated with 250 g of glass beads (size 31/10; Dragonwerk Wild, Bayreuth) in the cell homogenizer of Merkenschlager *et al.*[5] operated at 0° to 5° and run for 40 sec. The combined crude extracts were centrifuged at 15,000 *g* for 40 min.

Step 2. Ammonium-Sulfate Fractionation. The crude extract from step 1 was taken to 40% saturation by the addition of ammonium sulfate. The precipitate, which contained the acetyl-CoA carboxylase activity, was collected by centrifugation at 15,000 *g* for 60 min.

Step 3. Ultracentrifugation. The precipitate was dissolved in 1000 ml of 0.1 *M* potassium phosphate, pH 6.5, containing 1 m*M* 2-mercaptoethanol. The cloudy liquid was centrifuged at 100,000 *g* for 90 min, and the supernatant liquid, which contained the acetyl-CoA carboxylase activity, was saved.

Step 4. Ammonium-Sulfate Fractionation (0 to 35%). The supernatant fraction from stage 3 was taken to 35% saturation by the addition of ammonium sulfate. After stirring for 20 min, the precipitate was collected by centrifugation at 15,000 *g* for 40 min.

Step 5. Polyethylene Glycol Fractionations. The collected precipitate from step 4 was dissolved in 0.1 *M* potassium phosphate, pH 6.5, containing 1 m*M* 2-mercaptoethanol. The protein concentration was lowered to 15–20 mg/ml by addition of buffer (about 2000 ml); 230 ml of 50% (w/w) aqueous polyethylene glycol (average mw 1500) per 1000 ml of protein solution were added with stirring, and the solution was stirred for a further 30 min. The resulting precipitate was removed by centrifugation at 15,000 *g* for 40 min. An additional 220 ml of 50% aqueous polyethylene glycol solution per 1000 ml of initial volume of the protein solution was added to the supernatant. The resulting precipitate was collected by centrifugation at 15,000 *g* for 40 min. The pellet, which contained the enzyme, was dissolved in 0.05 *M* potassium phosphate, pH 6.5, to a protein concentration of about 10 mg/ml. For each 100 ml of protein solution, 5 g of solid ammonium sulfate and 10 ml of 50% (w/w) aqueous solution of polyethylene glycol (average mw 6000) was added. The resulting precipitate was removed by centrifugation at 15,000 *g* for 30 min. The acetyl-CoA carboxylase was then precipitated by further addition of 10 ml of polyethylene glycol solution per 100 ml initial volume. The sample was stirred for 30 min, and the resulting precipitate was collected by centrifugation at 15,000 *g* for 40 min.

Step 6. DEAE-Cellulose Chromatography. The pellet from step 5 was dissolved in approximately 20 ml of 0.02 *M* potassium phosphate, pH 7.5,

[5] M. Merkenschlager, K. Schlossmann, and W. Kutz, *Biochem. Z.* **329**, 332 (1957).

PURIFICATION OF ACETYL-CoA CARBOXYLASE FROM YEAST[a]

Fraction	Protein (mg)	Total activity (units)	Specific activity (mU/mg)	Recovery (%)
Crude extract	—	—	—	—
First $(NH_4)_2SO_4$	115,000	(3100)	27	100
Ultracentrifugation	60,000	3000	50	96
Second $(NH_4)_2SO_4$	20,400	2650	130	84
Polyethylene glycol	2,100	2100	1000	68
DEAE-cellulose	400	1450	3600	47
Phosphocellulose	175	1050	6000	34

[a] Yeast (4 kg, wet weight) was used. In the crude extract, reliable results could not be obtained using the spectrophotometric assay.

containing 20% glycerol and 2 m*M* $MgCl_2$. Water containing 20% glycerol was added to the solution until its conductance was equal to that of the equilibrating buffer. The solution was applied to a DEAE-cellulose column (4.6 × 30 cm) equilibrated with the above buffer. Elution was carried out with a linear concentration gradient established between 1.5 liters of buffer containing 50 m*M* NaCl and 1.5 liters containing 200 m*M* NaCl. The fractions of high specific activity (corresponding to those between approximately 1400 to 2100 ml of effluent volume) were taken to 50% saturation with solid ammonium sulfate and centrifuged at 15,000 *g* for 60 min. The precipitate was dissolved in a minimum volume of 20 m*M* potassium phosphate, pH 6.5, containing 20% glycerol.

Step 7. Cellulose-Phosphate Chromatography. Water containing 20% glycerol was added to the resulting protein solution until its conductance was equal to that of the equilibrating buffer: 20 m*M* potassium phosphate, pH 6.5, containing 20% glycerol. This solution was applied to a column (3.6 × 20 cm) of cellulose phosphate. Elution was carried out with a linear concentration gradient established between 1.2 liters of 50 m*M* KCl in buffer and 1.2 liters of 220 m*M* KCl in buffer. The acetyl-CoA carboxylase appeared in the eluate after about 1600 ml. The fractions containing acetyl-CoA carboxylase were pooled, and the protein was precipitated by addition of solid ammonium sulfate.

The results of a typical purification are summarized in the table.

Properties

Specificity and kinetic and some other properties of acetyl-CoA carboxylase were described previously in this series.[1]

Stability. The purified enzyme could be kept for at least 1 year without

loss of activity when stored in 0.3 *M* potassium phosphate, pH 6.5, containing 50% glycerol at −20°. Low ionic strength and alkaline pH favor the rapid inactivation of the enzyme.

Subunit Structure. The purified enzyme exhibits a single polypeptide band on SDS–polyacrylamide gels with an apparent molecular weight of 189,000.[6] The native enzyme was shown to be a tetramer of identical subunits with a C-terminal amino acid sequence -Leu-Lys-COOH and a sedimentation coefficient of $s_{20,w} = 15.5$ S.[6,7]

Dissociation and Reactivation. A rapid inactivation of acetyl-CoA carboxylase occurs at pH values above 8.0. This loss of enzymatic activity is accompanied by a change of sedimentation behavior, indicating a dissociation of the tetrameric native enzyme in a mixture of monomers, dimers, and trimers of the subunit polypeptide chain.[7] Inactivated acetyl-CoA carboxylase is reactivated upon transfer to concentrated (0.5 *M*) potassium phosphate buffer, pH 6.5, containing 20% glycerol and 10 m*M* dithiothreitol.

[6] J. Spiess, Doctoral thesis, University of Munich, 1976.
[7] M. Sumper and C. Riepertinger, *Eur. J. Biochem.* **29**, 237 (1972).

[5] Acetyl-CoA Carboxylase from *Candida lipolytica*

EC 6.4.1.2 Acetyl-CoA : carbon-dioxide ligase (ADP-forming)

By MASAYOSHI MISHINA, TATSUYUKI KAMIRYO, and SHOSAKU NUMA

$$ATP + HCO_3^- + \text{acetyl-CoA} \rightleftharpoons ADP + P_i + \text{malonyl-CoA}$$

Assay Methods

Several methods are available for the assay of acetyl-CoA carboxylase.[1–5] The activity of the *Candida lipolytica* enzyme is routinely determined by the $^{14}CO_2$-fixation assay or by the spectrophotometric assay in combination with the pyruvate kinase and lactate dehydrogenase

[1] M. Matsuhashi, this series, Vol. 14 [1].
[2] S. Numa, this series, Vol. 14 [2].
[3] H. Inoue and J. M. Lowenstein, this series, Vol. 35 [1].
[4] A. L. Miller and H. R. Levy, this series, Vol. 35 [2].
[5] T. Tanabe, S. Nakanishi, T. Hashimoto, H. Ogiwara, J. Nikawa, and S. Numa, this volume [1].

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