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# Acyl-Coenzyme A Carboxylase of the Free-Living Nematode *Turbatrix aceti*. 1. Its Isolation and Molecular Characteristics†

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ABSTRACT: A biotin containing enzyme which carboxylates acetyl-CoA has been isolated from the nematode *Turbatrix aceti* and purified to homogeneity as judged by the criteria of polyacrylamide gel electrophoresis and ultracentrifugation. The enzyme has a sedimentation coefficient of 18.0 S and a molecular weight of 667 000. It is composed of four protomers having a molecular weight of 140 000 each. Each protomer, in turn, consists of two distinct polypeptide chains (molecular weights 82 000 and 58 000) and one biotinyl prosthetic group which is linked to the 82 000 peptide. The amino acid composition of the nematode carboxylase has also been determined.

The enzymatic details of the conversion of acetyl-CoA to long-chain fatty acids have been studied in a wide range of organisms, including bacteria, yeasts, algae, plants, and higher animals (Vagelos, 1971). The essence of these studies is that all the fatty acid synthesizing systems are similar in the sequence of reactions they catalyze but different in their physical properties and the types of regulatory mechanisms to which they are adapted. A gap in our knowledge exists with regard to the lower invertebrates. Even though they surpass all other animal forms in numbers as well as in anatomical and functional diversity, a full effort has not been made until now to work out the details of their fatty acid synthesizing systems.

The helminths are a group of invertebrates which merit special attention, since previous studies have revealed that they vary greatly in their capacities to synthesize fatty acids de novo. For instance, among the flatworms so far investigated, both the free-living as well as the parasitic forms fail to synthesize fatty acids (Meyer et al., 1966, 1970; Meyer & Meyer, 1972; Ginger & Fairbairn, 1966). Among the nematodes, the free-living organisms such as *Turbatrix aceti* and *Caenorhabditis briggsae* can synthesize fatty acids (Rothstein & Götz, 1968), whereas the parasitic organism *Ascaris lumbricoides* can synthesize these compounds only at a very reduced level, if at all (Beames et al., 1967). Several possibilities have been advanced to account for this metabolic deficiency. They include a repression of enzyme systems at particular stages of the parasite's life cycle (Fairbairn, 1970), an adaptation to a lack of oxygen in the environment, and a general adaptation to a parasitic or symbiotic mode of life (Meyer et al., 1966; Meyer & Meyer, 1972). Clearly, a critical evaluation of these hypotheses requires knowledge of the properties of the enzyme systems associated with fatty acid biosynthesis and the physiological factors which control their activities.

Based on these considerations, we have undertaken the isolation and characterization of acetyl-CoA carboxylase from the free-living nematode *Turbatrix aceti*. We have focused initially on this enzyme, since it catalyzes the first committing step in the reaction sequence leading to long chain fatty acids, and since it plays a critical role as a regulatory enzyme in the control of this process in animals.

In this paper, we describe the purification to homogeneity of this enzyme, its subunit structure, and its amino acid composition.

## Experimental Procedure

*Materials.* Soy peptone was obtained from Humko Sheffield Co.; acyl-CoA esters were from P-L Biochemicals; radioactively labeled chemicals were from New England Nuclear; 4-hydroxyazobenzene-2′-carboxylic acid was from Sigma; β-galactosidase (*E. coli*), alcohol dehydrogenase (horse and yeast), catalase (beef), urease (jack bean), and aldolase (rabbit) were from Worthington Biochemical Corp.; Cellex E and gel electrophoresis reagents were from Bio-Rad; and Sepharose 4B was from Pharmacia Fine Chemicals.

Cellex E (anion-exchange cellulose) was washed for 30 min with 0.25 M NaOH containing 0.25 M NaCl, for another 30 min with 0.25 M HCl, and was then suspended in 10 mM EDTA for several days. Finally, it was washed with 0.3 M phosphate buffer and stored in water at 3 °C.

*Cultivation of T. aceti. Turbatrix aceti* was obtained from Dr. Morton Rothstein of the State University of New York at

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Buffalo. Stock cultures of this organism were maintained at 30 °C in a medium composed of 4% soy peptone, 3% acetic acid, 10% heated lamb's liver extract, and "EM" (a mixture of vitamins, nucleotides, and salts; Rothstein & Cook, 1966; Hieb & Stokstad, 1970). Mass cultures were grown in a modified stock culture medium composed of 4% soy peptone, 3% acetic acid, 0.75% yeast extract, 2.5% heated liver extract, 0.03% myoglobin, and 0.005% cholesterol dissolved in Tween 80 (0.5 g/25 mL). Although this mass culture medium will not support growth of *T. aceti* in serial cultures, it will support its growth in single step cultures. The number of organisms grown in this medium increased on the average from 200 to 40 000 per mL amounting to a wet mass of 150 g per 10-L culture. Acyl-CoA carboxylase preparations from *T. aceti* grown in either medium were comparable in terms of their physical and catalytic properties.

The mass culture medium was usually prepared in 10-L batches and distributed as 100-mL portions into flat milk bottles of 1000-mL capacity each. After each bottle had been inoculated with 2 mL from a *T. aceti* stock culture, the lot was stacked horizontally on shelves in a 30 °C warm-room. Checks for possible microbial contamination were routinely performed by inoculating Sterility Test Medium (Difco Laboratories) with small samples from the cultures.

*Collection of T. aceti. T. aceti* was harvested during the late logarithmic growth phase, about 17 to 20 days after inoculation. The entire culture (10 L) was first placed into an ice bath for 30–40 min to immobilize the organisms. The organisms were then centrifuged down at 8000*g* for 10 min, resuspended in 2 L of a chilled 50% sucrose solution, and recentrifuged at 6000*g* for 10 min (Rothstein et al., 1970). *T. aceti,* now floating on top of the sucrose solution, was collected, washed first with 3 L of chilled distilled water, and then with 300 mL of 0.1 M potassium phosphate buffer, pH 7.0, containing 1 mM EDTA plus 3 mM dithiothreitol. Finally, the organisms were suspended in a minimal volume of the same buffer and used either immediately or after storage for up to 2 months at −18 °C.

*Assay of the Enzyme.* The fixation of $H^{14}CO_3^-$ into malonyl-CoA was determined by measuring the incorporation of radioactivity into the acid stable reaction product (described in detail by Meyer & Meyer, 1978).

*Polyacrylamide Gel Electrophoresis.* Electrophoresis of the native carboxylase (5–50 μg) was performed at 2 mA per tube in 4% acrylamide gel columns (5 × 90 mm) with 0.375 M Tris–glycine buffer, pH 8.3, 1 mM EDTA plus 1 mM mercaptoethanol in the sample and electrode vessel and with Tris buffer alone in the gel. Electrophoresis of the $NaDodSO_4$[1] or guanidine dissociated carboxylase was carried out according to Weber & Osborn (1969) in 5 and 8% acrylamide gels containing 0.1 M sodium phosphate buffer, pH 7.2, and 0.1% $NaDodSO_4$.

*Dissociation of the Enzyme by $NaDodSO_4$ or Guanidine Hydrochloride.* The various conditions employed for dissociating the enzyme were those of Weber et al. (1972): (1) The enzyme (0.3 mg) in 0.5 mL of 0.01 M sodium phosphate buffer, pH 7.2, containing 1% $NaDodSO_4$ and 1% mercaptoethanol, was heated in boiling water for 3 min. (2) The enzyme in the same $NaDodSO_4$ buffer system was incubated at 37 °C for 2 h. (3) The enzyme was first dialyzed for 5 h at 25 °C against 0.1 M Tris-HCl buffer, pH 8.6, containing 8 M urea and 1% mercaptoethanol and then dialyzed for 5 h against 0.01 M sodium phosphate buffer, pH 7.2, containing 1% $NaDodSO_4$ and 1% mercaptoethanol. (4) The enzyme in 0.01 M sodium phosphate buffer, pH 7.2, containing 8 M urea, 1% $NaDodSO_4$, and 1% mercaptoethanol was incubated at 37 °C for 2 h. (5) The enzyme in 0.1 M Tris-HCl buffer, pH 8.5, containing 7 M guanidine hydrochloride and 1.6% mercaptoethanol was heated in boiling water for 5 min and then incubated at 37 °C for 2 h. The denatured enzyme was alkylated with iodoacetic acid.

*Determination of the Biotin Content.* Two milliliters of the purified carboxylase (4 mg/mL) was dialyzed against 0.05 M potassium phosphate buffer, pH 7.0, until free of EDTA, dithiothreitol, and glycerol. The dialyzed enzyme (4 mg) was heated in boiling water for 5 min and then treated with 50 μg of Pronase for 16 h at 37 °C (Inoue & Lowenstein, 1972). The biotin content in the digest was determined colorimetrically with the avidin dye complex, 4-hydroxyazobenzene-2′-carboxylic acid (Green, 1970).

In a second procedure, the enzyme (2 mg) was mixed with an equal volume of 6 N $H_2SO_4$ and autoclaved at 120 °C with 120 lb of pressure for 1 h. The free biotin in the hydrolysate was assayed microbiologically with *Lactobacillus plantarum,* ATCC 8014 (Shull et al., 1942). The protein content was determined by the biuret method with roughly 2 mg of enzyme and with bovine serum albumin as standard.

*Molecular Weight Determination.* The molecular weight of the enzyme was determined by high-speed sedimentation equilibrium centrifugation according to the procedure of Yphantis (1964). After the enzyme was dialyzed for 48 h against 0.2 M potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 5 mM mercaptoethanol, it was adjusted to protein concentrations ranging from 0.6 to 0.9 mg/mL. Centrifugation was carried out at 20 °C in a Spinco Model E analytical ultracentrifuge equipped with a RTIC temperature control and electronic speed control. Rayleigh interference fringe patterns were photographed with Kodak Type II-G spectroscopic plates and were measured in duplicate with the aid of a Nikon profile projector. The data were processed in a SEL 810A computer with a Fortran IV program as suggested by Yphantis (1964).

*Amino Acid Analysis.* Amino acid analysis was performed according to the method of Moore & Stein (1963) employing a Beckman-Spinco Model 120B amino acid analyzer. Samples of the purified enzyme, dialyzed extensively against 0.1 M potassium phosphate buffer, were hydrolyzed in 6 N HCl at 110 °C for 24, 48, and 72 h under vacuum. Cystine and cysteine were identified as cysteic acid by performic acid oxidation of the amino acids prior to acid hydrolysis (Moore, 1963). The amounts of threonine and serine were determined by extrapolating the hydrolysis time to zero. The values for valine and isoleucine were taken from the 72-h hydrolysis sample. Tryptophan was determined after 48-h alkaline hydrolysis according to Hugli & Moore (1972).

Protein was measured by the biuret reaction using bovine serum albumin as standard.

## Results

*Isolation and Purification of the Enzyme.* As Table I shows, we have isolated and purified to homogeneity an enzyme from *T. aceti* which carboxylates acetyl-CoA. The purified enzyme had a total activity of 146 units, which amounts to 27% of the initial activity. It had a specific activity of 6.4 units per mg of protein, which amounts to approximately a 300-fold increase in the specific activity over that of the crude high-speed supernatant fraction.

The purification of the enzyme involved nine steps.

[1] Abbreviation used: $NaDodSO_4$, sodium dodecyl sulfate.

TABLE I: Purification of Acyl-CoA Carboxylase from *T. aceti*.[a]

| Purification steps | Total protein (mg)[b] | Total act. (units)[c] | Spec act. (units/mg) |
|---|---|---|---|
| 1. High speed centrifugation | 26 000 | 549 | 0.02 |
| 2. 1st $(NH_4)_2SO_4$ precipitation | 9 200 | 843 | 0.1 |
| 3. Calcium phosphate gel adsorption | 3 908 | 610 | 0.2 |
| 4. 2nd $(NH_4)_2SO_4$ precipitation | 2 030 | 400 | 0.2 |
| 5. 1st Cellex E cellulose chromatography | 447 | 369 | 0.8 |
| 6. 2nd Cellex E cellulose chromatography | 188 | 234 | 1.3 |
| 7. 3rd Cellex E cellulose chromatography | 74 | 229 | 3.1 |
| 8. Sepharose 4B gel filtration | 34 | 209 | 6.1 |
| 9. 3rd $(NH_4)_2SO_4$ precipitation | 23 | 146 | 6.4 |

[a] *T. aceti* was grown in a 20-L culture yielding 270 g wet weight of organisms. Details of the purification procedure are described under Results. All manipulations were carried out below 4 °C. All buffers contained 1 mM EDTA and 5 mM mercaptoethanol or 3 mM dithiothreitol. [b] Assayed by the biuret method. [c] Assayed by measuring the carboxylation of acetyl-CoA. One unit = 1 μmol of $HCO_3^-$ fixed per min under the assay conditions described in Experimental Procedure.

1. Preparation of Crude Homogenate and High-Speed Supernatant. Frozen organisms from a 20-L culture were thawed and diluted to 600 mL with 0.2 M potassium phosphate buffer, pH 7.5, containing 1 mM EDTA and 3 mM dithiothreitol. The mixture was passed through a French Pressure Cell at 14 000 lb per in.$^2$. The homogenate was immediately adjusted to pH 7.0 and centrifuged at 66 000*g* for 60 min. The resulting supernatant was set aside while the pellet was washed once with 300 mL of 0.2 M potassium phosphate buffer, pH 7.0. After recentrifugation, the two supernatants were combined.

2. First $(NH_4)_2SO_4$ Fractionation. The high-speed supernatant was fractionated by the stepwise addition of solid ammonium sulfate (Enzyme Grade). During this process, the pH of the mixture was maintained at 7.0. The proteins precipitating from 0 to 30% ammonium sulfate saturation were discarded. The proteins precipitating from 30 to 55% saturation were dissolved in a minimal volume of 0.05 M phosphate buffer and dialyzed against the same buffer until free of ammonium sulfate. Steps 1 and 2 were carried out within the same day to minimize the risk of enzyme degradation.

3. Calcium Phosphate Gel Adsorption. The enzyme solution was diluted to a phosphate concentration of 0.02 M and a protein concentration of 20 to 30 mg per mL. To this solution, calcium phosphate gel suspended in water (Kunitz & Simms, 1928) was added in the ratio of 1.8 g of dry gel per g of protein. The mixture, totaling 800 to 1000 mL, was stirred for 10 min, and the calcium phosphate gel to which the enzyme was bound was collected by centrifugation at 7000*g* for 10 min. The gel was resuspended in 500 mL of 0.05 M phosphate buffer and homogenized gently with a magnetic stirrer. The suspension was centrifuged and the supernatant discarded. After this washing process was repeated, the enzyme was eluted from the gel with 300-mL portions of 0.25 M phosphate buffer by a repeated homogenization–extraction process.

4. Second $(NH_4)_2SO_4$ Fractionation. When the sample was again fractionated with solid ammonium sulfate, most of the enzyme precipitated in the range from 35 to 55% ammonium sulfate saturation. The precipitate from this fraction was dissolved in 0.05 M phosphate buffer, pH 7.0, and dialyzed against the same buffer until free of ammonium sulfate.

5. First Cellex E Column Chromatography. The enzyme solution containing about 2 g of protein was diluted to 0.02 M phosphate and applied to a Cellex E column (5 × 10 cm) previously equilibrated with 0.02 M phosphate buffer, pH 7.3. The sample was eluted stepwise with three column volumes each of 0.02 M, 0.06 M, and 0.1 M phosphate buffer, pH 7.3. Over 90% of the initial enzyme activity was recovered from the column with 0.06 M phosphate buffer. The fractions containing the enzyme were pooled and concentrated to about 40 mL with an Amicon Xm 50 ultra filter.

6. Second Cellex E Column Chromatography. The enzyme solution containing about 400 mg of protein was diluted to 0.02 M phosphate and loaded onto a second Cellex E column (2.5 × 20 cm). One liter of a 0.02 to 0.08 M linear phosphate gradient, pH 7.3, was applied to the column to elute the enzyme. A large protein peak without carboxylase activity appeared in the eluent at a phosphate concentration of 0.028 M (conductivity: $3.3 \times 10^{-3}$ mho); the enzyme appeared at a phosphate concentration of 0.035 M (conductivity: $4.0 \times 10^{-3}$ mho).

7. Third Cellex E Column Chromatography. Chromatography on Cellex E was repeated as described in step 6 to separate fully the carboxylase peak from a nearby contaminating protein peak. The enzyme was then precipitated with ammonium sulfate at 80% saturation, and the precipitate was dissolved in 3 mL of 0.1 M phosphate buffer.

8. Sepharose 4B Gel Filtration. A 2.5 × 60 cm column of Sepharose 4B was equilibrated with 0.2 M potassium phosphate buffer, pH 7.0. The enzyme was applied to the column and eluted with the same buffer. The enzyme-containing fractions, which were eluted with approximately twice the void volume of the column, were combined and concentrated to 20 mL by filtration.

9. Third $(NH_4)_2SO_4$ Fractionation. Saturated ammonium sulfate, pH 7.0, was added to the enzyme solution until it became slightly turbid. At this point, the precipitate was removed by centrifugation and saturated ammonium sulfate was added for a second time until the solution became slightly turbid. The sample was then allowed to stand for 15 to 20 h at 3 °C. The precipitate formed during this step was collected by centrifugation at 60 000*g* for 30 min and redissolved in 2 to 3 mL of 0.2 M phosphate buffer. After dialysis against the same buffer, the now purified enzyme was stored at −18 °C in 0.2 M phosphate buffer, pH 7.0, containing 1 mM EDTA, 3 mM dithiothreitol, and 30% glycerol.

*Enzyme Stability.* The enzyme which had been purified through the third step retained 80 to 90% of its initial activity when stored for 3 weeks at 3 °C in 0.05–0.25 M potassium phosphate buffer, pH 6.5 to 7.5, containing 1 mM EDTA and 5 mM mercaptoethanol. The fully purified enzyme retained all of its activity for at least 3 months when stored at −18 °C, at a protein concentration of 1 to 5 mg/mL in 0.2 M phosphate buffer, pH 7.0, containing 1 mM EDTA, 3 mM dithiothreitol, and 30% glycerol.

*Evidence of Homogeneity.* The purified enzyme was tested for homogeneity by subjecting it to acrylamide gel electro-

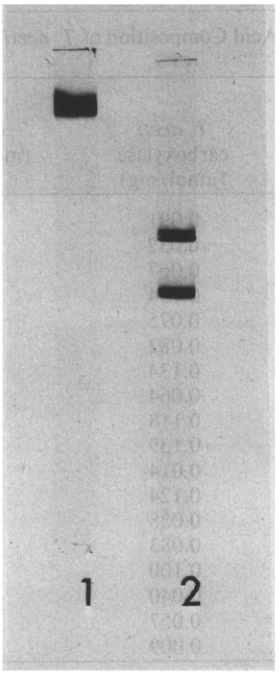


FIGURE 1: Polyacrylamide gel electrophoresis of native and dissociated nematode carboxylase. (1) Native enzyme on 4% acrylamide gel with 0.375 M Tris–glycine buffer, pH 8.3, stained with Coomassie Blue for 17 h. (2) $NaDodSO_4$ dissociated enzyme on 5% acrylamide gel with 0.1 M sodium phosphate buffer, pH 7.2, and 0.1% $NaDodSO_4$.

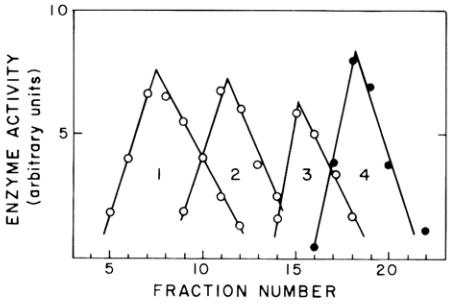


FIGURE 2: Sucrose density gradient centrifugation of acyl-CoA carboxylase. A mixture of 0.05 mg each of (1) yeast alcohol dehydrogenase; (2) beef catalase; (3) *E. coli* K-12-3300 β-galactosidase; and (4) *T. aceti* acyl-CoA carboxylase in 0.2 mL of 0.05 M Tris-HCl, pH 7.5, containing 10 mM $K_2HPO_4$, 1 mM EDTA, and 5 mM mercaptoethanol, was layered onto a 12-mL linear 5–30% sucrose gradient containing the same buffer. The sample was centrifuged for 11 h at 36 000 rpm in a Spinco SW 36 rotor at 3 °C. Thirty fractions were collected and assayed for individual enzyme activities. The relative distances sedimented by the enzymes were time independent under the experimental conditions. The calculated sedimentation coefficient of the carboxylase was 18.8 S relative to alcohol dehydrogenase 17.8 S relative to catalase, and 18.9 S relative to β-galactosidase.

phoresis under nondissociating and dissociating conditions. As shown in Figure 1, the enzyme migrated as a single component under nondissociating conditions in 4% acrylamide gels and as two components under dissociating conditions in $NaDodSO_4$–acrylamide gels. This basic pattern did not change when the gel concentration was varied or the amount of enzyme was scaled up to 100 μg per gel.

The enzyme (5.2 mg of protein per mL) in 0.2 M potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 5 mM mercaptoethanol was centrifuged at 56 000 rpm for 32 min at 21 °C in a Spinco Model E ultracentrifuge equipped with electronic speed control and schlieren optics. Under these conditions, the carboxylase sedimented as a single boundary with a sharp and symmetrical peak.

*Molecular Weight of the Native Enzyme.* The acyl-CoA carboxylase behaved as a single molecular species throughout the isolation and purification process. When followed by enzymatic assays, both the crude and purified carboxylases migrated with identical mobilities through sucrose density gradients and Sepharose 4B gel columns. An aggregated structure as it is encountered in the acetyl-CoA carboxylases of higher animals in the presence of tricarboxylic acid activators (Lane et al., 1974) was not encountered in the nematode enzyme. Thus, when the enzyme was centrifuged in sucrose density gradients in the presence or absence of 20 mM of citrate or isocitrate, no change in the migration pattern was observed.

Several methods were employed to determine the molecular size of the nematode carboxylase. Gel filtration of the carboxylase on a Sepharose 4B column (Andrews, 1970) with aldolase, catalase, urease, and β-galactosidase as standards yielded a Stokes' radius of 82 Å. Sucrose density gradient centrifugation of the enzyme (Figure 2) using alcohol dehydrogenase, catalase, and β-galactosidase (*E. coli* K-12-3300, mol wt 540 000; Craven et al., 1965) as references yielded an average sedimentation coefficient of 18.8 S and a molecular weight ranging from 620 000 to 700 000 (Martin & Ames, 1961). Velocity sedimentation of 5.2 mg of protein per mL in a Spinco Model E analytical centrifuge gave a sedimentation coefficient of 18.0 S.

Finally, with equilibrium centrifugation of the enzyme, an average molecular weight of 667 000 (651 000 to 686 000) was obtained using as partial specific volume a value of 0.736 mL/g. This value was calculated from the amino acid composition of the enzyme presented in Table II (Cohn & Edsall, 1943). Neither molecular association nor dissociation occurred during centrifugation, since plots of the logarithm of the fringe displacement against the square of the distance from the axis of rotation always yielded a straight line.

*Subunit Structure.* When the purified carboxylase was dissociated with $NaDodSO_4$ in the presence of dithiothreitol at 100 °C for 3 min and subjected to $NaDodSO_4$–acrylamide gel electrophoresis, it migrated as two components. The molecular weight of each component was estimated by comparing its electrophoretic mobility with those of seven $NaDodSO_4$-dissociated reference proteins (serum albumin, phosphorylase A, pyruvate kinase, fumarase, ovalbumin, horse liver alcohol dehydrogenase, and chymotrypsinogen). A plot of log molecular weight vs. electrophoretic mobility of the seven reference proteins yielded a nearly straight line. From several such plots, the molecular weights of the two carboxylase peptides were estimated as 82 000 and 58 000.

The presence of two distinct polypeptides in the nematode carboxylase was corroborated by dissociating the enzyme under several different conditions, including treatment with $NaDodSO_4$–urea, $NaDodSO_4$ at 37 °C, or guanidine hydrochloride (for details, see Experimental Procedure). Under all dissociating conditions, the enzyme yielded only two polypeptides upon acrylamide gel electrophoresis. The enzyme treated with $NaDodSO_4$–urea occasionally produced small quantities of several additional peptides ranging in molecular weight from 30 000 to 45 000. Since these minor components were also produced after prolonged storage of the $NaDodSO_4$-treated enzyme at −18 °C, they are most likely degradation products of the main peptides rather than true structural components of the enzyme. None of the dissociating procedures produced a low molecular weight polypeptide in the range of 10 000 to 20 000. Such small peptides are typical components of bacterial carboxylases where they function as biotinyl carrier proteins (Alberts & Vagelos, 1972; Woods &

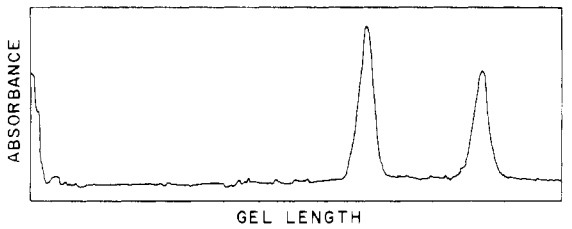


FIGURE 3: Scan of a $NaDodSO_4$–polyacrylamide gel of acyl-CoA carboxylase. $NaDodSO_4$-dissociated carboxylase (15 μg) was run on a 5% acrylamide gel. The gel was stained with Coomassie Blue overnight and scanned at 570 nm in a Gilford spectrophotometer, Model 240, provided with a linear transport. The molar ratio of the two polypeptides was determined by measuring each peak area and dividing its value by the peptide's molecular weight. The estimated ratio was 1:0.97.

Zwolinski, 1976). The molar ratio of the two carboxylase peptides was estimated from densitometric scans of the gels stained with Coomassie Blue (Figure 3). At low protein concentrations (5 to 20 μg), the relationship between optical density and protein concentration was linear, and the molar ratio of the two $NaDodSO_4$ peptides was close to one.

*Biotin Content.* Since the nematode carboxylase is sensitive to the biotin inhibitor avidin (see Meyer & Meyer, 1978), it most probably contains a biotinyl prosthetic group. This assumption was confirmed by determining the enzyme's biotin content via a colorimetric method entailing the formation of a complex with an avidin-azo dye (Green, 1970) and a microbiological method using *Lactobacillus plantarum* as the test organism (Shull et al., 1942). Assays employing both methods on several different enzyme batches yielded an average value of 1.58 μg of biotin per mg of protein. This is equivalent to 0.91 mol of biotin per mol of carboxylase protomer (mol wt 140 000). Since the carboxylase protomer consists of two polypeptide chains, studies were undertaken to determine which one of the two polypeptides contains the biotin prosthetic group. The biotin containing peptide was identified by running the $NaDodSO_4$-dissociated carboxylase on acrylamide gels, staining, and slicing the gels into sections and assaying each section microbiologically for biotin. It was found that 87% of the biotin was associated with the 82 000 peptide, 5% was associated with the 58 000 peptide and that 8% was randomly distributed over the gel. The low levels of biotin detected along the gel, including the section containing the 58 000 peptide, were most likely produced by some degradation of the $NaDodSO_4$-dissociated enzyme during electrophoresis or by handling the gels after electrophoresis. On these grounds, we assume that only the large peptide contains biotin.

*Amino Acid Composition.* An analysis of the amino acid composition of the purified *T. aceti* enzyme is presented in Table II. The nematode enzyme is marked by a low content of tryptophan, cystine, methionine, and histidine. Hydrophobic amino acids constitute 43% of the total amino acid residues. This ratio is close to that found in other acetyl-CoA and propionyl-CoA carboxylases (Inoue & Lowenstein, 1972; Kaziro & Ochoa, 1964).

TABLE II: Amino Acid Composition of *T. aceti* Acyl-CoA Carboxylase.[a]

| Amino acid | *T. aceti* carboxylase (μmol/mg) | *T. aceti* carboxylase (mol wt 667 000) (mol/mol) |
|---|---|---|
| Lys | 0.091 | 343 |
| His | 0.032 | 120 |
| Arg | 0.067 | 252 |
| Asp | 0.154 | 580 |
| Thr | 0.075 | 283 |
| Ser | 0.083 | 313 |
| Glu | 0.134 | 505 |
| Pro | 0.064 | 234 |
| Gly | 0.118 | 445 |
| Ala | 0.139 | 524 |
| ½-Cystine | 0.014 | 53 |
| Val | 0.124 | 467 |
| Met | 0.028 | 105 |
| Ile | 0.083 | 313 |
| Leu | 0.100 | 377 |
| Tyr | 0.040 | 151 |
| Phe | 0.057 | 215 |
| Trp | 0.009 | 34 |

[a] For analytical methods, see Experimental Procedure.

## Discussion

The *T. aceti* acyl-CoA carboxylase behaved as a stable, single molecular species throughout the purification procedure. High molecular aggregates of the kind occurring in animal acetyl-CoA carboxylases in the presence of tricarboxylic acid activators (Lane et al., 1974) were not observed with the nematode enzyme. Accordingly, neither citrate nor isocitrate increased its catalytic activity (see Meyer & Meyer, 1978).

In $NaDodSO_4$, the enzyme dissociates into two distinct polypeptides, a smaller 58 000 mol wt peptide and a larger 82 000 mol wt peptide which alone carries the biotinyl prosthetic group. Several pairs of these peptides or protomers constitute the oligomeric structure of the native enzyme. The number of protomers per mole of enzyme (mol wt 667 000) is 4.3, based on the biotin content of the enzyme (1.58 μg/mg), and 4.7, based on the molecular weight of the protomer (140 000). This leaves two possible subunit structures, one tetrameric and the other pentameric. The tetrameric structure is more likely, because it is frequently encountered in nature, whereas the pentameric structure is rare (Klotz et al., 1975). Moreover, several biotin containing enzymes are known to be tetramers, such as β-methylcrotonyl-CoA carboxylase (Apitz-Castro et al., 1970), pyruvate carboxylase (Scrutton & Yong, 1972), and chicken liver acetyl-CoA carboxylase (Lane et al., 1974). Another example may be porcine propionyl-CoA carboxylase, where a tetrameric structure is presumed on the basis of its biotin content (Kaziro & Ochoa, 1964).

The inherent functions of the two peptides constituting the nematode protomer remain to be elucidated. An analogy can perhaps be drawn to the wheat germ acetyl-CoA carboxylase (Heinstein & Stumpf, 1969) and to the well-characterized *Escherichia coli* acetyl-CoA carboxylase (Alberts & Vagelos, 1972), both of which are dissociable into enzymatically active subunits. From this analogy, it might be inferred that the larger biotin containing nematode peptide corresponds functionally to the biotin carboxylase–biotin carrier protein component of the plant and *E. coli* enzymes and that the smaller nematode peptide corresponds to their transcarboxylase component.

The nematode enzyme, as we will show in the accompanying paper (Meyer & Meyer, 1978), carboxylates propionyl-CoA more efficiently than acetyl-CoA. This prompted us to compare the percentage amino acid composition of the *T. aceti* enzyme with those of the chicken acetyl-CoA carboxylase (Gregolin et al., 1966), rat acetyl-CoA carboxylase (Inoue & Lowenstein, 1972), and porcine propionyl-CoA carboxylase (Kaziro & Ochoa, 1964) by the statistical method of Marchalonis & Weltman (1971). The results of this approach, though speculative, suggest that the *T. aceti* acyl-CoA car-

boxylase is in its primary structure more closely related to the propionyl-CoA carboxylase than to the acetyl-CoA carboxylases.

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