Vol. 82, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 14, 1978 Pages 834-839

# ISOLATION OF AN ACYL-CoA CARBOXYLASE FROM THE TAPEWORM SPIROMETRA MANSONOIDES

Haruko Meyer, Justus Mueller, and Franz Meyer

Department of Microbiology, Upstate Medical Center,
State University of New York, Syracuse, N.Y. 13210

Received April 18,1978

## Summary

An acyl-CoA carboxylase, which catalyzes the carboxylation of acetyl-, propionyl-, and butyryl-CoA, has been isolated from the tapeworm Spirometra mansonoides. The enzyme has an absolute requirement for ATP, $Mg^{2+}$, and $HCO_3^-$ and, in addition, requires $K^+$ for full catalytic activity. The enzyme has been purified 50-fold by a combination of calcium phosphate gel adsorption, ion-exchange column chromatography, and gel filtration. In its substrate specificity, $K^+$ requirement, molecular size, and antigenic behavior, the tapeworm enzyme is similar to the acyl-CoA carboxylase of another helminth--the free-living nematode Turbatrix aceti.

## Introduction

Recently we have isolated a biotin-containing acyl-CoA carboxylase from the free-living nematode Turbatrix aceti, and have characterized some of its physical and kinetic properties (1,2). These investigations revealed that the enzyme embodies a composite of properties which are generally considered typical of either propionyl-CoA carboxylases or acetyl-CoA carboxylases. The enzyme has highest catalytic activity with propionyl-CoA as substrate, requires $K^+$ for full activity, and resembles animal propionyl-CoA carboxylase in its amino acid composition. At the same time, the enzyme is located in the cytoplasm of the cell and presumably functions as an acetyl-CoA carboxylase in this organism. To evaluate whether these properties are merely features peculiar to the T. aceti enzyme, or features common to the acyl-CoA carboxylases of helminths in general, we have begun to look for similar enzymes in other helminths.

In this communication, we describe the isolation of an acyl-CoA

0006-291X/78/0823-0834$01.00/0

carboxylase from the tapeworm Spirometra mansonoides, and some properties of that enzyme. As will be shown, the tapeworm enzyme has several features in common with the T. aceti acyl-CoA carboxylase.

## Materials and Methods

The materials and methods used for purifying and assaying the tapeworm enzyme were similar to those described earlier (1,2). S. mansonoides larvae, reared in mice (3), were aseptically collected and washed with saline.

The antiserum against T. aceti acyl-CoA carboxylase was obtained as follows: Each of two New Zeanland female rabbits was injected at multiple subcutaneous sites with 2.0 mg of purified T. aceti acyl-CoA carboxylase, which was dissolved in 1.0 ml of 0.1 M phosphate buffer (pH 7.0), and emulsified with an equal volume of complete Freund's adjuvant. After a series of six such injections, spaced at ten day intervals, each animal received one intravenous injection of 1.5 mg of enzyme in 1.0 ml of phosphate buffer. Five days following the last injection, blood was removed by cardiac puncture. Before immunization, control blood had been taken from the same rabbits.

## Results

The procedures for the isolation and purification of the tapeworm acyl-CoA carboxylase were based on methods worked out for the purification of the T. aceti acyl-CoA carboxylase (1). However, some modifications were necessary to overcome the significantly greater instability of the tapeworm enzyme: 1.) Only freshly harvested larvae were used for preparing the enzyme. 2.) Homogenization of the larvae in a French Pressure Cell was conducted at pressures less than 5,000 pounds per square inch. 3.) Glycerol (5 to 15% by volume) was added to all buffer solutions to minimize loss of the enzyme's activity. 4.) Fractionation with $(NH_4)_2SO_4$ was omitted because this procedure lead to inactivation of the enzyme. 5.) Enzyme samples were not concentrated by filtration; instead, they were placed into dialysis tubes and then embedded in dry Sephadex.

Partial purification of the enzyme, as outlined in Table I, resulted in a 50-fold increase in the enzyme's specific activity over that of the crude high speed supernatant. The enzyme from the last purification step retained its full activity for at least one week when stored in the presence of 30%

Table I: Purification of Acyl-CoA Carboxylase from S. mansonoides*

| Purification steps | Total protein (mg) | Total activity** (units) | Specific activity (units/mg) |
|---|---|---|---|
| High speed centrifugation | 1,800 | 5.40 | 0.003 |
| Calcium phosphate gel adsorption | 257 | 3.86 | 0.015 |
| Cellex E cellulose chromatography | 74 | 2.96 | 0.040 |
| Sepharose 4B gel filtration | 16 | 1.38 | 0.086 |

* As starting material, 200 larvae having a wet weight of 8 g were used.

**Total activity was assayed by measuring the incorporation of $[^{14}C]HCO_3^-$ into propionyl-CoA (2). The reaction mixture had a final volume of 0.4 ml and contained: 60 mM $K_2HPO_4$ (pH 7.5), 5 mM $MgCl_2$, 1 mM ATP (Na-salt), 1 mM propionyl-CoA (Li-salt), 20 mM $[^{14}C]NaHCO_3$ (0.2 mCi/ mmol), and enzyme. Reactions were run for 10 min at 35°C. One unit = 1 µmol of $HCO_3^-$ fixed per min under the assay conditions.

glycerol at -18°C.

The tapeworm acyl-CoA carboxylase catalyzed the carboxylation of propionyl-CoA in the presence of $HCO_3^-$, ATP, and $Mg^{2+}$ (Table II). In addition to propionyl-CoA, the enzyme carboxylated butyryl-CoA and acetyl-CoA. The relative carboxylation rates for these substrates were: propionyl-CoA, 100%; butyryl-CoA, 60%; and acetyl-CoA, 30%. To determine whether the tapeworm enzyme, like the T. aceti enzyme (2), requires $K^+$ for full activity, the enzyme was freed from this cation by subjecting it to gel filtration (Table II, footnote). The $K^+$-free enzyme carboxylated propionyl-CoA at one fifth the rate of the enzyme with $K^+$. The activity of the $K^+$-free enzyme could be restored to its normal value by the addition of 10 to 40 mM of KCl. This restoration could not be accomplished by the addition of NaCl.

Rabbit antiserum, against pure T. aceti acyl-CoA carboxylase, inhibited the T. aceti enzyme and the tapeworm enzyme as well (Table III). Moreover, when the antiserum was first absorbed with the tapeworm enzyme, its activity against the T. aceti enzyme diminished. These observations suggest that the

Table II: Reactants Required by the Tapeworm Acyl-CoA Carboxylase*

| Assay system** | [$^{14}$C]Incorporated (cpm) |
|---|---|
| Complete | 5,280 |
| Minus ATP | 120 |
| Minus $Mg^{2+}$ | 169 |
| Minus propionyl-CoA | 107 |
| Minus propionyl-CoA; plus acetyl-CoA | 1,626 |
| Minus propionyl-CoA; plus butyryl-CoA | 3,376 |
| Minus $K^+$ | 1,074 |

* Enzyme from the last purification step (Table I) was freed from potassium phosphate by chromatography on a Sephadex G25 column (1x30 cm) equilibrated with buffer containing 0.2 M Tris-HCl (pH 7.2), 0.1 mM EDTA, 3 mM dithiothreitol, and 5% glycerol. The $K^+$-free enzyme was assayed immediately.

**The complete assay mixture contained 0.1 mg $K^+$-free enzyme, 50 mM Tris-HCl (pH 7.5), 20 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 1.5 mM propionyl-CoA, and 20 mM [$^{14}$C]$NaHCO_3$. Where indicated, 1.5 mM of either acetyl-CoA or butyryl-CoA was added to the reaction mixture. Reactions were run for 10 min at 35°C.

T. aceti acyl-CoA carboxylase and the tapeworm acyl-CoA carboxylase are immunochemically related.

## Discussion

The tapeworm acyl-CoA carboxylase resembles the nematode acyl-CoA carboxylase in its catalytic and physical properties. Both enzymes require $K^+$ for activation and carboxylate acetyl-, propionyl-, and butyryl-CoA at comparable rates. Both enzymes have similar affinities to calcium phosphate gel as well as to Cellex E ion-exchange cellulose, and also elute from a Sepharose 4B gel filtration column with comparable elution volumes ($V_e/V_o$ 2.00 and 2.14). Moreover, both enzymes are inhibited by antiserum against nematode acyl-CoA carboxylase.

Table III: Inhibition of the Tapeworm Enzyme with Antiserum against *T. aceti* Acyl-CoA Carboxylase*

| Serum added | Serum dilution | [$^{14}$C]incorporated (cpm) |
|---|---|---|
| None | | 3,080 |
| Anti-carboxylase | 1 : 90 | 1,138 |
| Anti-carboxylase | 1 : 60 | 632 |
| Anti-carboxylase | 1 : 30 | 468 |
| Control serum | 1 : 90 | 3,484 |
| Control serum | 1 : 60 | 3,835 |
| Control serum | 1 : 30 | 3,779 |

*Each sample had a final volume of 0.5 ml and contained: 0.1 mg enzyme, 0.1 M $K_2HPO_4$ (pH 7.3), 0.2 mM EDTA, 0.2 mM dithiothreitol, 12 mM NaCl, and varying amounts of antiserum against *T. aceti* acyl-CoA carboxylase. Samples were kept in an ice bath overnight. After addition of the appropriate reactants, residual enzyme activity was measured by assaying carboxylation of propionyl-CoA. Controls were prepared and assayed identically to the samples with one exception; in place of antiserum, either saline or control serum (described under Materials and Methods) was used.

Although the tapeworm and nematode enzymes have many properties in common, they most likely participate in different physiological processes. *S. mansonoides*, unlike *T. aceti* (4), lacks mechanisms for *de novo* fatty acid biosynthesis (5). Accordingly, the tapeworm's acyl-CoA carboxylase ought to be involved in processes other than the carboxylation of acetyl-CoA to malonyl-CoA—the committing step in the pathway of fatty acid biosynthesis. Clues as to the enzyme's role in the tapeworm, may be found within the already available evidence that propionyl-CoA carboxylase participates in the anaerobic energy metabolism of parasitic helminths. To account for the excretion of propionate by many parasitic helminths, Saz et al. proposed that propionate arises from succinyl-CoA *via* methylmalonyl-CoA, through the activities of methylmalonyl-CoA mutase and propionyl-CoA carboxylase (6,7).

The existence of the pathway, including the enzymes associated with it, has subsequently been shown in extracts of the liver fluke Fasciola hepatica (8), and recently in extracts of the tapeworm Spirometra mansonoides (9). On the basis of this information, it is likely that the acyl-CoA carboxylase described here is involved in the decarboxylation of methylmalonyl-CoA to propionyl-CoA.

The presence of carboxylases with similar properties in helminths of diverse metabolic capacities may be a sign that the enzymes' broad substrate specificity is not an accidental trait, but an essential quality which enables the enzyme to participate in a number of metabolic processes. This hypothesis leads us to further speculate that the evolutionary specialization of acyl-CoA carboxylases has not progressed as far within helminths as within higher animals.

Acknowledgements

This research was supported by Grant AI-0748610 from the National Institutes of Health, United States Public Health Service. We wish to thank Dr. Americo Marucci for his help and advice in setting up the immunological experiments.

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