Short Communication

Acetoacetyl-CoA Transferase and Thiolase Reactions Are Catalyzed by a Single Enzyme in *Clostridium acetobutylicum* ATCC824

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Clostridium acetobutylicum metabolizes carbohydrates anaerobically to acetone and butanol as the main fermentation products. A batch-wise acetone-butanol fermentation by *C. acetobutylicum* can be divided into two distinctive phases, an acid production phase and a solvent production phase. Organic acids produced during the former phase are taken up from the medium into cells and metabolized further in the latter phase. The final products are acetone and butanol, accompanied with ethanol, carbon dioxide, and hydrogen.

Acetoacetyl CoA: acetate CoA-transferase (acetoacetyl-CoA transferase) converts acetoacetyl-CoA to acetoacetate by transferring a CoA-moiety to acetate.^{1–5)} The acetoacetate produced is further irreversibly decarboxylated to acetone. So this enzyme seems to have a key role to produce acetone. Several kinds of CoA-transferase which transfer the CoA-moiety among organic acids have been observed in cell-free extracts of *C. acetobutylicum* and some of them have been purified. A CoA-transferase, however, which is responsible for converting acetoacetyl-CoA to acetoacetate (acetoacetyl-CoA transferase) has not been purified. We believe that purification and characterization of acetoacetyl CoA transferase is indispensable for the understanding of regulatory factors which affect the fermentation pattern of *C. acetobutylicum*. This is the first

report on the purification of acetoacetyl-CoA transferase of *C. acetobutylicum*. Surprisingly, the purified enzyme has been shown to have “thiolase” activity, which synthesizes acetoacetyl-CoA from two molecules of acetyl-CoA, in spite of the fact that the substrate specificity of the purified enzyme was highly restricted.

The enzyme activity of acetoacetyl-CoA transferase was assayed in the direction for acetoacetate formation with acetoacetyl-CoA as a CoA donor and acetate as a CoA acceptor by the method described by Andersch *et al.*²⁾ Thiolase activity was measured by the method of Hartmanis *et al.*³⁾ *Clostridium acetobutylicum* ATCC824 was grown at 35°C and pH 4.3 on a medium described by Andersch *et al.*²⁾ Cells at the middle of solvent production phase were harvested by centrifugation and stored at –40°C. The frozen cells were made into a cell-free extract by sonication (10% of maximum power, 30S, 10 times, Sonifier model 350, Bramson). The enzyme was purified by ammonium sulfate fractionation, column chromatographies on Toyopearl HW-40F, Q Sepharose Fast Flow, Sephacryl S-200HR, Dye Martrex Blue A, and Butyl-Toyopearl, followed by polyacrylamide gel electrophoresis. The overall purification of the enzyme was 55-fold with an activity yield of about 3%. A summary of the purification procedure is shown in Table I.

The purified CoA transferase is homogeneous on non-dissociating polyacrylamide gel electrophoresis. The molecular weight of the native CoA transferase was about 200,000 by Sephacryl S-200HR chromatography. Substrate specificity for several kinds of acyl-CoA are summarized in Table II. In these experiments, initial concentrations of acyl-CoA and carboxylic acids were 0.2 mM and 10 mM, respectively.

The purified enzyme was highly specific to acetyl-CoA and acetoacetyl-CoA. This result indicates that the enzyme is really responsible for acetoacetate formation from acetoacetyl-CoA. In spite of the narrow specificity of the purified enzyme, this enzyme also had thiolase

Table I. PURIFICATION OF ACETOACETYL-CoA TRANSFERASE

Step	Protein (mg)	Activity (units)	Specific activity (units/mg)	Purification (fold)	Recovery (%)
Cell-free extracts	1013	13.4	0.01		
Ammonium sulfate fractionation	212	20.3	0.1		
Q-Sepharose	28.3	39.6	1.4	1	100
Sephacryl S-200HR	8.3	9.2	1.1	0.8	23
Dye Martrex Blue A	4.0	4.3	1.1	0.8	11
Butyl Toyopearl	1.2	2.4	2.0	1.4	6
PAGE	0.01	1.3	76.4	54.6	3

Table II. SUBSTRATE SPECIFICITY OF PURIFIED CoA TRANSFERASE

Method	Acyl CoA (CoA donor)	Carboxylic acid (CoA acceptor)	Relative rate (%)
A ^a	Acetoacetyl CoA	Acetate	100
	Butyryl CoA	Acetate	<2
	Crotonyl CoA	Acetate	<2
B ^b	Acetyl CoA	Acetoacetate	100
	Acetyl CoA	Acetate	95

All rates are expressed relative to the first entry in each group of experiments.

^a A method described by Andersch *et al.*²⁾ (acetoacetyl-CoA assay) was used.

^b A method described Sramek *et al.*⁶⁾ (thiolase assay) was used.

activity, an ability to produce an acetoacetyl-CoA from 2 molecules of acetyl-CoA. Recently Wiesenborn *et al.*⁷⁾ reported on the purification of thiolase from *C. acetobutylicum* cells, but they did not mention acetoacetyl-CoA transferase activity of the purified enzyme. Some properties of our enzyme, such as molecular weight, subunit structure, and effects of inhibitors are similar to those of the purified thiolase (Nakamura, unpublished data). The enzyme purified in this report might be similar to the "thiolase" reported by

Wiesenborn *et al.*⁷⁾

Our finding shows that two important reactions related to CoA transferase, localized successively in the metabolic pathway of *C. acetobutylicum*, can be catalyzed by a single enzyme. This finding indicates that the formation of acetone and butyrate is metabolically coupled, and is very important for the understanding of the regulatory mechanisms which decide fermentation phases and end product patterns in *C. acetobutylicum*.

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