Note

Measurement of Intracellular Acetyl-CoA and Malonyl-CoA in Bacteria and Yeasts Using Acyl-CoA Cycling

Goro NOMURA, Yukihiro YOSHIDA
and Yoshichika TAKAMURA

Department of Agricultural Chemistry,
Faculty of Agriculture, Ibaraki University,
Ami-machi, Ibaraki 300-03, Japan

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Acetyl-CoA plays central role in the regulation of various metabolic processes such as glycolysis, fatty acid synthesis and energy yielding metabolic pathways. Recently, we established a new micro-method for the measurement of acyl-CoAs using malonate decarboxylase¹⁾ (acetyl-CoA: malonate CoA transferase [EC 2.8.3.3.]) (called "acyl-CoA cycling").²⁾ This method is sufficiently sensitive and simple for determining intracellular levels of acetyl-CoA and malonyl-CoA in routine experiments using a spectrophotometer. However, when biological fluids extracted by mechanical disruption of bacterial cells in aqueous buffers were subjected to the assay, the accuracy of quantification sometimes fluctuated and the linearity was disturbed. This problem was thought to be due to rapid metabolism of the CoA-thioesters and interference by acetate kinase originally present in the samples. Thus, acid extraction procedure was examined as to the maximal release of CoA-thioesters, prompt and irreversible inactivation of relevant enzymes and long-term stability of the extracted CoA-thioesters.

Escherichia coli K-12 was grown at 30°C aerobically in a synthetic medium consisting of 5 g KH₂PO₄, 13 g K₂HPO₄, 0.2 g MgCl₂, 2 g (NH₄)₂SO₄ and 5 g glucose in 1 l of distilled water, pH 7.0. *Brevibacterium ammoniagenes* was cultured aerobically at 30°C in a medium consisting of 1% meat extract, 1% peptone and 0.5% NaCl, pH 7.2. *Saccharomyces cerevisiae* was cultured with shaking at 30°C in a medium consisting of 2% malt extract, 0.1% peptone and 2% glucose, pH 6.0. Microbial cells were harvested by centrifugation at 12,000×g and 2°C for 10 min, and then ice-cold sulfuric acid, perchloric acid (PCA) and trichloroacetic acid (TCA) were added. After standing for 30 min at 0°C, the cold-acid extracts obtained on centrifugation were neutralized and then subjected to measurement of total acyl-CoA, the sum of acetyl-CoA and malonyl-CoA, by the acyl-CoA cycling method.²⁾ As shown in Table I, 0.6 N cold-sulfuric acid gave the optimal acyl-CoA extraction from *E. coli* K-12. The amount of intracellular total acyl-CoA extracted

under these conditions was similar to that on 0.01 N cold sulfuric acid extraction with sonic disruption, and little authentic acetyl-CoA was decomposed by the 0.6 N sulfuric acid. However, on 0.1 N sulfuric acid extraction with

TABLE I. EXTRACTION OF CELLULAR ACYL-CoA FROM *Escherichia coli* K-12 AND RECOVERY OF AUTHENTIC ACETYL-CoA

To 7 mg (dry weight) of *E. coli* cells, 10 ml of inorganic and organic acids was added at the concentrations indicated and then extraction was carried out at 0°C, followed by standing for 30 min. For the authentic acetyl-CoA recovery test, 2 nmol acetyl-CoA was dissolved in 1 ml cold sulfuric acid and then incubated for 30 min at 0°C, and then 20 μl of the solution, corresponding to 40 pmol of acetyl-CoA, was assayed.

Extraction procedure	Total acyl-CoA determined	Recovery of acetyl-CoA
H ₂ SO ₄ 0.01 N & sonic disruption	650 ^a (100%)	
H ₂ SO ₄ 0.1 N	548 (84)	40 ^b (100%)
0.6 N	662 (102)	39 (98)
1.2 N	582 (89)	21 (53)
PCA 0.1 N	32 (5)	
0.4 N	26 (4)	
0.6 N	0 (0)	
TCA 0.1 N	40 (6)	
0.4 N	18 (3)	
0.6 N	0 (0)	

^a pmol/mg dry weight.

^b pmol/tube.

FIG. 1. Linearity of the Amount of Cellular Acyl-CoA Determined as a Function of the Amount of the Cold-acid Extract from *Escherichia coli* K-12.

Intracellular total acyl-CoA extracted with 0.6 N cold-sulfuric acid from *E. coli* K-12 was measured by the acyl-CoA cycling method. The relationship between acyl-CoA determined and dry cell weight is shown.

FIG. 2. Long-term Stability of the Extracted Acyl-CoA. Intracellular acyl-CoA extracted from *E. coli* K-12 with 0.6 N cold-sulfuric acid was neutralized to pH 6.0 and then stored at 0°C as indicated. Intact cells were stored at the same temperature as pellets in centrifuge tubes, and intracellular acyl-CoA was extracted and then measured at the times indicated. ●, extracted total acyl-CoA with 0.6 N cold-sulfuric acid; ○, total acyl-CoA in the intact cells.

standing for 30 min, the release of cellular acyl-CoA was incomplete, while 1.2 N sulfuric acid was so strong that 49% of the authentic acyl-CoA was decomposed. PCA and TCA, which are most commonly used for the extraction of nucleotides and related compounds from microbial cells,³⁾ could not be utilized for this method. As shown in Fig. 1, there was a linear relationship between the amount of the cold-acid extract prepared from *E. coli* cells and total acyl-CoA determined, ranging from 5 to 30 µg dry cell weight per tube. This result indicated that quantitative measurement of the intracellular concentration of total acyl-CoA can be performed by the cold-acid extraction procedure. The intracellular acyl-CoA level in intact cells of *E. coli* K-12 decreased to less than 50% of the initial level during storage at 0°C for 24 hr (Fig. 2). On the other hand, the amount of total acyl-CoA extracted with 0.6 N cold sulfuric acid remained unchanged for more than 50 hr, indicating the long-term stability of the extracted CoA-thioesters.

For extraction of total acyl-CoA from Gram-positive bacteria and yeasts, the extraction procedure established for *E. coli* K-12 was not sufficient unless combined with mechanical disruption (Table II). For *Brev. ammoniagenes*, which was selected as a representative Gram-

TABLE II. MEASUREMENT OF THE INTRACELLULAR CONCENTRATION OF TOTAL ACYL-CoA EXTRACTED BY DIFFERENT PROCEDURES FROM GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA, AND YEASTS

Extraction	Organisms		
	Total acyl-CoA ^a		
	A	B	C
0.6 N Sulfuric acid, standing for 30 min	331	52	14
0.6 N Sulfuric acid, sonic, 50 W, 3 min	325	272	149
0.6 N Sulfuric acid, Mix-tower, 20 min	294	261	184

^a pmol/mg dry weight.

A, *Escherichia coli* K-12; B, *Brevibacterium ammoniagenes*; C, *Saccharomyces cerevisiae*.

positive bacterial species, sonic disruption (Branson Sonifier; 50 W, 3 min at 0°C) was indispensable; the intracellular level of total acyl-CoA in *Brev. ammoniagenes* was five times that extracted with 0.6 N cold-sulfuric acid on standing for 30 min. For extraction from *S. cerevisiae*, which was selected as a representative yeast, stronger mechanical breakdown, that is shaking of the cells suspended in 0.6 N cold-sulfuric acid with glass beads for 20 min on a tower-shaped mixer (Taiyo Mix-Tower Model A-14),⁴⁾ gave a good yield.

The procedures described in this paper gave excellent results as to recovery, long-term stability and linearity on determination of the extracted acyl-CoA. The advantages of the present method are that it requires only a small sample of microbial cells, it does not require the complicated isolation of individual components and it allows the handling of a large number of samples at one time.

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