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Received April 20th, 1959

Direct enzymic conversion of malonic semialdehyde to acetyl-coenzyme A

An enzyme obtained from a strain of *Pseudomonas fluorescens* has been reported[1] to catalyze the hydration of acetylenemonocarboxylic acid with the formation of malonic semialdehyde. The present communication is prompted by the discovery of a novel reaction for the further utilization of malonic semialdehyde. This involves a CoASH- and DPN-linked conversion to form acetyl-SCoA. The system is experimentally irreversible:

$$OHCCH_2COOH + DPN^+ + CoASH \rightleftharpoons CH_3COSCoA + CO_2 + DPNH + H^+ \qquad (1)$$

If the reaction proceeds by way of a CoASH-linked aldehyde dehydrogenase[2] followed by decarboxylation[3], malonyl-SCoA would be the expected first product. The system described is unique in that malonyl-SCoA has been ruled out as a free intermediate.

The product of reaction (1) has been shown to be a thiol ester which was identified as acetyl-SCoA by arsenolysis with phosphotransacetylase[4], and chromatography of the hydroxamate derivative formed from the isolated thiolester, with the solvent system of VAGELOS*. The last method definitively separates acetylhydroxamate (R_F, 0.62) from malonylmonohydroxamate (R_F, 0.14). The enzyme has been purified 200-fold by a combination of salt fractionation, elution from calcium phosphate gel and chromatography on N,N-diethylaminocellulose. Combination of eluate fractions produced only additive effects on activity; the system behaves as a single enzyme.

The stoichiometry of the reaction, detailed in Table I, is that expected for

TABLE I

STOICHIOMETRY OF THE REACTION

Incubations were carried out in the presence of the following, expressed as μmoles/ml: K-phosphate, pH 7.0, 50; mercaptoethanol, 5; an excess of the enzyme. Expts. 1 and 2 included CoASH, 0.3; malonic semialdehyde, 0.4; limiting quantities of DPN. When the reaction was completed as judged by following the formation of DPNH spectrophotometrically, aliquots were removed and assayed for aldehyde and thioester. Expts. 3 and 4 were conducted in manometric vessels at 30° and contained DPN, 4 and CoASH, 4. Expts. 3 and 4 included 4.0 and 4.2 μmoles, respectively, of malonic semialdehyde. After 20-min incubation, the reaction was terminated by the addition of 0.1 ml 1 *N* H_2SO_4 and the CO_2 evolved was measured. Aliquots were taken for aldehyde and hydroxamate determinations. All results are expressed in μmoles.

Expt.	*Malonic Semialdehyde*	*DPNH*	*Thiol ester*	CO_2
1	— 0.096	+ 0.089	+ 0.082	
2	— 0.150	+ 0.162	+ 0.156	
3	— 4.0			+ 3.6
4			+ 3.5	+ 3.8

Abbreviations: CoASH, coenzyme A; DPN+, diphosphopyridine nucleotide.
* Personal communication from Dr. P. R. VAGELOS.

reaction (1). Neither succinic semialdehyde nor acetaldehyde is oxidized in this system. When malonyl-SCoA (3 μmoles) is substituted for malonic semialdehyde* in the incubation system used in Table I (Expt. 3), acetyl-SCoA is not formed and malonyl-SCoA is recovered. When malonyl-SCoA and DPNH are incubated with the enzyme, DPN^+ is not formed. Malonyl-SCoA (1 μmole) does not inhibit the formation of acetyl-SCoA. These data support the contention that malonyl-SCoA is not a free intermediate in the conversion of malonic semialdehyde to acetyl-CoA.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. (U.S.A.)

WILLIAM B. JAKOBY
ESTHER W. YAMADA**

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Received April 16th, 1959

* Malonic semialdehyde was a gift from Dr. W. G. ROBINSON and Dr. M. J. COON.
** Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

Enzymic synthesis of thiamine

WOOLLEY[1] suggested that thiamine is synthesized enzymically from the free pyrimidine and thiazole moieties by utilizing the energy of a glycolytic system. It has been recently reported[2] that the reaction catalyzing the condensation of the pyrimidine OMP and the thiazole Th in cell-free yeast extracts involves the formation of a monophosphate ester of OMP. In the present study, a cell-free extract from "Oriental" baker's yeast has been obtained which catalyses the synthesis of thiamine (measured by the thiochrome assay) after incubation of OMP and Th with ATP and Mg^{++}.

The enzyme was extracted from quick-frozen yeast by the method used to extract acetyl CoA kinase[3]: the yeast was frozen with dry-ice and ether, and the frozen yeast (200 g) extracted overnight with 400 ml 0.05 *M* Na_2HPO_4 in the cold room. The suspension was centrifuged at 6500 × *g* for 20 min and the supernatant brought to 0.65 saturation by adding solid $(NH_4)_2SO_4$ with stirring. The solution was kept neutral by adding 2.0 *M* KOH and 0.2 *M* EDTA. After centrifugation the protein was dissolved in 0.05 *M* Tris buffer, pH 7.6. The enzyme preparation was then generally stored in a deep freeze. For further use the frozen material was dialyzed against 0.01 *M* Tris, 10^{-4} *M* EDTA, at pH 7.6, for 3 h in the cold. The solution still contained a small amount of thiamine. As shown in Table I, ATP and Mg^{++} are required for the synthesis of thiamine. ADP has a slight effect, due probably to the presence of myokinase, while other nucleotide polyphosphates have no effect.

When the enzyme preparation is incubated with OMP, ATP and Mg^{++} in the absence of Th for 30 min, an intermediate "active OMP compound" appears to be

Abbreviations: OMP, 2-methyl-4-amino-5-hydroxymethylpyrimidine; OMP-P, OMP monophosphate ester; OMP-PP, OMP pyrophosphate ester; Th, 4-methyl-5-hydroxyethyl thiazole; Th-P, Th monophosphate ester; Th-PP, Th diphosphate ester; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate.