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**

[1] E. W. YAMADA AND W. B. JAKOBY, *J. Biol. Chem.*, 235 (1959) in the press.
[2] R. M. BURTON AND E. R. STADTMAN, *J. Biol. Chem.*, 202 (1953) 873.
[3] O. HAYAISHI, *J. Biol. Chem.*, 215 (1955) 125.
[4] E. R. STADTMAN, *J. Biol. Chem.*, 196 (1952) 527.

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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.

TABLE I

REQUIREMENTS FOR THIAMINE SYNTHESIS

The incubation mixture (10 ml) contained 200 μmoles Tris buffer (pH 7.0), 10 μmoles ATP, 10 μmoles $MgCl_2$, 10 μmoles cysteine, 0.1 μmole OMP, 0.1 μmole Th and 5 mg protein. After 2 h incubation at 38°, 0.3 ml 1 *N* HCl was added and the mixture heated for 15 min at 80°. The synthesized thiamine was measured by the thiochrome method[4] and compared with a blank without incubation.

	Thiamine formed mμmoles 2 h
Complete system	10.7
No ATP	0
No $MgCl_2$	0
No cysteine	10.3
Boiled enzyme	0

formed. This is indicated by the fact that, on addition of Th to the previously incubated samples, the initial formation of thiamine is more rapid than without preincubation. In this case, the preincubation with Th in the absence of OMP has also a similar effect on the formation of thiamine though the rate is not as great as obtained with OMP preincubation (Fig. 1). This suggests that the formation of thiamine is preceded by activation of OMP and Th, presumably phosphorylation by ATP, followed by enzymic condensation of the two activated compounds to form the thiamine molecule.

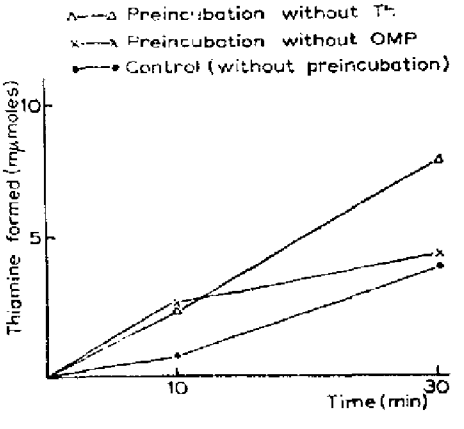

Fig. 1. Activation of OMP and Th by ATP. The complete system contained 200 μmoles Tris buffer (pH 7.0), 10 μmoles ATP, 10 μmoles $MgCl_2$, 10 μmoles cysteine, 0.1 μmole OMP, 0.1 μmole Th and 5 mg protein. Preincubation without Th or OMP for 30 min, followed by addition of Th or OMP, and further incubation for 10 and 30 min. Temperature, 38°.

In order to clarify the nature of the activated compounds, phosphate esters of OMP and Th were prepared by a slight modification of the method of WEIJLARD[5]. OMP-P, OMP-PP, Th-P and Th-PP were isolated respectively by means of column chromatography with Dowex-1 resin (formate form). The enzyme preparation is able to condense these OMP- and Th-phosphate esters without ATP and Mg^{++}. The rate of formation of thiamine was estimated as shown in Table II.

It can be concluded that two steps are required for the synthesis of thiamine. The first step involves the formation of phosphate esters of OMP and Th by an ATP-linked reaction. The second step is the condensation of such phosphate esters with the formation of thiamine monophosphate or thiamine pyrophosphate. No decision can yet be made concerning the nature of the phosphorylated product, since the

TABLE II

THIAMINE SYNTHESIS FROM OMP- AND TH-PHOSPHATE ESTERS WITHOUT ATP

The complete system contained 200 μmoles Tris buffer (pH 7.0), 10 μmoles cysteine, 0.1 μmole OMP-P or OMP-PP, 0.1 μmole Th, Th-P or Th-PP and 5 mg protein.

Substrates	*Thiamine formed mμmole 2 h*
OMP-P + Th-P	1.4
OMP-P + Th-PP	0.4
OMP-P + Th	0
OMP-PP + Th	1.7
OMP-PP + Th-P	63.2
OMP-PP + Th-PP	22.2
OMP* + Th	11.3

* Original complete system containing 10 μmoles ATP and 10 μmoles $MgCl_2$.

thiamine formed consists of free and phosphorylated thiamine and the enzyme system contains phosphatase activities.

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YOSHITSUGU NOSE
KIYOSHI UEDA
TAKASHI KAWASAKI

Biochemical Institute, Kyoto Prefectural University, School of Medicine, Kyoto (Japan)

1 D. W. WOOLLEY, *Nature*, 171 (1953) 323.
2 D. L. HARRIS AND J. YAVIT, *Federation Proc.*, 16 (1957) 192.
3 M. E. JONES AND F. LIPMANN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, Inc., New York, 1955, p. 585.
4 A. FUJITA, Y. NOSE, S. KOZUKA, T. TASHIRO, K. UEDA AND S. SAKAMOTO, *J. Biol. Chem.*, 196 (1952) 289.
5 J. WEIJLARD, *J. Am. Chem. Soc.*, 64 (1942) 2279.

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On the biosynthesis of felinine

DATTA AND HARRIS[1] noted the existence of a new ninhydrin-positive spot upon paper chromatography of cat urine. WESTALL[2] isolated this material, which he called felinine, and obtained evidence indicating that its structure was that of S-(3-hydroxy-1:1-dimethylpropyl)cysteine

$$HOOC\text{-}CH(NH_2)CH_2\text{-}S\text{-}C(CH_3)_2\text{-}CH_2\ CH_2OH$$

This structural assignment was confirmed by synthesis by TRIPPETT[3].

We have developed an analytical method for felinine based on an ion-exchange chromatographic separation, followed by development of the ninhydrin color in the appropriate fractions, and have obtained evidence that cystine on the one hand, and either leucine or mevalonic acid on the other, can contribute to the formation

Abbreviation: MVA, mevalonic acid.