THIAMINE KINASE IN THE MEMBRANE FRACTION OF *Escherichia coli**

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Received April 12, 1967

In recent studies on the transport of thiamine in *Escherichia coli*, we found that this compound was accumulated by the cells as thiamine pyrophosphate (TPP). The details of these experiments will be presented elsewhere, but TPP was detected at the earliest times used for the transport studies. These results suggested that *E. coli* contained thiamine kinase (EC 2.7.6.2) and that the kinase probably participated in the transport of thiamine.

While thiamine kinase has been isolated from yeast (Kaziro, 1959) and liver (Mano, 1960), it has not been previously detected in microbial cells. In the present report, evidence is presented that this enzyme, which catalyzes the formation of TPP from thiamine, is present in *E. coli*, and is located in the membrane fraction of spheroplast preperations. No activity was detected in the soluble fraction.

Materials and Methods

The disodium salt of ATP, the sodium salt of ADP, thiamine pyrophosphate chloride, and lysozyme from egg white were purchased

*This investigation was supported by the Scientific Research Fund of the Ministry of Education in Japan.

from Sigma Chemical Company. The cells of a thiamine, thiazole-lacking mutant of E. coli W were grown in the minimal medium of Davis and Mingioli (1950) in the presence of 0.01 μM thiamine. They were harvested at the stationary phase and washed once with a saline solution, and then suspended in a solution of 0.05 M Tris-HCl, pH 7.5, and 1 mM 2-mercaptoethanol. The cell suspension was treated for 10 min at 2° in a sonic oscillator (10 kc), then centrifuged for 15 min at 15,000 Xg, and the sediment was suspended in the same buffer used above. This suspension was employed as the "membrane fraction." Spheroplasts were prepared from the washed intact cells of E. coli W, thiazoleless strain, by treatment with lysozyme and EDTA (Nagata, Mizuno and Maruo, 1966). Spheroplasts were then lysed in a solution of 0.05 M Tris-HCl, pH 7.5, and 10 mM $MgCl_2$, centrifuged for 20 min at 15,000 Xg to collect the membranes, and the latter were suspended in a solution containing 0.05 M Tris-HCl, pH 7.5, and 1 mM 2-mercaptoethanol.

Thiamine kinase activity was measured according to the procedures described by Kaziro (1959). The reaction mixture contained: 100 μmoles Tris-HCl, pH 7.5; 10 μmoles ATP; 10 μmoles $MgCl_2$; 30 mμmoles thiamine; and the enzyme preparation (5 mg protein) in a total volume of 4 ml. After incubation for 60 min at 37°, the reaction was stopped by heating for 5 min at 90°, followed by centrifugation to remove denaturated protein. Thiamine pyrophosphate in the supernatant fluid was determined manometrically by carbon dioxide evolution with yeast apocarboxylase and sodium pyruvate (Aoshima, 1958).

Results

As shown in Table I, the enzyme activity was detected in

Table I

Thiamine kinase activity in the membrane fraction of E. coli

Reaction system	TPP formed
	mμmole/mg/hr
Complete	0.091
" -thiamine	0.010
" -ATP	0.059
" -ATP, +ADP	0.101
" +KCN	0.069
" -ATP, +KCN	0.021
" -ATP, +ADP, +KCN	0.058
" -membrane fraction, +soluble fraction	0
" but boiled enzyme	0

Complete reaction mixture contained: 100 μmoles Tris-HCl, pH 7.5, 10 μmoles ATP, 10 μmoles $MgCl_2$, 30 mμmoles thiamine, and enzyme preparation (5 mg protein) in a total volume of 4 ml.

the membrane fraction, but not in the soluble fraction of the sonicated cells. Although omission of ATP from the complete system lowered the activity by only 38%, the activity in the absence of ATP decreased markedly by adding 3.3 mM KCN. Substitution of ATP by ADP gave full activity, and this activity was again depressed in the presence of KCN. This result shows that ATP is required for TPP formation from thiamine. ATP is apparently generated from either endogenous or exogenous ADP

through oxidative phosphorylation; the latter system appears to be tightly associated with the membrane fraction.

Activity of the same order of magnitude as that of the thiazoleless mutant of E. coli W was observed in a mambrane fraction obtained from a mutant of E. coli W which was unable to synthesize the thiamine pyrimidine moiety and which was grown in the presence of 0.01 μM thiamine. On the other hand, a comparable membrane fraction of E. coli K12, a wild strain, showed a low activity of TPP formation, 0.02 - 0.03 mμmole/mg protein/hr.

The initial rate of TPP formation was proportional to the amount of enzyme added, up to 7.5 mg protein. Measurement of the time course of TPP formation showed that the reaction proceeded linearly for 60 min when 5 mg enzyme protein was used. The initial reaction rate was also dependent upon the amount of thiamine used in the range 0.1 - 1.0 mμmole; the apparent Km for thiamine was 0.7×10^{-6} M, plotting the results according to the method of Lineweaver and Burk (1934).

In order to confirm the existence of thiamine kinase in the membrane fraction of E. coli cells, we prepared spheroplasts from intact cells of E. coli W, thiazoleless strain (grown in the presence of 0.01 μM thiamine) by treatment with lysozyme and EDTA. The membrane fraction of the spheroplasts was collected by centrifugation after lysis in a hypotonic buffer solution. The results given in Table II show that thiamine kinase is located in the membrane fraction, but not in the soluble fraction of the spheroplasts.

Attempts to solubilize the enzyme from the sonically-prepared membrane fractions using deoxycholate or Tween 80, or by using alkali with the spheroplast membrane fractions, have been unsuccessful.

Table II

Thiamine kinase activity in the spheroplast membrane fraction of *E. coli*

Enzyme preparations	TPP formed
	mμmole/mg/hr
Cell membrane fraction	0.110
Cell soluble fraction	0
Spheroplast membrane fraction	0.040
Spheroplast soluble fraction	0

Discussion

Thiamine kinase activity was detected in the membrane fraction of *E. coli* cells; the enzyme has not yet been solubilized. The detection of thiamine pyrophosphate, not free thiamine, in *E. coli*, can now be explained. While the enzymes involved in thiamine biosynthesis from its pyrimidine and thiazole moieties are extractable into the soluble fraction of *E. coli* cells (Nose *et al.*, 1964), the physiological significance of thiamine kinase in the membrane fraction of bacteria is unknown. Possibly, the enzyme participates in thiamine transport.

The enzyme in the membrane fraction of *E. coli* required either ATP or ADP, but the effect of ADP was markedly inhibited by adding KCN, suggesting generation of ATP from ADP through oxidative phosphyorylation in the membrane fraction. There is ample evidence that shows the existence of electron transport and oxidative phosphorylation systems in the membrane fraction of many bacteria including *E. coli* (Asnis, Vely and Glick, 1956).

Furthermore, amino acid incorporation into proteins of the membrane fraction of E. coli (Nisman, 1959) and of Pseudomonas fluorescens (Yoshida et al., 1960) proceeded equally well in the presence and absence of ATP, and the incorporation was inhibited by addition of HCN. These observations support the view that endogenous and exogenous ADP is utilized for TPP formation after conversion to ATP through oxidative phosphorylation in E. coli membrane preparations.

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