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Inhibition of Thiamine Transport by Chloroethylthiamine in *Escherichia coli*

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Chloroethylthiamine was found to inhibit an entrapment of thiamine as thiamine monophosphate by blocking thiamine monophosphokinase in the cytoplasm after thiamine was taken up by the cells of *Escherichia coli*.

Chloroethylthiamine is a new, effective coccidiostatic agent which has been assumed to act on the coccidium by inhibiting thiamine uptake from the host cells (9).

During an investigation into the mechanism of thiamine transport in *Escherichia coli*, we found that chloroethylthiamine markedly inhibits ^{14}C-thiamine uptake by the cells. In *E. coli*, exogenous thiamine has been known to be accumulated intracellularly in the form of thiamine pyrophosphate (TPP) (3). It was recently demonstrated that TPP is formed via thiamine monophosphate (TMP) from thiamine after thiamine has passed through the cell membrane of *E. coli* (6) and that the cells possess both thiamine monophosphokinase (2) and TMP kinase (7) which are responsible for the conversion of thiamine into TPP. It was therefore of interest to determine whether chloroethylthiamine inhibits the entry of free thiamine into the cells or its accumulation as thiamine phosphates in the cells. In this communication we report that chloroethylthiamine primarily inhibits entrapment of thiamine as TMP in *E. coli*.

A mutant of *E. coli* K-12 that is auxotrophic for thiamine thiazole (KG 33) was used in this experiment (3). Figure 1 shows the effect of chloroethylthiamine on ^{14}C-thiamine uptake by KG 33. The rates of the uptake were inhibited 77.5% and 85.0% at 5 min after incubation at 37 C by the addition of chloroethylthiamine at molar ratios to thiamine of 10:1 and 100:1, respectively.

To investigate the effect of chloroethylthiamine on the initial uptake of ^{14}C-thiamine by the cells, the uptake was assayed at shorter time intervals for the initial 5 min of incubation with glucose or without glucose. As shown in Fig. 2, in the presence of chloroethylthiamine and glucose, the rate of ^{14}C-thiamine uptake reached a peak at 2 min after incubation at 37 C, and

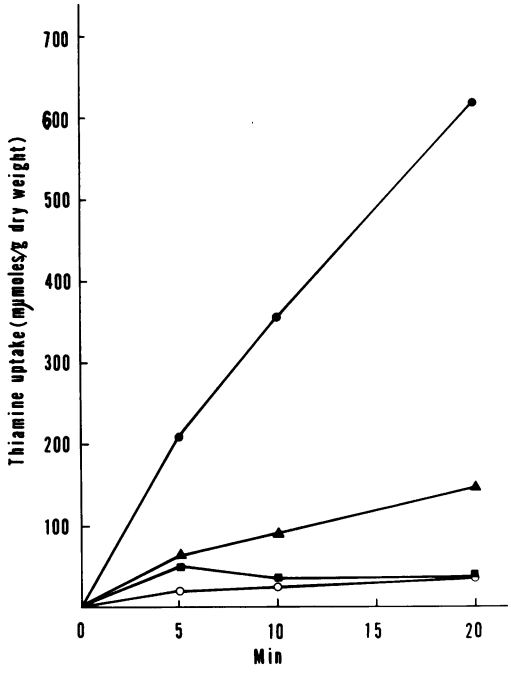

FIG. 1. *Effect of chloroethylthiamine on ^{14}C-thiamine uptake in Escherichia coli KG 33. E. coli KG 33 was grown at 37 C on 500 ml of Davis and Mingioli minimal medium (1) containing 0.2% glucose and 0.04 μM thiamine thiazole. The cells were harvested at stationary growth phase and washed once with minimal medium. Assay of ^{14}C-thiamine uptake was carried out as previously reported (3). The cell suspensions containing 0.4% glucose were incubated with 1 μM ^{14}C-thiamine (14.0 mCi/mmole) in the presence or absence of chloroethylthiamine. Symbols: ●, control at 37 C; ○, control at 0 C; ▲, 10 μM, or ■, 100 μM chloroethylthiamine added to the reaction mixture and incubated at 37 C.*

thereafter it decreased slowly to the same level as that in the cells incubated with ^{14}C-thiamine at 0 C. On the other hand, in the absence of glucose, the rate of the uptake by control cells was

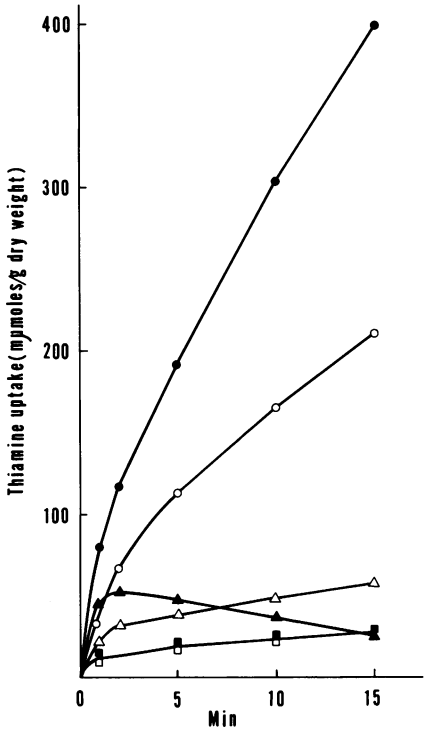

FIG. 2. *Effect of chloroethylthiamine on the time course of ^{14}C-thiamine uptake in the presence or absence of glucose. Cell suspensions containing 1 μM ^{14}C-thiamine (14.0 mCi/mmole) and 100 μM chloroethylthiamine were incubated with or without 0.4% glucose. Closed symbols represent the uptake in the presence of 0.4% glucose, and open symbols, in the absence of glucose. Symbols: ● and ○, control at 37 C; ■ and □, control at 0 C; ▲ and △, 100 μM chloroethylthiamine added and incubated at 37 C.*

approximately 55% of that found in its presence, and the initial uptake of ^{14}C-thiamine was also inhibited by chloroethylthiamine. However, the level of initial uptake was maintained during the incubation period even in the presence of chloroethylthiamine.

Recently, Kawasaki and Yamada reported that free thiamine taken up by mutants of *E. coli* auxotrophic for TMP passed outward through the cell membrane when glucose was present (4). The data reported above show an analogous finding suggesting that ^{14}C-thiamine taken up by the cells may be lost to the external medium through the membrane in the presence of both chloroethylthiamine and glucose as exogenous energy source.

To confirm this possibility, the fate of ^{14}C-thiamine transported into the cells in the presence or absence of chloroethylthiamine was analyzed by paper chromatography. As shown in Fig. 3B, in the presence of chloroethylthiamine, the intracellular form of thiamine was found to be free ^{14}C-thiamine exclusively after 1 min of incubation at 37 C, and it was reduced to the same level as that found in the cells incubated at 0 C for 15 min. In the control cells, phosphorylated forms of ^{14}C-thiamine were found in addition to free ^{14}C-thiamine even at 1 min of incubation, and the former were much increased by longer incubation (Fig. 3A).

Furthermore, it was demonstrated that chloroethylthiamine actually inhibits thiamine monophosphokinase in the extract of KG 33 which catalyzes the conversion of thiamine to TMP, although there was less inhibition than that observed with ^{14}C-thiamine uptake by the cells (Table 1).

Recently, it has been reported that thiamine transport from chick small intestine was inhibited by chloroethylthiamine although the drug has no inhibitory effect on rat liver thiamine pyrophosphokinase which may play an important role in the active transport of thiamine in mammalian tissues (5). On the other hand, the results described above suggested that, in *E. coli*, chloroethylthiamine inhibits thiamine monophosphokinase, resulting in a reduction of entrapment of thiamine taken up by the cells.

As shown in Fig. 2, however, chloroethylthiamine appears to inhibit the thiamine uptake process itself in addition to the phosphorylation step. Further investigation is now in progress to verify this possibility, since chloro-

TABLE 1. *Inhibition by chloroethylthiamine of thiamine monophosphokinase in Escherichia coli KG 33*[a]

Addition	Chloroethylthiamine/thiamine	TMP formed (nmoles/mg/hr)	Inhibition (%)
None	0	0.223	
Chloroethylthiamine	10	0.123	44.8
	100	0.045	79.8

[a] *E. coli* KG 33 was grown at 37 C on Davis and Mingioli (1) minimal medium (500 ml) containing 0.2% glucose and 0.04 μM thiamine thiazole. The cells were harvested in stationary growth phase, washed once with a saline solution, and then suspended in a solution of 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride (*p*H 7.5), 2 mM 2-mercaptoethanol, and 1 mM ethylenediaminetetraacetic acid. The cell suspension was treated for 10 min at 4 C in a sonic oscillator (10 kc) and centrifuged at 15,000 × *g* for 20 min, and the supernatant fluid was used as enzyme solution (0.9 mg of protein). The activity of thiamine monophosphokinase was measured, as previously reported (2), in the presence or absence of chloroethylthiamine.

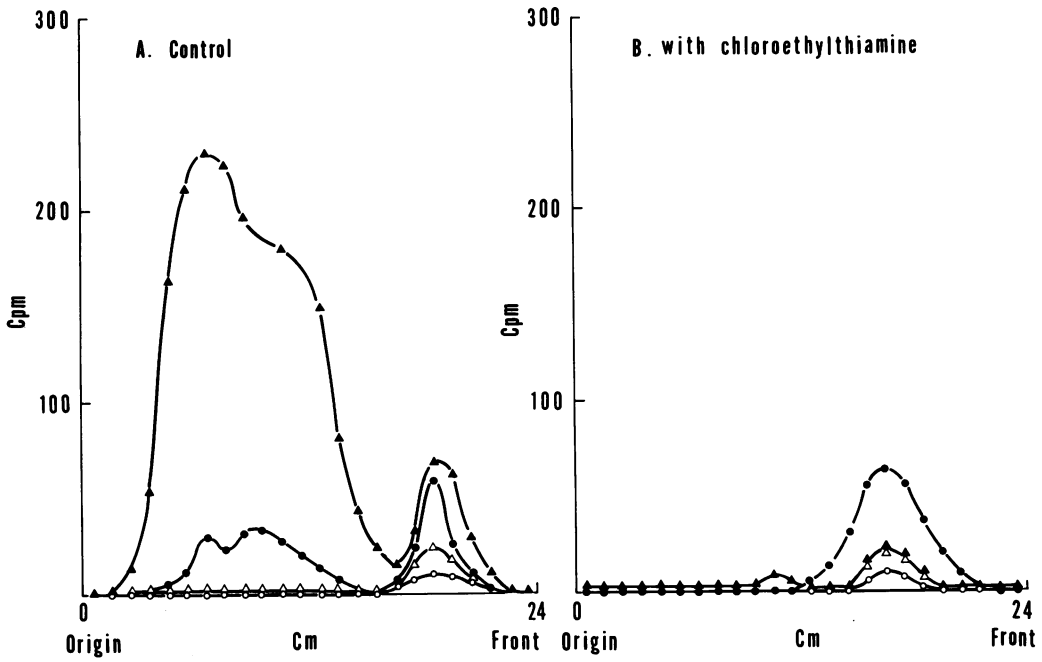

FIG. 3. *Chromatographic analysis of intracellular thiamine transferred in the presence of chloroethylthiamine. Cell suspensions containing 1 μM ^{14}C-thiamine were incubated with 0.4% glucose in the presence or absence of 100 μM chloroethylthiamine at 0 and 37 C. Three samples (2 ml) of reaction mixture at each reaction period were independently collected on membrane filters (Millipore Corp.), and the filters from the same reaction period were combined in 5 ml of 0.01 M acetate buffer (pH 4.5), heated at 85 C for 15 min to extract intracellular thiamine, and then centrifuged at 3,000 × g for 20 min. The supernatant fluids were lyophilized and dissolved in 0.1 ml each of distilled water. Each 50 μliters of the concentrated samples was spotted on Toyo filter paper (no. 50; 2 by 40 cm) and developed by ascending method in a solvent system consisting of isopropylalcohol-0.5 M acetate buffer (pH 4.5)-water (65:15:20, v/v). The papers were cut into segments (1 cm long), and all areas of the paper were quantitatively assayed for radioactivity in 10 ml of Bray solution. The R_F values for authentic thiamine, its monophosphate, and diphosphate were 0.72, 0.41, and 0.24, respectively, with this solvent system. Symbols: ●, 1 min at 37 C; ▲, 15 min at 37 C; ○, 1 min at 0 C; △, 15 min at 0 C.*

ethylthiamine inhibits the binding of thiamine to thiamine-binding protein from *E. coli* KG 38 (8).

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LITERATURE CITED

1. Davis, B. D., and E. S. Mingioli. 1950. Mutants of Escherichia coli requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
2. Iwashima, A., H. Nishino, and Y. Nose. 1972. Conversion of thiamine to thiamine monophosphate by cell-free extracts of *Escherichia coli*. Biochim. Biophys. Acta **258**:333-336.
3. Kawasaki, T., I. Miyata, K. Esaki, and Y. Nose. 1969. Thiamine uptake in *Escherichia coli*. I. General properties of thiamine uptake system in *Escherichia coli*. Arch. Biochem. Biophys. **131**:223-230.
4. Kawasaki, T., and K. Yamada. 1972. The uptake system of free thiamine in mutants of *Escherichia coli*. Biochem. Biophys. Res. Commun. **47**:465-471.
5. Komai, T., and H. Shindo. 1972. Metabolic fate and mechanism of action of chloroethylthiamine. III. Active transport of thiamine from chick intestine and competitive inhibition by chloroethylthiamine. J. Vitaminol. (Kyoto) **18**:55-62.
6. Nakayama, H., and R. Hayashi. 1972. Biosynthesis of thiamine pyrophosphate in *Escherichia coli*. J. Bacteriol. **109**:936-938.
7. Nishino, H., A. Iwashima, and Y. Nose. 1971. Biogenesis of cocarboxylase in *Escherichia coli:* a novel enzyme catalyzing the formation of thiamine pyrophosphate from thiamine monophosphate. Biochem. Biophys. Res. Commun. **45**:363-368.
8. Nose, Y., A. Iwashima, H. Nishino, and A. Matsuura. 1972. Thiamine uptake in *Escherichia coli* and thiamine-binding protein. Vitamins (Kyoto) **45**:222-223.
9. Shindo, H., and T. Komai. 1972. Metabolic fate and mechanism of action of chloroethylthiamine. I. Absorption of chloroethylthiamine from chick intestine and its competition with thiamine. J. Vitaminol. (Kyoto) **18**:41-47.