THE UPTAKE SYSTEM OF FREE THIAMINE IN MUTANTS OF ESCHERICHIA COLI

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SUMMARY

Mutants of Escherichia coli auxotrophic for thiamine monophosphate took up ^{14}C-thiamine as its free form from the external medium against a concentration gradient regardless of the presence of glucose. The radioisotope was then transported outward in the presence of glucose, but not in its absence. 2,4-Dinitrophenol and sodium azide eliminated the effect of added glucose. The other mutant auxotrophic for thiamine pyrophosphate showed the same profile of uptake as the parent strain except for the accumulation of the radioisotope as ^{14}C-thiamine monophosphate in the cytoplasm.

The uptake of thiamine in Escherichia coli proceeds in a manner similar to active transport (1, 2) and it was proposed (2) that the uptake process is composed of at least two steps: first, thiamine passes through the cell membrane as its free form, which is mediated by a specific carrier. Thiamine is then accumulated as thiamine pyrophosphate (TPP) in the cytoplasm in the second step, which is probably catalyzed by a membrane thiamine pyrophosphokinase (3).

It is not conclusively established, however, whether free thiamine crosses the membrane by a process of facilitated diffusion or of active transport. It is also not clear (2, 4) whether the accumulation reaction as TPP is involved in the

thiamine uptake. In order to solve these problems, an attempt to separate these two processes of the thiamine uptake has been tried with mutants of E. coli that lack the ability to form either thiamine monophosphate (TMP) or TPP.

The results obtained suggest that thiamine itself is actively transported across the cell membrane in the presence of either an endogenous or exogenous energy source and that thiamine monophosphokinase (5, 6) may play a role to hold thiamine in the cytoplasm as TMP. When this enzyme is deficient, thiamine taken up passes outward through the membrane probably depending on energized state in the cell.

MATERIALS AND METHODS

Strains used in the experiments are all derivatives of E. coli K12. The isolation and properties of the parent strain (KG1673) lacking thiaminephosphate pyrophosphorylase (EC 2.5.1.3) have been described (7). Mutants derived from KG1673 have been isolated after mutagenesis with nitrosoguanidine (8) as strains which grow on either 1×10^{-8}M TMP or TPP (KG1674 - 1678) or TPP alone (KG1679), but not on 1×10^{-5}M thiamine. Cells were grown on a minimal medium (9) supplemented with 1×10^{-8}M TPP unless otherwise indicated and harvested at the stationary phase. The assay method of ^{14}C-thiamine uptake has been described previously (1). Chromatographic analysis of ^{14}C-thiamine transported into the cell has been carried out in the same manner as described previously (1). Preparations of TMP and TPP used are the products of Sigma. Thiamine-^{14}C (thiamine thiazole-2-^{14}C; 14.0 mCi/mmole) hydrochloride was obtained from the Radiochemical Centre, England.

RESULTS AND DISCUSSION

The parent strain (KG1673) and its TPP-requiring mutant (KG1679) took up ^{14}C-thiamine from the external medium essentially in the same fashion with time regardless of the presence of glucose, although the rate of the uptake in the absence of glucose was approximately 50% of that in its presence, as shown in Fig. 1.

The uptake of ^{14}C-thiamine by KG1675, a TMP auxotroph, showed a characteristic profile with time; in the absence of glucose it was essentially the same as that of the parent strain or the TPP auxotroph. In the presence of glucose a rapid initial uptake higher than that shown in its absence occurred, reaching a peak at 2 min, and then the rate of uptake decreased slowly. After 10 - 15 min of incubation at 37°, the amount of ^{14}C-thiamine in the cell of KG1675 was reduced to the same level as that found in the cell incubated with the radioisotope at 0°. This suggests that ^{14}C-thiamine taken up by KG1675 was lost to the external medium through the cell membrane when glucose was present.

The intracellular form of thiamine formed in the presence of glucose by cells of KG1673, 1675, and 1679 was found to be ^{14}C-TMP and ^{14}C-TPP, free ^{14}C-thiamine, and ^{14}C-TMP, respectively. These results were obtained by chromatographic analysis of these cells incubated with ^{14}C-thiamine for 2 min at 37°.

At the maximal level of the uptake (2 min), the concentration of free ^{14}C-thiamine in the cell of KG1675 was calculated to be 2.4×10^{-5}M when the cellular water space of *E. coli* is taken as 2.55 ml per g dry weight (10), which is 24 times the concentration of ^{14}C-thiamine in the external medium. This suggests an active transport of thiamine as its free form in this KG1675 cell.

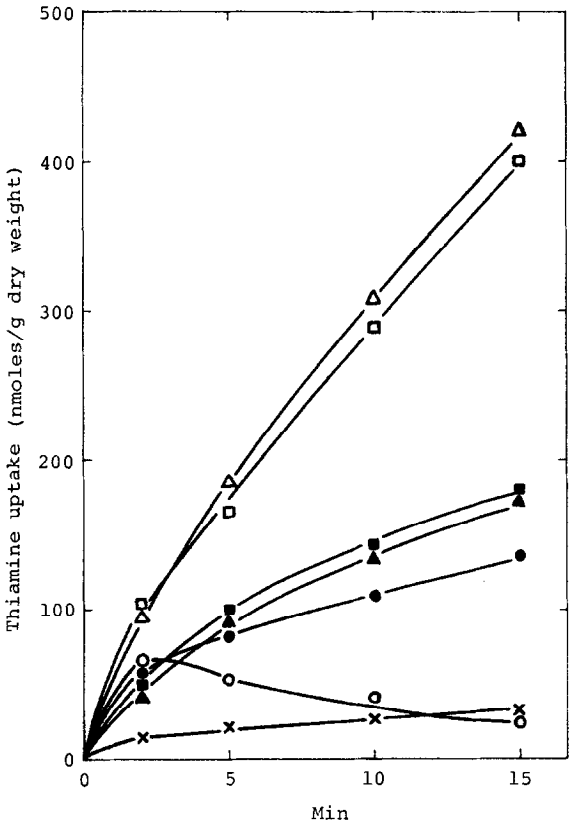

Fig. 1 (left). Time course of ^{14}C-thiamine uptake by three mutants of E. coli. Cells of three mutants grown and harvested as given in the text were washed and resuspended in minimal medium (9) containing 100 µg/ml chloramphenicol with or without 0.4% glucose. The uptake of ^{14}C-thiamine was measured in the same manner as described previously (1). The concentration of ^{14}C-thiamine (specific activity, 14.0 mCi/mmole) added to the uptake medium was 1 x 10^{-6}M. Open symbols represent the uptake in the presence of 0.4% glucose and closed symbols, in the absence of glucose. Δ, ▲: KG1673; o, ●: KG1675; □, ■: KG1679. x: uptake of ^{14}C-thiamine at 0°, which was practically identical in these three strains regardless of the presence of glucose.

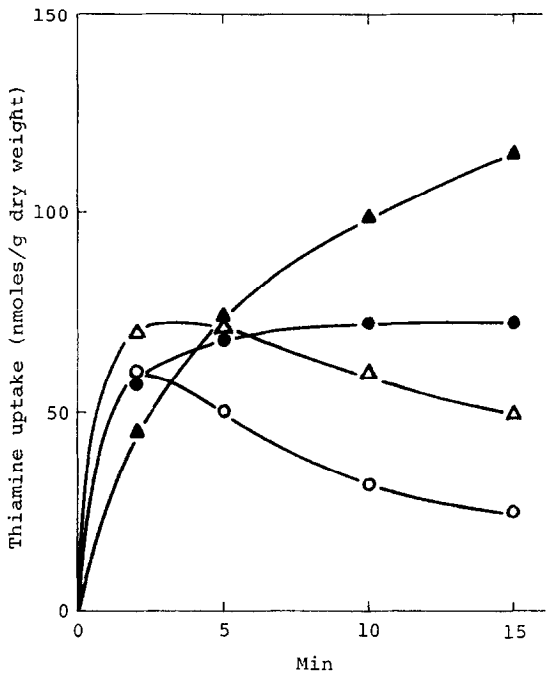

Fig. 2 (right). Uptake of ^{14}C-thiamine by 4 auxotrophs for TMP. Uptake of thiamine was carried out under the same conditions as shown in Fig. 1. These 4 strains could be divided into 2 groups (KG1674 and 1678; KG1676 and 1677), which show slightly different patterns of uptake. Open symbols represent the uptake in the presence of glucose and closed symbols, in the absence of glucose. o, ●: KG1674 and 1678; Δ, ▲: KG1676 and 1677.

In order to show that the loss of ^{14}C-thiamine once taken up is specific for mutants auxotrophic for TMP, the uptake of thiamine was determined with cells of 4 additional TMP auxotrophs (KG1674, 1676, 1677, 1678). All strains tested showed the same characteristics of thiamine uptake as found with KG1675, although the pattern of the uptake was not completely identical among these strains (Fig. 2).

The loss of the radioactivity which occurs in these mutants auxotrophic for TMP is assumed to be due to the presence of glucose as an exogenous supply of energy. This is illustrated in Fig. 3. When glucose was added to the uptake medium containing no glucose at 10 min after incubation at 37°, a rapid decrease in amount of the uptake was observed with cells of KG1675 and a rapid increase in the rate of the uptake was conversely demonstrated with cells of both KG1673 and 1679.

This assumption was further supported by the experiment in which 2,4-dinitrophenol (DNP) was added (Fig. 4). When DNP was present in the uptake medium without glucose, the rate of thiamine uptake was reduced approximately by 40%. Addition of DNP to the uptake medium containing glucose resulted in a time course of uptake essentially the same as that obtained in the absence of glucose and DNP. The addition of the uncoupler at 2 and 10 min after the incubation brought about a rapid rise in the rate of the uptake when glucose was present, although this is not illustrated. Sodium azide at 0.02M concentration showed the same effect as DNP.

Separation of free thiamine transport system from the coupled phosphorylating systems has been established by isolating mutants of E. coli auxotrophic for TMP. The energy-dependent loss of ^{14}C-thiamine taken up by these mutants is

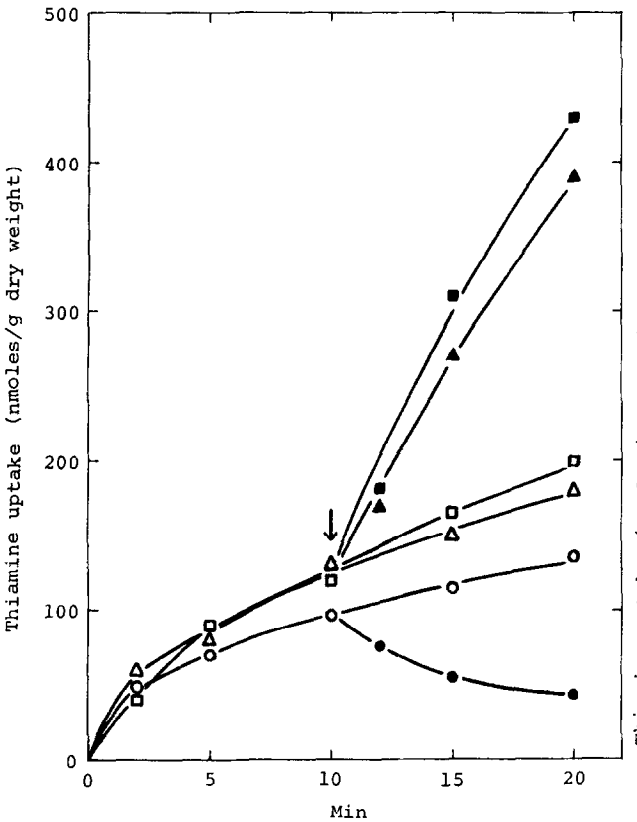

Fig. 3 (left). Effect of adding glucose on the uptake of thiamine by three mutants. At the time indicated by arrow, glucose was added at 0.4% concentration to the uptake medium without glucose and incubation was continued for another 10 min. Closed symbols show the uptake after addition of glucose. △, ▲: KG1673; o, ●: KG1675; □, ■: KG1679. The other conditions for the uptake were same as shown in Fig. 1.

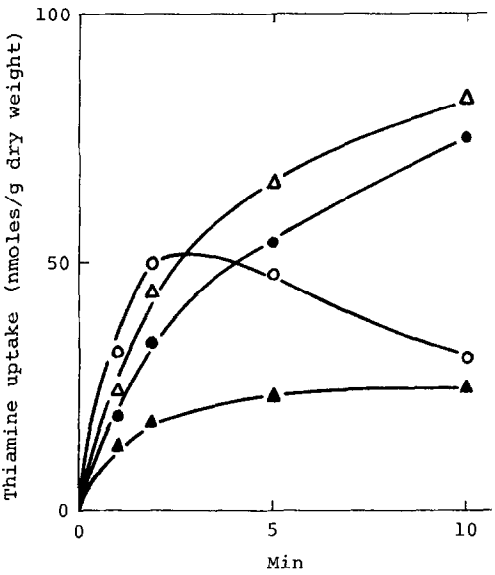

Fig. 4 (right). Effect of 2,4-dinitrophenol (DNP) on the uptake by KG1675. DNP was added to the uptake medium at 2×10^{-3}M. The medium was preincubated for 5 min at 37° prior to addition of ^{14}C-thiamine. The other conditions for the uptake were same as given in Fig. 1. o: glucose added; ●: both glucose and DNP added; △: no glucose added; ▲: no glucose, but DNP added.

assumed to be due to 1) the lack of thiamine monophosphokinase, and 2) energetic state of the cell membrane in which the specific carrier protein for thiamine is functioning.

It has been shown recently that active transport of amino

acids (11), sugars (12, 13), and manganese (14) is coupled to a D-lactate dehydrogenase via an electron transport chain. Although it was not determined whether or not the concentrative uptake of free thiamine is coupled to the same system as above mentioned, the results described suggest that a high energy compound probably generated by coupling to electron transport system is involved in transport of free thiamine. This is supported by the experiments demonstrated in Fig. 4.

When enzymes that phosphorylate thiamine are present, ATP generated through oxidative phosphorylation system is utilized to trap the transported thiamine to the cytoplasm concentratively either as TMP or TPP. TMP or TPP thus formed might not be removed from the cell either due to the negatively charged property of these compounds or the binding with cellular proteins (enzymes) and, therefore, accumulate in the cytoplasm.

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