JOURNAL OF BACTERIOLOGY, Jan. 1980, p. 254-261
0021-9193/80/01-0254/08$02.00/0

Vol. 141, No. 1

Properties of the Thiamine Transport System in *Escherichia coli*

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Thiamine transport was studied with a mutant (KG1976) of *Escherichia coli* K-12 deficient in thiamine kinase (EC 2.7.1.89), which catalyzes the formation of thiamine monophosphate from thiamine. Mutant cells accumulated thiamine 390-fold as the free form against a concentration gradient in the absence of added carbon sources at the steady state. Thiamine taken up from the medium, or thiamine preloaded in the absence of glucose, was expelled into the medium when glucose, D-lactate, or succinate was added, whereas exit in the absence of glucose was very slow. The rate of thiamine entry was therefore determined in the absence of glucose, and that of thiamine exit was followed by the addition of glucose to thiamine-preloaded cells. The activities of thiamine entry and exit were optimal at 42 and 37°C, respectively. Hyperbolic saturation kinetics were obtained for the entry rate with a K_m value of 5.9 nM. The exit rate showed a sigmoidal dependence on cellular thiamine concentrations, and a half-maximal velocity was observed at 31 μM. The rates of both entry and exit were lowered by electron transport inhibitors and uncouplers, suggesting that the energy coupled to both processes was provided through substrate oxidation. Thiamine exit from K^+-depleted cells was enhanced by K^+ alone and by Na^+ to a much lesser extent, and K^+ and glucose were found to be synergistic for thiamine exit. These cations had no effect on the entry of thiamine into KG1676 cells in the absence of glucose. These properties of the entry and exit of thiamine in KG1676 are discussed from the standpoint of the possible involvement of different membrane components or different sites of identical thiamine carrier protein.

Thiamine is transported into cells of *Escherichia coli* by a process of active transport (12, 13). Transported thiamine is found mostly as thiamine pyrophosphate (TPP) with a small amount of free thiamine, suggesting that thiamine passes through the cell membrane in the form of free thiamine, and is then converted to TPP in the cytoplasm (12). However, it remains to be elucidated whether phosphorylation of thiamine is actually required for the passage process in a manner similar to group translocation (24) or is involved in the accumulation process.

Recent studies on thiamine metabolism in *E. coli* have revealed that TPP is synthesized via TMP from thiamine by two phosphorylation steps (19, 20, 22). Thiamine kinase (EC 2.7.1.89), catalyzing TMP formation from thiamine, and TMP kinase (EC 2.7.4.16), catalyzing TPP synthesis from TMP, were partially purified (8, 21, 22). We confirmed these results by isolating mutants of *E. coli* K-12 deficient in either thiamine kinase or TMP kinase. It was, therefore, expected that a mutant of *E. coli* lacking thiamine kinase might transport thiamine into the cell without phosphorylation of this molecule.

In our preliminary report (16), it was shown that cells of such a mutant strain (KG1675) accumulated thiamine against a concentration gradient at 37°C in the absence of glucose and then excreted that thiamine in the presence of an exogenous energy supply. Although this could be explained by inhibition of the steady-state level of uptake, the effect of glucose of inducing thiamine exit could be useful to analyze the exit process.

A carrier-mediated transport system contains the entry and exit processes, which are assumed to be catalyzed by different components in some transport systems. The exit process of transport has not been analyzed sufficiently compared with that of entry, except for glutamate (5), galactose (6), and β-methylgalactoside (29) exit from *E. coli*, riboflavin efflux from yeast cells (23), and proline exit from *Mycobacterium phlei* (7). In this study, therefore, the exit of thiamine in a mutant of *E. coli* K-12, KG1676, was studied to elucidate the properties of the exit system and to compare it with the entry system.

MATERIALS AND METHODS

Organisms and growth medium. All strains used were derived from *E. coli* K-12. The parent strain (KG1673) was a thiamine auxotroph lacking thiamine

phosphate pyrophosphorylase (EC 2.5.1.3), and the properties of this strain have been described previously (14). Two kinds of mutants were isolated from KG1673: one type of mutant (KG1674–1678) was deficient in thiamine kinase, and the other was deficient in TMP kinase (KG1679). Sites of the enzymatic block in the strains used are shown in Fig. 1.

Growth medium was a minimal medium (4) containing 0.2% glucose as the carbon source with appropriate supplements. Sodium citrate was omitted from minimal medium.

Isolation of mutants. A logarithmic-phase broth culture of KG1673 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the method of Adelberg et al. (1). Survivors were grown overnight at 37°C in minimal medium containing 0.2% glucose supplemented with 0.01 μM TMP or TPP. Cells were harvested, washed, and suspended in minimal medium containing 0.01 μM thiamine, followed by penicillin selection (17). Survivors grown overnight in minimal medium supplemented with 0.01 μM TMP or TPP were diluted and then plated on the same supplemented minimal agar medium. After overnight growth at 37°C, mutants that could grow on either TMP or TPP but not on thiamine were screened by the replica method (17) and then purified. Five TMP auxotrophs were designated strains KG1674 to -1678, and a TPP auxotroph was designated KG1679. Most experiments were done with KG1676, which requires either TMP or TPP for growth in minimal medium; its enzymatic block sites are thiamine phosphate pyrophosphorylase and thiamine kinase, as shown in Fig. 1.

Growth of bacteria. Strains KG1673, KG1676, and KG1679 were aerobically grown at 37°C on minimal medium supplemented with 0.01 μM thiamine, TMP, or TPP, respectively, and 0.2% glucose as a carbon source, unless otherwise indicated. Cells were harvested at an absorbancy of 0.35 at 560 nm for assay of transport activity and at the late exponential growth phase for assay of enzyme activities. The cell suspension at an absorbancy of 0.5 at 560 nm gives 0.35 mg (dry weight) per ml (22).

Assay of the activities of thiamine kinase and TMP kinase. Harvested cells were washed in 50 mM Tris-hydrochloride (pH 7.5) containing 5 mM 2-mercaptoethanol, suspended in this buffer, and then disrupted in a Tominaga sonic oscillator (10 kc) for 5 min at 0°C. The disrupted cells were centrifuged for 20 min at 15,000 × *g* and 4°C, and the supernatant fluid, which was passed through a column of Sephadex G-25 (2 by 50 cm) equilibrated with the same buffer, was used for the enzyme assay. Protein concentration was measured by the method of Lowry et al. (18).

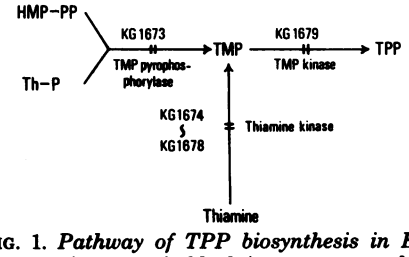

FIG. 1. *Pathway of TPP biosynthesis in E. coli and sites of enzymatic block in mutants used in the study. HMP (thiamine pyrimidine), 2-Methyl-4-amino-5-hydroxymethylpyrimidine; HMP-PP, HMP pyrophosphate; Th (thiamine thiazole), 4-methyl-5-hydroxyethylthiazole; Th-P, Th phosphate.*

TMP kinase activity was determined as described previously (15). Thiamine kinase activity was measured in the same manner as TMP kinase activity, except for the use of thiamine as substrate instead of TMP, which was also described elsewhere (26). To assay thiamine kinase activity in the extract of KG1679 deficient in TMP kinase, crude extract (4 mg of protein) of KG1676 lacking thiamine kinase was included in the reaction mixture.

Assay of entry and exit of thiamine. The entry rate of thiamine was determined by the following procedure. Harvested cells were washed twice and suspended in 50 mM potassium phosphate buffer (pH 7.0) containing 10 mM $MgSO_4$ to a final absorbancy of 0.35 at 650 nm. Glucose was added at 2 mM when required. These cell suspensions were incubated for 5 min at 37°C, and uptake was initiated by the addition of [^{35}S]thiamine (0.1 μM, 87 mCi/mmol). At the indicated time, 0.5-ml portions of the cell suspension were removed, immediately filtered on 0.45-μm membrane filters (Millipore Corp.), and washed with 5 ml of the same buffer as used for cell suspension; radioactivity retained by the filters was counted as previously described (12). Thiamine uptake for 1 min at 37°C in the absence of exogenous energy supply was found to be proportional to cell concentration in the range of absorbancy from 0.15 to 0.6 at 560 nm. With a cell suspension of absorbancy of 0.35, uptake proceeded linearly at least for 2 min.

The exit rate was measured by thiamine exit from preloaded cells. Cells were preloaded with [^{35}S]thiamine until the steady-state level was reached (30 min at 37°C in the absence of glucose), and then they were collected by filtration on membrane filters and washed with 50 mM potassium phosphate buffer (pH 7.0)–10 mM $MgSO_4$. The preloaded cells were suspended in fresh prewarmed buffer to the same cell density as that in the preincubation medium. After the cell suspension was incubated for 1 min at 37°C, glucose was added at appropriate concentrations, and portions were withdrawn and filtered at different times. Thiamine exit after the addition of glucose was linear for at least 1 min under these conditions. Specific transport activity of thiamine entry and exit was expressed as nanomoles of thiamine taken up or thiamine expelled, respectively, per minute per gram (dry weight) of cells.

Chromatographic analysis of [^{35}S]thiamine taken up and of the exit filtrate. [^{35}S]thiamine transported into cells was analyzed by paper chromatography according to the procedure described previously (12, 30). After exit of [^{35}S]thiamine from preloaded cells of KG1676 was induced by addition of glucose, the filtrate was also analyzed by the paper chromatographic technique.

Chemicals. TMP, TPP and oxythiamine hydrochlorides, and pyrithiamine hydrobromide were provided by S. Yurugi (Takeda Pharmaceutical Industries, Ltd., Osaka); chloroethylthiamine was provided by T. Yusa (Central Research Laboratory, Sankyo Co., Ltd., Tokyo); and nigericin was obtained from J. W. Chamberlin (Lilly Research Laboratories). ATP

and NADH were purchased from Sigma, alcohol dehydrogenase (yeast, crystalline) came from Boehringer, carbonylcyanide *m*-chlorophenylhydrazone and valinomycin were obtained from Calbiochem, and [^{35}S]thiamine (thiamine [^{35}S]thiazole, 87 mCi/mmol) hydrochloride was obtained from the Radiochemical Centre, Amersham, England. All other chemicals were analytical grade.

RESULTS

***E. coli* mutants lacking thiamine kinase.** Mutants isolated from KG1673 requiring either TMP or TPP for growth were tested for thiamine kinase and TMP kinase activities. The activities of thiamine kinase and TMP kinase were (nmol/mg of protein per h): 0.310 and 1.22 in parent strain; 0 and 1.49 in KG1676; and 0.362 and 0 in KG1679, respectively. Thiamine kinase activity in KG1679 was determined in the presence of crude extract of KG1676. Other TMP auxotrophs were comparable to KG1676 in both kinase activities.

The fate of labeled thiamine taken up by these mutants was analyzed by paper chromatography. [^{35}S]thiamine incubated with the parent cells for 2 min at 37°C in the presence of glucose was converted to two large radioactivity peaks, corresponding to TMP and TPP, and a small peak of [^{35}S]thiamine. In KG1676 cells [^{35}S]thiamine alone was found, whereas a large radioactivity peak of TMP and a small peak of [^{35}S]-thiamine were observed for KG1679 cells (data not shown). These results indicate that thiamine is taken up from the medium without chemical modification by cells of *E. coli* mutants lacking thiamine kinase. Thiamine transport uncoupled to phosphorylation of thiamine could therefore be studied with a mutant (KG1676) of this type.

Specificity of the transport system. Thiamine uptake in the absence of glucose increased with time and reached an equilibrium after 20 min (Fig. 2), showing a cellular thiamine level of 39.2 μM when cellular water space was taken as 2.55 ml per g (dry weight) of *E. coli* cells (27). This level was 390-fold higher than that of [^{35}S]thiamine in the uptake medium.

To test whether thiamine was transported by a specific carrier protein, exchange of cellular [^{35}S]thiamine by unlabeled thiamine added to the medium after 10 min was followed. Cellular [^{35}S]thiamine decreased rapidly depending on the concentration of thiamine added (Fig. 2), so that all of the intracellular thiamine was available for exchange. Thiamine analogs pyrithiamine, oxythiamine, and chloroethylthiamine inhibited the rate of thiamine uptake by 36.3, 64.5, and 60.2%, respectively, when added to the uptake medium at molar ratios of 100, 100, and 10, respectively.

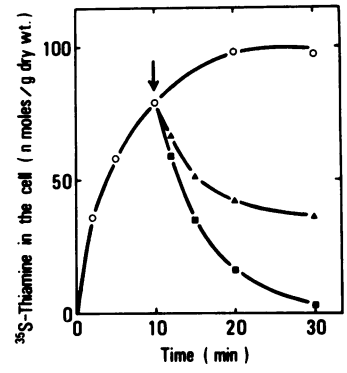

FIG. 2. *Effect of unlabeled thiamine on [^{35}S]thiamine transported by KG1676 cells. [^{35}S]thiamine transport in the absence of glucose was carried out under the standard assay conditions, and 1 μM or 10 μM unlabeled thiamine was added to the medium after 10 min, as shown by the arrow. Thiamine levels in the cell were followed with time.* (○) *None;* (▲) *1 μM thiamine;* (■) *10 μM thiamine.*

These results indicate that thiamine is transported by a carrier-mediated mechanism in this mutant.

Stimulation of thiamine exit by glucose or by other carbon sources. Since thiamine uptake by KG1676 cells in the presence of glucose reached a low steady state one-sixth of that in its absence, the effect of glucose was determined. The addition of glucose resulted in an immediate loss of radioactivity from the preloaded cells (Fig. 3), whereas the exit was very slow without glucose. The effect of glucose was dependent on its concentration: 20 μM was enough to bring about an initial rapid loss of thiamine, and the glucose effect was saturated at 200 μM. The radioactive compound that was extruded from the preloaded cells after the addition of glucose was identified solely as [^{35}S]thiamine by paper chromatographic analysis, as described in Materials and Methods.

When fructose, galactose, or glycerol was added at 10 mM to a cell suspension prepared after growth on the corresponding carbon source, thiamine exit was observed (data not shown). It was then found that D-lactate and succinate added at 10 mM induced a rapid exit of thiamine at a nearly equal rate, which was half as fast as with 10 mM glucose (Fig. 3). Since D-lactate is known to be an efficient electron donor for active transport in *E. coli* whole cells as well as membrane vesicles (10), these results indicate that thiamine exit is driven by substrate oxidation or perhaps by the energy derived therefrom. However, it is difficult to choose between these two possibilities.

Effect of pH and temperature. When trans-

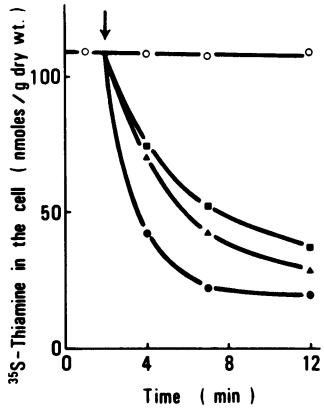

FIG. 3. *Effect of D-lactate and succinate on thiamine exit. To [^{35}S]thiamine-loaded cells was added glucose (●), D-lactate (▲), or succinate (■) at 10 mM each at the time shown by the arrow. Incubation at 37°C was then continued, and cellular [^{35}S]thiamine was determined as described in the text. (○) No carbon source.*

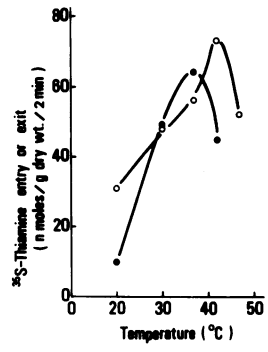

FIG. 4. *Optimal temperatures for the rates of thiamine entry (○) and exit (●). Effect of temperature on the rates of thiamine entry and glucose-induced exit was determined under the standard assay conditions.*

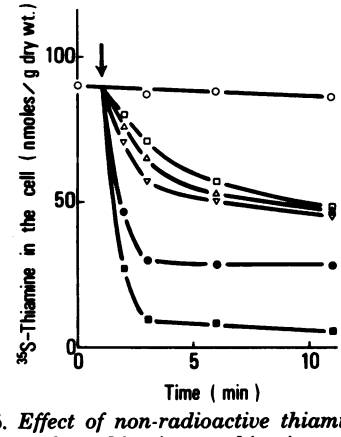

FIG. 5. *Effect of non-radioactive thiamine, pyrithiamine, and oxythiamine on thiamine exit. Unlabeled thiamine or its analogs were added at 10 μM with or without 2 mM glucose to thiamine-loaded cells 1 min after incubation at 37°C. (○) None; (□) thiamine; (▽) pyrithiamine; (△) oxythiamine; (●) glucose alone; (■) glucose with unlabeled thiamine, pyrithiamine, or oxythiamine.*

port activity of thiamine was determined at 37°C in 50 mM potassium phosphate buffer (pH 5.5 to 8.0) containing 10 mM $MgSO_4$, no detectable optimal pH was found for the initial velocity of both thiamine entry and exit. However, the steady-state level of thiamine entry was highest at pH 6.0, whereas that of the exit showed a broad peak of optimal pH. An optimal temperature of 42°C for the entry rate was observed when determined at pH 7.0; the optimal temperature for the rate of thiamine exit from the preloaded cells at pH 7.0 was 37°C (Fig. 4).

Exit of thiamine in the presence of the analogs. Glucose-stimulated exit of riboflavin (23) and β-methylgalactoside (29) resembles the exit of thiamine in KG1676 cells, but differs in the effect of unlabeled substrate or analogs. The addition of non-radioactive thiamine, pyrithiamine, or oxythiamine each at 10 μM to the preloaded cell suspension, which contained a cellular thiamine concentration of 35.5 μM, caused loss of thiamine (Fig. 5). Glucose and these analogs were additive in the effect.

Effect of metabolic inhibitors. Based on the results obtained with carbon sources, the effect of metabolic inhibitors on the rates of entry and of glucose-induced exit of thiamine was determined (Table 1). Electron transport inhibitors and uncouplers of oxidative phosphorylation inhibited both rates, but the arsenate effect was relatively minor. These results suggest that the energy coupled to entry and exit was provided through substrate oxidation.

Effect of sulfhydryl reagents. *N*-Ethylmaleimide at 1 mM and *p*-chloromercuribenzoate at 0.5 mM inhibited the entry rate by 40.0 and 13.5%, respectively, and inhibited the exit rate by 70.8 and 28.5%, respectively, suggesting that both processes of thiamine transport are sensitive to sulfhydryl reagents.

Repression of thiamine entry and exit. Thiamine uptake in *E. coli* K-12 and a thiamine-requiring mutant of *E. coli* was found to be repressible (11). The thiamine transport system in KG1676 showed a similar reduction in both entry and exit activities when cells were grown in increasing concentrations of TMP (Table 2). Cells loaded with unlabeled thiamine had the same rate of [^{35}S]thiamine uptake as unloaded cells, and the reduction in the activities was observed only when TMP or TPP was added in

TABLE 1. *Effect of metabolic inhibitors on the rates of entry and of glucose-induced exit of thiamine in KG1676*[a]

Addition	Concn (mM)	Relative rate (%)	
		Entry	Exit
None		100	100
KCN	0.1	70	92
	1	37	22
Amytal	1	40	76
	5	4	24
Sodium azide	10	51	76
	50	49	21
2,4-Dinitrophenol	0.1	52	69
	1	13	39
CCCP[b]	0.001	53	94
	0.01	10	65
Arsenate[c]	1	101	102
	10	73	67

[a] The rates of thiamine entry in the absence of glucose and of glucose-induced exit from [^{35}S]thiamine-preloaded KG1676 cells were determined under the standard assay conditions described in the text. Inhibitors were added to the reaction medium just before entry or exit of thiamine. The inhibition is expressed as rates in the presence of inhibitors relative to those in the absence of inhibitors.

[b] CCCP, Carbonylcyanide *m*-chlorophenylhydrazone.

[c] The effect of arsenate was tested with cells suspended in 50 mM Tris-hydrochloride buffer (pH 7.3)–10 mM $MgSO_4$ to avoid a competition of arsenate with phosphate.

TABLE 2. *Effect of TMP concentrations added to the growth medium on the rates of thiamine entry and exit*

TMP added to growth medium[a] (μM)	Thiamine transport[b] (nmol/g (dry wt) per min)	
	Entry (%)	Exit[c] (%)
0.01	16.3 (100)	36.4 (100)
0.05	5.60 (34.4)	18.9 (51.9)

[a] KG1676 was grown on minimal medium containing 0.01 and 0.05 μM TMP, respectively, with 0.2% glucose as the carbon source.

[b] The rates of thiamine entry and exit were measured under the standard assay conditions described in the text.

[c] To minimize the difference in the level of [^{35}S]-thiamine after loading, the suspensions of 0.01 μM TMP- and 0.05 μM TMP-grown cells of KG1676 were incubated for 30 min at 37°C with 1 μM and 10 μM [^{35}S]thiamine, respectively, and initial cellular levels of labeled thiamine were 83.0 and 113 nmol/g (dry weight), respectively. The other conditions to assay the exit were the same as described in the text.

excess to the growth medium, as has been described in a previous paper (11) with *E. coli* K-12. The observed reduction in entry and exit activities is therefore assumed to be due to repression of the thiamine transport system.

Kinetic properties. The entry process of thiamine transport was characterized by hyperbolic saturation kinetics, with a K_m value for thiamine of 5.9 nM at 37°C (data not illustrated). Thiamine taken up by cells was less than 13% of the substrate added at the lowest concentration (1 nM), indicating that under the conditions employed the rate of entry in the absence of carbon sources was adequately followed.

To determine the rate of glucose-induced exit at different intracellular thiamine concentrations, preloaded cells of KG1676 incubated with 5 to 80 μM [^{35}S]thiamine were prepared. Thiamine exit for 30 s after the addition of glucose was followed and plotted as a function of internal thiamine concentrations. A sigmoidal curve was obtained (Fig. 6), which was different from the entry kinetics, and the half-maximal velocity for exit was calculated to be 31 μM.

Effect of monovalent cations. Monovalent cations affect many transport systems in microorganisms, and in some studies (5, 23) K^+ stimulated the entry of solutes without affecting the exit. However, no evidence has been obtained as far as cation effects on the exit process are concerned. The effect of monovalent cations on thiamine transport was determined with K^+-depleted cells of KG1676. The depletion of intracellular K^+ was carried out by washing cells with 50 mM $MgSO_4$ according to the method of Thompson and MacLeod (28), which removed approximately 80% of cellular K^+ when measured by flame photometry (data not shown).

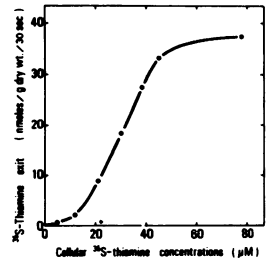

FIG. 6. *Effect of intracellular [^{35}S]thiamine concentrations on thiamine exit. Cell suspension of KG1676 was prepared and then incubated for 30 min with [^{35}S]thiamine ranging from 0.01 to 20 μM, resulting in loading of cells to levels of 5 to 80 μM concentrations. The cell suspensions thus obtained were incubated for 1 min at 37°C, and then 2 mM glucose was added. Radioactive thiamine exit for 30 s after the addition of glucose was determined as described in the text.*

The initial rate of thiamine entry in the absence of glucose was not altered by the addition of K^+, Na^+, Li^+, Cs^+, and Rb^+ at 10 mM, and the steady-state level was reduced only in the presence of K^+.

When K^+ or Na^+ with or without glucose was added to the K^+-depleted and [^{35}S]thiamine-loaded cell suspension, K^+ in the absence of glucose increased the exit at a rate slower than that shown with glucose alone, and the effect of K^+ with glucose appeared to be synergistic (Fig. 7). The effect of Na^+ to enhance the exit was minor at 10 mM and less than that of 1 mM K^+, indicating that the cation effect is not due to an increase in osmolarity of the medium. When the effect of other monovalent cations at 10 mM each on the exit from the preloaded cells was tested in the presence of 2 mM glucose, Cs^+ and Rb^+ had as slight an effect as Na^+ in stimulating the exit, and Li^+ was completely inactive.

DISCUSSION

Uptake of thiamine in its unphosphorylated form was demonstrated with cells of an *E. coli* K-12 mutant lacking thiamine kinase, and transported thiamine was detected actually as its free form. Thus, properties of the thiamine transport system could be studied in detail.

One of the interesting characteristics was that thiamine uptake against a concentration gradient occurred in the absence of exogenous carbon sources (Fig. 2). Cellular thiamine concentration at the steady-state level of uptake was calculated to be approximately 39 μM, which was 390-fold higher than that in the medium. On the other hand, a low steady-state level of uptake was demonstrated in the presence of glucose which was one-sixth of that in its absence. This was due to an enhanced exit of thiamine induced by carbon sources added, as shown in Fig. 3. These results indicate an involvement of entry and exit processes in the thiamine transport system of *E. coli*.

Thiamine entry into cells of KG1676 in the absence of glucose is mediated by a carrier specific for the substrate, since thiamine analogs show *trans*-stimulation of the exit (Fig. 2) and *cis*-inhibition of the entry as described in the text. The glucose-induced exit of thiamine appears to be also catalyzed by a carrier. Evidence supporting this is shown by the facts that the exit process is dependent on temperature (Fig. 4), is sensitive to sulfhydryl reagents, is repressible by TMP added to the growth medium (Table 2), and shows sigmoidal kinetics (Fig. 6).

Consistent with the description of other transport systems (5, 7, 23, 29), the results described above suggest that entry in the absence of an

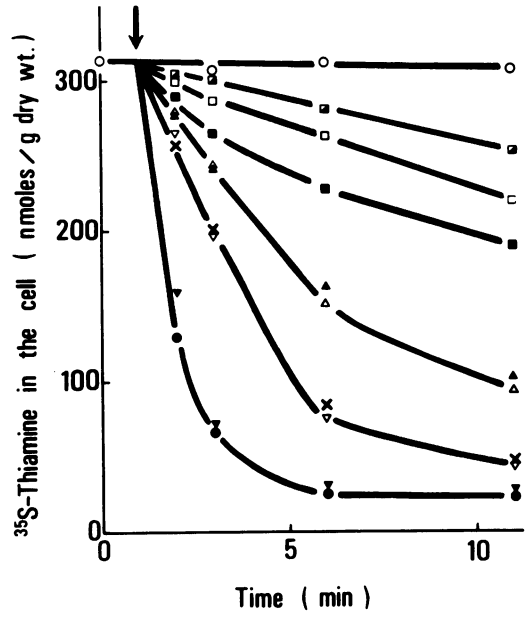

FIG. 7. *Effect of K^+ or Na^+ on thiamine exit from [^{35}S]thiamine-preloaded cells. K^+-depleted cells of KG1676 were prepared by the method of Thompson and MacLeod (28), and preloading with [^{35}S]thiamine was carried out as described in the text. KCl or NaCl and glucose in various combinations were added to the preloaded cell suspension 1 min after the incubation at 37°C. Assay of cellular radioactive thiamine at the time intervals indicated was carried out as described in the text.* (○) *None;* (△) *2 mM glucose;* (□) *1 mM KCl;* (■) *10 mM KCl;* (◪) *10 mM NaCl;* (▲, ▽, ▼, ●, *and* ×) *glucose plus 0.01 mM KCl, 0.1 mM KCl, 1 mM KCl, 10 mM KCl, and 10 mM NaCl, respectively.*

energy source and glucose-induced exit of thiamine in KG1676 cells are mediated by different membrane components or by different sites of identical thiamine carrier protein. The facts to support this assumption might be that properties of entry and exit are different in: (i) optimal temperature (Fig. 4) and pH; (ii) the K^+ effect in stimulating the exit (Fig. 7) without affecting the entry; and (iii) saturation kinetics (Fig. 6). However, these facts do not entirely prove that the two processes are carried out by different membrane components.

Based on the effect of inhibitors of electron transport and of uncouplers of oxidative phosphorylation on the entry and exit rates, endogenous and exogenous energy coupled to thiamine entry and exit, respectively, appear to be provided through substrate oxidation (Table 1). This is supported by the results obtained with an energy-uncoupling and neomycin-resistant mutant (NR157) isolated from KG1676. NR157,

which was found to be deficient in Mg^{2+}, Ca^{2+}-ATPase (EC 3.6.1.3) and was similar in its properties to strain NR76 of Rosen and Adler (25), showed markedly reduced activities of proline transport as well as thiamine entry and exit (unpublished data). Since proline transport in *E. coli* has been reported to couple to an energized state of cell membrane through substrate oxidation or hydrolysis of ATP (3), thiamine transport appears to be energized by a mechanism similar to that for proline transport. However, the involvement of ATP as energy source in both the entry and exit processes was not obvious in light of the minor effect of arsenate (Table 1). The observation that 2-deoxyglucose, a nonmetabolized analog of glucose, did not cause thiamine exit from the preloaded cells indicated that glucose should be oxidized before inducing the exit.

Thiamine transport in mutant KG1676 was inhibited competitively by TMP and TPP at the same rate as unlabeled thiamine, and, furthermore, the phosphorylated forms of thiamine showed increased exit upon addition of glucose in the same manner as nonradioactive thiamine, as shown in Fig. 5 (data not shown). These results suggest that the effect of energization by glucose is peculiar to the transport system and not to the transport substrate, thiamine.

The effect of K^+, which stimulated thiamine exit in KG1676 without affecting thiamine entry, was not due to an increase in glucose uptake, since K^+ alone at 10 mM induced exit of thiamine from the preloaded cells (Fig. 7). Indirect evidence obtained suggested that the effect of K^+ was not electrogenic. However, the precise role of K^+ in increasing thiamine exit remains to be elucidated.

With regard to the physiological significance of the glucose-induced exit process in KG1676, a profile of thiamine uptake practically identical to that in KG1676 has been reported with a mutant of *E. coli* K-12 which is phenotypically a wild strain for thiamine uptake system (9). In the presence of chloroethylthiamine, an antimetabolite of thiamine, and glucose, thiamine uptake showed an overshoot phenomenon. This is due to inhibition by this analog of thiamine kinase, which functions to entrap transported thiamine into the cytoplasm as TMP. That result (9) together with our data presented in this paper suggests that the exit process induced by carbon sources is present not only in a thiamine kinase-deficient strain (KG1676) but also in a wild-type strain of *E. coli*. The glucose-induced exit process might be concerned in regulating cellular thiamine level under conditions in which entrapment of thiamine as TMP is inhibited in the cytoplasm.

The effect of glucose to induce exit of a solute was reported previously in histidine transport (2) and β-methylgalactoside transport (29).

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