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Dual Mechanism for Stimulation of Glutamate Transport by Potassium Ions in *Streptococcus mutans*

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An ATP-driven primary transport system operative for L-glutamate or L-aspartate in *Streptococcus mutans* is, through the entire pH range from 5.5 to 8.5, specifically stimulated by extracellular potassium ions. The stimulation by potassium ions observed in the low pH range between 5.5 and 7 has been interpreted to be due to potassium ion-dependent regulation of the intracellular pH (the first mechanism). In the high pH range from 7 to 8.5, on the other hand, the present study demonstrates that potassium stimulation is essentially not associated with such intracellular pH regulation. This conclusion is based on our observation that potassium stimulation in the high pH range is insensitive to a proton conductor, carbonyl cyanide-*p*-trifluoromethoxyphenyl-hydrazone. Since none of the other monovalent cations, including sodium, rubidium, ammonium, and Tris ions, could replace potassium ions in significantly stimulating glutamate transport, it is most likely that the influx of potassium ions specifically cancels the membrane potential derived by movement of glutamate with the net negative charges across a membrane and thus facilitates transport (the second mechanism). The second mechanism appears to be operative even in a low pH range, in addition to the first mechanism.

We previously demonstrated that *Streptococcus mutans*, a major pathogen in the etiology of dental caries (7), accumulates L-glutamate and L-aspartate in cells through some ATP-driven transport system (12). In the present study, we found that potassium ions stimulate glutamate uptake in the entire pH range between 5.5 and 8.5. A probable mechanism for stimulation in the low pH range from 5.5 to 7.0, has already been proposed as follows (12). Cells cannot adjust intracellular pH without extracellular potassium ions, resulting in lowering the intracellular pH, with a consequent decrease of the transport activity. The addition of potassium ions was actually demonstrated to increase intracellular pH to a normal level (12, 15) and subsequently to activate glutamate transport. A similar observation was reported in *Streptococcus faecalis* (8) and *Streptococcus lactis* (13, 14).

We observed that potassium ions stimulate glutamate transport in *S. mutans* even in the high pH range between 7 and 8.5, in which the pH difference across the cell membrane was measured to be within 0.2 pH unit, as reported briefly in the previous paper (12). This fact implied an alternative and unique role of potassium ions in transport in the high pH range. In the present paper, we demonstrate the distinctive roles of potassium ions in the ATP-driven primary transport system predominantly operative for glutamate, as previously demonstrated (12).

MATERIALS AND METHODS

Organisms and medium. *S. mutans* (strain Ingbritt, serotype *c*) from the culture collection at the Department of Microbiology of Tokyo Dental College was cultured in a modified Berman broth (4) (2% Trypticase, 0.1% yeast extract, 25 mM potassium phosphate [pH 7.2], 34 mM NaCl, 1 mM $MgSO_4$, 0.1 mM $MnSO_4$, 0.05% sodium thioglycolate, 0.5% glucose) to mid-log phase in a test tube with a screw cap at 37°C. Cell density was measured turbidometrically as described previously (12).

Measurements of L-glutamate transport. Cells were harvested by centrifugation (8,000 × *g* for 10 min) and then washed three times with 100 mM sodium phosphate or 1 mM potassium phosphate buffer (varied pH) containing 1 mM $MgSO_4$. Unless otherwise noted, the washed cells were suspended in 100 mM sodium phosphate buffer containing 1% glucose, 0.1 mg of chloramphenicol per ml, and 1 mM $MgSO_4$. The procedures for the measurement of accumulation rates with L-[^{14}C]glutamic acid were the same as those described in the previous paper (12). The final concentration of glutamate was 0.6 mM, which is much higher than the Michaelis constant of 0.024 mM for a primary transport system for glutamate or aspartate (12).

Measurements of intracellular potassium ions. The concentration of potassium ions in cells was determined by the method of Zarlengo and Schultz (16) with an atomic absorption analyzer (Shimadzu model AA-640-12). The value of the intracellular water space was measured to be 1.60 ± 0.14 μl/mg (dry weight), as described previously (12).

Measurement of intracellular pH. The intracellular pH was measured with [^{14}C]benzoic acid by the method of Kashket et al. (9), as described previously (12).

Chemicals. L-[U-^{14}C]glutamate (280 mCi/mmol) and other radioisotopes were purchased from Amersham Japan Co. (Tokyo, Japan). All other chemicals were analytical reagent grade and used without further purification.

RESULTS

Effect of cations on L-glutamate transport. The effects of Na^+ or K^+ ions on the glutamate transport were examined for cells suspended in 100 mM potassium phosphate or sodium phosphate buffer, respectively. For Na^+ ions, no significant stimulation was observed in the concentration range from 0 to 100 mM. On the other hand, considerable stimulation was observed by exogenous K^+ ions. Figure 1 shows the effect of K^+ ions on the time course of intracellular accumulation of glutamate in the presence of 100 mM Na^+ ions. The addition of 20 mM KCl significantly enhanced the uptake of glutamate at pHs 6 and 8. The rate observed depends on conditions of cell preparation, especially on the

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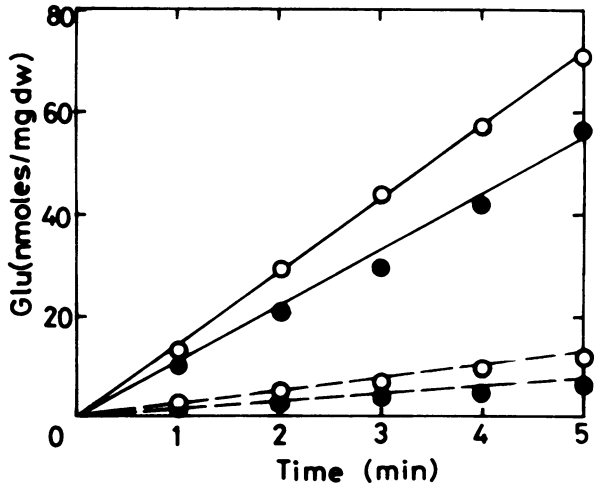

FIG. 1. Time course of glutamate transport in 100 mM sodium phosphate buffer containing 0 (– – –) or 20 (——) mM KCl at pH 6 (○) or 8 (●). Cells were washed with 100 mM sodium phosphate buffer containing 1 mM $MgSO_4$. dw, Dry weight.

components of washing buffers. The accumulation rate for cells washed with 100 mM sodium phosphate buffer was about half of that for cells washed with 1 mM potassium phosphate buffer. The former cells were found to retain 200 ± 40 mM K^+ ions inside the cell after being washed three times with 100 mM sodium phosphate. On the other hand, the intracellular concentration of K^+ ions for the latter cells was estimated to be 400 ± 80 mM, in agreement with the reported value of 400 mM for the normal intracellular level in *S. faecalis* (2). Even with the K^+-loaded cells, the rate of glutamate uptake was increased by the externally added K^+ ions in a manner similar to that seen when the potassium concentration was increased (Fig. 2). Thus, the extracellular potassium ions are concluded to be indispensable for stimulation of the transport.

Effects of other monovalent cations, like Rb^+, $Tris^+$, and NH_4^+ ions, on glutamate transport were examined for cells washed with 1 mM potassium phosphate at pH 6 or 8. The results are summarized in Table 1. Rb^+, $Tris^+$, or NH_4^+ ions stimulated transport but less effectively than that observed for K^+ ions at pH 6. Although at pH 8 the transport rate without the addition of K^+ ions was higher than that at pH 6, the effect of Rb^+ ions was similar to those of other cations, except for K^+ ions at both pH values. Thus, extracellular K^+ ions are demonstrated to be an indispensable cation quite specifically correlated with glutamate transport at both pH values.

TABLE 1. Effects of various cations on the transport rate of glutamate in *S. mutans*[a]

Cations and concn (mM)	Transport rate[b] at pH:	
	6	8
K^+ (10)	16.5	16.5
Rb^+ (10)	5.7	11.2
NH_4^+ (10)	4.8	11.8
$Tris^+$ (10)	4.3	10.7
None	4.2	10.3

[a] Cells were washed with 1 mM potassium phosphate buffer containing 1 mM $MgSO_4$

[b] The rate was expressed in nanomoles per minute per milligram (dry weight).

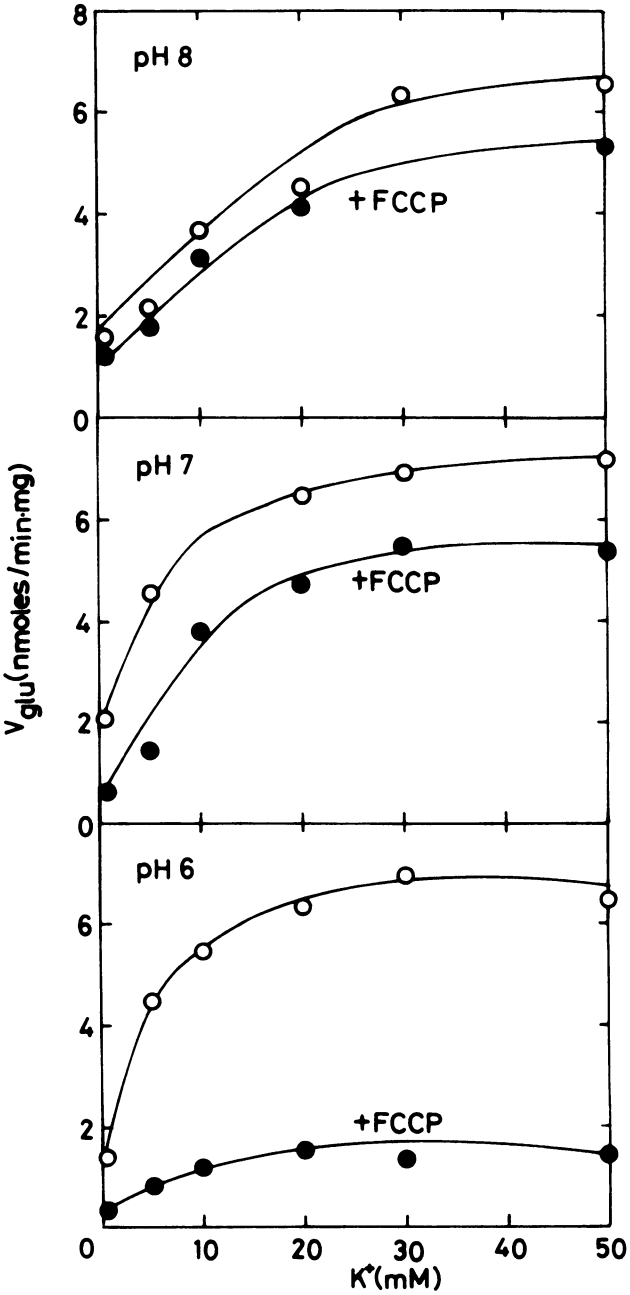

FIG. 2. Dependence of glutamate transport rate, V_{glu}, on potassium concentration at pHs 6, 7, and 8 in the absence (○) or presence (●) of 10 μM FCCP. Other conditions were the same as described in the legend to Fig. 1.

Dependence of glutamate transport on potassium ion concentration at pHs 6, 7, and 8. The characteristic pH dependence of the glutamate transport systems in *S. mutans* was already demonstrated (12). The rate of the glutamate transport was measured with varied K^+ ion concentrations at a pH of 6, 7, or 8. Figure 2 shows the dependence of glutamate transport on the concentration of potassium ions externally supplied for cells washed with 100 mM sodium phosphate buffer. Although the dependence at pH 6 differed slightly from that at pH 8, stimulation of the transport was observed at every pH value tested. The similar dependence on K^+ ion concentration was also observed in other buffer systems

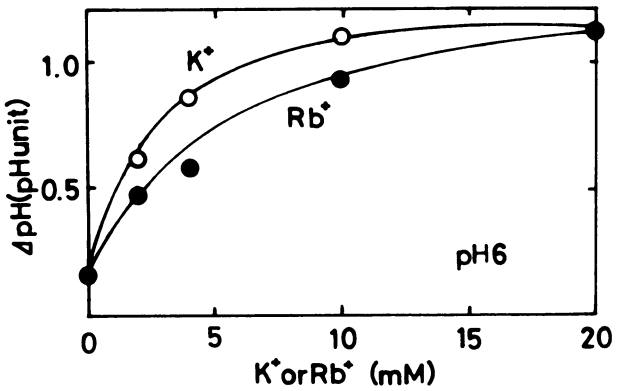

FIG. 3. Dependence of pH difference, ΔpH (the difference between the inside and outside of cells), on concentration of potassium or rubidium ions at pH 6. Cells were washed with 1 mM potassium phosphate buffer containing 1 mM $MgSO_4$.

such as Tris-HEPES (*N*-hydroxyethylpiperazine-*N*'-2-ethane-sulfonic acid) and Tris-maleate with slight changes in the observed rate of transport, indicating no participation of the present anion species in the stimulation by K^+ ions.

Effect of FCCP on stimulation of glutamate transport by K^+ ions. If the stimulation were at least due to adjustment of intracellular pH by uptake of K^+ ions, no stimulation by K^+ ions would be expected when carbonyl cyanide-*p*-trifluoromethoxyphenyl-hydrazone (FCCP), a proton conductor, was used to permeate protons across the cell membrane. It should be noted that potassium uptake in *S. mutans* is also inhibited in the presence of FCCP at pH 6, but not at pH 8 (15). A similar phenomenon was reported in *S. faecalis* (2). The inhibitory effect of FCCP was actually observed only at pH 6 (Fig. 2). In the low pH range around 6, stimulation of transport has been demonstrated to be primarily due to regulation of intracellular pH by K^+ ions (12), as has been previously observed in several other organisms (1–3, 5, 6, 9, 11). If glutamate transport depends on intracellular pH level only, it is expected that Rb^+ ions would stimulate glutamate transport as well as K^+ ions do, because Rb^+ ions are mostly replaceable with K^+ ions for pH gradient formation (Fig. 3). However, Rb^+ ions hardly stimulated glutamate transport (Table 1). These results clearly indicate that the stimulatory effect of K^+ ions on glutamate transport is, even at pH 6, not attributed solely to regulation of intracellular pH. The potassium uptake is considered indispensable for stimulation of glutamate transport.

In a higher pH range between 7 and 8, on the other hand, K^+ ions stimulated glutamate transport even in the presence of FCCP, which dissipated the pH gradient (a small reversed pH gradient [−0.1 to −0.2] observed at an external pH of 7 [15]). These results indicate that the stimulation by K^+ ions at pH 7 to 8 is independent of the intracellular pH or proton movement. Since movement of the net negatively charged glutamate generates a membrane potential that is negative inside the cell, potassium ions are likely to cancel the membrane potential derived by the charge movement and thus to facilitate transport.

DISCUSSION

We previously demonstrated that *S. mutans* accumulates glutamate through an ATP-driven primary system (12). In this system, potassium ions were found to be required to transport glutamate in the entire pH range from 5.5 to 8 (12). In the low pH range around 6, adjustment of intracellular pH by potassium ions has also been demonstrated to be essential for glutamate transport in *S. mutans* (12). In the present study, however, we showed that the stimulation of glutamate transport by K^+ ions could not be interpreted solely by adjustment of intracellular pH. Thus, it was necessary to take an alternative role of K^+ ions in the stimulation of glutamate transport into account. We supposed that the role is to cancel membrane potential derived by movement of net negatively charged glutamate molecules, resulting in facilitation of the transport. The following two mechanisms are conceivable for canceling the potential. (i) Influx of K^+ ions through a K^+ channel cancels the membrane potential generated by the glutamate transport. (ii) One (at least) K^+ ion is cotransported with a glutamate molecule. The specificity of cations for the stimulation seems to support the second mechanism. However, the affinity of K^+ ions to glutamate seems too low for K^+ ions to be cotransported, judging from the K^+ dependence of glutamate transport (the concentration of K^+ ions at half saturation was higher than 5 mM [Fig. 2]). Furthermore, potassium specificity can be interpreted by the preference of K^+ ions over Rb^+ ions in a K^+ transport system (10). Thus, the present results seem to support the first mechanism as the mechanism for stimulation of glutamate transport. In any case, we propose here a dual mechanism for potassium stimulation of glutamate transport: one is due to adjustment of intracellular pH by movement of potassium ions, and the other is due to cancellation of the membrane potential derived by movement of net negative charges of glutamate molecules through the corresponding channel(s). The first mechanism should predominate in stimulation in a lower pH range than 7. Nevertheless, the second mechanism may somewhat contribute to stimulation even in the low pH range, judging from the effect of Rb^+ ions on transport (Table 1). In contrast, stimulation in the high pH range between 7 and 8 is mainly attributed to the second mechanism.

Gale and Llewellin (6) reported that K^+ ions are required in glutamate transport driven by the proton motive force in *Staphylococcus aureus*. In this case, K^+ ions are concerned directly with formation of the proton motive force. Harold and Spitz (8) have found that K^+ ions are required in the transport of phosphate or arsenate. They proposed that phosphate, for example, is transported with an OH^- ion in the opposite direction, whereas H^+ ions are extruded with influx of K^+ ions so as to maintain OH^- ions inside the cell. Recently, Poolman et al. (14) reported stimulation of glutamate transport by potassium ions in *S. lactis*. They attributed the dependence of glutamate transport on the accumulation of potassium ions to the regulation of intracellular pH by potassium movement. It should be noted that glutamate is transported only in the acidic form in *S. lactis* (13, 14). In this case, the transport becomes inactive at a higher pH than 7. In contrast, this is not the case in *S. mutans*. Thus, glutamate seems transported without protons; it may, for example, be transported as a dissociated form in *S. mutans*. The observations of potassium stimulation reported so far have been primarily interpreted as evidence for the first mechanism. However, this does not necessarily exclude the possibility that the second mechanism contributes to stimulation in other organisms.

In conclusion, potassium ions are indispensable for the transport of glutamate in *S. mutans* cells. A dual mechanism for stimulation of glutamate transport by potassium ions both regulates intracellular pH and cancels the membrane

potential derived by movement of net negatively charged glutamate.

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