Roles of Na\(^+\) and K\(^+\) in \(\alpha\)-Aminoisobutyric Acid Transport by the Marine Bacterium *Vibrio alginolyticus*

Hajime Tokuda, Manabu Sugasawa, and Tsutomu Unemoto

From the Department of Membrane Biochemistry, Research Institute for Chemobiodynamics, Chiba University, 1-8-1 Inohana, Chiba, Japan

Effects of monovalent cations on \(\alpha\)-aminoisobutyric acid (AIB) transport were examined in the marine bacterium *Vibrio alginolyticus*. In Na\(^+\)-containing cells, AIB was actively accumulated only in the presence of Na\(^+\), and the addition of K\(^+\) had essentially no effect. On the other hand, K\(^+\)-depleted and Na\(^+\)-loaded cells required K\(^+\) as well as Na\(^+\) for the accumulation of AIB against its concentration gradient. The characterization of the roles of Na\(^+\) and K\(^+\) in AIB transport was performed by manipulation of intra- and extracellular cation compositions. K\(^+\) concentration gradient (K\(^+\)\(_{in}\) > K\(^+\)\(_{out}\)) was not essential for the Na\(^+\)-dependent AIB uptake. Na\(^+\) extrusion against its concentration gradient in Na\(^+\)-loaded cells occurred only in the presence of K\(^+\)({Rb}^+), Half-maximal stimulations of the Na\(^+\) extrusion and AIB uptake by K\(^+\) were observed at K\(^+\) concentration near apparent K\(_m\) for K\(^+\) transport. Finally, in the presence of the Na\(^+\) electrochemical gradient (toward the inside), K\(^+\) was not necessary for AIB uptake.

From these results, it was concluded that the Na\(^+\)-dependent AIB uptake is driven by the Na\(^+\) electrochemical gradient across the membrane and that K\(^+\) is required for AIB uptake only for the generation of the Na\(^+\) electrochemical gradient.

The ion coupling in active transport systems has a long history of study (see recent reviews in Refs. 1–4). In nonhalophilic bacteria, many transport systems involve H\(^+\) as a coupling ion. Lactose transport in *Escherichia coli* is the most extensively studied system and it is now well established that the driving force for the lactose uptake is the electrochemical potential difference of H\(^+\) across the membrane (6–8) as postulated by the chemiosmotic hypothesis of Mitchell (9–11).

In animal cells, another type of ion coupling is common to various transport systems, i.e., transports of amino acids and sugars are driven by the Na\(^+\) electrochemical gradient (4). Although a considerable number of transport systems have also been reported to be dependent on Na\(^+\) in nonhalophilic bacteria (12–21), these systems are rather exceptional in such cells. The electrochemical gradient of Na\(^+\) as a direct driving force in such systems is generated by the specific Na\(^+\) extrusion mechanism, a Na\(^+\)/H\(^+\) antiport system, which is fueled by the proton motive force (22, 23).

Halophilic bacteria, like animal cells, require Na\(^+\) rather than H\(^+\) for many amino acid transports. A marine bacterium *Alteromonas haloplanktis* (24–27), formerly referred to as pseudomonad B-16, and *Halobacterium halobium* (28, 29) take up various amino acids depending on Na\(^+\). Lanyi et al. have demonstrated in their series of papers (see a review in Ref. 30) that the driving force for amino acid transports in *H. halobium* membrane vesicles is the electrochemical gradient of Na\(^+\). Since these bacteria have adapted to salt-rich environments, it may be more advantageous to use Na\(^+\) than to use H\(^+\) as a coupling ion. The recent discovery of a primary Na\(^+\) pump, halorhodopsin (31–34), suggests that the role of Na\(^+\) in energetics is more fundamental in *H. halobium* than in nonhalophilic bacteria.

K\(^+\) has also been reported to affect many transport systems (for a review, see Ref. 11) although its mechanism is yet to be clarified and may not be common to all systems. K\(^+\) indirectly affects active transport systems by changing metabolisms in some cases and the charge-balancing function of K\(^+\) has been speculated upon in other cases. The requirement for K\(^+\) is known in some Na\(^+\)-dependent active transport systems. Uptake of \(\alpha\)-aminoisobutyric acid by the marine bacterium *A. haloplanktis* (35, 36) and uptake of glutamate by *E. coli* (37) do not function in the absence of K\(^+\) when K\(^+\) is depleted from cells by an osmotic shock treatment. Although Thompson and MacLeod (26) once concluded that neither a Na\(^+\) nor a K\(^+\) concentration gradient is necessary for the AIB\(^+\) uptake by *A. haloplanktis*, Niven and MacLeod (27) reached the conclusion from recent experiments that the AIB uptake is driven by the electrochemical gradient of Na\(^+\). However, the role of K\(^+\) in this system is still ambiguous. Using membrane vesicles isolated from *H. halobium*, MacDonald et al. (29) have shown that many Na\(^+\)-dependent amino acid transports are stimulated by K\(^+\). The stimulatory effect of K\(^+\) has been attributed to its function as a counter ion permitting the overall electro-neutral extrusion of Na\(^+\). However, in a more recent paper (38), Lanyi et al. have shown that the permeability of membrane vesicles to K\(^+\) is not sufficiently great to compensate for the loss of intravesicular Na\(^+\). Although the data described above indicate the necessity of K\(^+\) in some transport systems, the role of K\(^+\) in the active transport is still an open question.

In order to characterize the role of K\(^+\) in transport systems, the following precautions seem to be important. 1) K\(^+\) depletion must be performed by an isoosmotic treatment. The osmotic treatment, as used in the case of *A. haloplanktis*, causes a plasmolysis of cells (39), and uptake of solutes is generally dependent on the size of intracellular space. Furthermore, K\(^+\) uptake causes a deplasmolysis of such cells (39). Therefore, the use of plasmolyzed cells may make it difficult to distinguish whether the stimulatory effect of K\(^+\) is derived

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom all the correspondence should be addressed.
from its osmotic effect or others. 2) So far reported, no membrane vesicles show the active accumulation of K+ (38-42). If the effect of K+ involves the K+ transport system, it should be examined in whole cells. 3) The technique which allows the manipulation of intracellular cation compositions is useful since the artificial ion gradients can be imposed across the membrane.

The technique which we have recently developed (43) fulfills the points discussed above and using this method the roles of Na+ and K+ in AIB transport by the marine bacterium Vibrio alginolyticus are presented in this paper.

MATERIALS AND METHODS

Growth of Cells—The marine bacterium V. alginolyticus 138-2 was grown aerobically on a synthetic medium (43) at 37°C to the late logarithmic phase of growth. The cells were harvested by centrifugation at 4°C.

Preparation of Cells Loaded with Specified Cations—The technique developed recently (43) was modified slightly and extended to prepare cells depleted of K+ and/or Na+ and loaded with various cations. In brief, the harvested cells were resuspended twice at 25°C for 10 min with 50 mM diethanolamine-Cl, pH 8.5, containing 0.4 mM desired cation(s) as a chloride salt. The cells loaded with the specified cation were washed twice with 50 mM HEPES, pH adjusted to 7.0 as specified, containing 0.4 mM of the same salt as those used in the loading. In our previous paper (43), the K+ depletion and Na+ loading were performed in 0.9% saline and these conditions decreased neither the cellular activity to generate a proton motive force (43) nor the ability to take up AIB. However, for the loading with various alkali metal cations other than Na+, pH during the loading was lowered to 5.0 since the former conditions significantly reduced the activity of AIB uptake even after washing the cells with the buffer at pH 7.0. For the loading with choline+, pH was lowered to 8.0. Detailed experimental results and mechanisms involved will be discussed elsewhere. The above modifications gave satisfactory results regarding the depletion of K+ and Na+ and the loading with specified cations.

The cation-loaded cells were resuspended in the buffer (50 mM HEPES, 0.4 mM salt) at pH 7.0 and kept on ice until use.

Assay of AIB Uptake—Unless otherwise specified, AIB uptake was performed after dilution of cells to 50 µl of 50 mM HEPES-Na, pH 7.0, containing 0.4 mM NaCl, 20 mM glycerol, and [3H]AIB (20 nCi/µmol, 0.1 mM final concentration) at 25°C. The uptake was terminated by addition of 2 ml of 50 mM HEPES-Na, pH 7.0, containing 0.4 mM NaCl at room temperature and by filtration with Millipore Celotape filters (pore size, 0.5 µm). The filters were washed once with 2 ml of the above buffer and radioactivities were determined with 3 ml of Bray's scintillation liquid.

Assay of 22Na+ Exclusion—The Na+-loaded cells in 50 mM HEPES-Na, pH 7.0, containing 0.4 mM NaCl were equilibrated with 22NaCl (carrier-free) for 2 to 3 h on ice. Experiments of 22Na+ exclusion were started by either transfer of aliquots (50 µl) to 25°C or dilution of cells into 0.4 ml of 50 mM HEPES-choline, pH 7.0, containing 0.4 mM choline chloride at 25°C. Glycerol was added prior to the start of experiments to give a final concentration of 20 mM. Where cited, the addition of salt was made at appropriate times. The level of 22Na+ retained by the cells was determined at given times by the filtration method as described above for the uptake of AIB.

Determination of Cations—Na+ and K+ were analyzed by atomic absorption spectrophotometry and Li+ and Cs+ were determined by flame photometry. In order to determine the intracellular concentrations of cations, concentrated cell suspensions (about 60 mg of protein/ml) were extracted with 5% trichloroacetic acid. When the extracellular concentration of cation to be determined was high, the cells were filtered with Millipore Celotape and washed with 50 mM HEPES-choline, pH 7.0, containing 0.4 mM choline chloride prior to the extraction with trichloroacetic acid. In all determinations, intracellular cation concentrations were calculated based on a calibration performed under appropriate standard salt solutions and using a value of 3.3 µl of intracellular water space/mg of cell protein (43).[14C]Choline+-loaded cells were prepared in the presence of [14C]choline chloride (9.26 pCi/µmol) throughout the procedure, and the intracellular concentration of choline+ was determined from the radioactivity retained by the cells after filtration and washing with nonradioactive 50 mM HEPES-choline, pH 7.0, containing 0.4 mM choline chloride.

Determination of Proteins—Protein was determined as described by Lowry et al. (44) using bovine serum albumin as a standard.

Materials—[3H]AIB and 22NaCl were purchased from New England Nuclear, CCCP was obtained from Sigma. HEPES was a product of Nakarai Chemical Co. Ltd.

RESULTS

AIB Uptake in K+-containing V. alginolyticus—Fig. 1 represents the uptake of AIB, a nonmetabolizable substrate, examined in the presence of various cations using cells washed with 50 mM HEPES-choline, pH 7.0, containing 0.4 mM choline chloride. Intracellular concentrations of K+ and Na+ in these cells were found to be 355 and 22 mM, respectively. Only Na+ (open circles) could support the uptake among ions tested, Li+ being known to substitute for Na+ in Na+-dependent transport systems, had no effect (closed triangles). The addition of KCl at a final concentration of 10 mM had essentially no stimulatory effect (closed circles). Moreover, 0.4 mM KCl, choline chloride, or CsCl with or without 10 mM KCl did not stimulate the uptake (results not shown). CCCP, a proton conductor, collapsed membrane potential (ΔΨ) and ΔpH under all the conditions shown in this paper and caused a significant inhibition of the Na+-dependent AIB uptake (open triangles). Although the results shown were obtained with the cells preincubated for 5 min, the same results as described above were obtained without the preincubation. The intracellular concentrations of K+ and Na+ after the preincubation were similar to those determined before the preincubation even in the presence of CCCP. The addition of CCCP after the start of AIB uptake immediately caused the efflux of accumulated AIB. A half-maximal stimulation of the uptake was obtained with about 80 mM of Na+ and kinetic examinations revealed that both Vmax and apparent Km for AIB uptake were affected by the concentration of Na+. In the presence of 0.4 mM NaCl, Vmax and apparent Km for AIB uptake were 90 nmol/min/mg of cell protein and 43 µM, respectively.

AIB Uptake in K+-depleted and Na+-loaded Cells—Fig. 2 shows the AIB uptake by K+-depleted and Na+-loaded V. alginolyticus that contained about 5 mM K+ and 0.4 mM Na+. In contrast to the results presented in Fig. 1, the Na+-loaded cells showed little AIB uptake even in the presence of 0.4 mM NaCl (open circles). However, the preincubation of the Na+-loaded cells with K+ (closed circles) or Rb+ (triangles) prior to the assay caused a dramatic stimulation of the AIB uptake. The addition of K+ at 0 time to the cells preincubated without K+ also gave a similar stimulation of the uptake. However, in this case, the uptake always occurred after a certain length of lag (see Fig. 7). Neither the addition of other cations (Li+ or choline+)) at a final concentration of 10 mM nor the combined addition of 10 mM KCl and 0.4 mM of salts (choline chloride, LiCl, CaCl2, or KCl) caused the stimulation of AIB uptake. The preincubation of Na+-loaded cells in 0.4 mM NaCl without KCl induced no change in the intracellular level of cations. After a 5-min preincubation at 25°C with 10 mM KCl, the intracellular concentrations of K+ and Na+ became about 0.5 and 0.1 mM, respectively. These values were reproducible and similar to those found in the control cells shown in Fig. 1. Since further incubation caused little change in the intracellular levels of K+ and Na+, these values represented the steady state level of such cations. The preincubation with Rb+ also

1 In order to check degradation, [3H]AIB was recovered after transport experiments and analyzed on a paper chromatogram using the solvent system 1-butanol/acetic acid/water, 25:4:10. All the radioactivity was found in a position of authentic AIB.
caused the accumulation of Rb+ with the concomitant extrusion of Na+.

**Roles of Na+ and K+ in AIB Transport by V. alginolyticus.** Cells were harvested at the late logarithmic phase of growth and were washed twice with and suspended in 50 mM HEPES-choline, pH 7.0, containing 0.4 M choline chloride to give a final concentration of 4.7 mg of protein/ml. An aliquot (1 μl) of the cell suspension was assayed for AIB uptake after a 5-min preincubation in 50 μl of 50 mM HEPES-Na, pH 7.0, containing 0.4 M NaCl (■, ○, △) or 50 mM HEPES-Li, pH 7.0, containing 0.4 M LiCl (▲). Where specified, 10 mM KCl (●) or 10 μM CCCP (△) was present in the reaction mixture. AIB uptake was started by the addition of [3H]AIB (20 μCi/μmol, 0.1 mM) and determined by the filtration method as described under “Materials and Methods.”

**Fig. 2 (center).** K+ (Rb+) requirement for Na+-dependent AIB uptake. K+-depleted and Na+-loaded cells were prepared as described under “Materials and Methods” and suspended in 50 mM HEPES-Na, pH 7.0, containing 0.4 M NaCl to give a final concentration of 3.7 mg of protein/ml. AIB uptake was assayed after a 5-min preincubation of the cell suspension (1 μl) in 50 μl of 50 mM HEPES-Na, pH 7.0, containing 0.4 M NaCl, 20 mM glycerol (●). Where specified, 10 mM KCl (●) or 10 mM RbCl (△) was present in the reaction mixture. AIB uptake was started and determined as described in Fig. 1. AIB uptake in the presence and absence of K+ was determined at 20 min after the addition of Rb'.
K" concentration required to produce a half-maximal stimulation when initial rates of Na" extrusion were times by the fitration method as described under "Materials and Methods." KC1 or RbCl was added at 5 min at a final concentration of 10 mM. The values obtained were corrected for background radioactivity which was determined with samples boiled for 5 min prior to assay. The results are given in percentage of radioactivity at 0 time (9700 cpm).

Fig. 5 (center). Na" extrusion in the presence of various concentrations of K". Na"-loaded cells were prepared and suspended in 50 mM HEPES-Na, pH 7.0, containing 0.4 mM NaCl in a final concentration of 18 mg of protein/ml. Equilibration of the cells with 22Na" (3.1 x 10" cpm/μmol of Na") was performed on ice for 3 h. An aliquot (1 μl) was diluted into a series of reaction vessels containing 0.4 ml of 50 mM HEPES-choline, pH 7.0, 0.4 mM choline chloride, 20 mM glycerol. Incubations were performed at 25 °C. At 3 min, KCl was added to give the specified final concentration of K". Levels of 22Na" retained by the cells were determined by the filtration method as described in Fig. 4 using 50 mM HEPES-choline, pH 7.0, containing 0.4 mM choline chloride as a termination and washing buffer. The values shown were treated as described in Fig. 4. The value obtained at 0 time (100%) was 7030 cpm/assay. Intracellular concentrations of Na" and K" were about 0.4 M and 4 mM, respectively, at 0 time, 0 mM KC1; ♦, 0.5 mM KC1; Δ, 1.0 mM KC1; ▲, 99 mM KC1.

Fig. 6 (right). Effect of K" on the rate of Na" extrusion. The rates of "Na" extrusion determined as described in Fig. 5 are plotted as a function of K" concentration. The initial rates of Na" extrusion were determined at 1 min after the addition of KCl and corrected for K"-independent efflux of Na" (open circles in Fig. 5). The values obtained are presented as a double reciprocal plot in the inset.

It should be noted that the steady state levels of Na" obtained with K" and Rb" were the same. Although results are not presented, choline chloride, LiCl, or CsCl added at 10 mM as a final concentration did not cause the extrusion of 22Na".

Effect of K" on the Extrusion of Na"—Rates of Na" extrusion in the presence of various concentrations of K" were examined (Fig. 5). Because of technical difficulties in obtaining accurate rates, especially in the presence of limited concentrations of K", the experimental conditions like those in Fig. 4 could not be used and were modified as follows. 1) The concentration of cells in the assay buffer was reduced and the volume of the assay buffer was increased to avoid a decrease in the internal K" concentration due to the accumulation of K" by the cells. 2) In order to reduce the concentration of radioactivity in the assay system, the cells equilibrated 22Na" were diluted into nonradioactive 0.4 mM choline chloride instead of the transfer of cells to 25 °C without dilution. The level of background due to nonspecific binding to filters under such experimental conditions was much lower than that in Fig. 4. Under the conditions described above and in Fig. 5, the Na" concentration gradient was imposed and the rate of Na" release from the cells in the absence of K" was faster than that obtained in Fig. 4 (compare open circles in Figs. 4 and 5). Nevertheless, the K"-dependent extrusion of Na" could be observed (Fig. 5). The stimulatory effect of K" exhibited a saturation kinetics when initial rates of Na" extrusion were determined as a function of K" concentration (Fig. 6), and the K" concentration required to produce a half-maximal stimulation is 3.5 ± 0.7 mM (Fig. 6, inset) which is consistent with the apparent K_m (3.0 ± 0.2 mM) for K" transport determined in the presence of choline chloride.

Kinetics of K" Requirement in Na"-dependent AIB Uptake—The attempts to find the relationship between initial rates of the Na"-dependent AIB uptake in Na"-loaded cells and the concentration of K" were unsuccessful. Once the uptake started, the initial rates were essentially identical over the range of K" concentrations examined. Instead, the K" concentration dependence was clearly observed in the length of lag required for the start of AIB uptake as shown in Fig. 7. The lower that a K" concentration was, the longer a lag became. On the other hand, the linear uptake of AIB after the lag proceeded in the same speed in all cases. The rates obtained after the lag were the same as that obtained after a 5-min preincubation with the saturating concentration of K" (closed circles). When reciprocals of the length of lag were plotted as a function of K" concentration, the Michaelis-Menten-type kinetics was obtained as shown in Fig. 8. A half-maximal effect of K" was obtained with 1.2 ± 0.1 mM KCl (inset) which is consistent with the apparent K_m (1.65 ± 0.5 mM) for K" transport determined in the presence of NaCl/choline chloride. The minimum value of a lag time was 2.3 min which was very close to the time required in the presence of a saturating concentration of K" to lower the intracellular Na" to the steady state level (Fig. 4, closed circles). Similarly, in the presence of a saturating concentration of Rb", the
minimum value of a lag time (about 5 min, results not shown) was essentially the same as the time necessary to reach the steady state level (Fig. 4, triangles). As described in Fig. 4, the levels of Na⁺ at the steady state obtained with K⁺ and Rb⁺ were the same. The correlation between the lag time and the time required to reach the steady state level of Na⁺ was also observed in the presence of 1 mM K⁺. Furthermore, the level of Na⁺ at the steady state was approximately 0.1 mM which was similar to those obtained with saturating concentrations of K⁺ and Rb⁺. These results suggested that the lag period corresponded to the time required to reach the steady state level of Na⁺ and that the generation of a certain magnitude of Na⁺ chemical potential was necessary for AIB uptake. The fact that the steady state levels of Na⁺ were essentially the

**Fig. 7. Effect of K⁺ on the length of lag in Na⁺-dependent AIB uptake.** Na⁺-loaded cells (3.47 mg of protein/ml) in 50 mM HEPES-Na, pH 7.0, containing 0.4 mM NaCl were assayed for AIB uptake at 25 °C. Experiments were started by the addition of 1 μl of the cell suspension to 50 μl of 50 mM HEPES-choline, pH 7.0, containing 0.2 mM choline chloride, 0.2 mM NaCl, 20 mM glycerol, 0.1 mM [³H]AIB (20 μCi/μmol), and a given concentration of KCl. AIB uptake was determined by the filtration method. The results shown by closed circles of the cells were obtained with the cells preincubated for 5 min with 9.4 mM KC1 prior to the addition of [³H]AIB. Initial K⁺ and Na⁺ concentrations of the cells were 2.5 mM and about 0.4 mM, respectively. The symbols used represent 0 (■), 0.5 (△), 1.0 (○), 2.0 (□), and 9.4 (▲) mM of KCl.

**Fig. 8. Relationship between K⁺ concentration and the length of lag in AIB uptake.** The experiments shown in Fig. 7 were performed over the range of K⁺ concentrations of 0 to 20 mM. The length of lag was determined in each K⁺ concentration by extending the linear portion of AIB uptake to the abscissa. The reciprocal of lag time is plotted against K⁺ concentration. In the inset, the values of lag time are plotted against the reciprocals of K⁺ concentration.

**Fig. 9. AIB uptake in the presence of Na⁺ concentration gradient.** Cells loaded with various cations were prepared as described under "Materials and Methods" and AIB uptake by such cells was assayed in 50 μl of 50 mM HEPES-Na, pH 7.0, containing 0.4 mM NaCl, 20 mM glycerol, 0.1 mM [³H]AIB (20 μCi/μmol) at 25 °C. A, AIB uptake by Cs⁺-loaded cells was started by the addition of 1 μl of the cell suspension (2.9 mg of protein/ml) to the reaction mixture with (■, △) or without (○, □) 10 mM KCl. Where specified, 10 μM CCCP was added immediately after the start of experiments (△, □). The Cs⁺-loaded cells contained 1.5 mM Na⁺, 1.0 mM K⁺, and about 0.4 mM Cs⁺. B, an aliquot (1 μl) of choline⁺-loaded cells (5.6 mg of protein/ml) was preincubated for 5 min at 25 °C in the reaction mixture and [³H]AIB was added to start the assay. The symbols used are the same as those in A. The choline⁺-loaded cells initially contained 1.9 mM Na⁺, 1.0 mM K⁺, and about 0.4 mM choline⁺. C, AIB uptake by Li⁺-loaded cells was assayed with (○) or without (■) preincubation for 5 min in the reaction mixture. The assays without the preincubation were started by the addition of the cell suspension and those with the preincubation were started by the addition of [³H] AIB. The final concentration of the cells was 0.13 mg of protein/ml. The dashed line shows the rate of AIB uptake obtained by the cells preincubated for 5 min in the presence of 10 mM KCl. AIB uptake by the Na⁺-loaded cells with (△) or without (□) 5-min preincubation were also shown for comparison. The Li⁺-loaded cells contained 2.6 mM K⁺, 0.8 mM Na⁺, and about 0.4 mM Li⁺.
Roles of Na\(^+\) and K\(^+\) in AIB Transport by V. alginolyticus

same under the conditions examined may be the reason why the AIB uptake after various lengths of lag proceeded at the identical speed including the case of Rb\(^+\) (Fig. 2).

AIB Uptake in the Presence of Na\(^+\) Concentration Gradients—The results described above revealed that the concentration gradient of Na\(^+\) but not of K\(^+\) is necessary for AIB uptake and that K\(^+\) is required only for the generation of a Na\(^+\) chemical potential. However, since the Na\(^+\) extrusion is tightly coupled to K\(^+\) uptake under the conditions employed, it is not clear whether the intracellular accumulation of K\(^+\) is essential or not. In order to clarify this, the cells loaded with cations other than K\(^+\) or Na\(^+\) were prepared and assayed for AIB uptake in the presence of Na\(^+\) (Fig. 9). Since these cells contained less than 3 \(\mu\)M intracellular Na\(^+\), the dilution of cells into 0.4 \(\mu\)M NaCl imposed a large concentration gradient of Na\(^+\) across the membrane. The intracellular concentration of Na\(^+\) was found to be less than 0.1 \(\mu\)M even after a 10-min incubation of Cs\(^-\) - or choline\(^-\)-loaded cells with 0.4 \(\mu\)M NaCl, and K\(^+\) retained by these cells was negligible in all cases.

The uptake determined in the absence of K\(^+\) with Cs\(^-\)-loaded cells (Fig. 9A, open circles) was comparable to that in the control cells (Fig. 1) and to that in the Na\(^+\)-loaded cells assayed in the presence of K\(^+\) (Fig. 2). Furthermore, the addition of KC1 had essentially no effect (closed circles). Under both conditions, the uptake was abolished by CCCP (triangles). The Cs\(^-\)-loaded cells generated no \(\Delta\Psi\) and about -100 mV of \(\Delta\Omega\) that was completely collapsed by CCCP. The addition of K\(^+\) was without effect on the magnitude of \(\Delta\Psi\) and \(\Delta\Omega\) generated by the Cs\(^-\)-loaded cells. It is also noteworthy that no detectable lag was observed in the AIB uptake by the cells. Choline\(^-\)-loaded cells assayed after a 5-min preincubation in 0.4 \(\mu\)M NaCl also accumulated AIB in the absence of K\(^+\) (Fig. 9B). Similar results were obtained without the preincubation. It should be pointed out that Cs\(^-\)- or choline\(^-\)-loaded cells accumulated little K\(^+\) under similar conditions to those shown in Fig. 9.

The uptake of AIB by Li\(^+\)-loaded cells in the absence of K\(^+\) lasted for only a short period (Fig. 9C, closed circles). Although the initial rate determined at 30 s (about 16 nmol/min/mg of protein) was comparable to that determined after the preincubation with K\(^+\) (dashed line, 20 nmol/min/mg of protein), the accumulation of AIB stopped within 2 or 3 min. When the Li\(^+\)-loaded cells were preincubated for 5 min in the absence of KCl after the dilution into Na\(^+\) (open circles), the uptake of AIB was significantly reduced and became indistinguishable from that in the Na\(^+\)-loaded cells assayed in the absence of K\(^+\) (triangles). On the contrary, the AIB uptake by the Cs\(^-\)-loaded cells assayed after the preincubation was similar to that shown in Fig. 9A. These results suggested the possibility that the internal Li\(^+\) rapidly exchanges with the external Na\(^+\) and, as a result, the Na\(^+\) concentration gradient was collapsed within a few minutes. Indeed, the dilution of Li\(^+\)-loaded cells into Na\(^+\) or Na\(^+\)-loaded cells into Li\(^+\) caused much faster efflux of internal cations than the dilution of these cells into Cs\(^-\) or choline\(^-\). Although results are omitted from the figures, none of these cells showed AIB uptake in the absence of Na\(^+\).

**DISCUSSION**

The results presented in this paper led us to the conclusions that the driving force for AIB uptake is the electrochemical potential gradient of Na\(^+\) across the membrane and that K\(^+\) is required only for the generation of Na\(^+\) chemical potential.

In a previous paper (43), we have reported that the K\(^+\)-depleted and Na\(^+\)-loaded cells generate a large \(\Delta\Psi\) (-145 mV, negative inside, at pH 7.0) and only a small \(\Delta\Omega\) (15 mV, alkaline inside, at pH 7.0) in the absence of K\(^+\) and that by the addition of K\(^+\) to such cells the \(\Delta\Psi\) is partially collapsed to -100 mV with the concomitant generation of \(\Delta\Omega\) (40 mV). Therefore, it may be possible that K\(^+\) is required for AIB uptake to generate \(\Delta\Omega\). However, the facts that AIB uptake by Na\(^+\)-loaded cells assayed at pH 8.0, where no \(\Delta\Omega\) is present (43), still required K\(^+\) and that Cs\(^-\)-loaded cells accumulated AIB in the absence of \(\Delta\Omega\) exclude the generation of \(\Delta\Omega\) as the explanation for the K\(^+\) requirement. Therefore, the strong inhibitory effect of CCCP under all conditions examined (for example, Figs. 1, 3, 9A, and 9B), indicates that AIB uptake is coupled to \(\Delta\Psi\).

The examination of Na\(^+\) extrusion clearly demonstrated the K\(^+\) requirement for the active extrusion of Na\(^+\), namely the generation of the Na\(^+\) chemical potential. The stimulatory effect of K\(^+\) appeared to involve the K\(^+\) transport system since a half-maximal stimulation of Na\(^+\) extrusion was obtained with K\(^+\) at the concentration near \(K_m\) for K\(^+\) transport. When AIB uptake in Na\(^+\)-loaded cells was examined as a function of K\(^+\) (Fig. 7), the length of lag was inversely dependent on K\(^+\) concentration. Since Cs\(^-\)-loaded cells showed no lag whether in the presence or absence of K\(^+\), the intracellular accumulation of K\(^+\) cannot be the reason for the lag. Instead, the lag is likely to represent the time required for the extrusion of a certain amount of intracellular Na\(^+\). The results presented in Figs. 5 and 7 are indicative of the gating effects on AIB uptake brought about by the magnitude of the Na\(^+\) chemical potential or by the intracellular concentration of Na\(^+\). The effect of Na\(^+\) chemical potential as an essential factor for AIB uptake can be seen in the case of Li\(^+\)-loaded cells (Fig. 9C). Since the presence of Li\(^+\) inside the cells facilitates the equilibration of Na\(^+\) across the membrane, AIB uptake lasted for only a short period and the preincubation of cells inhibited the uptake significantly. From these points discussed above, it is clear that the Na\(^+\) chemical potential as well as \(\Delta\Psi\) is necessary for AIB uptake.

The function of K\(^+\) as a counter ion for Na\(^+\) extrusion has been demonstrated in a wide variety of microorganisms (48-52; for a review, see Ref. 53). Such a mechanism maintaining overall electroneutrality is indispensable to the generation of ion gradients. Although the role of K\(^+\) in the Na\(^+\)-dependent AIB uptake by V. alginolytica was clearly characterized in this paper, it is still equivocal whether or not the K\(^+\) requirement reported in A. haloplanktis (35, 36) can be attributed solely to the function of K\(^+\) as a counter ion. The K\(^+\)-dependent deplasmolysis may also play an important role in the Na\(^+\)-dependent AIB uptake by osmotically shocked A. haloplanktis. Lanyi et al. (38) have shown that K\(^+\) does not serve as a counter ion for Na\(^+\) extrusion in membrane vesicles isolated from H. halobium. Although it was shown that K\(^+\) serves as a counter ion for Na\(^+\) extrusion in whole cells of H. halobium (52), further investigations are necessary to reveal the role of K\(^+\) in Na\(^+\)-dependent amino acid transports in the membrane vesicles.

Our attempts to detect AIB-dependent uptake of Na\(^+\) under various conditions as an evidence for Na\(^+\)/AIB co-transport were unsuccessful. Such a failure does not exclude the possibility of co-transport since it may be difficult to detect the rather small portion of Na\(^+\) movement in a high concentration of Na\(^+\) and with cells possibly having a high capacity to bind Na\(^+\).

**REFERENCES**

1. Rosen, B. P., and Kashket, E. R. (1978) in Bacterial Transport (Rosen, B. P., ed) pp. 559-620, Marcel Dekker, Inc., New York
2. Harold, F. M. (1977) Curr. Top. Bioenerg. 6, 84-149
3. Harold, F. M. (1978) in The Bacteria (Sokatch, J. R., and Ornston, L. N., eds) Vol. 6, pp. 463-521, Academic Press, New York
4. Crane, R. K. (1977) Rev. Physiol. Biochem. Pharmacol. 78, 99-
Roles of Na\(^+\) and K\(^+\) in AIB Transport by \textit{V. alginolyticus}

159
5. Hirata, H., Altendorf, K., and Harold, F. M. (1973) *Proc. Natl. Acad. Sci. U. S. A.* 70, 1804–1808
6. Flagg, J. L., and Wilson, T. H. (1976) *J. Bacteriol.* 125, 1235–1236
7. Ramos, S., and Kaback, H. R. (1977) *Biochemistry* 16, 854–859
8. Kaback, H. R., Ramos, S., Robertson, D. E., Stzobant, P., and Tokuda, H. (1978) in *Molecular Specialization and Symmetry in Membrane Function* (Solomon, A. K., and Karnovsky, M., eds) pp. 160–188, Harvard University Press, Cambridge, MA
9. Mitchell, P. (1976) *Biochem. Soc. Trans.* 4, 399–430
10. Mitchell, P. (1968) *Chemiosmotic Coupling and Energy Transduction*, Glynn Research, Bodwin, Cornwall, England
11. Harold, F. M. (1972) *Bacteriol. Rev.* 36, 172–230
12. Stock, J., and Roseman, S. (1971) *Biochem. Biophys. Res. Commun.* 44, 132–138
13. Tokuda, H., and Kaback, H. R. (1977) *Biochemistry* 16, 2130–2136
14. Tsuichiya, T., Raven, J., and Wilson, T. H. (1977) *Biochem. Biophys. Res. Commun.* 76, 26–31
15. Kitada, M., and Horikoshi, K. (1977) *J. Bacteriol.* 131, 784–788
16. Frank, L., and Hopkins, I. (1969) *J. Bacteriol.* 100, 329–336
17. Hoshino, T., and Kageyama, M. (1979) *J. Bacteriol.* 137, 73–81
18. Hirata, H., Kosmakos, F. C., and Brodie, A. F. (1974) *J. Biol. Chem.* 249, 6965–6970
19. Ariel, M., and Grossowicz, N. (1974) *Biochim. Biophys. Acta* 352, 122–126
20. Koyama, N., Kiyomiya, A., and Nosoh, Y. (1976) *FEBS Lett.* 72, 77–78
21. Guffanti, A. A., Susman, P., Blanco, R., and Kruilich, T. A. (1978) *J. Biol. Chem.* 253, 708–715
22. West, L. C., and Mitchell, P. (1974) *Biochem. J.* 144, 87–90
23. Schuldiner, M., and Fishkes, H. (1978) *Biochemistry* 17, 706–711
24. Sprott, G. D., Drozdzowski, J. P., Mannin, E. L., and MacLeod, R. A. (1975) *Can. J. Microbiol.* 21, 43–50
25. Sprott, G. D., and MacLeod, R. A. (1972) *Biochem. Biophys. Res. Commun.* 47, 838–845
26. Thompson, J., and MacLeod, R. A. (1973) *J. Biol. Chem.* 248, 7116–7111
27. Niven, D. F., and MacLeod, R. A. (1980) *J. Bacteriol.* 142, 603–607
28. MacDonald, R. E., and Lanyi, J. K. (1975) *Biochemistry* 14, 2882–2889
29. MacDonald, R. E., Greene, R. V., and Lanyi, J. K. (1977) *Biochemistry* 16, 3227–3235
30. Lanyi, J. K. (1979) *Biochim. Biophys. Acta* 559, 377–397
31. Matsuno-Yagi, A., and Mukohata, Y. (1977) *Biochim. Biophys. Res. Commun.* 78, 237–243
32. Lindley, E. V., and MacDonald, R. E. (1979) *Biochim. Biophys. Res. Commun.* 88, 491–499
33. Greene, R. V., and Lanyi, J. K. (1979) *J. Biol. Chem.* 254, 10986–10994
34. Lanyi, J. K. (1981) *Trends Biochem. Sci.* 6, 60–62
35. Thompson, J., and MacLeod, R. A. (1971) *J. Biol. Chem.* 246, 4066–4074
36. Drapeau, G. R., Matula, T. I., and MacLeod, R. A. (1966) *J. Bacteriol.* 92, 63–71
37. Halpern, Y. S., Barash, H., Druck, K., and Silver, S. (1973) *J. Bacteriol.* 114, 53–58
38. Lanyi, J. K., Helgerson, S. L., and Silverman, M. P. (1979) *Arch. Biochem. Biophys.* 193, 329–339
39. Thompson, J., Costerton, J. W., and MacLeod, R. A. (1970) *J. Bacteriol.* 102, 843–854
40. Bhattacharyya, P., Epstein, W., and Silver, S. (1971) *Proc. Natl. Acad. Sci. U. S. A.* 68, 1488–1492
41. Lombardi, F. J., Reeves, J. P., and Kaback, H. R. (1973) *J. Biol. Chem.* 248, 3551–3565
42. Sprott, G. D., and MacLeod, R. A. (1974) *J. Bacteriol.* 117, 1043–1054
43. Tokuda, H., Nakamura, T., and Numoto, T. (1981) *Biochemistry* 20, 4196–4203
44. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
45. Rudnick, G., and Nelson, P. J. (1978) *Biochemistry* 17, 4739–4742
46. Kanner, B. I., and Sharon, I. (1978) *Biochemistry* 17, 3949–3953
47. Schneider, E. G., and Sacktor, B. (1980) *J. Biol. Chem.* 255, 7645–7649
48. Zarleno, M. H., and Schultz, S. G. (1966) *Biochim. Biophys. Acta* 126, 308–320
49. Slayman, C. W., and Slayman, C. W. (1968) *J. Gen. Physiol.* 52, 424–443
50. Schultz, S. G., Epstein, W., and Solomon, A. K. (1963) *J. Gen. Physiol.* 47, 329–346
51. Harold, F. M., Baarda, J. R., and Pavlasova, E. (1970) *J. Bacteriol.* 101: 152–159
52. Wagner, G., Hartmann, R., and Oesterhelt, D. (1978) *Eur. J. Biochem.* 89, 169–179
53. Harold, F. M., and Altendorf, K. (1974) *Curr. Top. Membr. Trans.* 5, 2–50
Roles of Na+ and K+ in alpha-aminoisobutyric acid transport by the marine bacterium Vibrio alginolyticus.
H Tokuda, M Sugasawa and T Unemoto

J. Biol. Chem. 1982, 257:788-794.

Access the most updated version of this article at http://www.jbc.org/content/257/2/788

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/257/2/788.full.html#ref-list-1