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Na^+—and K^+-Gradient Stimulated ATPase Activity in an Alkalophilic *Bacillus.*

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ABSTRACT

ATPase activity of membrane vesicles prepared from an alkalophilic *Bacillus* sp. A-007 was stimulated by Na^+ (in> out)-and K^+ (in < out)-gradient. Translocation of Na^+ from inside to outside of the membrane was obligately coupled to ATPase reaction, while K^+ moved electrophoretically to compensate membrane potential. The cation-gradient stimulated ATPase activity was electrogenic and inhibited by ouabain.

INTRODUCTION

According to chemiosmotic theory, active transport of solutes are driven by electrochemical gradient of proton (proton motive force; $\Delta p = \Delta\psi - 2.3\,RT/F \cdot \Delta$ pH) (MITCHELL, 1966; HAROLD, 1977). Δp is generated via primary pumps (e.g. electron transport system) and used for other chemical or physical works via secondary pumps (e.g. Na^+/H^+ antiporter and amino acid $-H^+$ (or $-Na^+$) cotransporter). Many bacteria, which prefer to grow at near neutral pH, maintain their intracellular condition to be more alkaline and negative than those of extracellular environments through primary pumps. Optimal growth pH of an alkalophilic *Bacillus* sp. A-007 is pH 10.5, while its intracellular pH is maintained at nearly neutral (ANDO *et al*, 1981 a). So, magnitude of Δp under optimal growth condition is considered to be much smaller than those of the above bacteria (GUFFANTI *et al*, 1978).

Active transport of the alkalophile for solutes, so far examined, are driven by Na^+—gradient (ANDO *et al*, 1981 b and 1982). But Na^+—gradient generating mechanism is not clarified. In animal cells, Na^+—gradient is generated by $Na^+ \cdot K^+$—ATPase exists in bacterial cell membranes, cation (K^+) stimulated ATPase activity was reported in the previous study (ANDO *et al*, 1983). In this paper, effect of cation gradient on the ATPase activity of membrane vesicles prepared from the alkalophile was examined. Some data that suggested the existence of Na^+—translocating ATPase were obtained.

MATERIALS AND METHODS

1. Cultivation of bacterium and preparation of membrane vesicles.

Bacillus sp. A-007 was grown aerobically and membrane vesicles were prepared according to lysozyme-protoplasts method as described earlier (ANDO *et al*, 1982), except 25 mM Tris-HCl buffer pH 7.4 containing 0.4 M salt (NaCl, KCl, LiCl, RbCl or choline-Cl), 2 mM ATP and 2.5 mM $MgCl_2$ was used instead of 25 mM buffer containing 0.4 M choline-Cl and 10 mM $MgCl_2$. Prepared vesicles were washed once with the above buffer without ATP by centrifugation (30, 000× g, 10 min).

2. Assay method for ATPase activity.

Assay mixture (total 3.0 m*l*) contained 25 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, 0.4 M salt (LiCl, NaCl, KCl, RbCl or choline-Cl), and ATP-loaded vesicles (37°C). Other assay procedure was similar to that of described earlier (ANDO *et al*, 1983). Protein was estimated by the Lowry method (LOWRY *et al*, 1951) using bovine serum albumin as a standard.

3. Chemicals

ATP (Tris salt) and ouabain were obtained from Sigma Co. (USA). Monensin was from Calbiochem-

ATP; Adenosine-5′-triphosphate.

Tris; Tris (hydroxymethyl) aminomethane.

FCCP; carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

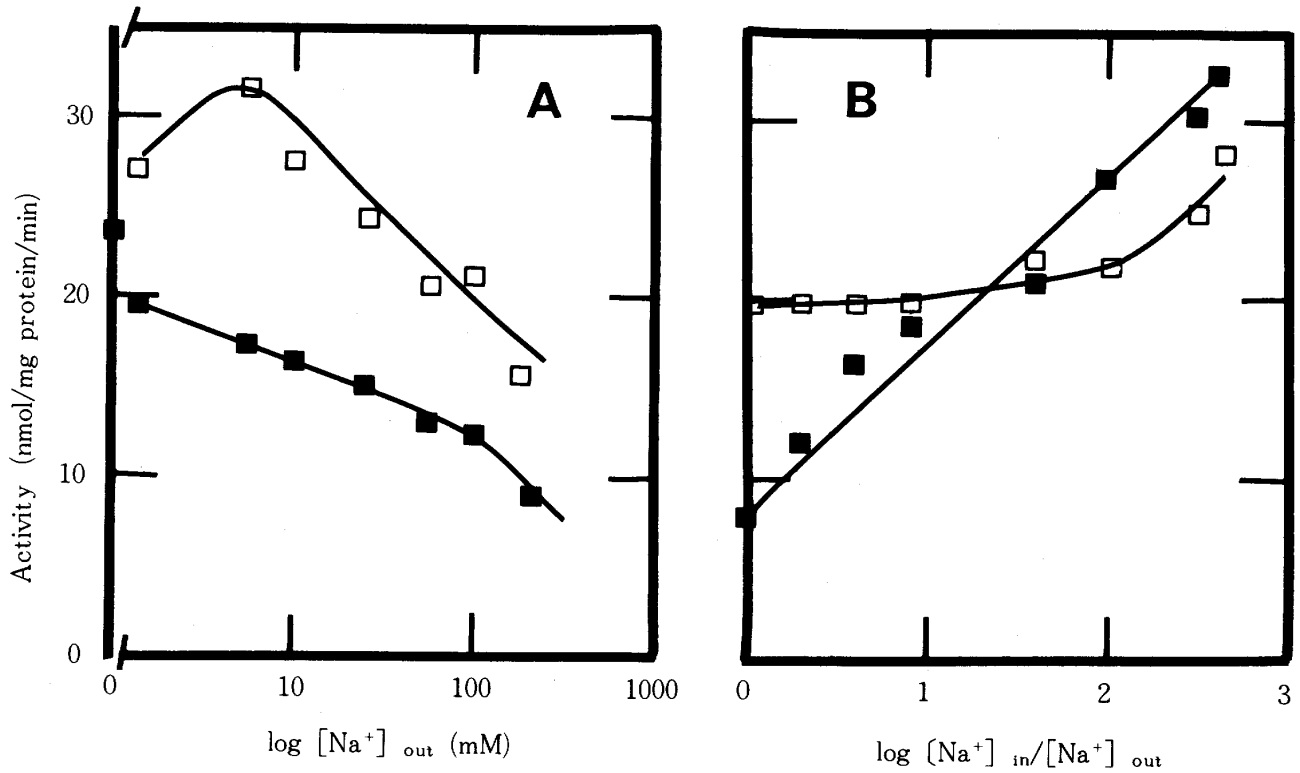

Fig. 1　Effect of Na^+-gradient on ATPase activity of membrane vesicles.
Membrane vesicles were prepared in 25 mM Tris-HCl pH 7.4 containing 2 mM ATP, 2.5 mM $MgCl_2$, and 0.4 M choline-Cl (A) or NaCl (B). Assay mixture (total 3 ml) contained 25 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, NaCl/ choline-Cl in total 0.4 M, and membrane vesicles (1.5 mg protein). With (□) or without (■) monensin (10 μg/ml).

Behring Co. (USA). Other chemicals were of the best grade commercially obtainable.

Results

1. Effect of Na^+-gradient on ATPase activity of membrane vesicles.

ATPase activity of membrane fraction prepared from an alkalophilic *Bacillus* sp. A-007 was stimulated by the presence of K^+ (Ando *et al*, 1983) or Na^+ (unpublished data). Experiments using membrane fraction were not enough for further inspection of ATPase system, because the system were thought to translocate cations by the expence of ATP. Then, we examined ATPase activity of membrane vesicles (right-side out vesicle), in which ATP was loaded.

At first, effect of cation gradient on ATPase activity was examined. When Na^+-gradient (in < out) was imposed across the membrane, ATPase activity was decreased (Fig. 1 A). While, reverse Na^+-gradient (in > out) clearly stimulated the activity (Fig. 1 B). These results suggested that there was other type of ATPase in addition to H^+-ATPase. Na^+-ionophore (monensin) not only abolished the effect of Na^+-gradient (in < out, 1—50mM). Presence of Na^+ inside the vesicle might stimulated the activity. Of cations tested, Li^+ showed similar effect to that of Na^+ (data not shown).

2. Effect of K^+-gradient on ATPase activity of membrane vesicles.

Effect of K^+-gradient on ATPase activity was definitely different from that of Na^+-gradient. As shown in Fig. 2 A, K^+-gradient (in < out) did not inhibit ATPase activity, rather stimulated the activity at a range of 10 to 100 mM of K^+ outside vesicles. While, reverse K^+-gradient (in > out) did not stimulate the activity (Fig. 2 B). Rb^+-gradient also showed similar effect to that of K^+-gradient (data not shown).

3. Effect of Na^+-gradient (in > out) and K^+-gradient (in < out) on ATPase activity.

As shown in the previous section, Na^+-gradient (in > out) and K^+-gradient (in < out) stimulated ATPase activity. We also examined the effect of K^+-gradient (in < out) on ATPase activity in the presence of Na^+-

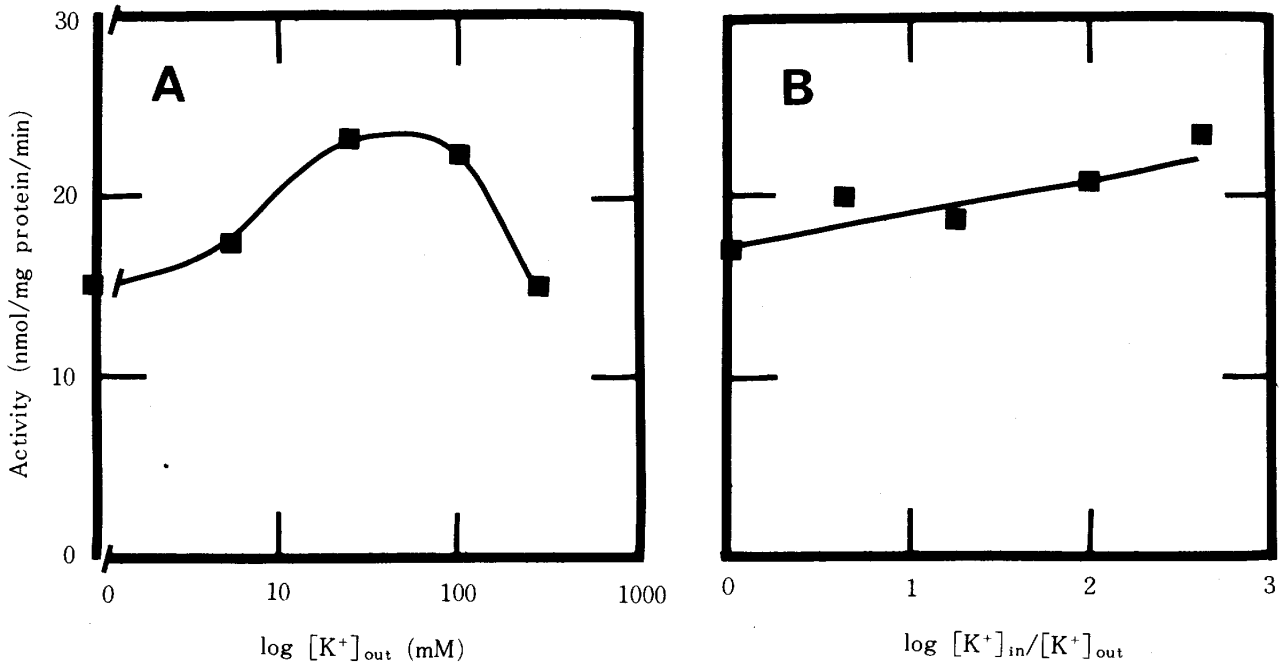

Fig. 2 Effect of K^+-gradient on ATPase activity of membrane vesicles.
Membrane vesicles were prepared in 25 mM Tris-HCl pH 7.4 containing 2 mM ATP, 2.5 mM $MgCl_2$, and 0.4 M choline-Cl (A) or KCl (B). Assay mixture (total 3ml) contained 25 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, KCl/choline-Cl in total 0.4 M, and membrane vesicles (1.5 mg protein).

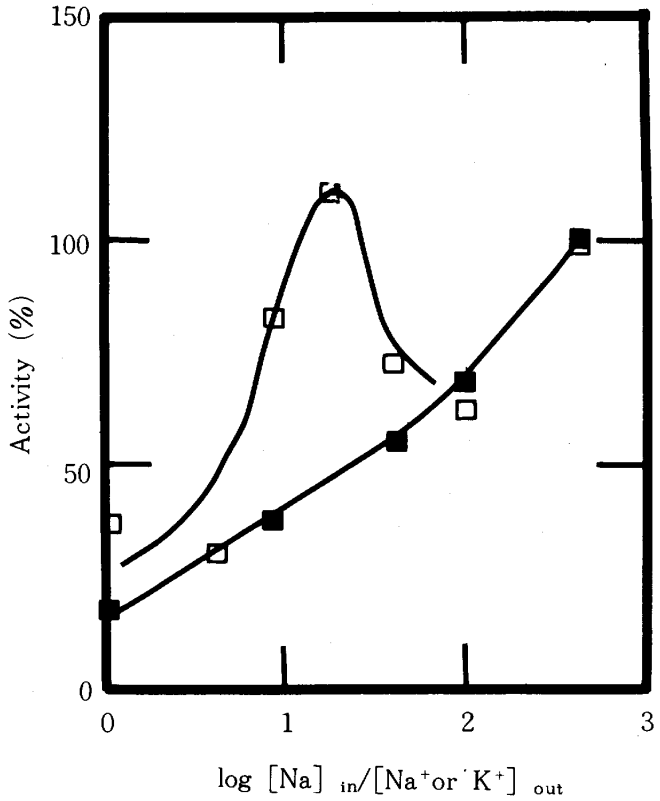

Fig. 3 Effect of K^+-gradient (in < out) on Na^+-gradient (in > out) stimulated ATPase activity.
Membrane vesicles were prepared in 25 mM Tris-HCl pH 7.4 containing 2 mM ATP, 2.5 mM $MgCl_2$ and 0.4 M NaCl. Assay mixture (total 3 ml) contained 25 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, KCl/choline-Cl in total 0.4 M, and membrane vesicles (1.5 mg protein). With (□: $\log[Na^+]_{in}/[K^+]_{out}$) or without (■; $\log[Na^+]_{in^+}]_{out}$) K^+-gradient (in < out).

gradient (in > out). As shown in Fig. 3, K^+-gradient (optimal conc. of extravesicular conc.; 25 mM) stimulated as well Na^+-gradient stimulated ATPase activity. These results again suggested that the membrane had cation-gradient sensitive ATPase and H^+-ATPase. The former might function to expel Na^+

from the cell and to uptake K^+ into it. This kind of ATPase is known to exist in animal cell membranes ($Na^+ \cdot K^+$-ATPase).

4. Effect of ouabain on Na^+- and K^+-gradient stimulated ATPase activity.

Similarity of Na^+- and K^+-gradient stimulated ATPase to $Na^+ \cdot K^+$-ATPase was examined by using $Na^+ \cdot K^+$-ATPase specific inhibitor (ouabain). When ouabain was added, ATPase activity became insensitive to cation gradients (Fig. 4 A). Ouabain-sensitive ATPase activity (Fig. 4 B) showed clear Na^+-gradient dependency.

5. Effect of ionophores on Na^+- and K^+-gradient stimulated ATPase activity.

It was plausible that Na^+- and K^+-gradient stimulated ATPase, which was ouabain sensitive, expeled Na^+ from vesicles. When monensin was added in the assay mixture at 3.25 min after initiation of assay, ATPase activity was stimulated (Fig. 5). Monensin might abolish Na^+-gradient (in < out) which was generated by ATPase reaction.

While K^+ ionophore (valinomycin) stimulated

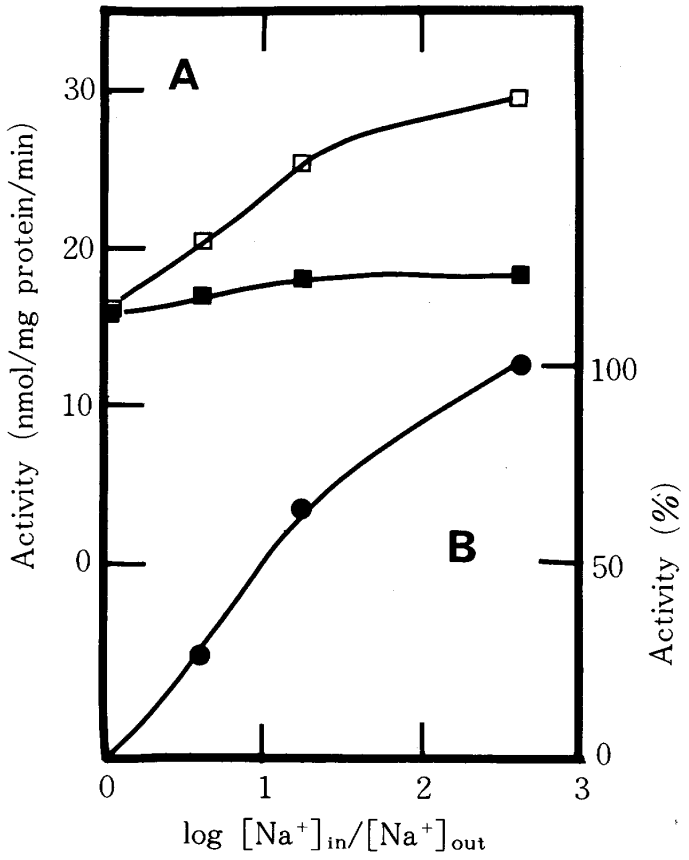

Fig. 4 Effect of ouabain on Na^+-gradient (in > out) and K^+-gradient (in < out) stimulated ATPase activity.
Membrane vesicles were prepared in the same way as that of Fig. 3. Assay mixture (total 3 ml) contained 25 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, 25 mM KCl, NaCl/choline-Cl in total 0.375M, and membrane vesicles (1.5 mg protein). A; with (■) or without (□) 1 mM ouabain. B; ouabain-sensitive ATPase activity.

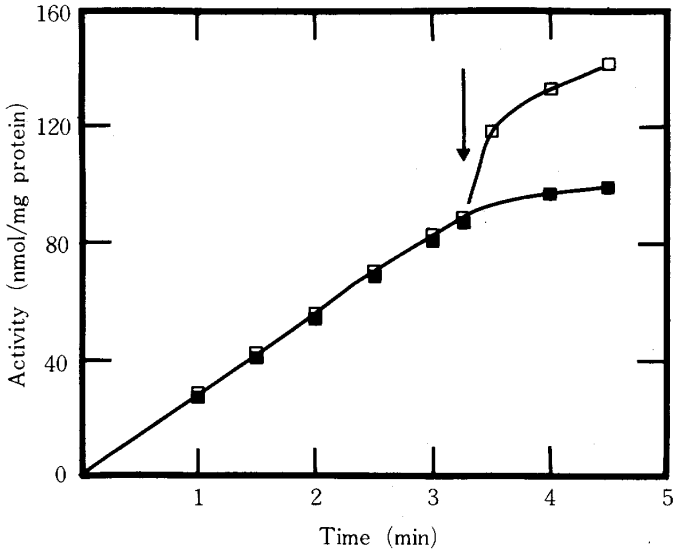

Fig. 5 Effect of monensin on Na^+-and K^+-gradient stimulated ATPase activity.
Membrane veiscles were prepared in the same way as that of Fig. 3. Assay mixture (total 6 ml) contained 25 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, 25 mM KCl, 0.375 M choline-Cl and membrane vesicles (3.0 mg protein). At the time shown in the figure by arrow, monensin (10 μg/ml) was added.

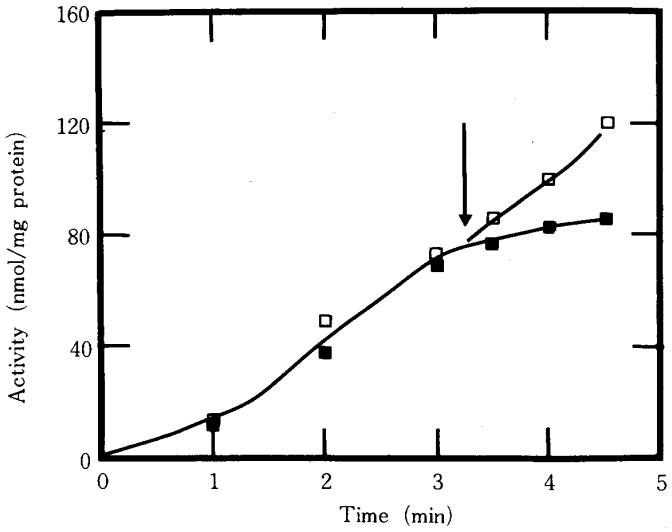

Fig. 6 Effect of valinomycin on Na^+-gradient stimulated ATPase activity. Experimental condition was similar to that of Fig. 5, except valinomycin (10μ g/ml) was used instead of monensin.

ATPase activity (Fig. 6). As already shown in Fig. 2, intravesicular K^+ did not stimulate the activity, valinomycin might compensate membrane potential, which was generated by extrusion of Na^+ from vesicles (inside negative), by permeating K^+ into vesicles. This idea was also supported by the experiment, in which FCCP stimulated the activity (data not shown).

Discussion

Experimental data obtained in the present study suggested the existence of Na^+-gradient sensitive ATPase in the membrane of alkalophilic *Bacillus* sp. A-007. Because the ATPase activity was stimulated by Na^+-gradient from inside and inhibited by the gradient from outside the vesicles, it appeared to expel Na^+ using chemical energy of ATP. K^+ might act as counter-flow ion to compensate membrane potential generated by the translocation of Na^+. This reaction mechanism and ouabain sensitivity are similar to that of $Na^+ \cdot K^+$-ATPase (Poole, 1978). Although the presence of both Na^+ and K^+ is needed for ATP hydrolysis by $Na^+ \cdot K^+$-ATPase (Skou, 1957), only presence of Na^+-gradient is essential for Na^+- gradient sensitive ATPase of Na^+-gradient is essential for Na^+-gradient sensitive ATPase reaction. The latter might have adapted to its cation (mainly Na^+) rich environment. As Na^+-gradient (in < out) is the driving force for uptake of nutrients (Ando *et al.*, 1981 b and 1982), Na^+-gradient sensitive ATPase may establish Na^+-gradient enough for the proliferation of the bacterium.

Na^+/H^+-antiporter is the only one transport system for Na^+ in bacteria (Abrams *et al*, 1960; Harold, 1977). In *Bacillus alkalophilus*, electrogenic Na^+/H^+-antiport system, which is driven by membrane potential, is known (Mandel *et al*, 1980). Thinking that intracellular pH of *Bacillus* A-007 is nearly neutral, it is not plausible to expel Na^+ via antiport with H^+. Rather, ATPase system seems to be more efficient device for the alklophile.

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好アルカリ性 *Bacillus* の $Na^{\pm}$ および $K^{\pm}$ 濃度勾配促進 ATPase について

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日本文摘要

好アルカリ性 *Bacillus* sp. A-007株より調製した膜小胞の ATPase 活性は Na^+ (内＞外) および K^+ (内＜外) 濃度勾配により促進された．Na^+ の膜の内側から外側への転位は ATPase 反応と共役しているが，K^+ は膜電位を打ち消す様に電気泳動的に動くらしい．このカチオン濃度勾配促進 ATPase 活性は電位差形成的でウアバインにより阻害された．