FLAGELLAR MOTORS OF ALKALOPHILIC *BACILLUS* ARE POWERED BY AN ELECTROCHEMICAL POTENTIAL GRADIENT OF Na^+

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Received 3 August 1981

1. Introduction

Flagellated bacteria such as *Bacillus subtilis* and *Escherichia coli* swim by rotating their flagella at the basal structures which are embedded in cytoplasmic membrane [1,2]. There is considerable evidence that the flagellar basal structure is a reversible rotary motor powered by the transmembrane electrochemical potential gradient of H^+, $\Delta\mu H^+$ [3–8]. The flagellar motor of *B. subtilis* requires at threshold $\Delta\mu H^+$ of about −30 mV for its rotation, and the rotation rate is saturated at a $\Delta\mu H^+$ of about −100 mV [9,10].

Some alkalophilic *Bacillus* whose optimal growth pH is between 9 and 11 swim vigorously at high pH [11,12]. However, in [13,14] the $\Delta\mu H^+$ of an alkalophilic *Bacillus* was only about −15 mV at pH 11. This result suggests that the flagellar motors of alkalophilic *Bacillus* may be powered by some energy sources other than $\Delta\mu H^+$.

This communication reports that Na^+ in the medium was required for the swimming of two independently isolated alkalophilic *Bacillus*. This suggests that the flagellar motors of these bacteria are powered by the transmembrane electrochemical potential gradient of Na^+, $\Delta\mu Na^+$.

2. Materials and methods

2.1. *Bacterial growth*

Alkalophilic *Bacillus* strains used were YN-1 [11] and no. 8-1 [12]. YN-1 was grown at pH 10.5 as in [11], and no. 8-1 was grown at pH 9 as in [15]. Cultivation was carried out at 35°C with shaking.

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2.2. *Measurement of swimming speed*

Cells (1–2 ml) were harvested at late-log phase, collected on Sartorius membrane filter (SM113, 0.45 μm) by filtration, and washed 3 times with 5 ml each of TG medium consisting of 25 mM Tris–HCl buffer (pH 9.0), 0.1 mM EDTA and 5 mM glucose. After resuspension in 10 ml TG medium, cells were kept at 30°C until use.

A drop of cell suspension was placed on a microscope slide and the cells swimming at 30°C were recorded by a video tape recording system as in [5]. Swimming tracks were then recorded by photographic method with 1 s exposure [9], and swimming speed of the cells were calculated by measuring the length of >50 tracks. A correction for average cell size (about 6 μm on the photograph) was made to get the average swimming distance.

2.3. *Reagents*

Monensin and nigericin were obtained as gifts from Dr S. Esumi of Kaken Kagaku Co. Ltd (Tokyo) and from Mr M. Arisawa of Nippon Roche Research Center (Kamakura), respectively. Valinomycin and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) were the products of Sigma Chemicals (St Louis MO).

3. Results and discussion

3.1. *Requirement of Na^+ for motility*

Cells of an alkalophilic *Bacillus*, YN-1, in the growth medium consisting of rich broth and NaCl showed vigorous motility between pH 8.5–11.5. This is a characteristic property of alkalophilic bacteria, because *B. subtilis* showed vigorous motility between pH 6–8 [9].

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50

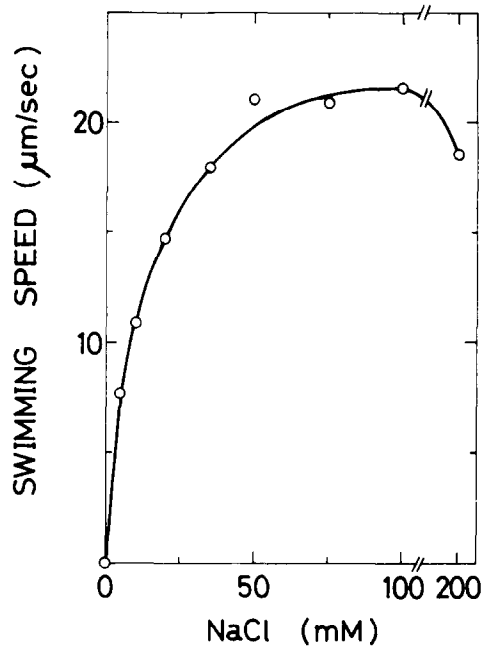

Fig.1. Effect of Na^+ concentration on the swimming speed of YN-1 cells. Cells in TG medium (pH 9.0) were mixed with various concentrations of NaCl as indicated, and the swimming speed at 30°C was measured.

When YN-1 cells were washed and resuspended in TG medium consisting of 25 mM Tris–HCl buffer (pH 9.0), 0.1 mM EDTA and 5 mM glucose, no translationally swimming cells were observed. Most of the cells showed only Brownian motion and some cells showed slow rotation. The addition of NaCl to the medium, however, caused a quick recovery of the translational swimming in almost all the cells. The swimming speed increased with increasing NaCl concentration, and >50 mM NaCl was required for the maximum speed (fig.1).

When various salts were examined for the effectiveness on the motility of YN-1 cells, it was found that only the salts containing Na^+ were effective (table 1). Other cations such as Li^+ and K^+, as well as anions, had no effect. Increase in the pH of the medium up to 11 did not affect the Na^+ requirement for motility. Similar results were obtained by another alkalophilic *Bacillus*, no. 8-1, although the cells showed vigorous motility between pH 8–9.5.

Since many amino acid transport systems in alkalophilic *Bacillus* require Na^+ as a coupling ion for symport [13,15,16], there is a possibility that the transport of the energy source contained in TG

Table 1
Ion specificity for the motility of YN-1

Salts added	Swimming speed (μm/s)
None	0
NaCl	16
$NaNO_3$	17
Na_2HPO_4	15
Na_2SO_4	18
NaSCN	18
Na-acetate	16
LiCl	0
KCl	0
NH_4Cl	0
RbCl	0
CsCl	0
$CaCl_2$	0
$MgCl_2$	0

YN-1 cells in TG medium (pH 9.0) were mixed with 15 mM of various salts, and the swimming speed was measured after 5 min incubation at 30°C. In the case of Na_2HPO_4 and Na_2SO_4, salt concentration used was 7.5 mM. When necessary, medium pH was adjusted to 9.0 by KOH or HCl

medium, namely glucose, is Na^+-dependent and therefore the requirement of Na^+ for motility is the secondary effect of this dependence. However, we found that this was not the case, because the elimination of glucose from TG medium did not affect the results. Of course, in the absence of glucose, the swimming speed was gradually decreased after 10 min at 30°C. In [16], the glucose transport system in YN-1 cells was considerably active without Na^+.

Thus, it is concluded that Na^+ is directly required for the motility of these two alkalophilic *Bacillus* strains. This may be a unique property of alkalophilic

Table 2
Effect of ionophores on the motility of YN-1

Additions	Swimming speed (μm/s)
None	22
Valinomycin (10 μM)	22
Valinomycin (10 μM) + KCl (60 mM)	0
KCl (60 mM)	22
CCCP (20 μM)	10
Nigericin (2 μM)	0
Monensin (6 μM)	6

Cells in TG medium (pH 9.0) containing 50 mM NaCl were mixed with ionophores as indicated, and the swimming speed was measured at 30°C within 1 min

bacteria, because neutrophilic bacteria such as *B. subtilis, E. coli* and *Streptococcus* do not require Na^+ for their motility [5,6].

3.2. *Effect of ionophores on motility*

In order to clarify the role of Na^+ on motility, the effect of various ionophores on motility was studied (table 2). Valinomycin, a K^+ ionophore, alone had no effect on the motility of YN-1 cells in TG medium containing 50 mM NaCl. However, the addition of 60 mM KCl in the presence of valinomycin resulted in an instantaneous and a complete inhibition of motility, indicating that the collapse of membrane potential of the cells caused the loss of motility. Similar motility inhibition was observed by CCCP or nigericin which are the ionophores effective to collapse the membrane potential, although quite high concentrations of the ionophores were required for the motility inhibition at pH 9 (cf. [3] on *B. subtilis* motility). Thus, it is clear that the membrane potential is a component of the energy source for the motility of alkalophilic *Bacillus.*

Monensin, which catalyzes Na^+/H^+ exchange, caused a strong inhibition of motility in YN-1 cells at 6 μM (table 2), although the same concentration of monensin gave only a slight inhibition of motility in *B. subtilis* at pH 8 or higher (not shown). These results suggest that the flux of Na^+ by the chemical potential gradient of Na^+ is coupled with the motility of YN-1 cells. Essentially the same results were obtained by no. 8-1 cells.

All of these results are well consistent with the idea that the flagellar motor of alkalophilic *Bacillus* is powered by $\Delta\mu Na^+$.

As discussed before, many transport systems in alkalophilic *Bacillus* require Na^+ as a coupling ion for symport. In [17] it was suggested that the Na^+-dependent transport systems in alkalophilic *Bacillus* share a common Na^+-translocating subunit with the Na^+/H^+ antiporter of the cell. It would be quite interesting to test whether or not the common Na^+-translocating subunit is involved in the energy coupling systems of flagellar motor in alkalophilic *Bacillus.*

Acknowledgements

We are grateful to Drs K. Horikoshi and Y. Nosoh for provision of alkalophilic *Bacillus* strains and to Drs F. Oosawa and S. Asakura for their helpful discussions and encouragement during this study. We also thank to Dr S. R. Zukin of Mount Sinai Medical Center, New York, for his critical reading of the manuscript.

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