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Quantitative Measurements of Proton Motive Force and Motility in *Bacillus subtilis*

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The proton motive force of metabolizing *Bacillus subtilis* cells was only slightly affected by changes in the external pH between 5 and 8, although the electrical component and the chemical component of the proton motive force contributed differently at different external pH. The electrical component of the proton motive force was very small at pH 5, and the chemical component was almost negligible at pH 7.5. At external pH values between 6 and 7.7, swimming speed of the cells stayed constant. Thus, either the electrical component or the chemical component of the proton motive force could drive the flagellar motor. When the proton motive force of valinomycin-treated cells was quantitatively decreased by increasing the external K^+ concentration, the swimming speed of the cells changed in a unique way: the swimming speed was not affected until about −100 mV, then decreased linearly with further decrease in the proton motive force, and was almost zero at about −30 mV. The rotation rate of a flagellum, measured by a tethered cell, showed essentially the same characteristics. Thus, there are a threshold proton motive force and a saturating proton motive force for the rotation of the *B. subtilis* flagellar motor.

Peritrichously flagellated bacteria such as *Bacillus subtilis* and *Escherichia coli* possess a number of flagella originating from the cell body and swim by rotating their flagella as a bundle. Each flagellum is driven at its base by a reversible rotary motor which is composed of ring structures inserted in the cytoplasmic membrane (3, 4, 7, 28).

In a previous paper (27), we have shown that the motility of *B. subtilis* cells is equally supported either by transmembrane electrical potential or by transmembrane pH gradient. According to Mitchell's chemiosmotic hypothesis (22, 23), this means that the energy source for bacterial motility is the electrochemical potential of protons across the membrane, the so-called proton motive force (Δp). To directly support this idea, we succeeded in developing a protocol for inducing translational swimming in starved *B. subtilis* cells by an artificially created Δp (18, 19). Similar systems were also developed by Manson et al. (17) in a motile *Streptococcus* sp. and by Glagolev and Skulachev (5) in *Rhodospirillum rubrum*. From all these results, it can be concluded that the bacterial flagellar motor is powered by the Δp.

However, in relation to motility, no direct and quantitative measurement of the Δp in bacterial cells has been reported. Recent progress in the methods for quantitating Δp in bacterial cells by using membrane-permeable and radioactive cations and weak acids (15, 26) provides the means of clarifying this point. Miller and Koshland (20, 21) measured transmembrane electrical potential in *B. subtilis* cells by using cyanine dye fluorescence and a membrane-permeable cation in relation to swimming behavior, but they did not quantitate the motility and also did not measure transmembrane pH gradient.

In this paper, we describe the relationship between the measured Δp and motility in *B. subtilis*. We found that the rotation of the flagellar motor of *B. subtilis* required at least −30 mV of Δp, and the rotation rate was saturated at about −100 mV. Similar results have recently been reported by Khan and Macnab (9).

MATERIALS AND METHODS

Chemicals. Valinomycin was purchased from Calbiochem (La Jolla, Calif.) and was dissolved in methanol to a concentration of 10 mM. [^{14}C]salicylic acid (56 mCi/mmol) was obtained from New England Nuclear Corp. (Boston, Mass.). [^{3}H]triphenylmethylphosphonium bromide ([^{3}H]$TPMP^+$) (6 Ci/mmol) was a generous gift of R. M. Macnab of Yale University. Nonradioactive $TPMP^+$ was obtained as iodide salt from Tokyo Kasei Co. (Tokyo, Japan) and was passed through a Dowex AG1-X2 column to make the bromide salt (6). Sodium tetraphenylboron (TPB^-) was purchased from Katayama Chemicals (Osaka, Japan).

Motility medium. The K^+ motility medium contained 10 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA, and 10 mM potassium succinate. The Na^+ motility medium contained the same ingredients as above except that K^+ was replaced by Na^+ through-

out. To vary K^+ concentration in the motility medium, 1/20 volume of a mixture of 4 M KCl and 4 M NaCl in various ratios was added to the K^+ or Na^+ motility medium. Therefore, the total cation concentration in the medium was maintained at 240 mM regardless of K^+ concentration. The pH of these media was changed by the addition of HCl or NaOH.

Bacterial growth. *B. subtilis* BC26 (*phe-12 argA ery*) was grown at 35°C with shaking in tryptone broth containing 1% tryptone (Difco Laboratories, Detroit, Mich.) and 0.5% NaCl (27). At a cell concentration of about 2×10^8 cells per ml, cells were harvested either by centrifugation at 12,000 × *g* for 3 min at room temperature or by filtration through a membrane filter (pore size, 0.45 μm; Sartorius-Membrane Filter GmbH, Göttingen, West Germany). Cells were washed with motility medium and resuspended in the same medium.

Measurement of swimming speed. Cell concentration was adjusted to about 5×10^6 cells per ml, and cells were kept at 30°C in a water bath. A drop of cell suspension was transferred onto a glass slide, and the swimming cells were observed by a Nikon microscope at 30°C. The swimming tracks were recorded by the photographic method described previously (19). Swimming speed of the cells was calculated by measuring the length of the swimming tracks obtained by a 2-s exposure. The length of more than 50 tracks, including nonswimming cells, was measured, and a correction for average cell length (about 4 μm) was made to get the average swimming distance.

Valinomycin (final concentration of 1 μM) or suitable amounts of HCl or NaOH were added, if necessary, to the cells in a test tube, and the mixture was incubated for 3 min at 30°C before measuring the swimming speed.

Measurement of rotation rate of tethered cells. Cells in growth medium were sucked and blown out vigorously about 100 times through a Pasteur pipette to shear their flagella. Then, the cells were mixed with 1/1,000 volume of antiflagellar antiserum and transferred to a glass slide to tether them (10, 28). The serum was prepared by injecting purified *B. subtilis* flagella into a rabbit. The yield of tethered cells was, for unknown reason, quite small compared to that of *E. coli*. After the tethered cells were washed with a motility medium, rotation of the tethered cells was recorded at 30°C on a videotape. The rotation rate 1 min after a medium change was measured by methods described previously (10).

Measurement of Δp. According to Mitchell's chemiosmotic hypothesis, Δp, namely, the electrochemical potential difference of protons across the cell membrane, is given by $\Delta p = \Delta\psi - 2.3(RT/F)\,\Delta pH = \Delta\psi - Z\Delta pH$, where $\Delta\psi$ is the difference in electrical potential between inside and outside of the cell, R is the gas constant, T is the absolute temperature, F is the Faraday constant, and ΔpH is the difference in pH between the inside and outside of the cell. At 30°C, $2.3(RT/F)$ is 60 mV.

$\Delta\psi$ was estimated from the partitioning of a radioactive membrane permeable cation, $[^3H]TPMP^+$, inside and outside of the cell, by using the Nernst equation, $\Delta\psi = 2.3\ (RT/F)\log([TPMP^+]_{out}/[TPMP^+]_{in})$, where $[TPMP^+]_{out}$ and $[TPMP^+]_{in}$ are the concentrations of $TPMP^+$ in the external medium and in the cytoplasm, respectively. The method was essentially the same as that described by Miller and Koshland (20). Cells were harvested by centrifugation at 12,000 × *g* for 3 min at room temperature, washed once, and suspended in a motility medium at a cell density of 4×10^9 cells per ml (7 mg [dry weight] per ml or 11 μl of cell water per ml [19]). Along with 2 μM TPB^-, 10 μM $[^3H]TPMP^+$ (42 mCi/mmol) was added to the cell suspension, and the mixture was incubated at 30°C with shaking. After addition of 10 μM valinomycin, where used, the cell suspension was vigorously shaken for 3 min. A sample (50 μl) was withdrawn, poured into a test tube containing 2 ml of the motility medium, and rapidly filtered by using a membrane filter (cellulose acetate; pore size, 0.65 μm; Sartorius-Membrane Filter GmbH, Göttingen, West Germany). Membrane filters were presoaked in motility medium containing 10 μM nonradioactive $TPMP^+$. Cells were quickly washed with 2 ml of the motility medium. The filters were dried, and the radioactivity was measured by a scintillation spectrophotometer (Aloka LSC-671). Nonspecific binding of the radioactivity to the cells was small and was corrected by treating the cells with 1% toluene. To measure the total radioactivity in the system, a sample (50 μl) was put on a filter and dried without any washing, and the radioactivity was counted.

Alteration of the concentration of $[^3H]TPMP^+$ and TPB^- over a fivefold range did not cause any change in the estimated value of $\Delta\psi$. This means that the maximum amount of $[^3H]TPMP^+$ accumulated in the cells under the assay condition was apparently lower than the saturation level. Furthermore, the uptake of $[^3H]TPMP^+$ by the cells was completed within 15 s at 30°C. Therefore, the change of $\Delta\psi$ in the cell could be analyzed with a time resolution of less than 15 s. To get reproducible values of $\Delta\psi$, it was important to give enough aeration to the cells. In particular, values of $\Delta\psi$ higher than −100 mV tended to be underestimated by 10 to 20% when the cell density was greater than 10^9 cells per ml. For $\Delta\psi$ lower than −100 mV, however, cell densities up to 4×10^9 cells per ml yielded the same estimate of $\Delta\psi$. Thus, for the measurement of $\Delta\psi$ higher than −100 mV, the cell density was adjusted to 10^9 cells per ml.

ΔpH was measured by the uptake of $[^{14}C]$salicylic acid into the cells by the method of Maloney (14). Since salicylic acid penetrates the cell membrane in its neutral form, the proton concentration inside and outside of the cell is given by the following equation (26):

$$\frac{[A^t]_{in}}{[A^t]_{out}} = \frac{1/[H^+]_{in} + 1/K_a}{1/[H^+]_{out} + 1/K_a}$$

where $[A^t]_{in}$ and $[A^t]_{out}$ are the total concentrations of salicylic acid in cytoplasm and in external medium, respectively, and K_a, the dissociation constant of salicylic acid, is 1.05×10^{-3}. ΔpH was measured by essentially the same method as described above for $\Delta\psi$, except that 10 μM $[^{14}C]$salicylic acid (56 mCi/mmol) was used instead of radioactive $TPMP^+$. A sample (50 μl) was withdrawn and immediately filtered. Cells were then quickly washed with 3 ml of the motility medium.

Measurement of intracellular K^+ concentra-

tion. Intracellular K^+ concentration was measured by an atomic absorption method as described previously (27). The K^+ concentration observed just after the cells were suspended in any motility medium was 450 mM and stayed constant for about 10 min. To calculate the K^+ diffusion potential of valinomycin-treated cells, K^+ activities were used.

RESULTS

Δp in *B. subtilis* cells as a function of external pH. The electrical and chemical components of the Δp of metabolizing cells of *B. subtilis* at 30°C were measured separately using [^{3}H]TPMP$^+$ and [^{14}C]salicylic acid, respectively (Fig. 1a). At pH 7.5, transmembrane electrical potential (Δψ) was about −140 mV. With decreasing external pH, Δψ decreased gradually, and at pH 4.5, almost no Δψ was detected. On the other hand, transmembrane pH gradient (ΔpH) was almost zero at pH 7.5 and increased with decreasing external pH. Thus, Δp was about −140 mV at pH 7.5 and slightly increased with decreasing external pH. At around pH 6, Δp showed the maximum value of about −160 mV.

The intracellular pH, which was calculated from the data of ΔpH, was about 7.4 and was

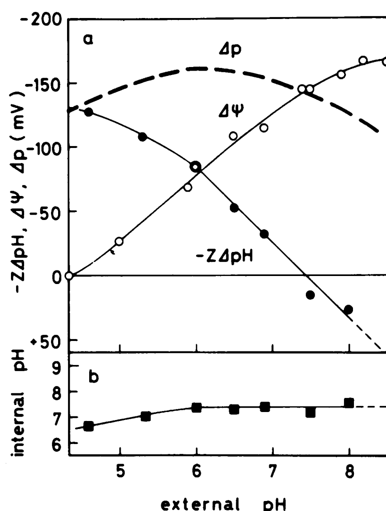

FIG. 1. *Effect of external pH on Δψ, ΔpH, and Δp of B. subtilis cells. Cells (1 × 10^9 to 4 × 10^9 per ml) in Na^+ motility medium were mixed with [^{3}H]TPMP$^+$ or [^{14}C]salicylic acid at 30°C, and then the mixture was divided into 0.5-ml portions. After the pH of the medium was changed by the addition of HCl or NaOH, the mixture was vigorously shaken at 30°C for 3 min. A sample (50 µl) was taken, and the radioactivity in the cells was measured. (a)* (○) *Δψ measured by [^{3}H]TPMP$^+$ distribution;* (●) *−ZΔpH measured by [^{14}C]salicylic acid distribution, Z = 2.3 RT/F;* (- - -), *Δp calculated as a sum of Δψ and −ZΔpH. (b) Intracellular pH calculated from external pH and ΔpH.*

independent of the external pH between 6 and 8 (Fig. 1b).

Swimming speed of *B. subtilis* cells as a function of external pH. Bacterial flagellar motors are driven by Δp. Although, as described in the previous section, Δp of *B. subtilis* cells was fairly constant with external pH, the contributions of the electrical and the chemical components were different. As shown in Fig. 2, the speed of translational swimming was almost constant between external pH 6 and 7.7. Thus, either the electrical component or the chemical component of Δp can equally support the swimming.

External pH values less than 6 or higher than 8 caused a drastic decrease in the swimming speed, although Δp stayed very high. Under these extreme pH conditions, the flagellar motor seems to be damaged. However, restoration of the external pH to neutral resulted in complete recovery of swimming speed (data not shown), indicating that the motility inhibition induced at very low or very high pH was reversible.

Neither TPMP$^+$ nor salicylic acid at the concentration used here had any effect on the swimming of *B. subtilis* cells. TPB$^-$ (2 µM) induced a drastic increase in tumble frequency for about 1 min at 30°C. The cells then returned to the normal swimming pattern as reported previously (25). Therefore, all of our experiments were carried out more than 3 min after the addition of TPB$^-$ to prevent the interference of the transient action of TPB$^-$ as a repellent.

Alteration of Δp in *B. subtilis* cells by valinomycin-K$^+$ treatment. Δp in valinomycin-treated cells at pH 7.5 was altered almost linearly by logarithmic changes in the K^+ concentration of the medium (Fig. 3). At a K^+ concentration of 1 mM, Δψ was about −130 mV and decreased to about 0 mV at a K^+ concentration of 450 mM. Under these conditions, ΔpH was very small. Accordingly, the Δp also decreased linearly with logarithmic increase of K^+ concentration from 1 mM to 540 mM.

Using the measured intracellular K^+ concentration of 450 mM for *B. subtilis* cells under these conditions, the K^+ diffusion potential of valinomycin-treated cells was calculated as a function of external K^+ concentration (dotted line in Fig. 3). The observed curve of Δψ as a function of external K^+ concentration fitted this theoretical curve very well, although a small deviation was observed at the region of high Δψ. This means that estimation of Δψ of *B. subtilis* cells using radioactive TPMP$^+$ is quite reliable up to values of about −120 mV.

Swimming speed of *B. subtilis* cells as a function of Δp. Swimming speed at various values of Δp was measured by using valinomy-

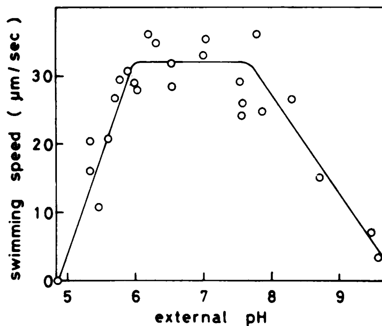

FIG. 2. *Effect of external pH on the swimming speed of B. subtilis cells. The experimental conditions were the same as described in the legend for Fig. 1, except that the cell density was 5 × 10^6 cells per ml, and the radioactive materials were omitted.*

cin-treated cells at pH 7.5 (Fig. 4). The addition of K^+ to the medium up to 7 mM ($\Delta p = -100$ mV) did not cause any change in swimming speed. Further increase in K^+ concentration resulted in a rather sharp decrease in swimming speed. At a K^+ concentration of 240 mM ($\Delta p = -25$ mV), almost all the cells lost their motility. Between -30 and -100 mV of Δp, the swimming speed seemed to change linearly. Thus, for translational swimming of *B. subtilis* cells, there was a threshold Δp of about -30 mV and a saturation Δp of about -100 mV.

Rotation rate of tethered *B. subtilis* cells as a function of Δp. *B. subtilis* cells are peritrichously flagellated. Cells form a flagellar bundle and rotate it for translational swimming. The results presented in the previous section showed that cells required at least -30 mV of Δp for rotation of a flagellar bundle and that Δp higher than -100 mV did not further accelerate the rotation of the bundle.

To clarify the characteristics of the individual flagellar motors, the rotation rate of tethered cells was examined. A cell which is tethered by a flagellum to a glass slide by antiflagellar antibody can rotate around its attachment point. The rotation rate of the cell body, therefore, is the rotation rate of the flagellar motor. Tethered cells were treated with valinomycin, and the Δp of the cells was varied by changing the K^+ concentration of the medium. Figure 5 shows the rotation rate of typical tethered cell samples as a function of the size of Δp. When Δp was decreased from -130 mV to about -80 mV, the decrease in the rotation rate of many cells was small. Further decrease in Δp caused a drastic decrease in rotation rate, and at around -30 mV, the rotation was stopped. Thus, just as in the case of the rotation of the entire flagellar bundle, each individual flagellar motor has a threshold and a saturation Δp for its rotation.

Restoration of motility by increasing Δp. At the external pH of 7.5, ΔpH in *B. subtilis* cells is so small that Δp is mainly determined by Δψ, as described before. When Δp of valinomycin-treated cells at pH 7.5 was decreased to about -25 mV by increasing the external K^+ concentration up to 240 mM, almost all the cells lost their motility. Motility was recovered when Δp was increased by lowering the external K^+ concentration. However, as shown in Fig. 6, the time courses for the re-increase in Δψ and for the recovery of translational swimming speed were different. Especially in the case of small increases in Δψ (that is, Δp), there was a delay for the appearance of translational swimming, although the time course of the increase in Δp was quite rapid. During the delay, however, cells showed rotational or jiggly swimming, indicating that at least some flagella of the cell surely started to rotate much earlier than the appearance of translational swimming. Thus, the formation of the flagellar bundle, which is necessary for translational swimming, seems to proceed during this delay period.

DISCUSSION

Δp in metabolizing *B. subtilis* cells. The Δp of metabolizing cells of *B. subtilis* stayed higher than -120 mV throughout the external

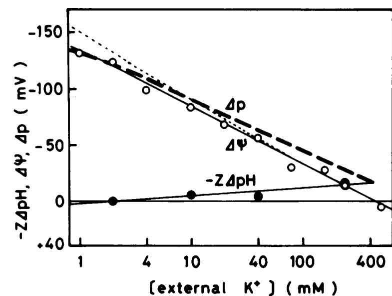

FIG. 3. *Changes in Δψ, ΔpH, and Δp of valinomycin-treated B. subtilis cells as a function of external K^+ concentration. Cells (1 × 10^9 to 4 × 10^9 per ml) were suspended in motility medium (pH 7.5) at various K^+ concentrations. Total cation concentration in the media was maintained at 240 mM by the addition of NaCl. Cells were mixed with either [^{3}H]-$TPMP^+$ and TPB^- or [^{14}C]salicylic acid at 30°C, and then valinomycin was added to 10 µM. After vigorous shaking for 3 min at 30°C, a sample (50 µl) was taken, and the radioactivity in the cells was measured. To make motility medium containing 540 mM K^+, 1/7 volume of 4 M KCl was added to the K^+ motility medium.* (○) *Δψ;* (●)– *ZΔpH;* (– – –) *Δp;* (-----) *a theoretical curve of K^+ diffusion potential based on an internal K^+ concentration of 450 mM. For this calculation, K^+ activities were used.*

pH range 5 to 8, although the contributions of $\Delta\psi$ and ΔpH to Δp at various pH values were different. In response to changes in external pH, *B. subtilis* cells have the ability to keep both the intracellular pH and Δp fairly constant. This homeostatic phenomenon is probably important to maintain the best conditions for cellular functions in spite of changes in the external environment.

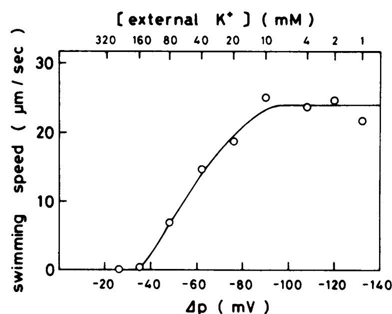

FIG. 4. *Swimming speed of valinomycin-treated B. subtilis cells as a function of Δp. Experimental condition was the same as described in the legend to Fig. 3, except that the cell density was 5 × 10⁶ cells per ml and the valinomycin concentration was 1 μM. Swimming speed at 30°C was measured 3 min after the addition of valinomycin. The values of the Δp at various K⁺ concentrations were obtained from the data of Fig. 3.*

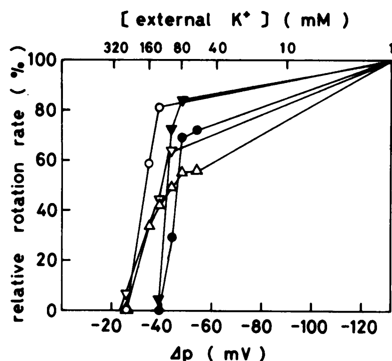

FIG. 5. *Rotation rate of tethered cells as a function of Δp. Tethered cells were washed with motility medium containing 1 mM K⁺ (pH 7.5, total cation concentration = 240 mM) and valinomycin (1 μM). After measuring the rotation rate at 30°C, the medium was changed to increase the K⁺ concentration and the rotation rate was measured. Valinomycin concentration was fixed at 1 μM. K⁺ concentration was increased step by step, and the rotation rate of the same cell was followed at each K⁺ concentration. Then the cells were washed with K⁺-free, valinomycin-free medium. Only the cells that showed a good recovery of rotation rate are shown in the figure. Each cell is denoted by a different symbol. The rotation rates were normalized to the values at 1 mM K⁺. The values of Δp at different K⁺ concentrations were obtained from the data of Fig. 3.*

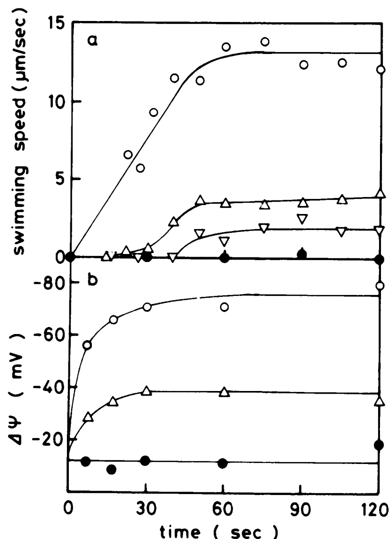

FIG. 6. *Recovery of translational swimming by restoring Δp. (a) Changes in swimming speed. Cells (5 × 10⁷ per ml) in motility medium (pH 7.5) containing 240 mM K⁺ were treated with 10 μM valinomycin for 3 min at 30°C. The suspensison was then diluted 10-fold by motility medium (pH 7.5) at various K⁺ concentrations. Swimming speed of the cells after dilution was followed by 30°C. (b) Changes in Δψ. Experimental condition was the same as above, except the cell density and valinomycin concentration after dilution were 2 × 10⁹ cells per ml and 10 μM, respectively. The experiment for the dilution to 80 mM K⁺ was not done. Final K⁺ concentrations were 24 mM (○), 60 mM (△), 80 mM (▽), and 240 mM (●).*

$\Delta\psi$ of the cell is a sum of the diffusion potential of permeable ions and the potential created by electrogenic H^+ pump. If H^+ is the major ionic species traveling through the bacterial membrane and the activity of the H^+ pump is pH independent, pH dependency of $\Delta\psi$ should be parallel with the pH dependency of the diffusion potential of H^+. As shown in Fig. 1a, with decreasing external pH, $\Delta\psi$ of *B. subtilis* cells decreased with a slope of about −50 mV per pH unit and was almost parallel with the decrease in the diffusion potential of H^+: $-Z\log[H^+]_{in}/[H^+]_{out} = +Z\Delta pH$. Therefore, it is likely that in *B. subtilis* H^+ is the major permeable ion and the activity of the H^+ pump is pH independent. From the data of $\Delta\psi$ at the external pH of 7.4 where no diffusion potential of H^+ was present, the activity of the H^+ pump is estimated to be enough to create a potential of about −140 mV.

B. subtilis cells are highly susceptible to valinomycin, and therefore the $\Delta\psi$ of valinomycin-treated cells can be varied simply by changing the external K^+ concentration. The good agree-

ment between the observed and the theoretical values of $\Delta\psi$, calculated from the K^+ diffusion potential, indicates that the method of measuring $\Delta\psi$ in *B. subtilis* cells by radioactive $TPMP^+$ is quite reliable.

Flagellar rotation of *B. subtilis* cells and Δp. From the quantitative measurement of Δp and swimming speed, it was shown that for translational swimming in *B. subtilis*, Δp of about −30 mV was the threshold value and Δp of about −100 mV was the saturation value. In a previous paper (27), we reported that the number of motile cells of valinomycin-treated *B. subtilis* began to decrease at a Δp of −90 mV, estimated from the K^+ diffusion potential. This value is consistent with a saturation Δp of −100 mV for swimming reported here. Miller and Koshland (20) reported an observation in which valinomycin-treated cells of *B. subtilis* showed paralysis at an external K^+ concentration where the depolarization of the cells, judged by the changes in cyanine dye fluorescence, was not complete. This result suggests the existence of a threshold Δp for swimming. Since Miller and Koshland did not quantitate motility and ΔpH, estimation of a threshold value of Δp for swimming is difficult.

B. subtilis is peritrichously flagellated, and cells swim by forming a flagellar bundle; therefore, the above values are for the rotation of the flagellar bundle. However, the rotation of an individual flagellum, measured by the rotation of tethered cells, showed essentially the same characteristics: rotation required a Δp of at least −30 mV and was saturated by a small increase in Δp. Thus, it is concluded that the flagellar motor of *B. subtilis* cells has a threshold Δp of about −30 mV and a saturating Δp of about −100 mV for its rotation.

It is interesting that there was no apparent difference between the threshold Δp for the rotation of the flagellar bundle and of each flagellum. This may indicate that no additional Δp is required for flagellar bundle formation (1, 12). When the swimming was restored by increasing the Δp of the cells, an apparent delay was observed for the appearance of translational swimming, although simultaneous measurement of Δp showed that it recovered rather quickly. Jiggly and rotational movement of the cells observed during the delay time indicated that the rotation of some flagella started much earlier. Thus, the flagellar bundle was formed after each flagellum had been rotating for a while. Either rotation of the cell body or water stream or both, which are induced by the rotation of each flagellum, should be necessary to form a flagellar bundle (1). Similar results were also observed when a small Δp was imposed on starved *B. subtilis* cells (18; S. Matsuura, J. Shioi, and Y. Imae, unpublished data).

For ATP synthesis by H^+-ATPase, it is reported that a threshold Δp of about −200 mV is required if the influx of two or three H^+ is coupled with the formation of one ATP (8, 23). In the case of the flagellar motor, we showed that a threshold Δp was only about −30 mV. This small threshold Δp is interesting in reference to the mechanism of flagellar rotation. Although there is no direct evidence, the simplest model for the coupling of H^+ influx and flagellar rotation is that H^+ influx directly drives the flagellar motor (2, 5, 11, 13). According to DePamphilis and Adler (4), the rotor of the flagellar motor is composed of 16 identical subunits. If we assume that each subunit corresponds to an H^+ channel for the chemomechanical coupling as proposed by Läuger (11), it is reasonable to speculate that the cooperative flow of H^+ through the channels is necessary to give an effective rotation of the motor. Thermal fluctuation in proton electrochemical potential is given by the expression $-kT/e$, where k is the Boltzman constant, T is the absolute temperature, and e is the elementary electric charge (24). At 30°C, $-kT/e$ corresponds to −26 mV. The presence of a threshold Δp of −30 mV, which is significantly higher than the value of thermal fluctuation, supports the idea that the unidirectional flow of a considerable amount of H^+ is necessary for the rotation of the flagellar motor. The presence of a saturation Δp for rotation may be due to a limitation of the flow rate of H^+ through each channel.

During the preparation of this manuscript, we learned that Khan and Macnab (9) measured the Δp of *B. subtilis* as a function of external pH and in relation to swimming speed. They observed that *B. subtilis* maintained a fairly constant Δp and intracellular pH as external pH was varied. They also observed a threshold Δp and a saturation Δp for the swimming of this species, when Δp was lowered by the use of an uncoupler. These results are in good agreement with the findings reported here, although their reported $\Delta\psi$ values are somewhat smaller than ours. The difference is probably due to the absence of TPB^- in their assay system (29).

In the case of *Streptococcus*, however, Manson et al. (16) obtained the result that, if a threshold Δp existed, it was apparently smaller than −25 mV; also, no saturation Δp was observed for the rotation of tethered *Streptococcus* cells. There may be some differences in the efficiency of Δp utilization between the motors of *B. subtilis* and of *Streptococcus*.

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