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The constitutive K^+ pump in *Serratia marcescens*

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Transport of K^+ and H^+ in the anaeronically and aerobically grown bacterium *Serratia marcescens* has been studied. The volumes of one cell of the anaerobically and aerobically grown bacterium were $3.7 \cdot 10^{-13}$ cm^3 and $2.4 \cdot 10^{-13}$ cm^3, respectively. Irrespective of the growth conditions the bacteria manifested the same respiration rate. However, the values of membrane potential for the anaerobically and aerobically grown bacterium were different and equal to -130 mV and -175 mV (interior negative), respectively, in the absence of an exogenic energy source. KCN + DCCD decreases $\Delta\Psi$ down to almost zero in both species. DCCD alone decreases $\Delta\Psi$ partially in anaerobes and increases $\Delta\Psi$ in aerobes, whereas KCN alone reduces $\Delta\Psi$ partially in both species. The introduction of glucose into the medium containing K^+ reduces the absolute value of $\Delta\Psi$ to $|-160|$ mV in aerobes and to $|-20|$ mV in anaerobes. The effect is not observed without external K^+. In the presence of arsenate a $\Delta\Psi$ is not reduced after the addition of glucose. At pH 7.5–7.8 the ATP level in aerobes grows notably faster than in anaerobes. The H^+ extrusion becomes intensified when K^+ uptake is activated by the increase in external osmotic pressure. Apparent K_m and V_{max} for K^+ accumulation are 1.2 mM and 0.4 mM $\cdot$ min$^{-1} \cdot$ g^{-1}. The decrease of $\Delta\Psi$ by glucose or KCN + DCCD have no effect on the K^+ uptake whereas CCCP inhibits potassium accumulation. At the same time, arsenate stabilizes the $\Delta\Psi$ value, but blocks K^+ uptake. The accumulation of K^+ correlates with the potassium equilibrium potential of -200 mV calculated according to the Nernst equation, whereas the $\Delta\Psi$ measured was not more than $|-25|$ mV. The calculated H^+/ATP stoichiometry was 3.3 for aerobes. It was assumed that a constitutive K^+ pump having a K^+/ATP ratio equal to 2 or 3 operates in *S. marcescens* membranes.

Introduction

The study of K^+ transport in aerobically grown facultative anaerobes *Escherichia coli* showed that the accumulation of these ions proceeds mainly through two transport systems [1]. The constitutive system is a K^+ ionophore, the so-called Trk system [2], for which $\Delta\Psi$ serves as the energy source and the functions of which are controlled by the intracellular concentration of ATP [3,4]. The repressible system is the K^+ pump (the Kdp system [5,6]), which is switched on only when

Abbreviations: $[K^+]_{in}$ and $[K^+]_0$, concentrations of K^+ in the cell and medium, respectively; ΔG_P, phosphate potential; E_{K^+}, potassium equilibrium potential; DCCD, *N,N'*-dicyclohexylcarbodiimide; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; H^+/ATP and K^+/ATP, ratios of the moles of ions transported via H^+ and K^+ pumps, respectively, to 1 mole of synthesized or hydrolyzed ATP; $\Delta\tilde{\mu}_{H^+}$, electrochemical potential difference for protons; TPP^+, tetraphenylphosphonium cation.

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$[K^+]_0$ is less than 0.02 mM. Also available are data on the existence of H^+/K^+-antiport [7,8], which may play an important role in the K^+ uptake [9,10] under defined conditions.

Along with the above findings, some experimental evidence was obtained during the last years, indicating that in *E. coli* grown without aeration in glucose medium, ECF_0F_1 may unite with the Trk system into a single supercomplex [10,11]. Such a supercomplex $ECF_0F_1 \cdot$ Trk functions as an ion-exchange H^+-K^+ pump with an $ATP/2H^+/K^+$ stoichiometry. This phenomenon is not observed in aerobically grown *E. coli* [12] and in mutant strains of *E. coli* with defects in ECF_0F_1 [13] or in the Trk system [9]. It was established that several properties, mentioned hereafter, should be consistent with the formation of such a supercomplex in anaerobically grown bacteria.

(1) The stoichiometry of ion exchange should be equal to $2H^+/K^+$ irrespective of the absolute values of ions fluxes, i.e., this stoichiometry must be independent of the experimental conditions (t^0, pH, $[K^+]_0$, etc.), although each flux taken separately might change. However, the change of fluxes must be proportional.

(2) The mentioned stability of exchange stoichiometry refers only to DCCD-sensitive components of H^+ and K^+ fluxes.

(3) In Gram-negative organisms this exchange must be activated only through the increase of osmotic pressure in the medium.

(4) The distribution of K^+ between a cell and the medium and hence E_{K^+} must considerably exceed the measured values of membrane potentials.

(5) In aerobically grown bacteria the DCCD-sensitive constant stoichiometry of $2H^+/K^+$ must not be observed.

(6) In mutant strains with defects in BF_0F_1 or in the K^+ ionophore the variation in the nature of H^+/K^+ exchange must be observed.

(7) For magnitudes of $\Delta\Psi - 2 \cdot 2.3 \cdot RT\Delta pH/F + E_{K^+}$ having the sum of nearly 500 mV, the reversal of H^+ and K^+ fluxes with the parallel synthesis of ATP and the stoichiometrical ratio of $ATP/2H^+/K^+$ must be observed [10,11].

(8) The DCCD-sensitive ATPase activity of membranes in the presence of K^+ occurs for anaerobic cells. This is not observed in the aerobically grown bacteria and mutant strains with defects in both ECF_0F_i and K^+ ionophore [14].

The first five conditions are already sufficient for the identification of $BG_0F_1 \cdot K^+$-ionophore supercomplex. Indeed, the anaerobically grown Gram-negative *Salmonella typhimurium, Proteus mirabilis* and Gram-positive anaerobes *Lactobacillus salivarius* or *L. lactis* and *Streptococcus faecalis* possess these properties, while they are lacking in the aerobically grown *S. typhimurium, P. mirabilis* and Gram-positive aerobes *B. flavum* and *C. glutamicum* [15].

The Gram-negative facultative anaerobe *S. marcescens* differs from the studied bacteria by a number of features [16]; in particular, they accomplish butanediol fermentation of glucose. It was nevertheless unexpected to detect in them an entirely different type of K^+ transport, namely, a constitutive electrogenic K^+ pump.

Materials and Methods

Serratia marcescens, strain ATCC 9986, was used in all the experiments. To obtain the cells in which the H^+-ATPase F_0F_1 acts in the mode of ATP hydrolysis, the bacteria were grown at 30 °C in medium containing 2% peptone, 0.5% NaCl, 0.2% K_2HPO_4 and 0.5% of glucose in flasks of 500 ml filled entirely, without aeration and shaking. We shall term these cells 'anaerobes'. To get aerobic cells in which BF_0F_1 works as an ATP synthase, the bacteria were grown at 30 °C in the liquid Glover medium (0.5% NH_4Cl; 0.1% NH_4NO_3; 0.2% Na_2SO_4; 0.3% $K_2HPO_4 \cdot 3H_2O$; 0.1% KH_2PO_4; 0.01% $MgSO_4$ and 0.5% sodium succinate) in flasks of 500 ml containing 20 ml of this medium under the vigorous agitation. After attaining the early stationary phase (18–20 h) the cells were centrifuged for 5000 r.p.m., washed by distilled water or by the solution with arabinose as a non-utilized sugar for the maintenance of appropriate osmotic pressure, and then resuspended. The pellet was transferred to the solution of the following composition: 50 mM Tris; 1–15 mM KCl; 1 mM NaCl; 0.4 mM $MgSO_4$. 11 M phosphoric acid was titrated to get the required value of pH. The tonicity of the solution was regulated by the addition of arabinose. The biomass was determined by the optical density of bacterial suspension, by the dry weight of the biomass or

the amount of viable cells in 1 ml of suspension (a titre of bacteria) depending on the purpose of the experiment. The size of cells was determined with the help of a phase-contrast microscope. The volume of one anaerobic cell was $3.7 \cdot 10^{-13}$ cm^3 and that of one aerobic cell was $2.4 \cdot 10^{-13}$ cm^3.

Ion fluxes, the value of $\Delta\tilde{\mu}_{H^+}$ and $[K^+]_{in}$

The ion fluxes were measured by means of cation-selective glass electrodes as described earlier [17]. The ΔpH value was measured with an ion-selective salicylate-sensitive electrode. The initial salicylic acid concentration in the medium was $6 \cdot 10^{-5}$ M. To measure the slope of electrode potential upon acid concentration, $1.5 \cdot 10^{-5}$ M acid was added and a change of potential was recorded. Using such a slope the ΔpH was calculated with the Nernst equation from the distribution of salicylate. The ΔpH was close to zero at external pH 7.5–7.8 just as in other bacteria [18,19]. All the measurements were carried out at pH 7.5–7.8. The value of $\Delta\Psi$ was determined by the distribution of TPP^+ as described [20]. The cells were preincubated with 100 mM Tris, 10 mM EDTA at pH 7.0 for 20 min. The assertion that TPP^+ is adsorbed on cell structures and exhibits an incorrect $\Delta\Psi$ value was examined in our laboratory in a series of aerobically grown bacteria, in which K^+ were distributed between a cell and the medium in accordance with $\Delta\Psi$. It was shown that the values of E_{K^+} and $\Delta\Psi$ coincide [12,15]. Electrodes for determination of TPP^+ and salicylate concentrations were prepared in the Vilnius State University and kindly given to us by Prof. L. Grinius. The kinetic curves shown in the figures represent one of 3–5 independent experiments.

The $[K^+]_{in}$ values were obtained from the amount of K^+ released from bacteria after the addition of toluene (1 µl/ml) to the medium. The toluene treatment of cell leads to the release of 95% of intracellular potassium [21].

Phosphate potential

The value of ΔG_P was calculated according to Eqn. 1

$$\Delta G_P = \Delta G_P^0 + 2.303RT \log \frac{[ATP]}{[ADP] \cdot [P_i]} \quad (1)$$

where R, T and F have their usual meanings; ΔG_P^0 indicates the standard free energy for ATP hydrolysis (or synthesis). $\Delta G_P^0 = 31$ kJ $\cdot$ mol^{-1} for pH 7.5 (see Table I in Ref. 22); $[Mg^{2+}] = 1$–10 mM; $t^0 = 30°$ and the ionic strength of cytoplasm of 0.02.

The extraction and quantitative determination of ATP was made by the method of Ref. 23 with slight modification of the absolute, not the relative amounts of reagents. The light emission proportional to the concentration of ATP was registered with an LKB-1250 luminometer in the mixture of 50 µl of ATP extract and 1 ml of luciferin-luciferase extract. The preparation of the luciferin-luciferase extract of fireflies was described in Ref. 24. The extract was used during 8 h after the preparation.

The determination of ADP was made after enzymatic conversion into ATP. The intracellular concentration of ADP by the difference of ATP concentrations prior to and after the conversion. For the analysis of experimental and control samples, 0.5 ml of the sample or of the ADP standard was mixed with 0.05 ml of solution containing 50 mM phospho*enol*pyruvate; 100 mM Tris (pH 7.4); 35 mM KCl; 6 mM $MgCl_2$ and 300 units per ml pyruvate kinase. The mixture was incubated for 40 min at room temperature and then exposed to the ice bath. The enzymatic activity was stopped by adding 25 µl of concentrated $HClO_4$. The perchlorate anions were neutralized by 3.5 µl of KOH (7.5 M) containing 50 mM K_2PO_4. The neutralized samples were kept on ice during 10 min up to the removal of precipitate by means of centrifugation. The supernatant (50 µl) was taken for the determination of ATP.

The inorganic phosphate was found by the method of Berenblume and Chaine [25] in the Weil-Malerbet modification [26].

Respiration of bacteria

The respiration was determined polarographically in nA O (10^{-9} atoms of oxygen) with the help of LP7 polarograph (Czechoslovakia). The bacteria were placed into thermostat-controlled sealed cell (1.5 ml) with a platinum cathode.

Results

Aerobically and anaerobically grown bacteria

As we have shown in the preceding work *E. coli* grown in minimal salt medium with succinate and

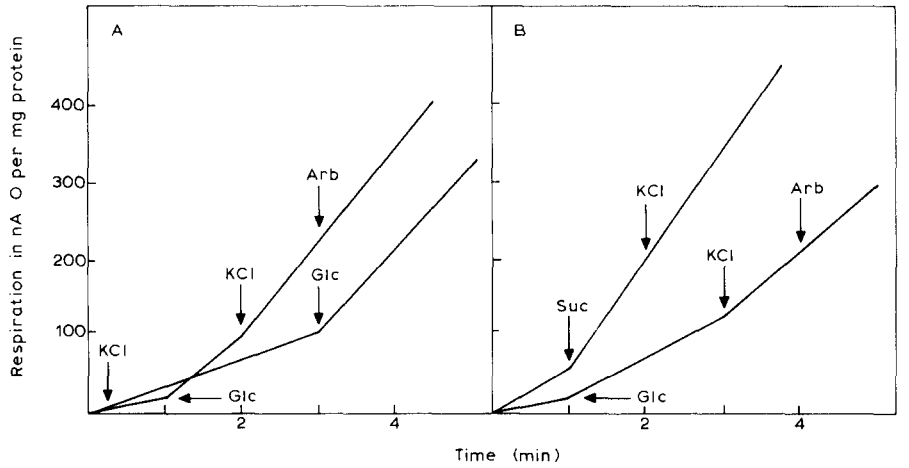

Fig. 1. The consumption of oxygen in anaerobically (A) and aerobically (B) grown *S. marcescens*. Experimental solutions contained Tris-phosphate buffer (pH 7.8); 1 mM NaCl; 0.4 mM $MgSO_4$ at an average protein concentration of 1.2 mg/ml. The additions introduced into the medium were: 10 mM KCl; 10 mM glucose; 200 mM arabinose; 10 mM sodium succinate.

forced aeration behave as aerobes while after growth in glucose medium without aeration the bacteria demonstrate the electrochemical properties of anaerobes [12].

The same procedure for the preparation of cells was adopted for the present work. One could conclude from Fig. 1 that both types of *S. marcescens* cells are real aerobes because both have the same respiration rates. Furthermore, in both cells the introduction of K^+ to the medium in the presence of an exogenic energy source increased the respiration notably.

However, DCCD that depresses the function of F_0F_1 increased the value of $\Delta\Psi$ in aerobes (Fig. 2A, curve 1). This result directly follows from the chemiosmotic theory. In the coupling membranes the F_0F_1 synthesizes ATP during aerobiosis and hence serves as an electrical shunt for a respiratory chain. The inhibition of this electrical shunt by DCCD leads to the increase of $\Delta\Psi$. To the contrary, in anaerobes DCCD lowered a $\Delta\Psi$ (Fig. 2A, curve 2) because F_0F_1 itself generates $\Delta\Psi$. In such conventional anaerobes the role of the respiratory chain seems to consist in the stabiliza-

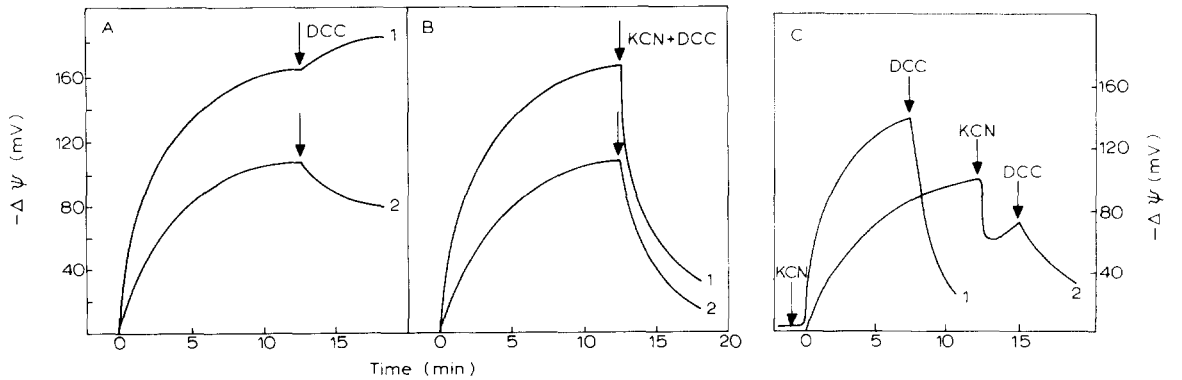

Fig. 2. The actions of DCCD (A), KCN + DCCD (B) and KCN (C) on the values of membrane potential of the Tris-EDTA-treated bacteria in the absence of exogenic energy source; the curves 1 and 2 refer to aerobically and anaerobically grown cells, respectively. KCN (Fig. 2C, curve 1) was present in the medium before bacteria were added. Experimental solutions contained Tris-phosphate buffer (pH 7.8); 3 mM KCl; 1 mM NaCl; 0.4 mM $MgSO_4$. The experiment was carried out in the aerobic condition, i.e., in the open chamber with a mixing medium. DCCD and KCN were added to get the final concentrations of $5 \cdot 10^{-4}$ M and 10^{-2} M, respectively. TPP was $5 \cdot 10^{-6}$ M [20].

tion of the $\Delta\tilde{\mu}_{H^+}$ value (in this case $\Delta\tilde{\mu}_{H^+} = \Delta\Psi$). At pH 7.5 DCCD is able to lower $\Delta\Psi$ just by 15–20 mV in such cells, while in strictly anaerobic organisms like *S. faecalis* DCCD completely blocks $\Delta\Psi$ [20,27]. This idea about the role of the respiratory chain in conventional anaerobes was confirmed experimentally (Fig. 2B). The combined action of KCN + DCCD reduced the absolute value of $\Delta\Psi$ down to almost zero in aerobes and anaerobes. This experiment also showed that in *S. marcescens* membranes as well as in many facultative anaerobes there are two $\Delta\tilde{\mu}_{H^+}$-generators: the respiratory chain and H^+-ATPase complex BF_0F_1 · KCN alone decreased partially a $\Delta\Psi$ in both species (Fig. 2C), since BF_0F_1 begins to function as an electrogenerator in the absence of respiration (Fig. 2C, curve 2).

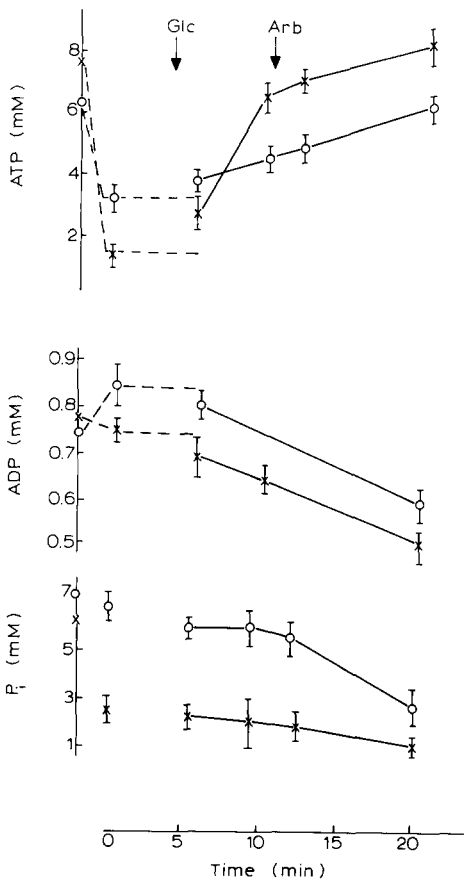

Fig. 3. The changes of intracellular ATP, ADP and P_i concentrations in anaerobically (○) and aerobically (×) grown *S. marcescens* after inoculation of experimental medium. Aerobic condition was used in the experiment (see Fig. 2). Additions: 10 mM glucose; 200 mM arabinose. Arabinose had no effect on nucleotides level. The symbols on the vertical axis are the meanings of ATP, ADP and P_i concentrations just after the growth of bacteria. The dotted lines relate to the control experiments, indicating the ATP, ADP levels in cells before they were used in the experimental solution with glucose (see Fig. 2). A solid line dates from the time after glucose addition.

Another feature of anaerobes is the lower value of membrane potential $|-131 \pm 11|$ mV in comparison to that for aerobic cells of *S. marcescens* ($|-176 \pm 8|$ mV). These experiments were carried out in the absence of exogenic energy source (see Fig. 2 and Table I).

And finally, the intracellular ATP concentration increase at the introduction of glucose to the medium showed that aerobes accumulate ATP noticeably faster than anaerobes (Fig. 3).

Kinetics of H^+/K^+-exchange and $\Delta\Psi$

The alteration in H^+ and K^+ fluxes and in the magnitude of $\Delta\Psi$ after the inoculation of the medium by anaerobes and aerobes are plotted against time in Fig. 4. In the glucose medium the uptake of K^+ and extrusion of H^+ proceeded at slow rate and were insignificant although the respiration for both types of cell increased up to the maximum value as soon as glucose and potassium were introduced into the medium (see Figs. 4A and 1). Under the same condition when the external osmotic pressure was low no uptake of K^+ was observed in *E. coli* grown anaerobically [17]. The introduction into the medium of non-utilized arabinose sugar creating hypertonical conditions activated the additional extrusion of H^+ and sharply increased the uptake of K^+ just as was the case in Gram-negative enterobacteria [15,17], although the variation in the external osmolarity did not affect the respiration (Fig. 1).

An important observation in this experiment was the reduction of the absolute value of $\Delta\Psi$ from $|-120-130|$ mV to $|-10-20|$ mV in anaerobes during the period of glycolysis without changes in K^+ uptake (Fig. 4B, curve 2; cf. Fig. 4A). Under the same conditions the aerobes retained the $\Delta\Psi$ at the level of -160 mV, but the uptake of K^+ in them was decreased (Fig. 4A and B, curves 1). When glucose and arabinose were initially present in the suspension of anaerobic cells the $\Delta\Psi$ was zero but an intense uptake of K^+

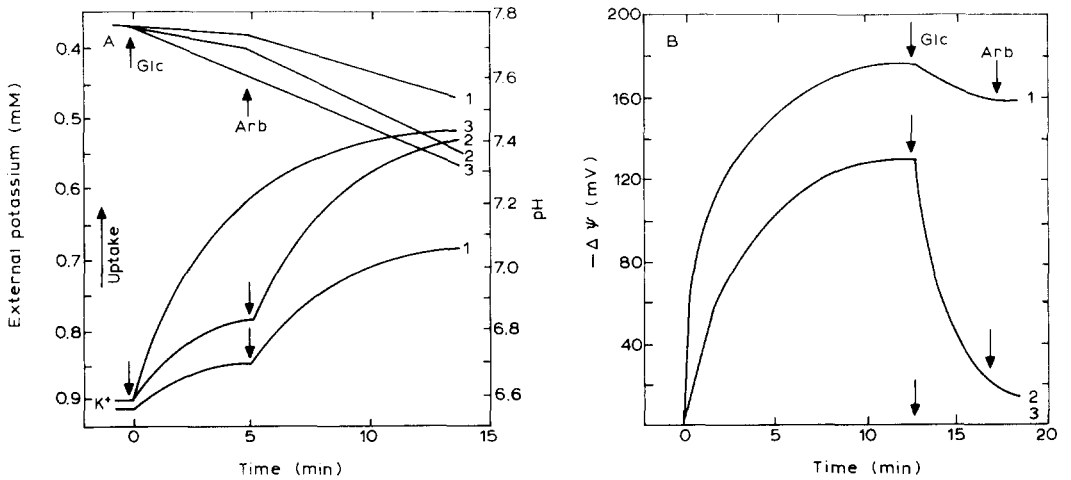

Fig. 4. The H^+ and K^+ counter fluxes (A) and membrane potentials (B) in anaerobically (curves 1 and 3) and aerobically (curves 2) grown *S. marcescens*. The measurements were carried out in aerobic condition (see Fig. 2). For experiment 3 arabinose (200 mM) was initially in the medium. Bacteria feel the osmotic shock after transferring them from washing distillate water into the hypertonic experimental solution. Addition for experiment 1 and 2 was 10 mM glucose; 200 mM arabinose. The measurements of ion fluxes and accumulation of TPP^+ were carried out in the separte chambers.

took place (Fig. 4A and B, curves 3). This experiment gives an extra indication of the independence of K^+ influx and H^+ efflux on the $\Delta\Psi$.

At the complete suppression of the membrane potential by means of combined application of KCN + DCCD (Fig. 2B) the rates of K^+ uptake

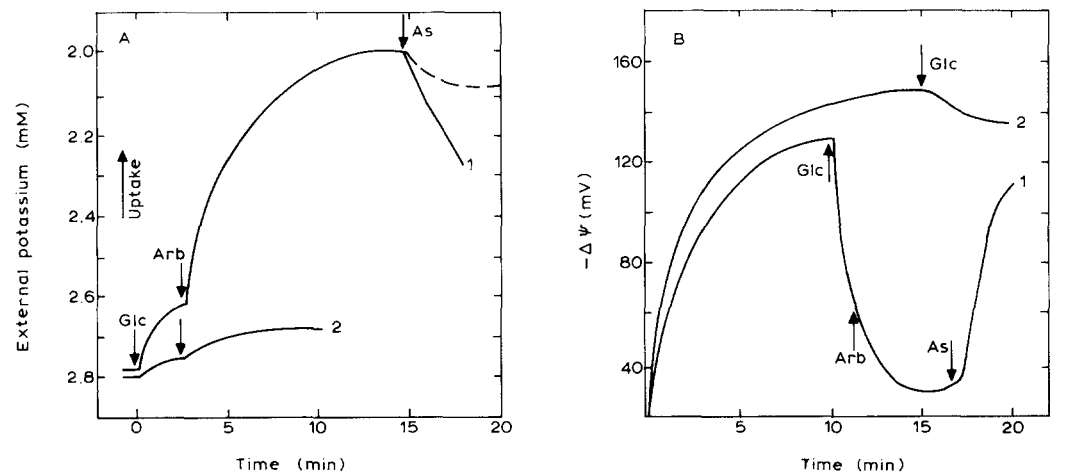

Fig. 5. The influence of arsenate, As, on K^+ influx (A) and on the values of $\Delta\Psi$ (B) in anaerobically grown *S. marcescens*. The curve 1 is the control experiment for K^+ influx. The addition of sodium arsenate (10 mM) leads to the abrupt exit of K^+ from cells, whereas the addition of 20 mM NaCl (dotted line) creates the expected slight alteration in the potential of potassium glass electrode according to the equation

$$\Delta\Psi = \Delta\Psi_0 + \frac{RT}{F} \ln\left(a_{K^+} + K_{K/Na} \cdot a_{Na}\right)$$

where $K_{K/Na}$ is a constant of electrode selectivity to Na^+ with reference to K^+. Sodium arsenate for the experiment 2 was initially in the medium. Glucose, 10 mM; arabinose, 200 mM.

TABLE I

THE STEADY-STATE MEANINGS OF SOME PHYSICO-CHEMICAL VALUES IN *SERRATIA MARCESCENS*

$E_{K^+} = \frac{RT}{F} \ln \frac{[K^+]_0}{[K^+]_{in}}$; $\Delta\Psi$ is given after glucose additions (at the 20th min, Fig. 4), in the brackets are represented the initial membrane potentials in the media without exogenic energy sources (see Fig. 2, 4 and 5).

Growth conditions	$[K_0^+]$ (mM)	$[K^+]_{in}$ (mM)	E_{K^+} (mV)	$\Delta\Psi$ (mV)	[ATP]/[ADP] $\cdot[P_i]$ (M^{-1})	$\Delta G_P/F$ (mV)	$[H^+]$/ [ATP]	$[K^+]$/ [ATP]	Number of experiments
Anaerobic	0.45 ± 0.05	890 ± 150	198	20 ± 4 (131 ± 11)	2797	500	–	2.5	6
Aerobic	0.50 ± 0.03	920 ± 140	195	160 ± 15 (176 ± 8)	7830	530	3.3	2.7	5

and acid production remained unchanged (not shown).

The absence of a correlation between the K^+ uptake and a $\Delta\Psi$ is most clearly seen in Fig. 5. Sodium arsenate was introduced into the medium when the most active uptake of K^+ and the essential decrease of $\Delta\Psi$ (from $|-125|$ mV to $|-30|$ mV) were observed. The value of membrane potential recovered almost to -110 mV (Fig. 5B, curve 1). In the meantime, the uptake of K^+ was completely ceased (Fig. 5A, curve 1). If arsenate was initially present in the medium the $\Delta\Psi$ reached -145 mV, while the K^+ uptake was blocked (Fig. 5A and B, curves 2).

In our opinion, the experiments showed that the transport of K^+ requires ATP and does not involve a $\Delta\Psi$. Actually, the intense electrogenic uptake of K^+ sharply decreased the membrane potential [28]. As potassium ions are transported into cells against the gradient of concentrations without using the electrochemical energy, one has to conclude that a K^+ pump is operative in *S. marcescens* membranes.

This conclusion is supported by the data of Table I. The internal potassium concentration was estimated by the amount of K^+ leaving the toluene-treated cells (see Materials and Methods). The potassium equilibrium potential (E_{K^+}) was calculated to be -200 mV both for anaerobes and aerobes, while the measured membrane potential was about -20 mV for anaerobes and -160 mV for aerobes.

The action of protonophores on the transport of K^+ in these bacteria was not at all different from the usual action of uncoupling agents (Fig. 6). However, it would be erroneous to suppose for *S. marcescens* that a protonophore reduces $\Delta\Psi$ and that the uptake of K^+ is inhibited after the decrease of the $\Delta\Psi$ value. All the previous experiments indicated that the $\Delta\Psi$ did not take part in the uptake and retention of K^+. It is noteworthy that the efflux of K^+ in the presence of CCCP is followed by the decrease of the coupled efflux of H^+ ($\Delta V_{K^+} = \Delta V_{H^+} = 1.16$ mmol $\cdot$ min^{-1}, where ΔV_{K^+} is the difference between the K^+ efflux and influx rates and ΔV_{H^+} is the difference between the final and initial efflux of H^+).

The nature of H^+/K^+ exchange

The kinetics of H^+ efflux reflects exactly the process of K^+ uptake. If K^+ was absent in the medium, the hypertonic condition did not already

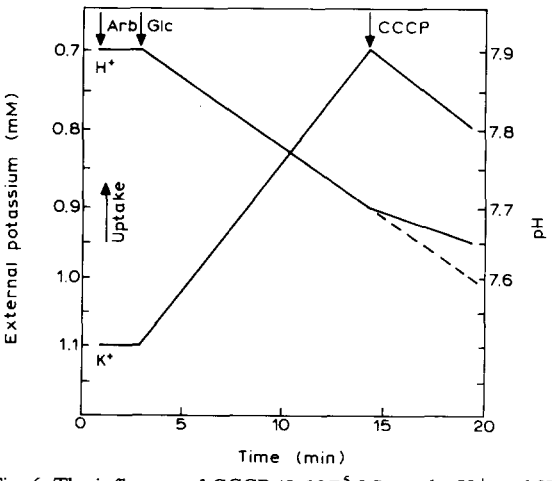

Fig. 6. The influence of CCCP ($5 \cdot 10^{-5}$ M) on the H^+ and K^+ fluxes. Dotted line is the control experiment. The condition was aerobic (see Fig. 2). Glucose, 10 mM; arabinose, 200 mM. Bacteria were grown anaerobically.

actuate the proton exit from the cells (not shown). This indicates the important role of external osmotic pressure for the stimulation of K^+ uptake and corresponding production of acid. However, unlike other Gram-negative organisms [12,15,17] the increase of osmotic pressure enhanced the H^+/K^+ exchange not only in anaerobically grown *S. marcescens*, but also in aerobic cells.

The rate of osmotic-sensitive K^+ uptake depended on the concentration of K^+ only up to $[K^+]_0 = 6$ mM (Fig. 7A) as was the case with *E. coli* [29,30]. The apparent values of K_m and V_{max} were 1.2 mM and 0.4 mM $\cdot$ min$^{-1} \cdot$ g^{-1}, respectively, i.e., a K^+ transport system has the low affinity to K^+ and low transport rate.

The most important factor for the nature of the link between H^+ and K^+ fluxes in bacteria is the ratio of rates of ion exchange under different conditions. When the ions H^+ and K^+ are translocated through separate mechanisms and fluxes are connected indirectly (for instance, via $\Delta\tilde{\mu}_{H^+}$), then each flux may be changed differently. This was observed in aerobically grown cells [12]. At the same time, if the special molecular mechanism accomplishes an ionic exchange this ratio remains strictly constant irrespective of conditions, as it was observed in anaerobic bacteria [10]. This simple physical interpretation of the properties of stoichiometric interrelation between hydrogen and potassium fluxes was more than once confirmed for a number of bacteria (see Introduction). The aerobically and anaerobically grown cells of *S. marcescens* lacked the stable V_{H^+}/V_{K^+} stoichiometry (Fig. 7B). From such an electrochemical point of view the electrogenic accumulation of K^+ and H^+ extrusion are accomplished via different mechanisms.

H^+/ATP and K^+/ATP stoichiometry

The estimate of H^+/ATP and K^+/ATP ratios given in Table I, was made according to the equations

$$\frac{H^+}{ATP} = \Delta\frac{G_P}{F}\,\Delta\Psi$$

$$\frac{K^+}{ATP} = \Delta\frac{G_P}{F}\,E_{K^+} \qquad (2)$$

The H^+/ATP stoichiometry is taken for aerobes, since anaerobes did not use BF_0F_1 for ATP synthesis (Fig. 2A, curve 2). Furthermore, the operation of electrogenic K^+ transport system diminishes the value of $\Delta\Psi$ down to almost zero in anaerobes. In these calculations we accepted $\Delta\tilde{\mu}_{H^+} = F \cdot \Delta\Psi$ as $\Delta pH = 0$ of pH 7.5–7.8. The $\Delta\Psi$ were taken -160 mV that we observed after the addition of glucose (see Table I). The H^+/ATP

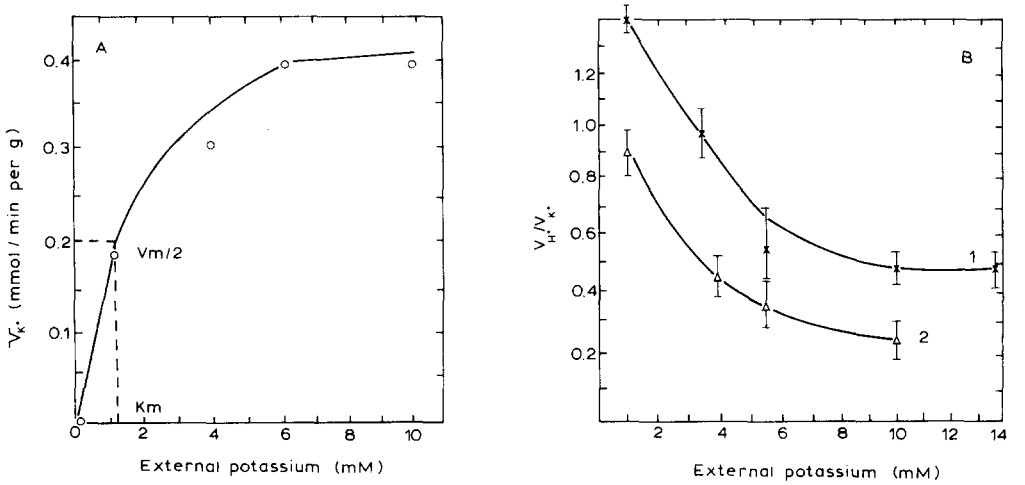

Fig. 7. The dependence of the rates of K^+ uptake (A) and the changes of H^+/K^+ stoichiometric ratio (B) upon external K^+ concentrations. The curves 1 and 2 refer to aerobic and anaerobic bacteria, respectively. At $[K^+]_0$ equal to 0.1 mM the K^+ accumulation is assumed to be not observed due to the insensitivity of cation selective glass electrodes method. V_{K^+} is the osmotic sensitive rate of K^+ uptake that revealed after the addition of arabinose. 1, aerobically; 2, anaeronically grown cells.

value of 3.3 is close to the value of 3.0 obtained for other bacteria and chloroplasts [31]. The value of K^+/ATP equal to 2.5–2.7 (Table I) is more than we have expected. There are good reasons to believe that the K^+/ATP-ratio is 2.

Discussion

As is known for a long time, the intracellular osmotic pressure of bacteria is maintained by the accumulation of K^+ [32]. The increase in the osmolarity of the medium leads to the abrupt uptake of K^+ in *E. coli* by two distinct transport systems: Trk-ionophore and Kdp-pump [5,33]. However, it was shown subsequently that the Trk system itself is not osmotically sensitive [10–13,34]. Only after the anaerobic growth of *E. coli* when the structural association of K^+-ionophore with ECF_0F_1 is assumed to take place (see 'Introduction') the apparent sensitivity of Trk-mechanism to osmotic pressure must be observed [10,13], whereas the Trk system remains indifferent to the variation of external osmolarity in aerobically grown *E. coli* [12]. It was successfully shown by using the *E. coli* mutant strains defective in the different subunits from the H^+-ATPase complex that the proton channel ECF_0 is osmotically sensitive and this sensitivity of ECF_0 is presumably connected with an unknown periplasmic protein valve [13,34]. A protein valve serves to open and close a gate of H^+-channel ECF_0 depending on the increase and decrease in the osmolarity of the medium. The osmotic sensitivity of K^+ accumulation was discovered also in other Gram-negative bacteria but only after the growth in glucose medium without aeration [15]. The osmotic regulation of the BF_0 function is neither observed in Gram-positive bacteria not in spheroplasts devoid of periplasmic proteins [10,34].

We assume that a cell needs regulating the consumption of ATP for transport, especially during the low-energetic process as glycolysis, since the ionic pump activity takes the greater part of metabolic energy [35].

In the present case, another osmotic sensitive pump transporting K^+ electrogenically is discovered in Gram-negative bacterium *S. marcescens*. At the same time, a K^+ pump which is assumed to function in *P. denitrificans* is considered to be insensitive to the variation of osmotic pressure in the medium [36]. One cannot exclude yet that *P. denitrificans* should be grown under anaeronic conditions; besides, these bacteria may require greater differences of osmotic pressure as it was observed in *P. mirabilis* (not shown).

If we compare the present situation with the transport of sugars and amino acids we can find certain similarities. In both cases the periplasmic regulation is essential for systems which use high-energy phosphorylated compounds as the source of energy and such a regulation is not needed for $\Delta\tilde{\mu}_{H^+}$-dependent transport systems [37,38].

ATP is probably the more expensive source of energy for a cell.

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