ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 8, pp. 917-924. © Pleiades Publishing, Ltd., 2012.
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Evaluation of the Electrical Potential on the Membrane of the Extremely Alkaliphilic Bacterium *Thioalkalivibrio*

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Received April 20, 2012

Abstract—The electrical potential on the membrane was measured in cells of strains AL2 and ALJ15 of the extremely alkaliphilic bacterium *Thioalkalivibrio versutus* using the penetrating cation tetraphenylphosphonium (TPP^+) and a TPP^+-selective electrode. The potentials were -228 ± 5 and -224 ± 5 mV, respectively, i.e. higher than in most alkaliphilic bacteria. Membrane potential in the cells was estimated by measuring the inner cell volume by two independent methods: (1) estimation of total cell volume by light microscopy and (2) estimation of the inner aqueous volume of the cells with allowance for the distribution difference of tritium labeled water penetrating through the membranes and a nonpenetrating colored protein. The inner cell volume was 2.4 ± 0.2 and 2.2 ± 0.1 μl/mg of cell protein by the two methods, respectively. Computer computation was used as an alternative to manual calculation to count the number of cells for estimation of total cell volume.

DOI: 10.1134/S0006297912080135

Key words: electrochemical potential of H^+ ions, transmembrane electric potential difference, alkaliphilic bacteria

More than four decades have passed since the publication of the chemiosmotic theory (1966) [1] and its verification by proving generation of transmembrane potential by mitochondria [2] and *Escherichia coli* cells [3] during the oxidation of respiratory substrates. The first works on visualization and measurement of the electric component of membrane potential in living objects (1967-1970) were based on the method of ion penetration. This method was devised and developed by E. A. Liberman et al. [4, 5]. Now it is undisputed that the electrochemical potential difference for hydrogen ions ($\Delta\overline{\mu}_{H^+}$, membrane potential) in the membranes of subcellular organelles (mitochondria and chloroplasts), as well as many bacteria and archaea, plays the key role in the energy supply of living cells. Cell functions such as movement, nutrient transport, transfer of the hereditary elements, ATP synthesis, etc., directly depend on membrane potential. Its two components, electric (transmembrane electric potential difference ($\Delta\psi$)) and concentration (the difference of H^+ ion concentrations on the two sides of the membrane (ΔpH)), are important energy characteristics in contemporary studies of bacteria and archaea as well as energy converting subcellular organelles. The nature of membrane potential and the contributions of its components can significantly vary depending on the type of energetics of an organism. The membrane potential of some bacteria and organelles consists mainly of the electric component. In some representatives of the living world, the concentration component of proton gradient (chloroplasts, acidophilic bacteria) makes a major contribution to the transmembrane potential of cells or organelles. In other organisms, the contributions of the two components are comparable. The objects of interest in our study were strains of the extremely alkaliphilic bacterium *Thioalkalivibrio versutus* isolated from highly saline and alkaline lakes [6]. These organisms attract attention due to a unique combination of two extremely high characteristics of the habitat: the extremely alkaline pH of the growth medium (10.7) and the extremely high concentrations of sodium salts (2-4 M). These bacteria are characterized by unique membrane composition [7] and possess previously unknown pigments apparently performing protective functions [8]. The energetics of these unique bacteria is still under study and has not yet been completely elucidated. The study of the nature and components of membrane potential makes it possible to determine the mechanisms providing active life under such unusual environmental conditions.

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

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MATERIALS AND METHODS

Measurement of protein concentration by light scattering in cell suspension. The culture of *Tv. versutus* was washed twice: first from the growth medium (Eppendorf microcentrifuge, 5000 rpm, 10 min) and then from elemental sulfur by precipitating the latter (2000 rpm, 5 min) in medium containing 50 mM Caps-NaOH, pH 10, 0.6 M NaCl in the case of the moderately salt-tolerant strain *Tv. versutus* AL2, or 50 mM Caps-NaOH, pH 10, 2 M NaCl in the case of highly salt-tolerant strain *Tv. versutus* ALJ15. Then the wet precipitate of each strain was resuspended with one or two volumes of the respective medium. The resulting cell suspension was used to prepare a series of dilutions (1 : 250, 1 : 500, 1 : 1000, 1 : 1500, 1 : 2000), where protein concentration was measured by the biuret method (with BSA as a standard) and light scattering was simultaneously measured with a Specol-20 at λ_{420}. The indicated values were used for plotting a calibration curve (Fig. 1) for further rapid assessment of cell protein concentration in the cell suspension by its light scattering. The values for the two studied strains were similar; therefore, protein in the cell suspensions was assayed using a single calibration curve.

Specific inner cell volume (ϑ) was determined in a Goryaev chamber using light microscopy. For this purpose, the ratio of total inner volume of counted cells in a given volume of suspension to total cell protein was determined. On the basis of geometrical dimensions of the cells of sulfur-oxidizing bacteria from alkaline lakes, including *Thioalkalivibrio* strains, the volume of a single average-sized cell was taken as $2.5 \cdot 10^{-10}$ μl. Also, it was assumed that the cell wall of Gram-negative bacteria is 20 nm on average and cell wall contribution would be 16% for rod-shaped cells with an average size of 0.5 × 1.5 μm. Taking into account these assumptions, we considered that the inner cell volume ϑ was 0.84 of the total cell volume.

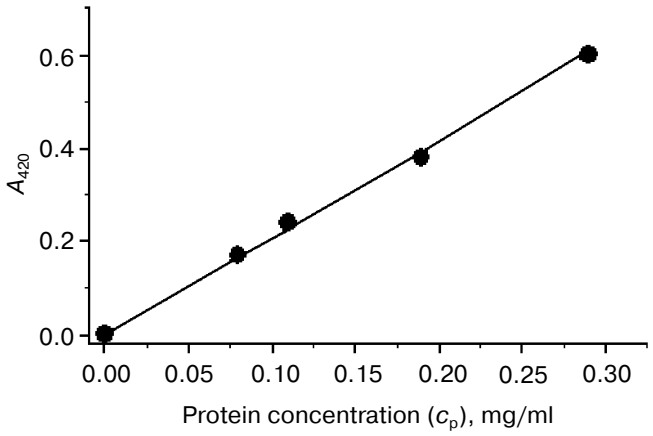

Fig. 1. Dependence of light scattering (A_{420}) on protein concentration in the cell suspension (c_p) of the bacterium *Tv. versutus*.

Cell counting in the Goryaev chamber. The number of bacterial cells in the specified volume of 0.1 mm deep Goryaev chamber was counted by assessing: (a) distribution of cells in the layers from top to bottom of the chamber and (b) the depth of field of bacterial cell images observed on microphotographs. For cell counting, the suspension of washed immobile cells depleted of endogenous substrates (diluted as required) was put into the Goryaev chamber and closed with a ground cover glass; the place of contact between the cover glass and the chamber was sealed with paraffin. The preparation was left in the chamber at rest for 2 h for bacterial cells to precipitate to the bottom of the chamber. The precipitation of the cells was monitored under the microscope using its microscrew. Microscopy and photography of the fields in a Nikon microscope (Japan) equipped with a Canon A620 digital camera were performed after precipitation of the cells to the bottom of Goryaev chamber. The sum of cells in the fields was considered the total cell number in the 10^{-6} ml volume limited by the chamber bottom glass from below, by the cover glass from above, and by an imaginary cubic cell with 0.1-mm facets on each side. Specific cell content at a given dilution was determined by the formula:

$$C_c = \frac{N}{V},$$

where N is the total cell number in the suspension volume under consideration and V is the Goryaev chamber volume under consideration. Cell number in the Goryaev chamber volume was determined with allowance for the fact that the ×40 objective lens of the Nikon E200 microscope had a numerical aperture of 0.65 and the ocular lens had a magnification power of 10. Different combinations of microscope objective and ocular lenses (or photographic camera lenses with a 35-mm objective instead of the ocular lens) during the photography give different zoom ranges during the microscopy: from 40 to 1500 for observations through the ocular lens and from 8 to 500 for photomicrography with the Canon A640 camera connected to the microscope.

The depth of field during microscopy was determined by the Berek formula:

$$\text{D.O.F.} = \frac{2500 \times \omega}{A_n \times M} + \frac{\lambda}{2 \times A_n^2}\ (\mu\text{m}),$$

where D.O.F. is the depth of field formula, ω is the resolution of the eye of 0.0014 (when the optical angle is 0.5°), M is the total magnification power (magnification of the objective lenses × magnification of the ocular lenses), A_n is the numerical aperture of the microscope objective lens, and $\lambda = 0.55$ μm is the visible light wavelength.

The depth of field for microscopy through the ocular lenses was:

$$\text{D.O.F.} = \frac{25\cdot10^5 \times 1.4\cdot10^{-3}}{0.65\cdot40\cdot10} + \frac{0.55}{2 \times 0.65^2} = 2\ (\mu\text{m}).$$

The depth of field for photomicrography was:

$$\text{D.O.F.} = \frac{25\cdot10^5 \times 1.4\cdot10^{-3}}{0.65\cdot40\cdot3} + \frac{0.55}{2 \times 0.65^2} = 5.1\ (\mu\text{m}),$$

where M is the total magnification power of the microscope objective lens (×40) and of the photographic camera (×3).

We established experimentally that the depth of field for photomicrography with these parameters for the optics is 5 μm, which is practically in agreement with data from calculation. This value was determined by focusing the camera objective lens on a cell attached to the slide and lying there flat. Then the microscope objective lens was sequentially raised by 1-μm steps by turning the microscrew and photographing the visible layers of cell suspension every 1 μm until the cell taken as a starting point moved out of the field of view. The moment of cell disappearance was taken as the upper limit of the depth of field during photomicrography.

Electronic cell counting. To facilitate the procedure of cell counting per volume, we used partial computer data processing in the Corel PHOTO-PAINT software, using the function of determining the total area of figures, each bacterial cell in the field of photographic image being replaced by a fixed-size white circle. For this purpose, it is convenient to use the "copy" operation for the preset circle image with a preselected standard diameter overlapping the area of a single bacterial cell. Then the cells were counted through arithmetic calculations by finding the area of all white circles in the field relative to the area of one circle corresponding to one bacterial cell. The area of all white circles in the field was determined as follows: (1) opening the file with the photographic image of the bacterial preparation of the tested dilution in the Goryaev chamber; (2) specifying the RGB mode in the editorial line of the "Image" option; (3) finding and clicking on the "Histogram" option at the end of all options in the "Image" option. A histogram with the underlying bar showing smooth distribution of color from black to white and with numerical expressions of various parameters below it was displayed in the window. The "Percent" window showed the entire surface of the figure (100%). The cursor was aligned exactly to the extreme right vertical line of the histogram and, accordingly, showed the percentage of white color in the figure. At the same time, the cursor was watched not to move to avoid capturing other colors on the bar of color distribution and counting the area of other colors besides the white used in the given task; and (4) the area of white color was calculated on the basis of the displayed white color percentage and total image area. The derived white color area was divided into the white circle area to obtain the number of circles in the field equal to the number of bacterial cells in the field.

Determination of specific inner volume of bacterial cells (ϑ) based on data on the distribution of tritium labeled water and nonpenetrating colored protein. Specific inner volume of all cells in the suspension (ϑ in μl/mg protein) was calculated by the difference between the volume accessible to tritium labeled water in the cell precipitate as described in [9] with our modifications and the volume of intercellular space accessible to horse heart cytochrome *c* in the precipitate as a ratio to total cell protein by the following formula:

$$\vartheta = \frac{(\text{volume accessible to } {}^3H_2O) - (\text{volume accessible to cytochrome } c)}{\text{mg protein}}.$$

The measurement is based on the fact that ^3H_2O penetrates through both membranes of bacterial cells (the outer membrane of the cell wall and the cytoplasmic membrane), while the low molecular weight water-soluble protein cytochrome *c* does not penetrate through the cytoplasmic membrane. The volume of the precipitate accessible to water was calculated by the ratio of ^3H_2O radioactivity in the supernatant and in the cell precipitate. The ratio of cytochrome *c* concentrations in the supernatant before and after mixing with the cell precipitate was used to calculate the volume of the precipitate accessible to cytochrome *c*. The difference between the precipitate volumes accessible to water and to cytochrome *c* was considered the inner volume of all cells.

Measurement of volume of intercellular space in the precipitate and cell volume using the nonpenetrating colored protein cytochrome c. The measurement included several successive steps:

1. The suspension of bacterial cells under study (630 μl), with protein content of 80 mg in each tube, was prepared in three repeats, and the cells were precipitated to obtain a dense precipitate in 1.5-ml Eppendorf tubes (8000 rpm, 15 min). Then the supernatant was thoroughly removed without brushing against the precipitate surface.

2. The volume of cell precipitate V_1 was determined by weighing the tubes at all stages of the experiment on a microbalance, with allowance for the experimentally determined density of the precipitate ($\rho = 1.03$ g/ml).

3. Alkaline buffer, 414 μl (volume V_2), containing the nonpenetrating colored protein (oxidized horse heart cytochrome *c*, 40 μM) was added to the tube with the pre-

cipitate (C_1 before mixing with the precipitate). Prior to that, the used cytochrome *c* was checked not to be adsorbed or reduced by the cells. The precipitate was thoroughly suspended on a vortex mixer to homogeneity. The cells in the suspension were precipitated by centrifugation under the same conditions as those specified in Step 1.

4. The supernatant was harvested, and the steady state concentration of cytochrome *c* was determined (C_2 after mixing with the precipitate). For this purpose, the cytochrome *c* concentration was determined by the optical density of the supernatant at 412 nm in several dilutions with four repeats each. Optical density values in the zone of linear dependence on dilution were used for calculations. Optical density of the solutions of oxidized cytochrome *c* in the maximum absorption region at 407-412 nm (absorption γ-band) in the used transparent media linearly depended on dilution in the range of cytochrome *c* final concentrations of at least 0 to 6 μM.

5. The volume V_m of intercellular space in the dense cell precipitate was calculated using the formula:

$$V_m = V_2 \cdot (\frac{C_1}{C_2} - 1).$$

The concentration of the nonpenetrating compound in the supernatant and in the intercellular space was assumed to be equal.

6. Cell volume V_{cell} in the precipitate was determined by the following difference: $V_{cell} = V_1 - V_m$.

Water-accessible volume in the cell precipitate based on the distribution of radiolabeled water was measured simultaneously with the volume of intercellular space in the suspension poured into a 1.5-ml Eppendorf tube. For this purpose, 414 μl (volume V_2) of the alkaline buffer containing, in addition to cytochrome *c*, 2 μl of tritium labeled water ^3H_2O (0.2 μCi) was introduced into the tube with the cell precipitate (see previous section, Step 3). The initial tritium-labeled reagent had specific activity of 115 μCi/ml (4.28 MBq/ml; Russia). The precipitate was thoroughly suspended until homogeneity and incubated for 15 min at room temperature. The cells in the suspension were precipitated by centrifugation. The cell precipitate was completely separated from the supernatant and thoroughly homogenized by adding 300 μl of the alkaline buffer. The exact volumes of the supernatant and homogenized precipitate were measured, followed by measurement of radioactivity in each of them, with no less than three repeats for the samples from each tube. For this purpose, 1 ml of 1 M $HClO_4$ and then 50 μl of the supernatant or 10 μl of the homogenized precipitate was introduced into a plastic scintillation vial. Upon complete dissolution of the precipitates under smooth rotation of the scintillation vials for 10-15 min, 9 ml of Ultima Gold scintillation liquid was added (High flash-point LSC-cocktail for counting aqueous and nonaqueous samples; Perkin Elmer, USA). The contents of scintillation vials were intensively stirred, and radioactivity was counted in a Delta 300 liquid scintillation counter (Tracor Analytic) in the signal accumulation mode so that the counting error 2σ would be less than 1%. The counting efficiency was 50%.

The accessible volume for radiolabeled ^3H_2O in the cell precipitate was calculated as the ratio:

$$\text{Volume accessible to } ^3H_2O = \frac{(\text{activity in precipitate [cpm]} \cdot V_2\,[\mu l])}{\text{activity in supernatant [cpm]}}.$$

The membrane potential of the cells was measured using tetraphenylphosphonium (TPP^+) penetrating cation at the final concentration of 1.6 μM and a TPP^+-selective electrode as described [10]. For the measurements, the TPP^+-selective electrode was connected to a PHM-220 Lab pH/millivoltmeter (Radiometer, Denmark), and the readings were displayed on a PC in the real-time mode using the PHM-220 software (N. Khitrin). The membrane potential was measured in the suspension of *Tv. versutus* AL2 or *Tv. versutus* ALJ15 cells diluted to a final density corresponding to 2-6 optical units at λ_{420} (Specol-20). The membrane potential of both strains was measured in a conical glass compartment at 22°C under intensive aeration and continuous stirring on a magnetic stirrer under two variants of conditions: in the medium used for cell washing or in the growth medium (pH 10) [6]. The working volume of the studied suspension was 1 ml. The zero electric potential value on the membrane of the bacteria, corresponding to de-energized state of the cells, was determined in the suspension with the following additives at final concentrations: nonionic detergent *n*-octyl-β,D-glucopyranoside (OG, 7 mM) [10] or a combination of the protonophore uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP) (1 μM) and the ionophore monensin (5 μM). The TPP^+-selective electrode was calibrated by measuring Δψ of known value preset in the cells by induced K^+-diffusion potential in the presence of 1 μM valinomycin and the known K^+ concentration as described [11]. The Δψ value was calculated by the modified Nernst equation:

$$\Delta\Psi = 2.3\frac{RT}{F}\log(\frac{\vartheta \cdot c}{V \cdot (10^{\Delta E/59} - 1)}),$$

where *R* is the universal gas constant (8.314 J/mol·K); *T* is the absolute temperature (K); *F* is the Faraday constant (96,485 C/mol); ϑ is the specific inner cell volume (μl/mg); *V* is the volume of the compartment (μl); ΔE is the difference in the readings of the TPP^+ electrode between the energized and de-energized states of the cells (mV); and *c* is the number of bacterial protein (mg) introduced into the compartment.

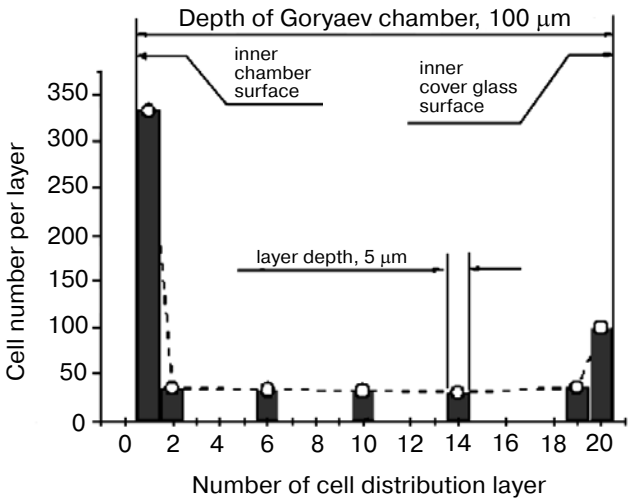

Fig. 2. Cell distribution of the halophilic strain of the sulfur-oxidizing bacterium *Thioalkalivibrio* from top to bottom in a Goryaev chamber after 2 h of rest.

RESULTS AND DISCUSSION

The calculation of electric potential of living cell membrane using the Nernst equation requires knowledge of the specific inner volume (ϑ) of the cells. The membrane potential of the extremely alkaliphilic bacterium *Tv. versutus* represented in this study by two strains (the moderately salt tolerant AL2 and the extremely salt tolerant ALJ15) was determined by measuring the specific inner cell volume in the AL2 strain by two independent methods: (a) light microscopy with cell counting in a Goryaev chamber and (b) the difference in distribution of tritium labeled water (^3H_2O) and nonpenetrating colored protein (cytochrome *c*). According to the data of electron microscopy, the two strains are of similar size and have similar inner cell structures; therefore, the specific inner volume measured for the AL2 strain was used for calculating the electric membrane potential of both strains: AL2 and ALJ15.

Cell counting in the Goryaev chamber and measurement of specific inner cell volume in suspension. Small bacterial cells of about 1 μm, confined to the habitats with extremely high salinity, do not completely precipitate to the chamber bottom in the media used for the study (Fig. 2). Total cell number in the chamber was the sum of cell number in each layer. The number of layers in the chamber depends on the depth of field of the analyzed layered images. In our experiment, the depth of field was 5 μm, so that the whole chamber volume was presented as 20 layers of 5 μm in height (Fig. 2). Because of the rod-like shape of the cells, correction coefficients (depending on rod size) were introduced for summing the cells in the layers to account for the doubling of cell number at the interface between the two neighboring conditionally isolated layers during direct counting. We counted the cells on photographic images manually and by an alternative semiautomatic method using a software procedure (Fig. 3). The results showed that the semiautomatic counting error is no more than 0.05% relative to the manual count-

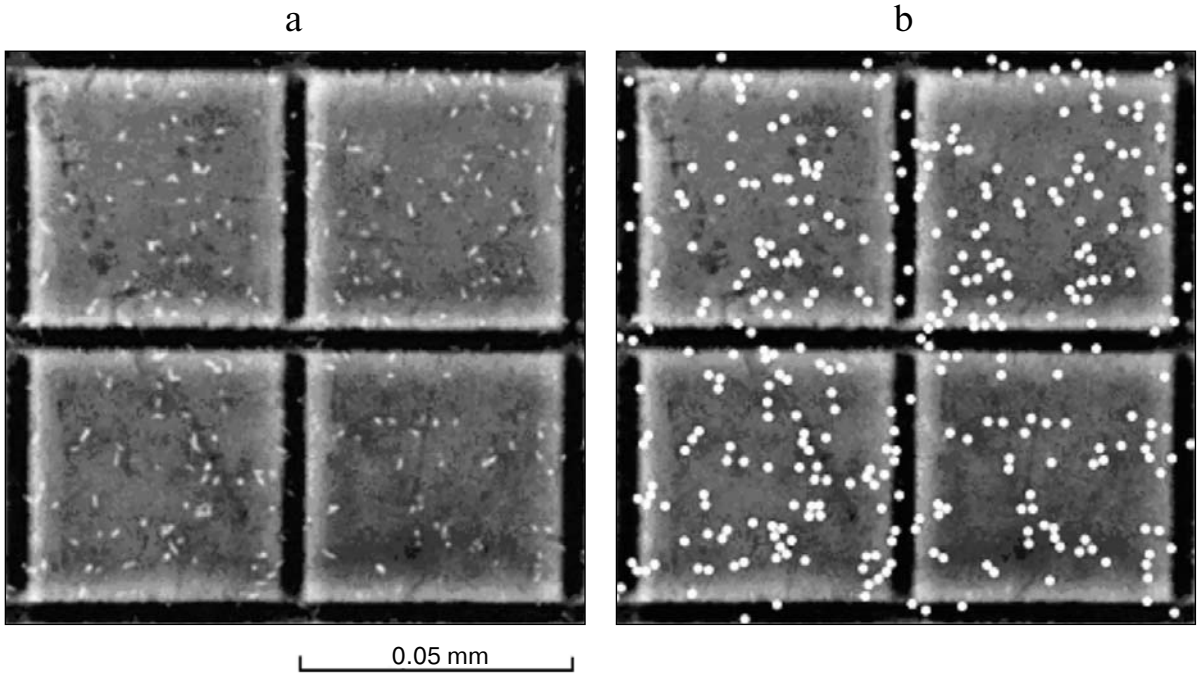

Fig. 3. Illustration of the method of cell counting in a Goryaev chamber by determining the area of figures when each cell on the photograph (a) is indicated by a white circle (b).

Table 1. Inner cell volume accessible to water and cell volume of the bacterium *Tv. versutus*

Method	Cell volume V_{cell}, μl/mg	Inner volume ϑ, μl/mg
Light microscopy	2.8 ± 0.2	2.4 ± 0.2
Distribution of ^3H_2O and cytochrome *c*	2.6 ± 0.1	2.2 ± 0.1

Table 2. Radioactivity counting in the tritium channel for the supernatant and precipitate after separation of the cell suspension by centrifugation

Phase	Counting result, cpm*		
	series 1	series 2	series 3
Supernatant	21648.2	22351.2	21508.8
Precipitate	1785.0	1926.3	1861.8

* Spread of the data in each series was below 2%.

ing. Cell number in the suspension, according to the data of three independent experiments, was 929 ± 17 per chamber volume of 10^{-6} ml. In our study, cell number was counted in a diluted suspension with predetermined protein concentration c_p = 0.082 mg/ml. Specific cell volume in the suspension of the strains was V_{cells} = 2.8 ± 0.2 μl/mg protein, and the inner cell volume was ϑ = $0.84 \cdot V_{cells}$ = 2.4 ± 0.2 μl/mg protein (see Table 1).

Determination of inner volume of bacterial cells based on data on distribution of tritium labeled water and non-penetrating colored protein. During determination of the volume of the intercellular space in the cell precipitate, the cytochrome *c* concentration in the supernatant before and after mixing with the precipitate was 40 ± 0.4 and 34.8 ± 0.3 μM, respectively. The intercellular volume was: V_I = 62.1 ± 0.4 μl. The V_{pH_2O} volume in the precipitate accessible to ^3H_2O was 242.3 ± 6 μl according to radioactive counting data upon averaging the results of all series (Table 2), and the average cell volume accessible to ^3H_2O was 180.2 ± 6 μl. Taking into account that the total cell protein was 80 mg in each series, the specific inner cell volume was ϑ = 2.2 ± 0.1 μl/mg and the mean specific cell volume was V_{cells} = 2.6 ± 0.1 μl/mg (see Table 1). The resulting specific inner cell volume of *Thioalkalivibrio* is close to the respective value for the cells of *Halobacterium halobium* (*salinarum*) (2.5 μl/mg protein) [9] and *Thiobacillus ferrooxidans* (2.4 μl/mg protein) [12], but lower than for the cells of *Bacillus acidocaldarius*

Table 3. Values of proton motive force components (electric membrane potential Δψ and pH gradient) on the membranes of bacteria with different habitat preferences

Bacterium	Additional characteristic	pH value of measurement	Δψ, mV	ΔpH, mV	Δp, mV	Source
Alkaliphilic						
B. pseudofirmus OF4	moderately salt tolerant	10.5	−180	+121	−46	[16]
B. firmus RAB	moderately salt tolerant	10.5	−179	+157	−22	[17]
B. alcalophilus	fresh water	8.5	−132	+81	−51	[14]
Tv. versutus AL2	moderately salt tolerant	10.0	−228	+165	⩾−80	this study
Tv. versutus ALJ15	highly salt tolerant	10.0	−224			this study
Neutrophilic						
E. coli		7.1	−137	−100	−237	[18]
Staphylococcus aureus		7.0	−128	−68	−196	[18]
S. faecalis		7.5	−135	−63	−197	[15]
Halobacterium salinarum	highly salt tolerant	8.2	−150	−47	−197	[19]
Acidophilic						
Thermoplasma acidophila		2.0	+110	−255	−145	[20]
Bacillus acidocaldarius		2.0	+34	−249	−215	[13]
Thiobacillus ferrooxidans		2.0	+10	−266	−256	[12]

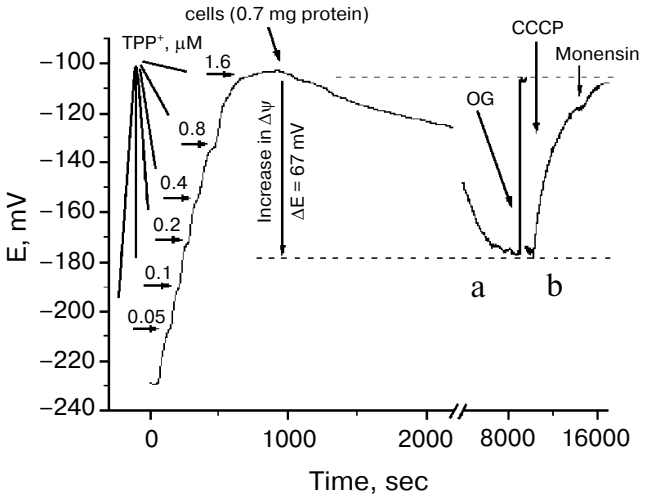

Fig. 4. Membrane potential measurement on intact *Tv. versutus* AL2 cells using the penetrating cation TPP^+. The measuring medium contained: Caps, 50 mM, pH 10, and NaCl, 0.6 M. The additives were introduced to the final concentrations: OG, 7 mM; CCCP, 1 μM; monensin, 5 μM.

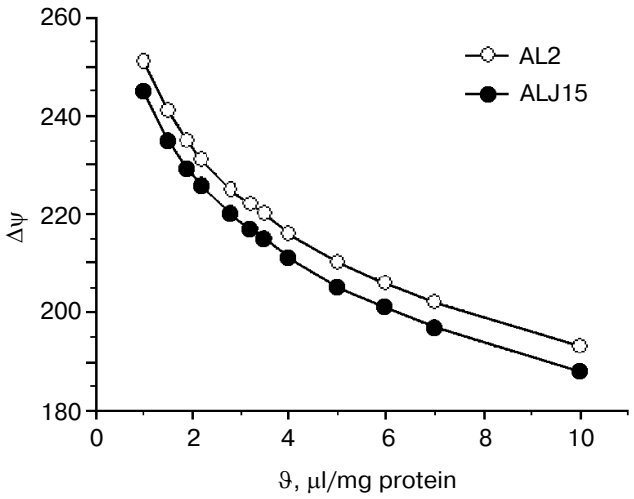

Fig. 5. Dependence of calculated Δψ on tentative inner cell volume ϑ values. The Δψ value was calculated from data on electric potential measurement on cells of strains *Tv. versutus* AL2 and ALJ15 and tentative ϑ values.

(3.6 μl/mg protein) [13] and *Bacillus alcalophilus* (17 μl/mg protein) [14] and higher than for the cells of *Streptococcus faecalis* (1.75 μl/mg protein) [15] and rat liver lysosomes (1.1 μl/mg protein) [9].

Cell membrane potential measurement using TPP^+. The difference of electric potential across the bacterial membranes was estimated immediately after growth of the culture. The activity of ion pumps on the cell membrane was maintained by adding 0.2 mM respiration substrate (sodium thiosulfate) to the measuring medium. When measuring the electric potential of living cells, culture dilution was increased to attain experimental approximation to constant Δψ value on cell membrane. Only the potential difference corresponding to the energized state of cells, without the contribution of nonspecific binding of lipophilic cations TPP^+, was taken into account. TPP^+ binding was considered nonspecific in the case of complete electric potential dissipation. The electric potential of the tested cells completely dissipated in the presence of a combination of the protonophoric uncoupler CCCP and the Na^+/H^+-exchanger monensin (Fig. 4a), as well as in the presence of nonionic detergent octyl glucoside disturbing the integrity of cell membranes (Fig. 4b). The measured Δψ values on the membrane of the strains are given in Table 3. According to the literature data, the values of membrane potential in alkaliphilic bacteria are higher than those in neutrophilic bacteria [21]. Our results show that the membrane potentials in the strains *Tv. versutus* AL2 (−228 mV) and ALJ15 (−224 mV) are notably higher compared to most of known alkaliphilic bacteria. Even assuming that the inner cell volume could be underestimated during our experimental determination, e.g. twofold, the electric potential for the cells with a twofold greater inner volume is still higher compared to the values for other known alkaliphilic bacteria (Fig. 5).

The H^+ (ΔpH) ion concentration difference on the membranes of alkaliphilic microorganisms, in contrast to acidophiles and neutrophiles (Table 3), has the opposite sign compared to the electric potential difference [21]. According to our preliminary data, the tested strains of the bacterium *Thioalkalivibrio* also have high H^+ gradient directed oppositely to Δψ, which results in low values of the calculated proton motive force (Δp) in this group of microorganisms (less than 80 mV in absolute values). As one can see, Δp values in alkaliphilic organisms are much less in magnitude than in neutrophilic and acidophilic organisms. For the two latter groups, Δp is from −250 to −150 mV. The question whether useful work such as ATP synthesis can be performed in the cells of alkaliphiles on account of low Δp at extremely high pH values (above 9.5) has not been solved. On one hand, ATP in alkaliphiles is synthesized in spite of low Δp; on the other hand, ATP synthesis does not occur in the cells and subcellular membrane vesicles with artificially induced diffusion potential at Δp = −162 mV [22]. In this context, it was supposed that alkaliphilic microorganisms inhabiting the environments with the high content of sodium ions could use the membrane electrochemical gradient of sodium ions instead of the proton gradient to perform useful biological work [23]. Our data are of consequence for further investigations of the problem of energetic coupling in extremely alkaliphilic microorganisms inhabiting alkaline lakes.

This work was supported by the Russian Foundation for Basic Research (project No. 11-04-00819a).

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