MEMBRANE POTENTIAL OF *THERMOPLASMA ACIDOPHILA*

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1. Introduction

Thermoplasma acidophila is a mycoplasma-like organism which grows optimally at 59°C and pH 2, and stops growing above pH 4 [1]. The intracellular pH lies between 6.4 and 6.9, as determined by the distribution of a radioactive weak organic acid, 5,5-dimethyl-2,4-oxazolidinedione (DMO). The cell can maintain this pH gradient of about 4.5 pH units when subjected to metabolic inhibitors, such as iodoacetate, NaN_3, and 2,4-dinitrophenol [2]. Because the cell can passively maintain such a huge pH gradient across the membrane, we propose that a Donnon potential exists, possibly generated by charged macromolecules impermeable to the cell membrane. To test this hypothesis, the membrane potential was measured by the distribution of radioactive $KS^{14}CN$, which is known to premeate biological membranes [3,4]. In addition, a radioactive lipophilic cation TEA^+ was used to determine the polarity of the cell.

2. Materials and methods

Thermoplasma acidophila was grown as described previously [2,5]. Aerated 18 l cultures were harvested at late log phase, after 22 h of growth, by centrifugation at 9000 × *g*, at 15°C for 5 min. Then, the cells were washed twice and resuspended in 1.5 mM $(NH_4)_2SO_4$, 4.2 mM $MgSO_4 \cdot 7\ H_2O$, 1.7 mM $CaCl_2 \cdot 2\ H_2O$, 0.02 M sucrose, and 0.01 M glycine buffer, pH 2. This medium has the same ionic strength, osmolarity, and divalent cation concentration as the growth medium. An 18 l culture usually yielded 35–40 ml of a cellular suspension containing 20–30 mg/ml protein. In aliquots of 1 ml, that cellular suspension was distributed into a set of centrifugation tubes (size 4 ml), followed by addition of 1 ml of the suspension buffer mentioned above to each tube.

In a typical experiment, 8 tubes were used for gravimetrically determining the total pellet water, V_t. Another 8 tubes were incubated with [^{14}C]dextran (mol. wt 16 000) to measure the extracellular pellet water, V_e. In addition, 8 tubes were incubated with ~ 0.1 μCi $KS^{14}CN$, 60 mCi/mM specific activity, and 8 tubes with ~ 0.1 μCi [^{14}C]TEAB. After incubation for one hour at 56°C (the amount of KSCN accumulated in the cells did not depend upon the length of incubation which was varied from 10 min to 2 h), all 32 tubes were chilled to 4°C, then centrifuged simultaneously at 9000 × *g* for 10 min. The supernatant was transferred to test tubes, each pellet was resuspended in 2 ml of the above mentioned suspension buffer in a separate test tube. 1 ml from each test tube was dried separately in a scintillation counting vial. After drying, 0.065 ml water and 0.5 ml NCS tissue solubilizer (Amersham/Searle) was added, followed by addition of 10 ml of a toluene solution containing PPO (3 g/l) and dimethyl-POPOP (0.1 g/l), and counted in a Packard Tricarb scintillation instrument. The intracellular water volume is $V_i = V_t - V_e$, where $V_e = C_p(\text{dextran})/C_s(\text{dextran})$. Here C_p are the counts of total pellet material, and C_s are

Abbreviations: TEA^+ = tetraethylammonium, TEAB = tetraethylammonium bromide, CCCP = carbonyl cyanide, *m*-chlorophenyl hydrazone, DNP = dinitrophenol

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the counts per millilitre of supernatant. The intracellular concentration of SCN^- is calculated from $[SCN^-]_{in} = \{C_p(SCN^-) - C_s(SCN^-) \cdot V_e\}/V_i$eq(1). The membrane potential ψ, positive inside, was calculated from $\psi = (RT/F) \cdot \ln \{[SCN^-]_{in}/[SCN^-]_{out}\}$.

3. Results and discussion

The SCN^- anion was accumulated in the cells whereas the TEA^+ cation was not accumulated in the cells. This demonstrates that the cells are positive inside. The membrane potential ψ lies between 109 and 125 mV, positive inside (table 1). Because ψ is a logarithm of a ratio, the variation within each set of experiments is small, at most 3–5 mV, but variations between different sets of experiments may be larger. This may arise from the error made when estimating the intracellular water space; *e.g.*, a 100% error causes only a 10% change in the calculated membrane potential.

In order that the ratio $[SCN^-]_{in}/[SCN^-]_{out}$ truly reports the membrane potential, the following conditions must be fulfilled: (1) KSCN should be mostly in the ionized form, (2) SCN^- is passively permeable to the cell membrane, in the sense that no substrate-specific active accumulation of SCN^- into the cell takes place, (3) the SCN^- concentration should be small enough such that the SCN^- itself does not perturb the membrane potential. On the other hand, a high external SCN^- concentration should depress the observed potential, because it is lowered by any passively permeable anion present at high concentrations.

Because HSCN is a rather strong acid with a $pK = -1.85$ [6], at pH 2, more than 99% of SCN^- should be in the ionized form. To test whether the SCN^- anion passively permeates the cell membrane, two kinds of experiment were performed. First, the SCN^- accumulated inside the cell can be readily washed out by the same buffer applying three washes (fig.1). For comparison, acetate cannot be washed out (fig.1). Secondly, increasing the external non-radioactive KSCN concentration to 1 mM, the amount accumulated depends linearly on the KSCN concentration (fig.1). However, upon increase of the external SCN^- concentration to 0.01 M, the membrane potential was reduced to 87 mV; upon further increase to 0.1 M, the membrane potential was lowered to 54 mV (table1). This behaviour confirms the notion that at lower concentrations the SCN^- distribution does indeed monitor the membrane potential as we proposed.

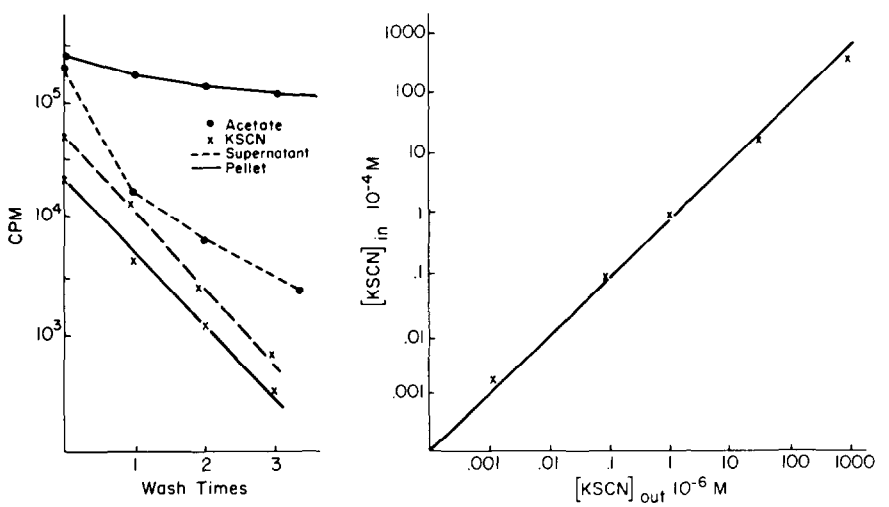

Fig.1 Left: removal of radioactive material by washing *Thermoplasma acidophila* with suspension buffer. Right: dependence of intracellular SCN^- concentration on the extracellular concentration in *Thermoplasma acidophila*.

Table 1

Determination of the membrane potential in *Thermoplasma acidophila* by measuring the distribution of $S^{14}CN^-$ across the plasma membrane[a]

	Expt. 1	Expt. 2	Expt. 3	Expt. 4 [Control]	0.01N KSCN	0.1N KSCN
C_s [^{14}C]Dextran cpm × 10^4	8.66	3.42	8.50	7.99	7.65	9.31
C_p [^{14}C]Dextran cpm × 10^3	4.66	2.49	4.89	4.79	4.45	4.64
V_t (ml)	0.0625 ± 0.001	0.0999 ± 0.002	0.0678 ± 0.001	0.074 ± 0.001	0.074 ± 0.001	0.065 ± 0.002
V_e (ml)	0.054 ± 0.003	0.073 ± 0.004	0.0576 ± 0.003	0.060 ± 0.005	0.058 ± 0.005	0.050 ± 0.003
V_i (ml)	0.009 ± 0.004	0.027 ± 0.006	0.0102 ± 0.004	0.014 ± 0.006	0.016 ± 0.006	0.015 ± 0.005
C_s ($KS^{14}CN$) cpm × 10^4	3.92	5.21	5.75	5.48	6.34	10.4
C_p ($KS^{14}CN$) cpm × 10^3	32.1	84.3	46.2	38.0	25	15.5
$[SCN^-]_{in}/[SCN^-]_{out}$	86 ± 10	57 ± 4	70 ± 4	45.5 ± 5	22.2 ± 2.2	6.9 ± 1.0
ψ	125 ± 5	114 ± 2	120 ± 2	109 ± 2.4	87.5 ± 3	54 ± 4.2

	Expt. 5 [Control]	10 mM NaN_3	Expt. 6 [Control]	1 mM 2,4-DNP	Expt. 7 [Control]	5 mM CCCP
	9.39	9.22	8.51	8.90	7.62	8.41
	4.18	4.0	4.37	4.45	2.48	2.48
	0.053 ± 0.001	0.0515 ± 0.001	0.061 ± 0.001	0.059 ± 0.001	0.036 ± 0.001	0.034 ± 0.001
	0.044 ± 0.003	0.043 ± 0.003	0.051 ± 0.004	0.050 ± 0.004	0.0325 ± 0.001	0.0295 ± 0.001
	0.009 ± 0.004	0.0084 ± 0.004	0.010 ± 0.005	0.009 ± 0.005	0.004 ± 0.002	0.0045 ± 0.02
	3.95	5.6	2.77	3.1	6.94	4.9
	22.9	23.5	18.3	19.7	21	20.3
	53.2 ± 8	45.5 ± 3.3	61 ± 7	65 ± 8	70 ± 9	79 ± 12
	112 ± 4.4	108 ± 2	116 ± 3	117 ± 8	118 ± 5	112 ± 5

[a]All experiments were done at 56°C and pH 2

Influence of high KSCN concentrations and inhibitors upon the membrane potential were measured parallel to the control from the same cell batch. Expt. 1–7 were each from different batches, but the experimental conditions in each were the same. C_s represents the counts per milliliter of supernatant, C_p represents the counts of total pellet material. They are typical data of a single tube selected from a group of 8 tubes.

V_t is the total pellet water space. V_e is the extracellular pellet water space. $V_i = V_t - V_e$ is the intracellular water space. The membrane potential was calculated from:

$$\psi = \frac{RT}{F} \ln \frac{[SCN^-]_{in}}{[SCN^-]_{out}} = 65 \frac{[SCN^-]_{in}}{[SCN^-]_{out}}$$

where the anion concentration $[SCN^-]_{in}$ was calculated from eq. (1).

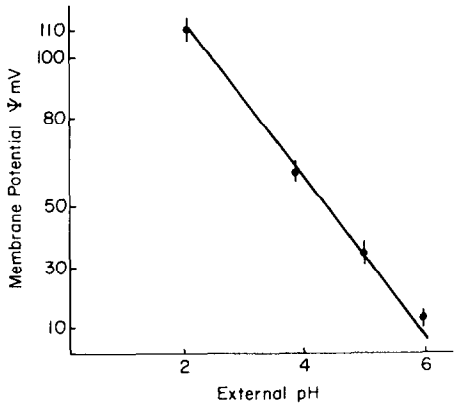

Fig.2. Dependence of membrane potential on the external pH of *Thermoplasma acidophila* suspended in suspension buffer (pH 2 and 3:8). For pH 5 and 6, the glycine buffer was replaced by citrate buffer.

In the presence of 10 mM NaN_3, an electron transport inhibitor, or 1 mM 2,4-dinitrophenol, or 5 mM CCCP, which are proton-conducting uncouplers, the measured membrane potential remains unchanged (table 1). As reported previously [2], similar inhibitors exerted no measurable influence upon the intracellular pH. Therefore, both the membrane potential and the pH gradient are maintained passively. The 120 mV membrane potential, positive inside, compensates only partially the pH gradient of 4.5 pH units. The membrane potential decreased linearly upon increase of the external pH (fig.2). At pH 6, the potential is diminished to less than 15 mV. From fig.2, one finds a slope of 28 mV/pH unit which is approximately one half of RT/F. We have presently no explanation for this factor of ½.

At the end we would like to raise the question whether the chemiosmotic theory of energy coupling, proposed by P. Mitchell [7–9] can be applied to this organism; *i.e.*, can the respiratory transport of H^+ and the return of H^+ to induce ATP synthesis be the mechanism for energy transduction in *Thermoplasma acidophila?* In this organism the huge pH gradient is partially balanced by the membrane potential, positive inside. The net electrochemical gradient in our microorganism at the resting amounts to approximately 170 mV, *i.e.*, 290 mV at 56°C for the pH gradient minus 120 mV for the Donnan potential.

Is the value of 170 mV incompatible with the Mitchell hypothesis? The answer is negative since the electrochemical potential of about 300 mV required for ATP synthesis is not the net electrochemical potential at the resting state but rather the proton motive force in the energized state [10]. Obviously our investigation does not provide any direct information in support of the chemiosmotic theory either.

Acknowledgement

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