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ζ-POTENTIAL AND SURFACE CHARGE OF *THERMOPLASMA ACIDOPHILA*

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SUMMARY

The surface charge density and the ζ-potential of *Thermoplasma acidophila* was estimated from microscopic electrophoresis experiments. The cells moved towards the positive electrode. The mobility remained constant from pH 2 to 5, and increased for pH values higher than 6. The mobility at pH 6 decreased dramatically with increased external Ca^{2+} concentration. At pH 2 and an ionic strength similar to that of the growth medium, the ζ-potential was about 8 mV, negative relative to the bulk medium; the surface charge density was 1360 esu/cm^{-2} which corresponds to one elementary charge per 3500 A^2.

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-oxazolidine-dione (DMO) [2]. The membrane potential had a value of 120 mV (positive inside) as determined by the distribution of radioactive SCN^-, an anion of a strong acid [3]. The cell can passively maintain the pH gradient and membrane potential when subjected to metabolic inhibitors, such as NaN_3, and 2,4-dinitrophenol [2, 3].

The membrane potential measured by the distribution of SCN^- is the potential difference between the bulk phase of the cytoplasm to that of the outside medium. The ζ-potential, i.e. the potential difference from the cell surface of shear relative to the bulk medium, and the surface charge density can be estimated from the electrophoretic mobility of the cell. The movement of the cell in an applied electric field can be observed directly under a microscope [4, 5]. The following equations can be used to calculate the ζ-potential and the surface charge density [4–6]:

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electrophoretic mobility $u = \frac{D\zeta}{4\pi\eta}$,

surface charge density $\sigma = \sqrt{\frac{NDkT}{2000\pi}} \cdot \sqrt{\Sigma c_i\,[\exp(-z_i e\zeta/kT)-1]}$.

Here, D is the dielectric constant, η is the viscosity of the suspension medium, c_i are molar concentrations of ions with charge z_i, N is the Avogadro number.

These equations are valid for large smooth particles at an ionic strength such that $\kappa\alpha > 100$. Here, α is the radius of curvature of the cell surface, κ is the Debye-Hückel constant defined as

$$\kappa = \sqrt{\frac{8Ne^2}{1000\,DkT}} \cdot \sqrt{\tfrac{1}{2}\Sigma c_i z_i^2}$$

For *T. acidophila* with a diameter from 0.5 to 1 μm, the condition $\kappa\,\alpha > 100$ holds for the ionic strength used in this investigation.

It is the purpose of this investigation to determine the potential profile between the two interfaces of the plasma membrane. This profile can be constructed from the bulk-to-bulk potential (already known from the SCN^- distribution experiments) and the ζ-potential described below.

MATERIALS AND METHODS

T. acidophila was grown in aerated 1-l cultures in a medium described previously [7]. After 11 h of growth, the cells were harvested at late log phase by centrifugation at $9000\times g$, at 15 °C for 5 min. Then the cells were washed twice and resuspended in TG buffer which consists of 0.01 M glycine buffer at pH 2, 0.02 M KCl and solution T containing 1.5 mM $(NH_4)_2SO_4$, 4.2 mM $MgSO_4 \cdot 7H_2O$, and 1.7 mM $CaCl_2 \cdot 2H_2O$. This TG buffer has the same ionic strength and divalent cation concentration as that of the growth medium.

The electrophoresis instrument used was a Northrop-Kunitz cataphoresis unit, purchased from Thomas Scientific Co., Philadelphia, Pa. The electrophoretic cell was mounted on the stage of a Zeiss photomicroscope which was equipped with a planachromatic objective $16\times/0.35$. The depth of the cataphoresis unit chamber was 380 μm as determined with the micrometer scale of the microscope. A Sony video camera was attached to the microscope and connected to a Sony-matic video recorder, model AV-3600, and also to a Sony Electrohome TV monitor. A Heathkit regulated power supply, model IP-17, was used as a constant voltage source. The distance between the two tips of the platinum electrodes in the centre chamber was 3.2 cm. The potential difference between these tips was monitored at the beginning, end, and occasionally during the experiments; it measured 36 V, when 200 V were applied, and 17.5 V, when 100 V were applied at the $Zn/ZnSO_4$ electrodes (0.5 M $ZnSO_4$).

The movement of the *T. acidophila* cells was visually observed and recorded on video tapes. The distance travelled was followed on the TV screen and calibrated with a micrometer. The mobility was measured at two stationary levels, which were calculated to lie at 0.211 and 0.789 of the total depth of the chamber. A further check with human red blood cells showed a mobility of -1.3 ± 0.13 $\mu m \cdot s^{-1} \cdot (V \cdot cm^{-1})^{-1}$

in 0.067 M phosphate saline, which value is consistent with the literature value [8]. All electrophoresis experiments were carried out at 24 °C.

The cells were harvested, then resuspended in TG buffer, then washed twice, then resuspended in the following electrophoresis medium. For pH values from 2 to 4, glycine buffer was used; for pH values from 4 to 10.5, glycyl-glycine buffer was used. For pH values from 2 to 6, three kinds of suspension media were employed, (a) plain 0.01 M buffer, (b) 0.01 M buffer with 0.02 M KCl, and (c) 0.01 M buffer with 0.02 M KCl plus solution T defined above. For pH 6 to 10.5 studies, only the third kind of suspension medium was employed. To investigate the dependence of mobility on the Ca^{2+} and K^+ concentrations, electrophoresis was carried out with 0.01 M glycine buffer at pH 2, and 0.01 M glycyl-glycine buffer at pH 6, over a concentration range varying from 10^{-5} to 10^{-1} M of the cation concentration.

RESULTS

T. acidophila cells moved from the negative to the positive electrode. Thus, the net surface charge is negative, with a surface ζ-potential negative relative to the bulk of the medium.

Table I lists the electrophoretic mobility, the ζ-potential, and the surface charge density σ at various ion concentrations. The mobility (Fig. 1) remained con-

TABLE I

ELECTROPHORETIC MOBILITY u, ζ-POTENTIAL, SURFACE CHARGE DENSITY σ OF *T. ACIDOPHILA* AT VARIOUS pH VALUES, IONIC STRENGTH, AND ION CONCENTRATIONS

The parameters μ, ζ, and σ were determined from the equations given in Introduction, using $D = 78$ and $\eta = 0.01$ cP.

Suspension medium	Ionic strength	$10^8/\kappa$ (cm^{-1})	$-u^*$ $\mu m \cdot s^{-1}/V \cdot cm^{-1}$	ζ^* (mV)	σ (esu/cm^{-2})
pH 2, 0.01 M glycine buffer	0.01	30.3	0.94	12.3	850
pH 2, 0.02 M KCl	0.03	17.5	0.80	10.6	1260
pH 2, 0.02 M KCl+solution T**	0.06	12.8	0.64	8.3	1360
pH 6, 0.01 M glycyl-glycine buffer	0.01	30.3	2.74	36.1	2677
pH 6, 0.02 M KCl	0.03	17.5	2.24	29.4	3677
pH 6, 0.02 M KCl+solution T**	0.06	12.8	1.27	16.7	2826
pH 2, 10^{-3} M KCl	0.01	28.9	0.86	11.3	817
pH 2, 0.05 M KCl	0.06	12.4	0.47	6.2	1035
pH 2, $5 \cdot 10^{-5}$ M $CaCl_2$	0.01	29.8	0.81	10.6	743
pH 2, $5 \cdot 10^{-4}$ M $CaCl_2$	0.01	26.6	0.71	9.3	745
pH 2, $5 \cdot 10^{-3}$ M $CaCl_2$	0.04	15.1	0.38	5.1	718
pH 6, 10^{-3} M KCl	0.01	28.9	2.5	32.9	2526
pH 6, 0.05 M KCl	0.06	9.9	2.2	29.2	5161
pH 6, $5 \cdot 10^{-5}$ M $CaCl_2$	0.01	29.9	2.4	31.6	2362
pH 6, $5 \cdot 10^{-4}$ N $CaCl_2$	0.01	26.6	0.98	12.9	1042
pH 6, $5 \cdot 10^{-3}$ M $CaCl_2$	0.04	15.2	0.47	6.2	882

* The standard deviation of u and ζ is about 10 % each.

** Solution T: 1.5 mM $(NH_4)_2SO_4$, 4.2 mM $MgSO_4 \cdot 7H_2O$ and 1.7 mM $CaCl_2 \cdot 2H_2O$.

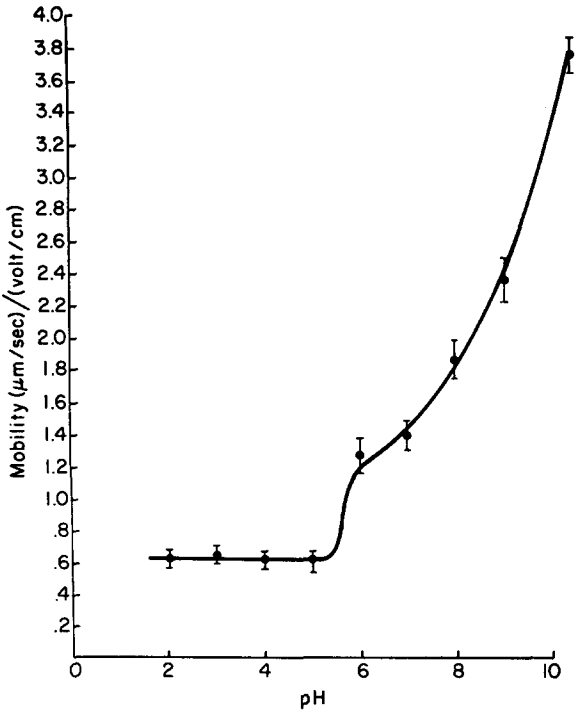

Fig. 1. pH dependence of electrophoretic mobility of *T. acidophila* cells. The suspension medium was 0.02 M KCl and solution T (1.5 mM $(NH_4)_2SO_4$, 4.2 mM $MgSO_4 \cdot 7H_2O$, 1.7 mM $CaCl_2 \cdot 2H_2O$), and 0.01 M glycine buffer (pH 2–4). For experiments from pH 4 to 10.5, the glycine buffer was replaced by 0.01 M glycyl-glycine buffer.

stant around $u = -0.6\ \mu m \cdot s^{-1} \cdot (V \cdot cm^{-1})^{-1}$ in the pH range from 2 to about 5. When the external pH was enhanced to pH 6, the mobility of the *T. acidophila* cells increased abruptly to $u = -1.3\ \mu m \cdot s^{-1} \cdot (V \cdot cm^{-1})^{-1}$, followed by a steady increase as the pH became alkaline (Fig. 1). In 0.01 M glycine buffer, pH 2, with 0.02 M KCl and solution T, which lies close to the ionic strength and divalent cation concentration of the growth medium, the surface charge density is 1360 esu/cm^{-2}. This charge density corresponds to $5.7 \cdot 10^4$ elementary charges per cell or about one elementary charge per 3500 A^2 (assuming a cell with a diameter of 0.8 μm).

The effects of Ca^{2+} and K^+ concentration on the mobility of the cell is shown in Fig. 2. At pH 2, and at concentrations below 1 mM, both K^+ and Ca^{2+} exert a slight effect on the mobility of the cell. The mobility was reduced by only 50 %, when the cation concentration was increased to 0.01 or 0.1 M, which is presumably an ionic strength effect. At pH 6, the effect of Ca^{2+} concentration on the electrophoretic mobility was dramatic. The mobility had a value of $u = -2.7\ \mu m \cdot s^{-1} \cdot (V \cdot cm^{-1})^{-1}$, when the Ca^{2+} concentration was lower than $2.5 \cdot 10^{-5}$ M, and changed to $u = -0.5\ \mu m \cdot s^{-1} \cdot (V \cdot cm^{-1})^{-1}$; i.e. a 5-fold decrease in mobility when the Ca^{2+} concentration increased to 0.01 M and higher. The Ca^{2+} concentration which reduced the mobility to half its value was $2.5 \cdot 10^{-4}$ M. At pH 6, K^+ concentrations similar to those of Ca^{2+} had little effect on the electrophoretic mobility of *T. acidophila* cells.

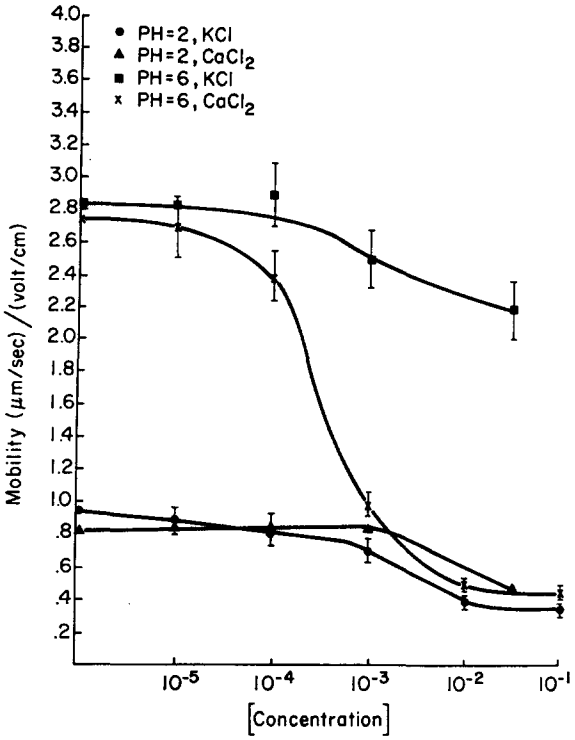

Fig. 2. Dependence of electrophoretic mobility of *T. acidophila* cells on the cation concentration. For pH 2, 0.01 M glycine buffer, for pH 6, 0.01 M glycyl-glycine buffer was used.

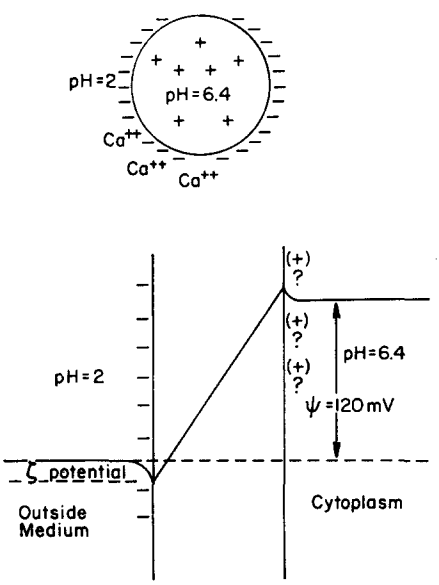

Fig. 3. pH and potential profile of *T. acidophila* cells. A ζ-potential of about 8 mV exists between the outer surface of shear to the bulk phase of the external medium.

DISCUSSION

The surface charge of *T. acidophila* was inferred to be negative. This conclusion is based on several facts: (a) The cells move from the negative electrode to the positive one; (b) when the external pH was raised above pH 6, the mobility increased. This resulted presumably from the fact that more negative charges were exposed by raising the pH, since the pK of some carboxyl groups lies around 4.5; (c) Ca^{2+}, in the concentration range of 1 mM and higher, reduced the electrophoretic mobility at pH 6. This can be explained by assuming that Ca^{2+} are specifically bound to the negatively charged cell surface, probably to the phosphate head groups of the phospholipids. This conclusion is based on two facts: First, K^+ concentrations of comparable ionic strength reduced the mobility only slightly. Secondly, the Ca^{2+} concentration needed in reducing the mobility was rather low, viz. $2.5 \cdot 10^{-4}$ M Ca^{2+} could reduce the electrophoretic mobility to half the value measured in the absence of the bivalent ion (Fig. 2). At pH 2, most of the binding sites are protonated, and therefore few Ca^{2+} can be bound. At pH 6, however, a high Ca^{2+} concentration (> 0.05 M) could reduce the mobility of *T. acidophila* cells to a level usually found at pH 2 (Fig. 2). Thus, H^+ and Ca^{2+} have similar and competitive effects in reducing the mobility of cells. This interpretation of Ca^{2+} binding to the cell surface is consistent with the Ca^{2+} effect on the phase transition temperature of *T. acidophila* membranes as determined by the EPR spin labelling technique [9].

On the basis of measurements of the membrane potential by the method of SCN^- distribution, the surface charge density, and the ζ-potential derived from the electrophoretic mobility, the pH and potential profile of *T. acidophila* cells may be drawn (Fig. 3).

The total charge of the cell is negative, the bulk potential across the membrane is positive inside, and the surface is highly negatively charged. Therefore, inside the cell some positive ions or macromolecules may exist which cannot penetrate the cell membrane. These particles are responsible for the Donnan potential which is positive inside the cell. We do not have any direct evidence for such an existence, but we do have some indicative evidence. From pH 7 to 10.5, the mobilities of the cells are rather high (Fig. 1), resulting probably from leakage of cytoplasmic or membrane proteins (the proteins do indeed leak out at pH > 7; see ref. 7). Since the mobility increased significantly, the materials which leaked out must carry a positive charge.

We do not have any information about the surface on the cytoplasmic side of the membrane; it may be positive or negative. In Fig. 3 it is drawn as positive for illustrative purposes. Thus, a ζ-potential may also exist on the inner surface of the membrane. Consequently, the "real potential across the bilayer" may be 15 mV higher than the bulk-to-bulk phase potential difference measured by SCN^- distribution.

The surface ζ-potential will also influence the local pH near the surface of the membrane [10]. If the cytoplasmic side of the membrane really has a surface potential more positive than that of the bulk of the cytoplasm, then the local pH near the inner surface is more alkaline than in the bulk phase, and the local pH at the external surface is lower than that of the bulk of the medium.

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