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CHANGE IN ZETA POTENTIAL AND MEMBRANE POTENTIAL OF SLIME MOLD *PHYSARUM POLYCEPHALUM* IN RESPONSE TO CHEMICAL STIMULI

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SUMMARY

Electrophoretic study of microplasmodia of the true slime mold *Physarum polycephalum* was carried out in the presence of various concentrations of inorganic salts, nucleotides and sugars, and the zeta potential at the surface of the plasmodia was determined from the electrophoretic mobilities. The membrane potential of the plasmodia was also measured under the same external conditions. It was shown that changes in the membrane potential induced by the chemical stimuli agreed approximately with those induced in the zeta potential in all cases examined. These results suggested that the phase boundary potential at the membrane-solution interface is mainly responsible for the membrane potential in the chemoreception of the slime mold.

INTRODUCTION

The plasmodium of the true slime mold exhibits positive or negative chemotaxis to various kinds of chemical substances such as inorganic salts, acids, nucleotides and sugars. Since the plasmodium of slime mold is a huge aggregate of cells having neither boundary membrane nor cell wall, the plasmodium provides a suitable preparation for studying the physicochemical mechanism of chemoreception and taxis at receptor membrane level. In a previous paper [1] concerned with the chemotaxis of the slime mold, we demonstrated that the motive force of tactic movement and the membrane potential changed simultaneously and discontinuously at a certain threshold for respective chemicals, and that the threshold concentration of the chemoreception is closely correlated to the instability of colloidal systems. Disclosures of the mechanisms of how a chemical stimulus induces a change in the membrane potential and how the membrane transmits the potential change to the motile system are indispensible for basic understanding on chemoreception and taxis in the slime mold.

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In the present study, spherical microplasmodia obtained with the submersed culture method are subjected to the electrophoretic measurements under the presence of various chemical stimuli. The zeta potential of the plasmodia, determined from the electrophoretic mobility, is compared to the membrane potential of the slime mold. The charge density of the surface membrane of the mold changes rather discontinuously at the threshold when the concentration of a chemical stimulus increases successively. The possible mechanism of generation of the potential change in response to chemical stimuli in the slime mold is then discussed.

EXPERIMENTAL

Materials. Microplasmodia of the slime mold *Physarum polycephalum* were kindly furnished by Dr. J. Ohta at the Ochanomizu University, in the form of shake flask cultures of actively growing plasmodia. Culture of the plasmodia was performed at 23 °C according to the method of Daniel and Rusch [2]. 8-ml portions of 4-day cultures were used to inoculate 500-ml shaking flasks containing 100 ml of the culture medium. The plasmodia used for the experiments were taken from 3- or 4-day cultures. The electrophoretic mobility of the plasmodia did not change appreciably with the length of growth period.

All chemicals used were analytical grade with no further purification. Water used as solvent was doubly distilled in an all glass vessel.

Experimental procedures and measurements of electrophoretic mobility. Transfer of the microplasmodia from the culture medium into distilled water caused disruption of the organisms. Therefore, a solution containing 10 mM sucrose and 2 mM NH_4Cl (hereafter referred to as the standard solution) was used as a basal medium in the subsequent experiments. The pH of the standard solution was 5.5. The plasmodia taken from the culture medium were washed with standard solution by centrifugation three times at about $1000 \times g$ for 30 s and suspended in the standard solution under continuous shaking in the dark for more than 5 h. This procedure was necessary to starve the microplasmodia, which had been adapted to the culture medium containing a relatively high concentration of nutrients, including 5.5×10^{-2} M glucose. The electrophoretic mobility of the microplasmodia did not change in at least 24 h in the standard solution so long as the suspension was shaken continuously.

The electrophoretic mobility was measured by a microelectrophoretic apparatus (Karl Zwiss, West Germany, Cytopherometer). At a given concentration of a chemical stimulus, 10–20 microplasmodia, the diameters of which were between 80 and 100 μm, were timed over 284 μm (142 μm in each direction) at the front "stationary layer" under the potential gradient between 3 and 8 V/cm applied to the vessel through a pair of platinum electrodes. The Pt electrode immersed in saturated KCl solution is separated from the test solution by a diaphragm. Variation both in potential gradient applied and in the size of plasmodia studied led to no appreciable change in the observed value of the electrophoretic mobility, u. These facts imply that the Helmholtz-Smoluchowski equation for the electrophoresis of a charged particle is applicable to the present system [3]. The medium was frequently changed in order to avoid contamination of the basal medium caused by any disruption of plasmodia. Temperature was controlled at a desired temperature (20 °C

in most cases) within ± 0.3 °C by circulating thermostatted water.

Determination of zeta potentials. Under the experimental conditions employed here, the product of the radius of the microplasmodia and the Debye-Hückel function (reciprocal of the thickness of ion atmosphere), κ, was much larger than 300. Therefore, the zeta potential, ζ, was safely evaluated from the observed electrophoretic mobility, u, with the aid of the Helmholtz-Smoluchowski equation [3];

$$\zeta = (4\pi\eta/D)u \tag{1}$$

where η and D are the viscosity and dielectric constant of the medium, respectively. The viscosity was determined by an Ostwald viscometer. The dielectric constant was assumed to be the same as that of pure water.

Calculation of surface charge density. The surface charge density of the microplasmodium was calculated from the observed zeta potential by use of the Gouy-Chapman equation [3];

$$\sigma = \sqrt{\frac{DRT}{2000\pi}} \cdot \sqrt{\Sigma C_c(e^{-z_c e\zeta/kT}-1)+\Sigma C_a(e^{-z_a e\zeta/kT}-1)} \tag{2}$$

where σ is the surface charge density in e.s.u./cm^2, e the electronic unit charge, z_a, z_c, C_a, and C_c are the valencies and concentrations of anion and cation species, respectively, and other symbols have their usual thermodynamic meanings. When the salt involved is symmetric one having a common valence $|z|$, Eqn. 2 is simplified to give

$$\sigma = 2\sqrt{\frac{DRT}{2000\pi}} \sqrt{c} \sinh\left(\frac{|z|e\zeta}{2kT}\right) \tag{3}$$

Measurements of the membrane potential. When a microelectrode is inserted in the plasmodia of the slime mold, the membrane is formed rapidly at the tip of the electrode, which makes it difficult to measure stable intracellular potential. The membrane formation at the tip of electrode was prevented by putting di-sodium ethylenediaminetetraacetate (EDTA) in the microelectrode. Hence, a microelectrode filled with a solution containing 3 M KCl and 0.1 M EDTA was used for the measurement of the intracellular potential. The membrane potential of the microplasmodia was -90 ± 10 mV in the standard solution. The change in the membrane potential in response to chemical stimuli is also observed with the plasmodia prepared by the surface culture method [4] with the aid of the double chamber method proposed by Kamiya [5], details of which were described in the previous paper [1]. The data of the membrane potential presented in this article were mainly obtained with the double chamber method, because the dependence of the membrane potential on the concentration of chemical stimuli determined by the microelectrode method gave essentially the same result as the double chamber method, so far as the change in the potential is concerned. This will be shown experimentally in Fig. 1.

All experiments were carried out at 20 °C unless otherwise noted.

RESULTS

Effect of inorganic salts.

Fig. 1 represents the zeta potential of the microplasmodia of slime mold as a function of the logarithmic concentration of various inorganic salts. As seen in the

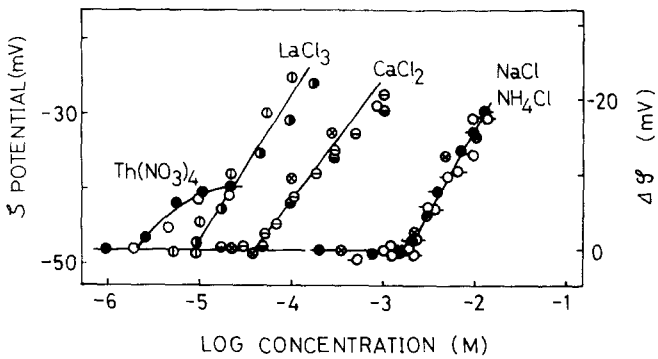

Fig. 1. Zeta potential, ζ, and membrane potential, $\Delta\varphi$, of plasmodia of slime mold as a function of salt concentration. ζ : ○, $Th(NO_3)_4$; ◫, $LaCl_3$; ⊖, $CaCl_2$; –○, NaCl; ○–, NH_4Cl. $\Delta\varphi$: ●, $Th(NO_3)_4$; ◑, $LaCl_3$; ◒, $CaCl_2$; –●, NaCl; ●–, NH_4Cl. ⊗ marks show the change in the intracellular potential for $CaCl_2$ and NaCl measured by a micro-electrode inserted into the plasmodia. Other $\Delta\varphi$ values were obtained by the double chamber method. The potential level in the standard solution is taken as zero for both cases (see text). Note that the membrane potentials observed with two methods agree quite well each other for all cases examined so long as the concentration dependences of the membrane potential is concerned. Temperature, 20 °C.

figure, the zeta potential stays at a nearly constant level, i.e. -48 ± 5 mV, until the concentration of added salt attains the threshold, C_{th}, for respective salt species. When the concentration, C, of the chemical exceeds the threshold, the zeta potential starts to change discontinuously in the positive direction. When the concentration rises to about 10 times higher than the threshold, aggregation of the microplasmodia becomes appreciable and the number of independent particles decreases. Hence the zeta potential in the high salt concentration media was hard to measure. As seen in the figure, the threshold concentration, C_{th}, decreases remarkably with increase of valences of cation, z_c. As pointed out in the previous paper [1], C_{th} was found to be proportional to z_c^{-6}, that is, the Shulze-Hardy rule [6] known in the field of colloid chemistry was applicable.

The microplasmodia have a membrane potential of about -90 mV in the standard solution, being negative on the inside of the cell with respect to the outside, and undergo depolarization when the plasmodia are subjected to various salts in concentration higher than their respective thresholds. The ordinate on the right hand side in Fig. 1 indicates the scale for the membrane potential, where the potential in the standard solution is taken as zero. Except in the case of $Th(NO_3)_4$, the curves in the figure are essentially identical to those for the membrane potential change shown in the previous paper [1]. The slope of the curve for $Th(NO_3)_4$ in the figure is much smaller than that given in the previous study. This discrepancy is probably attributable to the difference of the medium: 10 mM sucrose/2 mM NH_4Cl is used in the present study, while distilled water was taken as the basal medium in the previous study. The effects of sugars on the salt reception of the slime mold will be reported in detail in a subsequent paper (Terayama, K., et al., in preparation).

It is important to note that the change in membrane potential in response to salts agrees approximately with the corresponding change in the zeta potential. The implication of this fact will be discussed later.

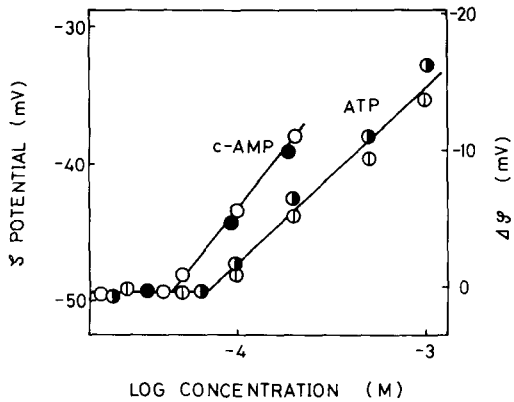

Fig. 2. Zeta potential and membrane potential of slime mold measured with the double chamber method as a function of cyclic AMP and ATP added in the external solution at 20 °C. ζ : ○, cyclci AMP; ⓘ, ATP. $\Delta\varphi$: ●, cyclic AMP; ◑, ATP.

Effect of nucleotides and sugars

Fig. 2 shows zeta potential and membrane potential as a function of log concentration of cyclic AMP and ATP, while Fig. 3 illustrates the same relation for glucose, galactose and mannose. As in the case of the salt reception mentioned above, both the membrane potential and the zeta potential remain at a constant level until the concentration of the reagent reaches its respective threshold, and then start to change in a positive direction as the concentration becomes greater than the threshold. As described previously, the chemotactic movement of the slime molds took place at the same threshold concentration as the potential change, e.g. 10^{-4} M for glucose, $6 \cdot 10^{-4}$ M for mannose, respectively (both act as attractants). Note that the data shown in Figs. 2 and 3 indicate that changes in zeta potential and membrane potential agree approximately with each other for the substances studied here.

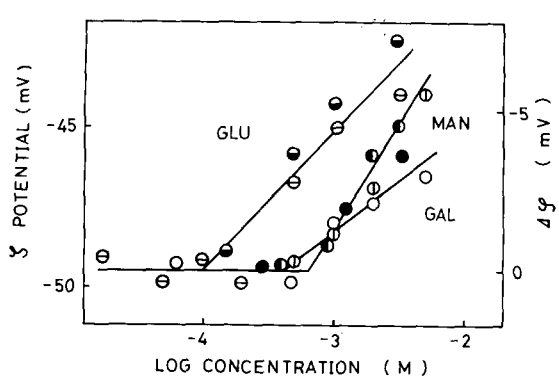

Fig. 3. Zeta potential, ζ, and membrane potential, $\Delta\varphi$, of slime mold measured with the double chamber method as a function of sugar concentrations in the external media. ζ : ⊖, glucose; ○, galactose; ⓘ, mannose. $\Delta\varphi$: ◒, glucose; ●, galactose; ◐, mannose. Temperature 20 °C.

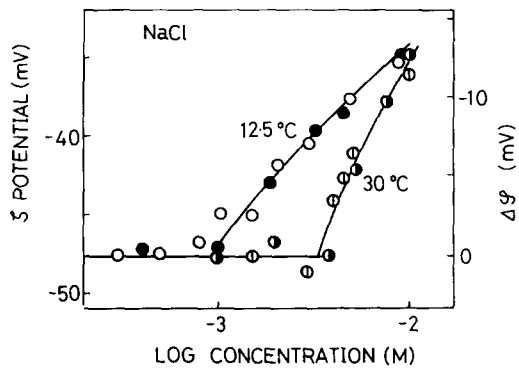

Fig. 4. Zeta potential, ζ, and membrane potential, $\Delta\varphi$, observed with the double chamber method as a function of NaCl concentration at different temperatures. ζ : ○, 12.5 °C; ⓘ, 30 °C. $\Delta\varphi$: ●, 12.5 °C; ◑, 30 °C.

Effect of temperature

Fig. 4 represents the effect of temperature on the relationship between the zeta potential or the membrane potential and the concentration of NaCl added to the basal medium. As seen in the figure, the constant level of the zeta potential below the threshold was practically independent of the surrounding temperature. The threshold for NaCl decreased with a decrease in temperature, i.e. the thresholds were 3 mM at 30 °C, 2 mM at 20 °C, and 1 mM at 12.5 °C. A similar temperature dependence was obtained with the membrane potential for NaCl, as illustrated in Fig. 4. An excellent agreement was obtained again between the zeta potential and the membrane potential observed at different temperatures.

DISCUSSION

Fig. 5 represents the surface charge density of the slime mold in the presence of various chemical stimuli, calculated from the data of zeta potential given in Figs. 1, 2 and 3. The surface charge density σ of the slime mold in the standard solution is calculated to give about -1800 e.s.u./cm^2. Note that the absolute value of σ depends strongly on the ionic strength in medium according to Eqn. 2 or 3. The charge density, σ, starts to change at the threshold concentration of the chemical stimulus except for the case of 1:1-type inorganic salts, e.g. NaCl. It is considered that the change in σ of the slime mold is attributable to the adsorption of ionic component on the receptor membrane or the conformational change in the membrane induced by the reception of chemical stimulus, or to both. For the case of sugars, the change in the surface charge density can probably be attributed to the conformational change of the receptor membrane because the sugars used are non-electrolytes. The data shown in the figure indicate that the surface charge density decreases with the the reception of non-electrolyte stimuli. For example, σ becomes approx. -1500 e.s.u./cm^2 in 1 mM glucose solution, which indicates that 10–15 % of the negative net charge is buried in the receptor membrane when a conformational change of the receptor membrane is induced by glucose reception. The gradual change in σ may be interpreted as a successive increase of the number of domains, the conforma-

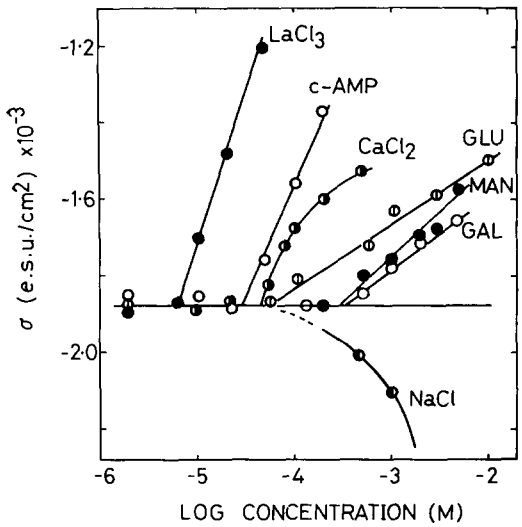

Fig. 5. The density of surface charge of slime mold, σ, in e.s.u./cm^2, as a function of concentrations of various chemical stimuli. Notations are given in the figure.

tional change of which was induced by sugar reception, with increase of sugar concentration.

The results obtained in the present study indicate that a good agreement exists between the changes in the zeta potential and the membrane potential in all cases examined. Furthermore, the electric resistance of surface membrane of plasmodia of slime mold in NaCl and $CaCl_2$ solutions remained constant within experimental accuracy unless the salt concentrations exceeded 10 times their respective thresholds. According to Teorell [7] and Meyer and Sievers [8], the membrane potential is represented by the sum of the intramembrane diffusion potential and the two phase boundary potentials at the membrane-solution interface. When the membrane under consideration is compact enough [9] and when the density of charges fixed in the membrane is much higher than the salt concentration in the external solution, permeation of ions across the membrane scarcely occurs. In such cases, the intramembrane diffusion potential becomes negligible in comparison with the potential difference at the membrane surface, and hence the phase boundary potential is mainly responsible for the membrane potential across the membrane [10]. Recently, we found that a model membrane made of a Millipore filter impregnated with the total lipids extracted from bovine tongue epithelium simulated well the taste receptor potential in response to electrolyte stimuli [11], and that the membrane potential of the model membrane stemmed from the electrical potential at the membrane-solution interface [12, 13]. From these results, together with various observations on taste reception in the frog, we proposed that taste receptor potential is ascribable to a change in electrical potential at the interface between gustatory receptor membrane and stimulating solution [12, 14, 15]. The present study has offered further strong evidence that the change in the membrane potential in response to chemical stimulations is attributable to the potential at the membrane-solution interface because the zeta potential is closely correlated with the phase boundary potential.

It is worthwhile to note that the changes in zeta potential of a suspension of a cation-exchange resin in response to various salts are similar to those in the phase boundary potential, i.e. the Donnan potential at the membrane surface, in spite of very low electrical resistance in the resin [16].

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