J. Membrane Biol. 37, 1–12 (1977)

Variation in Selectivity of Univalent Cations in Slime Mold *Physarum Polycephalum* Caused by Reception of Polyvalent Cations

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Received 3 January 1977; revised 30 March 1977

Summary. Specificity of reception on 1:1 electrolytes in the slime mold *Physarum polycephalum* was investigated in the presence of polyvalent cations in media. Membrane potential and motive force of tactic movement were examined with the aid of the double chamber method, and the zeta potential at the membrane surface of the slime mold was measured by electrophoretic mobility. The results obtained are summarized as follows: (1) The presence of polyvalent cations (e.g., Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, Th^{4+}) in medium led to an increase in threshold concentration, C_{th}, determined from the potential measurements for Na- or Li-salts, and to a decrease in C_{th} for K-, Rb- or NH_4-salts. C_{th} for 1:1 electrolytes changed discontinuously when the concentration of polyvalent cations in medium exceeded their respective thresholds. (2) The C_{th} determined from chemotaxis agreed with that from the potential response both in the presence and absence of polyvalent cations. (3) Sequence of selectivity of univalent cations varied extensively in the presence of polyvalent cations. (4) Changes in the zeta potential induced by NaCl reception agreed with those in the membrane potential even in the presence of Ca^{2+} in medium. (5) The C_{th} for reception of NaCl changed sharply at about 12 °C in the presence of polyvalent cations, while that for KCl was independent of the temperature.

Conformational changes in surface membrane of the slime mold in response to reception of polyvalent cations were then discussed in relation to the discrimination of univalent cations.

It is well known that divalent cations interact with univalent cations antagonistically in many biological membrane systems. For example, the responses to univalent cations are suppressed by the presence of divalent cations in a fish lateral line organ [15], and in frog taste organs [14, 17]. The abrupt depolarizations in cell potential in squid giant axons and in the excitable protoplasmic droplets isolated from *Nitella* caused by univalent cations are suppressed by Ca^{2+} added in media [11, 16, 19, 20]. The antagonistic interaction between univalent and divalent cations

is considered to play an indispensable role in the appearance of various functions in biological membranes, but the relevant physicochemical mechanism remains almost unknown at the present.

The plasmodium of the true slime mold *Physarum polycephalum* exhibits positive or negative chemotaxis to various kinds of chemical substances [1, 3, 9, 18, 21, 22, 23]. The plasmodium of the slime mold is a suitable experimental organism to study the physicochemical mechanism of chemoreception and taxis at the receptor membrane level, because the plasmodium is a large aggregate of protoplasm without boundary membrane separating the cellular components. In a previous paper [23], we demonstrated that the motive force of chemotactic movement and the membrane potential of the slime mold changed simultaneously at a certain threshold concentration, C_{th}, for respective chemicals, and that the slime mold discriminated various univalent cations. The sequence or the selectivity for univalent cations determined from the C_{th} values was $Li^+ < K^+ < Na^+ < Rb^+ < Cs^+ < NH_4^+$ in pure water. In the present study, we investigated the selectivity of 1:1 electrolytes reception of the slime mold in the presence of polyvalent cations in the external solution, and we examined variations of univalent cation selectivity accompanying the reception of polyvalent cations. The sequence of cation selectivity described above will be shown to change discontinuously when Ca^{2+} of other polyvalent cations exceeded their respective threshold concentrations. The implication of these results is discussed in relation to the structural change of the surface membrane of the slime mold.

Materials and Methods

The true slime mold *Physarum polycephalum* used in the present study was kindly furnished by Prof. N. Kamiya of Osaka University, and cultured by the method employed by Camp [2]. Microplasmodia of the slime mold *Physarum polycephalum* used in the electrophoretic study were a gift of Prof. J. Ohta at Ochanomizu University. Culture of the microplasmodia was performed according to the method proposed by Daniel and Rusch [5].

Measurements of the membrane potential and the motive force of protoplasmic movement were performed by using a double chamber method proposed by Kamiya [12, 13], the details of which were described in the previous paper [23]. Two pieces of plasmodia were placed in two compartments which were connected by a plasmodial strand through a narrow ditch. The opening of the ditch was filled with silicone grease and the chambers were covered by a slide glass so as to make the two compartments airtight and to insulate them electrically, except for the plasmodial strand. The basis of the method lies in the fact that the protoplasmic streaming can be controlled by applying a difference in hydrostatic pressure between the two compartments, and that the potential difference between them affords a measure of the membrane potential [21, 22, 23]. The

effect of the presence of polyvalent cations in medium on the response to 1:1 electrolytes was examined as follows: aqueous solution of a given concentration of a polyvalent cation was placed in both compartments, and then the solution in one compartment was replaced with a 1:1 electrolyte solution containing the same concentration of the polyvalent cation. The differences in the membrane potential and motive force of taxis were measured successively by increasing the added 1:1 salt concentration.

The zeta potential of the microplasmodia of the slime mold was measured by the method accomplished by Hato *et al.* [10], by using a microelectrophoretic apparatus (Carl Zeiss, Cytopherometer). In the present study, a solution containing 10 mM sucrose and 1 mM NaCl was used as the standard solution. The zeta potentials were measured by increasing concentration of 1:1 electrolyte with a given concentration of a polyvalent cation added to the standard solution.

All experiments were performed at a room temperature, 20 ± 0.5 °C, except the experiments studying the temperature effect. Dependency of the threshold on the temperature was examined by regulating the room temperature within ± 0.5 °C at a desired value ranging between 6 and 30 °C. All chemicals used were analytical grade without further purification. Water used as solvent was distilled twice by glass vessels.

Results

Effects of Ca^{2+} on Reception of 1:1 Electrolytes

Fig. 1 shows the dependences of the membrane potential, $\Delta\phi$, zeta potential, ζ, and of chemotactic motive force, $\overline{\Delta P}$, on NaCl concentration. In Fig. 1, $\Delta\zeta$ is defined by $\Delta\zeta = \zeta - \zeta_o$, where ζ_o stands for the zeta potential in NaCl solution whose concentration is lower than the threshold. The value of ζ_o depends slightly on the $CaCl_2$ concentration when it exceeds 6×10^{-5} M, e.g., $\zeta_o = -48$ mV in the standard solution and in 2×10^{-5} M, while $\zeta_o = -45$ mV in 10^{-4} M $CaCl_2$. In the absence of $CaCl_2$ in medium, $\Delta\phi$ started to change in the direction of depolarization at the concentration of 2.5×10^{-3} M NaCl as illustrated by ○ marks. The variation of the zeta potential, $\Delta\zeta$ (◐ marks), agreed with that of $\Delta\phi$. The plasmodium showed a negative taxis at the same concentration and the motive force of tactic movement $\overline{\Delta P}$ stayed at the level of about 10 cm H_2O with further increase of NaCl concentration. The negative value of $\overline{\Delta P}$ represents a negative taxis. Note that the presence of 2×10^{-5} M $CaCl_2$ in medium led to appreciable influence on the $\Delta\phi$, $\Delta\zeta$, and ΔP *vs.* log[NaCl] relations. In the presence of 10^{-4} M $CaCl_2$ in medium, however, $\Delta\phi$ and $\Delta\zeta$ started to change at the concentration of 10^{-2} M NaCl, and the plasmodium showed negative taxis at the same concentration (● marks). In other words, the presence of 10^{-4} M $CaCl_2$ in medium increased the threshold concentration, C_{th}, for NaCl from 2.5×10^{-3} to 10^{-2} M. The data shown in Fig. 1 illustrate that the changes in $\Delta\phi$ in response to NaCl agree with the corresponding

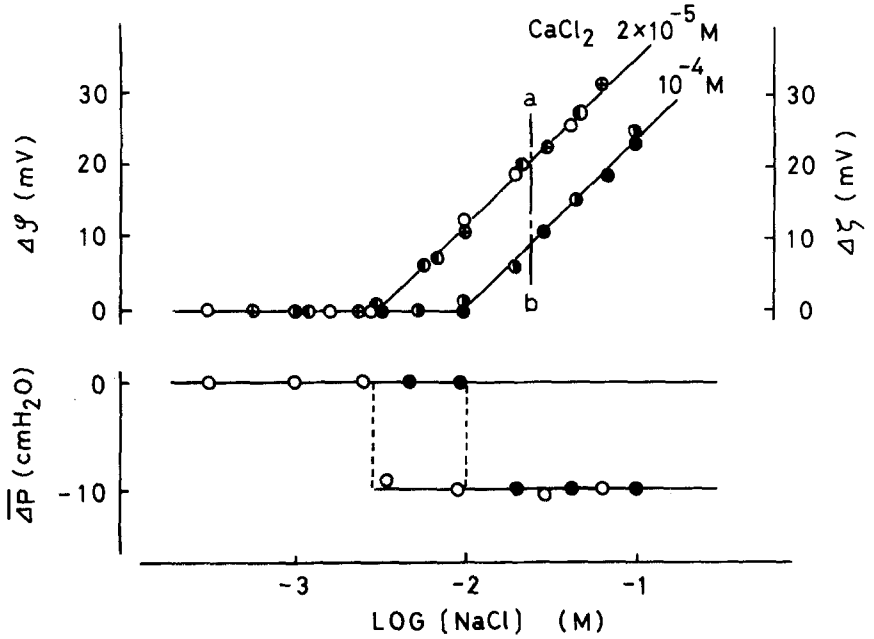

Fig. 1. Changes in membrane potential, $\Delta\phi$, zeta potential, $\Delta\zeta=(\zeta-\zeta_o)$, and motive force of taxis, $\overline{\Delta P}$, as a function of NaCl concentration in the presence and absence of $CaCl_2$. ○: $\Delta\phi$, $\overline{\Delta P}$ in the absence of $CaCl_2$ in media; ⊕: $\Delta\phi$ in the presence of 2×10^{-5} M $CaCl_2$; ◐: $\Delta\zeta$ in the presence of 2×10^{-5} M $CaCl_2$; ●: $\Delta\phi$, $\overline{\Delta P}$ in the presence of 10^{-4} M $CaCl_2$; ◑: $\Delta\zeta$ in the presence of 10^{-4} M $CaCl_2$. Depolarization of the membrane potential is taken as positive

changes in zeta potential in the absence and presence of $CaCl_2$. The agreement between zeta and membrane potentials was obtained in all concentrations of $CaCl_2$ examined, although a discontinuous change in the C_{th} for NaCl reception resulted at a certain concentration of $CaCl_2$. The implication of these results will be discussed later.

Fig. 2 shows a similar plot of $\Delta\phi$ and $\overline{\Delta P}$ as a function of KCl concentration in the absence and the presence of 3×10^{-4} M $CaCl_2$ in the external solution. In the absence of $CaCl_2$, the variation of $\Delta\phi$ took place at 10^{-3} M KCl, above which point the slime mold showed negative chemotaxis. Contrary to NaCl, the presence of 3×10^{-4} M $CaCl_2$ led to a marked decrease in the C_{th} for KCl reception where a depolarization of $\Delta\phi$ took place at about 10^{-4} M KCl, and $\Delta\phi$ changed in two steps, in the manner seen in Fig. 2. The slime mold showed a weak positive taxis (maximum of $\overline{\Delta P}\cong5$ cm H_2O) in the range of $10^{-4}-10^{-3}$ M KCl concentration. $\overline{\Delta P}$ became zero at 10^{-3} M KCl, and the slime mold showed negative taxis when the concentration of KCl increased further. Since the

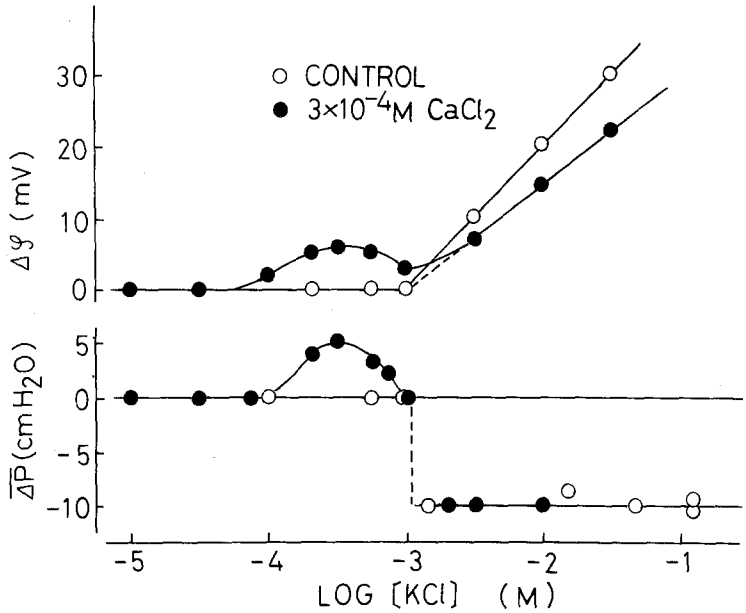

Fig. 2. Changes in membrane potential, $\Delta\phi$, and in chemotactic motive force, $\overline{\Delta P}$, as a function of KCl concentration in the presence of 3×10^{-4} M $CaCl_2$ (●) and in the absence of $CaCl_2$ (○)

slime mold showed appreciable changes both in $\Delta\phi$ and $\overline{\Delta P}$ at 10^{-4} M KCl, this point is refered to as the threshold for KCl in the presence of 3×10^{-4} M $CaCl_2$. Response to NH_4Cl or RbCl in the presence of $CaCl_2$ was essentially in a fashion similar to that of KCl, i.e., an appreciable decrease in C_{th} with a depolarization in membrane potential and a weak positive taxis. On the other hand, responses of the slime mold to LiCl were similar to those for NaCl when $CaCl_2$ concentration in medium exceeded 6×10^{-5} M, i.e., the threshold concentration of $CaCl_2$ itself.

Fig. 3 summarizes the change in the C_{th} for chloride salts of Li, K, Na, Rb and NH_4 as a function of $CaCl_2$ concentration in media both in logarithmic scales. When the $CaCl_2$ concentration exceeded the threshold for $CaCl_2$, the C_{th} for each chloride salt changed discontinuously. Fig. 3 shows that the C_{th} for NaCl and LiCl increased about 4 and 10 times, respectively, in the presence of $CaCl_2$ above 6×10^{-5} M, as compared with the cases with no $CaCl_2$ in media, i.e., 3×10^{-3} M for NaCl, and 5×10^{-4} M for LiCl. When the concentration of $CaCl_2$ exceeded 10^{-3} M in the case of NaCl, and 3×10^{-3} M in the case of LiCl, the C_{th} for these salts returned to their respective original levels. Contrary to NaCl or LiCl, the C_{th} for KCl, RbCl and NH_4Cl decreased

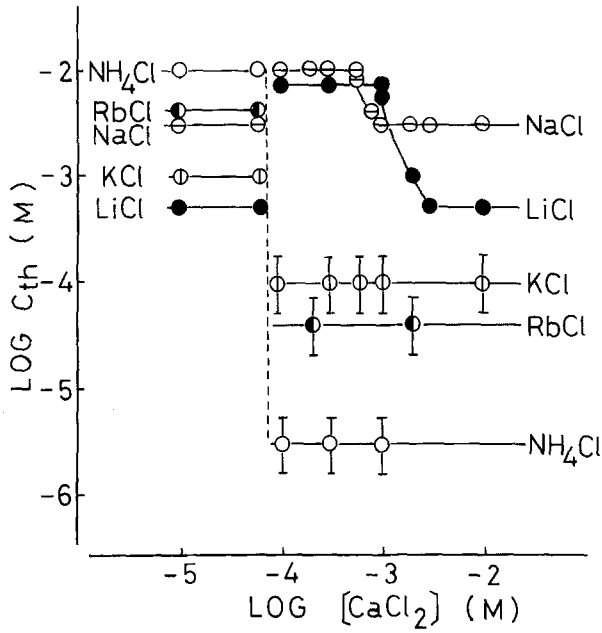

Fig. 3. The threshold concentrations, C_{th}, of LiCl, KCl, NaCl, RbCl and NH_4Cl as a function of concentration of $CaCl_2$ added in the external solution

discontinuously and stayed at a constant level of 10^{-4} M, 4×10^{-5} M and 3×10^{-6} M, respectively, with further increase of $CaCl_2$ concentration. The slime mold remarkably changed the selectivity to univalent cations when $CaCl_2$ concentration exceeded the threshold of $CaCl_2$, i.e., 6×10^{-5} M. The sequence of univalent cation selectivity in the absence of Ca^{2+} ($Li^+ < K^+ < Na^+ < Rb^+ < NH_4^+$) was changed to $NH_4^+ < Rb^+ < K^+ < Li^+ < Na^+$. When the concentration of Ca^{2+} in medium exceeded about 10^{-3} M, the sequence became $NH_4^+ < Rb^+ < K^+ < Li^+ < Na^+$.

Discrimination of Anions in 1:1 Electrolytes in the Presence of Ca^{2+}

In the previous paper [23], we showed that the C_{th} for various 1:1 salts carrying a common cation with different anions fell on a straight line when log C_{th} is plotted against the lyotropic number of anions, N. The lyotropic number of anions was determined from the relative concentration required to cause the coagulation of agar or protein solution [20, 24]. Fig. 4 shows the relation between log C_{th} and N for NaCl, LiCl, KCl and NH_4Cl in the presence of 3×10^{-4} M $CaCl_2$ and

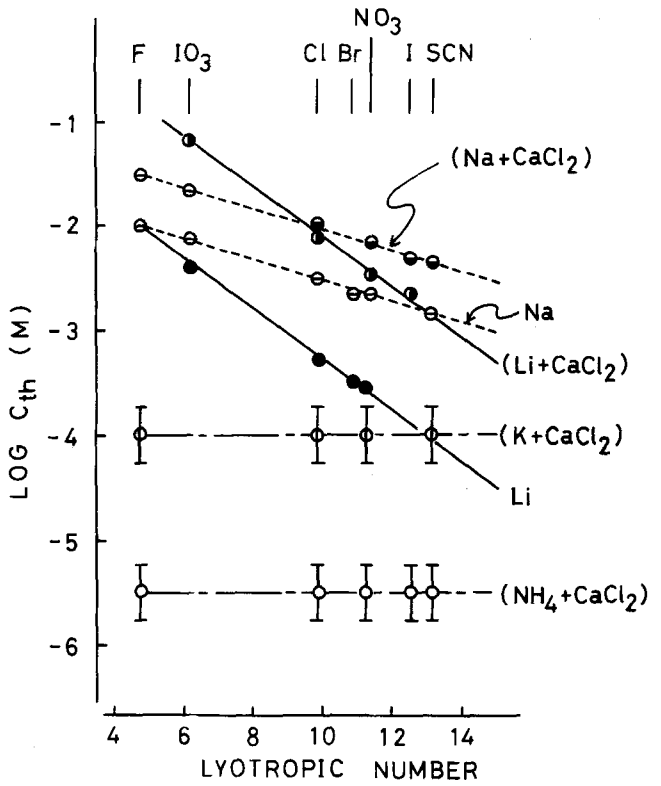

Fig. 4. Relations between the C_{th} for 1:1 type salts and the lyotropic number of anions in the presence of 3×10^{-4} M $CaCl_2$ and in the absence of $CaCl_2$. Anion species are indicated at the top in the figure, and cations used are noted at the right of respective lines

with no $CaCl_2$ in media. It is noted that the C_{th} of Na-salts with different anions increased the C_{th} by the same amount in the presence of $CaCl_2$ compared with those in the absence of $CaCl_2$; namely, the straight line in log C_{th} *vs.* N plots for Na-salts in the presence of $CaCl_2$ was shifted in parallel from the straight line for Na-salts without $CaCl_2$. The similar relation between log C_{th} *vs.* N relation was observed for Li-salts as illustrated in Fig. 4. On the other hand, the C_{th} for K-salts decreased remarkably in the presence of 3×10^{-4} M $CaCl_2$, and stayed at a constant level, 10^{-4} M, irrespective of the species of anions involved. NH_4-salts with different anions showed the similar dependency of C_{th} on N as shown in the figure. NH_4-salts with different anions showed the same value of C_{th}, 10^{-2} M, in pure water independent of anion species [23], which was changed to 3×10^{-6} M in the presence of $CaCl_2$.

As shown above, the C_{th} for receptions to 1:1 electrolytes of the slime mold in the presence of $CaCl_2$ differed from one salt to the other

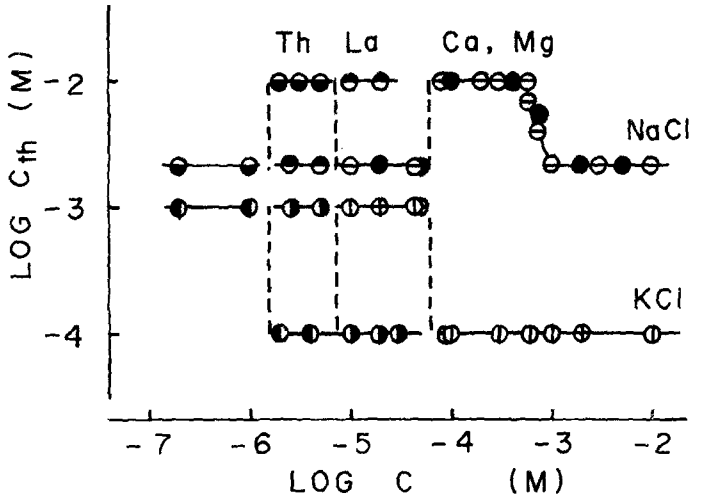

Fig. 5. The C_{th} of NaCl and KCl as a function of concentration of polyvalent cations added in media. ⊖: Th^{4+}, ●: La^{3+}, ○: Ca^{2+}, ●: Mg^{2+} for NaCl; ◐: Th^{4+}; ◑: La^{3+}; ⊕: Ca^{2+}; ⊖: Mg^{2+} for KCl

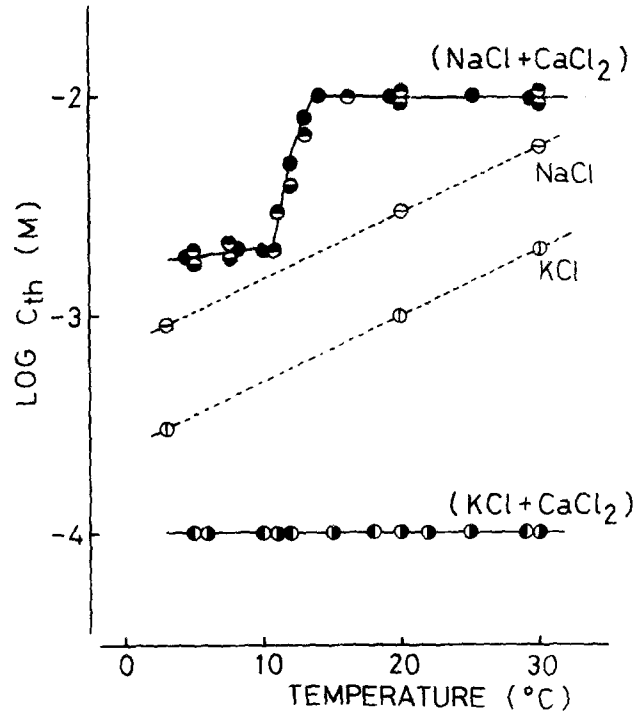

Fig. 6. Temperature dependences of the C_{th} for NaCl and KCl in the presence and absence of $CaCl_2$. $CaCl_2$ concentration are 10^{-4} M (⊖, ◐), 3×10^{-4} M (●, ◑), 6×10^{-4} M (⊖), and 0 M (⊖, ⊕). Salt species in each cases are indicated in the figure at the right of respective lines

depending on the species of cation involved, and which were quite different from those in the absence of Ca^{2+}. Similar results were observed not only with Ca^{2+} but also with the other polyvalent cations. Mg^{2+}, Sr^{2+}, and Ba^{2+} changed the selectivity for univalent cations discontinuously when the concentration exceeded 6×10^{-5} M as in the case of Ca^{2+}. La^{3+} and Th^{4+} changed the selectivity for univalent cations at 7×10^{-6} M and 1.5×10^{-6} M, respectively. The discontinuous changes in the C_{th} for KCl and NaCl in the presence of various polyvalent cations are illustrated in Fig. 5 as a function of polyvalent cation in media. Note that the concentration of polyvalent cations which induced the discontinuous changes in C_{th} for univalent cation reception agreed with the threshold concentration of respective cations [23].

Effects of Temperature on the C_{th} for NaCl and KCl

Fig. 6 shows the dependences of the C_{th} for NaCl and KCl on temperature in the absence and the presence of $CaCl_2$. The C_{th} for NaCl changed sharply at about 12 °C when the concentration of $CaCl_2$ exceeded its threshold, 6×10^{-5} M. Contrary to the C_{th} for NaCl, the C_{th} for KCl did not show any temperature dependency and stayed at a constant level of 10^{-4} M when the concentration of $CaCl_2$ in media exceeded 6×10^{-5} M. These temperature dependencies of the C_{th} for KCl and NaCl in the presence of $CaCl_2$ differ from those in the absence of $CaCl_2$, which are shown by broken lines in the figure. Similar results are obtained with polyvalent cations other than Ca^{2+}. The results shown above indicate that the surface membrane in the presence of polyvalent cations behaves in entirely different ways for reception of Na^+ and K^+.

Discussion

The results shown in Fig. 1 indicated that an increase of $CaCl_2$ concentration from 2×10^{-5} to 10^{-4} M at a given concentration of NaCl was associated with a large decrease in $\Delta\zeta = \zeta - \zeta_o$ of the slime mold when the concentration of NaCl was higher than the C_{th} (*see* chained line *a–b* in Fig. 1). Since the zeta potential is proportional to the surface charge density at the membrane surface at a given ionic strength in medium [10], the decrease in $\Delta\zeta$ corresponds to an increase in negative charge density at the membrane surface of the slime mold. This increase in the density of negative fixed charge caused by an addition of $CaCl_2$ is

not attributable to the Ca^{2+} adsorbed on the membrane surface because Ca^{2+} has positive charge. Therefore, the increase of the negative charge must be attributed to a conformational change of the surface membrane induced by reception of $CaCl_2$, where either an appearance of negative charge or a disappearance of positive charge is associated. It is interesting to note that the conformational change occuring at the membrane surface by reception of $CaCl_2$ could be observed only when the concentration of NaCl exceeded the C_{th}. An increase of the negative surface charge led to an increase of the C_{th} for NaCl in the zeta potential, which in turn led to an increase of the C_{th} for NaCl measured by the membrane potential, because the changes in the zeta potential coincided with those in the membrane potential both in the presence and the absence of $CaCl_2$, as pointed out previously.

The conformational change caused by reception of a chemical stimulus could also be observed when the slime mold was subjected to nonelectrolyte stimuli, such as sugars or alcohols. Although in these cases, the negative charge density at the membrane surface decreased by the reception of nonelectrolytes both for attractants and repellents [10].

The selectivity to univalent cations was affected remarkably by the presence of polyvalent cations. Also, the temperature dependences of the C_{th} for KCl and NaCl are different from each other when a polyvalent cation concentration in medium exceeded its own threshold (*see* Fig. 6). These facts imply that the characteristics of the membrane of the slime mold before and after the reception of a polyvalent cation are quite different from each other. If the concentration of Ca^{2+}, for example, in the external solution is decreased successively, the C_{th} for NaCl decreases while that for KCl increases discontinuously at 6×10^{-5} M $CaCl_2$ (*see* Fig. 3). Thus, the selectivity of the membrane to K^+ and Na^+ is changed in different directions by the elimination of Ca^{2+} from the membrane surface of the slime mold. The different responses of biomembranes between K^+ and Na^+ caused by Ca^{2+} concentration have been observed in various functions in biological systems as described in the introduction. The data presented here may throw a light on the mechanism of these antagonistic actions between uni- and divalent cations.

The selectivity of univalent cations in biomembranes is one of the central problems in the studies of living cells. Selectivity in the membrane system was analyzed theoretically by Eisenman some years ago [6, 7] (*see also* [8]) in terms of the hydration energy of cations and the coulomb force acting between ions and the anionic site in the membrane. He predicted that only 11 sequences of cation selectivity are possible in

nature. As shown in the previous paper [23], however, the sequence of cation selectivity observed with the slime mold in pure water was not included in the classifications proposed by Eisenman. The sequence of cation selectivity in the presence of polyvalent cations presented in this paper was also not in line with the Eisenman theory. It would be desirable to investigate more generally the origin of selectivity to cations in membrane systems.

The present study was supported partly by a grant-in-aid from the Ministry of Education in Japan.

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