

Threshold Phenomena in Chemoreception and Taxis in Slime Mold *Physarum polycephalum*

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ABSTRACT The plasmodium of *Physarum polycephalum* reacts to various kinds of chemical substances and moves towards or away from them. Threshold concentration of recognition of chemicals was examined in terms of membrane potential and of the averaged motive force of tactic movement by using a double-chamber method, i.e., a single plasmodium was placed between two compartments through a narrow ditch, and differences in membrane potential and in pressure between two compartments were measured. Results are summarized as follows: (a) By increasing the concentration of various substances in one compartment, the membrane potential started to change at a certain threshold concentration, C_{th} , for each chemical. Chemotactic movement of the plasmodium took place at the same threshold concentration. These results held both for attractants (glucose, galactose, phosphates, pyrophosphates, ATP, c-AMP, etc.) and for repellents (various inorganic salts, sucrose, fructose, etc.). (b) The threshold concentration, C_{th} , for inorganic salts decreased remarkably with increase of the valences of cations, z , and was proportional to z^{-6} , i.e., the Shultze-Hardy rule known in the field of colloid chemistry was found to be applicable. (c) The plasmodium distinguished the species of monovalent cations in the following order: H(Li(K(Na(Rb(Cs(NH₄). Plots of $\log C_{th}$ against the lyotropic number of anion fell on different straight lines for each monovalent cation species. (d) Plots of $\log C_{th}$ against the reciprocal of the absolute temperature followed different straight lines for different substances. The slopes of the lines were almost the same and gave a value of 12 kcal/mol for the enthalpy change. These results suggest that the recognition of chemical substances appears as the result of a structural change of the membrane at the threshold point, and that the change in membrane structure is transmitted simultaneously to the motile system of the plasmodium.

INTRODUCTION

Chemoreception and its transduction systems are widely distributed in nature from bacteria to mammalia. In higher vertebrates, chemoreception takes place at the receptor cells in olfactory and taste organs and the stimulus information received at the receptor cell is transduced into nerve impulses

transmitting to the brain. The most primitive type of chemoreception and its transduction system can be seen in a phenomenon called chemotaxis: i.e., motile bacteria or protozoa move towards or away from a variety of chemicals. The study of chemotaxis was initiated at the end of the 19th century by Engelmann and Pfeffer, but, with few exceptions, was not baptized with modern biochemical and genetical techniques until Adler began his study of *Escherichia coli* in the last decade. According to Adler and his co-workers, *E. coli* recognizes attractants without metabolizing and transporting stimulus substances. There exist at least eight different receptor sites for different groups of chemicals (Adler, 1969). One of the receptor molecules was isolated and identified as the galactose binding protein (Anraku, 1968; Hazelbauer and Adler, 1971).

Despite their extensive studies on the receptor sites of molecules for attractants, the transduction process of the information sensed at the cell surface into chemotactic response still remains almost unknown. In sensory cells of higher vertebrates, reception of chemical stimuli at receptor membrane generates a change in intracellular potential, called receptor potential, which induces neural responses (Beidler, 1971). Provided that a similar transduction mechanism in chemoreceptor systems of microorganisms exists, observation of various phenomena occurring at receptor membrane level (e.g., conformational changes of receptor membrane, changes in membrane potential, etc.) is indispensable for elucidating the transduction mechanism in chemotaxis. From this point of view, the true slime molds are a suitable experimental organism, because during the plasmodium stage the mold is a huge aggregate without boundary membranes around the cellular components. Measurements can be made quantitatively both of the motive force of tactic movement and of electrical properties reflecting a change in membrane potential by using a double-chamber method developed by Kamiya (Kamiya, 1942).

Chemotaxis of the true slime mold *Physarum polycephalum* was studied qualitatively by observing the behavior of the slime mold towards sugars and inorganic salts (Stahl, 1884; Anderson, 1964; Carlile, 1970; Coman, 1940; Harris, 1961; Rosen, 1962; Ziegler, 1962). Earlier investigators seemed to be interested chiefly in the motile mechanism of the mold. Later the tactic movement towards chemicals such as ATP became the object of their study (Kamiya, 1959). Thus, no systematic investigations have been performed aiming at elucidating the mechanism of chemoreception in the slime mold at the receptor membrane level. In the present study, the chemoreceptor mechanism in the true slime mold is studied on the basis of quantitative measurements of chemotactic motive force and electrical potential. The results suggest that there is a critical phenomenon, that occurs in the re-

ceptor membrane, which permits recognition of chemicals at the threshold concentration.

MATERIALS AND METHODS

The slime mold used was *P. polycephalum*, which was kindly furnished by Professor Kamiya at Osaka University. *P. polycephalum* was cultured by the method employed by Camp (Camp, 1936), i.e., the plasmodium was cultured on wet filter paper with tap water in a dark place and fed with oatmeal. Before use, the slime mold was allowed to move overnight on 1% agar gel with no food. During this procedure, the plasmodium differentiated into two portions, the tip and strand portions.

The membrane potential and the motive force of protoplasmic movement were measured by using a double-chamber method proposed by Kamiya (Kamiya, 1942), as schematically depicted in Fig. 1. The chamber was made of Lucite 5-mm thick. Each compartment was floored with 1% agar gel containing 10^{-3} M KCl. Changes

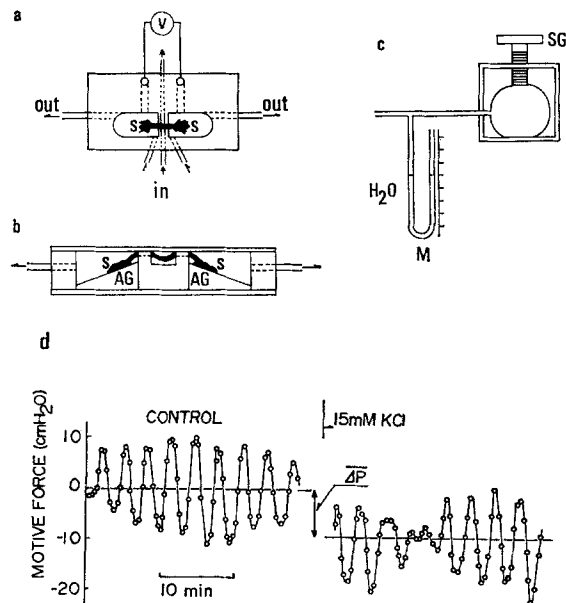


FIGURE 1. Schematic diagram illustrating the experimental setup for measuring membrane potential and motive force of protoplasmic streaming of slime molds: (a) the double chambers used. S: slime molds, V: potentiometer. Slime mold is placed between two compartments through a narrow ditch. Solution is exchanged as indicated by arrows. (b) Sideview of the double chambers. AG: agar gels. (c) Apparatus supplying the pressure difference between two compartments. M: U-tube manometer containing H_2O . Pressure difference is controlled by pressing rubber ball with screw, SG. (d) Dynamoplasmogram; solution in one compartment was exchanged from water to 15 mM KCl at the arrow indicated in the figure. Motive force of taxis of the plasmodium is the difference in the area average pressure, $\bar{\Delta P}$, as depicted in the figure.

of KCl concentration in the agar gel made no appreciable difference in the results obtained. The tips of plasmodium were separated from the strand portion and were put on the agar gel in each compartment. Then, the tips in two compartments were connected with a strand through a narrow ditch. Each compartment was air-tightened by using silicone grease or white Vaseline and a glass slide in order to apply the difference in pressure between two compartments.

The motive force of the protoplasmic streaming was measured by applying a difference in pressure between two chambers necessary just to stop the protoplasmic streaming in the strand portion, and the applied pressure difference was measured by a handmade U-tube manometer. The protoplasmic streaming at the middle portion of the strand was observed by a microscope. The characteristic periodical variations of the pressure difference, i.e., the pressure required for compensating the protoplasmic streaming, were observed as illustrated in the lower trace of Fig. 1. The area averaged difference of the pressure, $\overline{\Delta P}$, is referred to as the motive force of the tactic movement of the plasmodium and defined positive for attractants. Positive or negative taxis actually occurred when the pressure difference was not applied externally, and both the direction and the threshold of tactic movement of the slime mold agreed with those determined by $\overline{\Delta P}$.

The difference in the electromotive force, emf, between two compartments was measured by a potentiometer (Nihon Kohden Electrical Co., Tokyo, model MZ-4) conducted with a pair of calomel electrodes through salt bridges which are connected with each compartment as shown in Fig. 1 *a*, and was monitored by a channel of a synchroscope. Strictly speaking, the emf thus measured is not the membrane potential itself, but the difference in the emf between two parts of the slime mold. Since the composition of solution in one compartment was kept constant and that in the other side was changed successively, the observed emf seemed to reflect the variation of the membrane potential in the side of the test solution. Therefore, we will refer to the emf thus observed as the membrane potential and denote it as $\Delta\phi$ in this article. As shown in Fig. 1 *b*, the middle portion of the strand was washed by slowly flowing aqueous solution of sucrose or distilled water to minimize the effect of surface conductance of the measured emf. Preliminary experiments showed that no surface effect was observed if a thick strand is used. Therefore, apparatus with no middle channel was also used for measuring the variation of the membrane potential. Exchange of the external test solution was performed by flowing the test solution gently as illustrated in Fig. 1 *a* and *b*. The application of a microelectrode technique was impossible to use for the measurements of the membrane potential of the slime mold, because the rapid formation of the membrane at the tip of the electrode prevented measurement of the stable value of the membrane potential when the microelectrode was inserted in the slime mold.

All experiments were performed at room temperature, $20 \pm 1^\circ\text{C}$, except the experiments studying the temperature effect. Dependence of the membrane potential on the surrounding temperature was examined by carrying out the experiments in rooms thermostated at 3 ± 0.5 and $30 \pm 0.5^\circ\text{C}$. All chemicals used were analytical grade and used as delivered. Water used as solvent was distilled twice in glass vessels.

RESULTS AND DISCUSSION

Chemotaxis and Chemoreception for Sugars

Fig. 2 shows a typical example of variations of observed pressure difference as a function of time (usually referred to as dynamoplasmogram) where the compositions of the solutions in two compartments are either equal or different. Fig. 2 *a* illustrates the case where the solution in one compartment is replaced from H₂O to 3×10^{-4} M of glucose solution at the arrow indicated

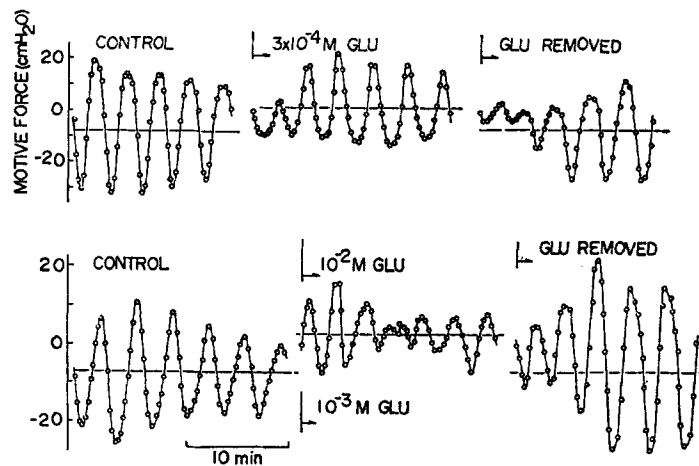


FIGURE 2. Dynamoplasmograms; upper trace shows that the plasmodium is attracted to 3×10^{-4} M glucose solution, where the plasmodium is placed between the glucose solution and H₂O. Lower one shows that the plasmodium is attracted to the glucose solution of higher concentration, where plasmodium is placed between 10^{-2} and 10^{-3} M glucose.

in the figure. The motive force is deviated to the side of the glucose solution on the average. The averaged pressure difference, $\overline{\Delta P}$, affords a quantitative measure of chemotactic motive force. Fig. 3 shows the average pressure difference, $\overline{\Delta P}$, as a function of concentration with various kinds of sugars. Glucose and galactose (○ and ⊕, respectively) attracted the plasmodium when the concentration exceeded about 10^{-4} M, whereas sucrose (●) repelled the mold when the concentration increased higher than about 3×10^{-2} M. It should be noted that the chemotactic movement took place in an all-or-nothing manner, i.e., even if the concentration of chemicals was increased further, the chemotactic motive force, $\overline{\Delta P}$, stayed at the constant level of about 10 cm H₂O, while $\overline{\Delta P}$ was zero in the region of concentration lower than C_{th} . This result does not imply that the plasmodium cannot distinguish the difference in concentrations of chemicals at concentrations

higher than C_{th} . In fact, when the plasmodium was placed between two solutions of 10^{-2} and 10^{-3} M of glucose, the plasmodium moved towards the 10^{-2} M solution side (Fig. 2 *b*). Note that even in this case, the chemotactic motive force, $\overline{\Delta P}$, remained at the same level of about 10 cm H₂O. The results described above indicate that the plasmodium has a mechanism for distinguishing the difference in concentration for each chemical above its respective threshold concentration. The increase of membrane potential with increase of concentration of chemicals, which will be described below, seems to be utilized for distinguishing the concentration difference.

Fig. 4 shows the dependence of the membrane potential, $\Delta\phi$, on concentrations of four kinds of sugars, where one side of the slime mold was immersed in H₂O. Symbols \circ and \oplus indicate glucose and galactose, respectively. In these cases, $\Delta\phi$ starts to change its value from a concentration of about 10^{-4} M. Symbols \bullet and \bullet show fructose and sucrose. Plots show that the

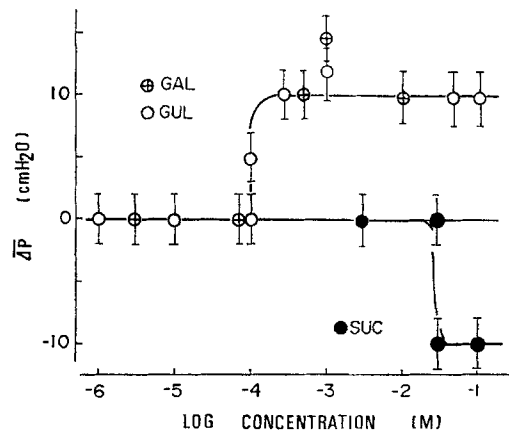


FIGURE 3. Tactic motive force, $\overline{\Delta P}$, as a function of concentration of sugars. Symbols \oplus , \circ , and \bullet indicate galactose, glucose, and sucrose, respectively.

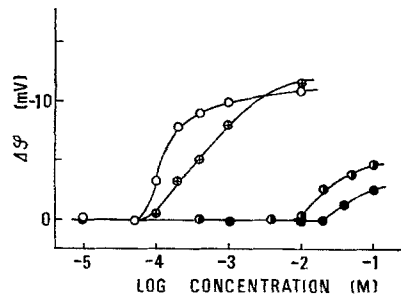


FIGURE 4. Membrane potential, $\Delta\phi$, as a function of concentration of sugars. Symbols \oplus , \circ , \bullet , and \bullet indicate galactose, glucose, sucrose, and fructose, respectively.

variation of the membrane potential took place at about 10^{-2} M (fructose) and 3×10^{-2} M (sucrose). Comparison of Figs. 3 and 4 indicates that the threshold concentration, C_{th} , determined by the membrane potential agreed with those determined from taxis measurements. It is noted that the membrane potential changes gradually in one direction (the direction of depolarization) with increase of concentration of chemicals for both attractants and repellents when the concentrations of chemicals, C , exceed their respective threshold, C_{th} . On the other hand, the motive force changes rather discontinuously either positive (attractants) or negative (repellents) at the threshold concentration and keeps a constant level with increase of concentration of chemicals above C_{th} , as seen in Fig. 3. Chemicals of high concentration were not always relevant to repellents. For example, 10^{-1} M of glucose attracted the plasmodium, whereas the same concentration of sucrose or fructose repelled the slime mold.

Reception and Taxis for Inorganic Salts

Fig. 5 shows the dependence of the membrane potential on salt concentrations, C , for various valences of cations with a common anion species. Fig. 5 refers to the case of Cl^- salts, except KH_2PO_4 and $\text{K}_4\text{Fe}(\text{CN})_6$, as indicated in the figure. The difference in anion species led to no appreciable difference in the observed membrane potential for polyvalent cations. On the other hand, the threshold concentrations for univalent cations were affected with anion species as will be described later. Note that in each case, the membrane potential changed rather discontinuously at the threshold concentration for different inorganic salts, and then increased almost linearly with $\log C$ by increasing the salt concentration. For a given ion species, of the salt concentration, C , more than two orders of magnitude greater than C_{th} , $\Delta\varphi$ approached a limiting value or reached a maximum and then decreased as C was further increased. At this stage, the membrane potential was not

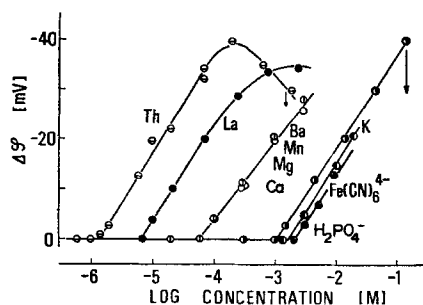


FIGURE 5. Membrane potential, $\Delta\varphi$, as a function of concentration of salts of various valences of cations. Anion species is Cl^- for all salts, except $\text{K}_4\text{Fe}(\text{CN})_6$ (\bullet) and KH_2PO_4 (\oplus).

stable and decreased gradually with time and approached zero within about 30 min, as indicated by arrows in Fig. 5. During this time-course, morphological changes of the plasmodium were observed: the plasmodium was separated into small droplets of protoplasm. These changes were similar to those observed in the presence of caffeine in the external medium (Hatano, 1970). It is important to note that in the linear region of $\Delta\varphi$ vs. $\log C$ relation, the membrane potential changed reversibly with the variation of salt concentration. Therefore, subsequent arguments will be limited to the threshold phenomena in order to avoid the complexity caused by secondary effects.

Fig. 6 shows the average motive force, $\overline{\Delta P}$, as function of $\log C$ for various salts. Note that the threshold decreased remarkably by increasing the valences of cations as in the case of the membrane potential. Notwithstanding the remarkable decrease of the threshold with increase of valences of cations, the

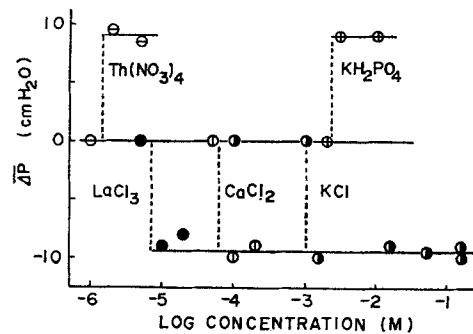


FIGURE 6. Tactic motive force, $\overline{\Delta P}$, as a function of concentration of salts of various valences of cations.

judgement of the slime mold to move toward or away from the salts seems to depend on anion species. All the chloride salts studied (K, Na, NH₄, Ca, Mg, La) acted as repellents, whereas thorium nitrate (Th(NO₃)₄) and phosphates of monovalent cations acted as attractants. It is generally believed that polyvalent cations have a toxic effect on living organs. Nevertheless, the plasmodium moved toward Th(NO₃)₄, and the plasmodium distinguishes NO₃⁻ as an attractant at a concentration as low as 10⁻⁶ M.

Phosphate Compounds as Attractants

All phosphate compounds studied were attractants for *P. polycephalum*. Fig. 7 shows the dependences of membrane potential, $\Delta\varphi$, and of tactic motive force, $\overline{\Delta P}$, on concentrations of c-AMP, ATP-Na, and pyrophosphate-Na. The critical concentrations determined by $\overline{\Delta P}$ measurements agreed with those determined by $\Delta\varphi$ as in the case of sugars (Figs. 2 and 3) and inorganic salts (Figs. 5 and 6). Cyclic AMP, known as the specific factor to cause ag-

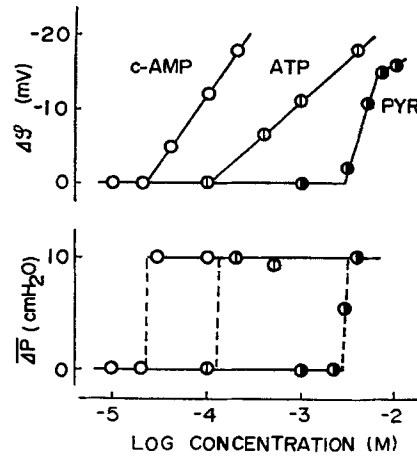


FIGURE 7. Concentration dependence of membrane potential, $\Delta\phi$, and tactic motive force, $\Delta\bar{P}$. The plasmodium is attracted to cyclic AMP, Na-ATP, and sodium pyrophosphate, PYR.

gregation of amoebae of cellular slime molds (Konijn et al., 1968), does not seem to be a special substance for *P. polycephalum* at least at the reception level, because the dependences of the membrane potential and the motive force on the concentration of c-AMP are not especially different from those observed with other phosphates and inorganic salts. This point will also be seen later when the effect of temperature on C_{th} is observed. In the case of pyrophosphate, structure of the plasmodium disrupted even in the slightly higher concentrations than C_{th} , where yellow pigments flowed out into the external solution. Notwithstanding the fatal rupture of the membrane, the plasmodium moved toward the solution of pyrophosphates.

Effect of Salts on the Recognition Threshold and Colloidal Instability

All results described above indicate that a threshold or critical process plays an essential role in chemoreception and chemotactic movement of the plasmodium. A threshold phenomenon is not always related to the transition or structural change of the membrane. The following results and arguments, however, suggest the occurrence of a structural change of the membrane at the threshold point. Fig. 8 shows the dependence of C_{th} on valences of cations, z , where $\log C_{th}$ is plotted against $\log z$. Linear relationship with a slope -6 holds for all cations studied. This is well known as the Shultze-Hardy rule in the field of colloid chemistry (Verway and Overbeek, 1948). In the same figure, flocculation values of various valences of cations for Au and AgI sols are plotted for the sake of comparison. According to the theoretical interpretations (Verway and Overbeek, 1948), the instability of lyotropic colloids appears as a result of competition between repulsive forces

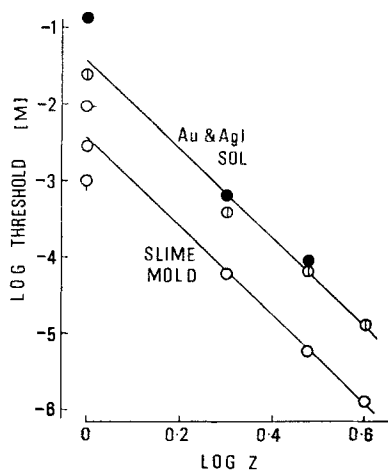


FIGURE 8. Dependence of the threshold concentration on valences, z , of cation of salts. Marks of O^- , O , and \circ indicate NH_4Cl , $NaCl$ and KCl , respectively. All data for multivalent cations were taken from Fig. 5 where the mean value of the thresholds for various salts of divalent cations was plotted. For the sake of comparison, flocculation values of Au (\oplus) and AgI (\bullet) sols are plotted in the figure, where the data were taken from Verway and Overbeek, 1948.

due to the electrical double layer and attractive force of the Van der Waals force. Although structural similarity between colloidal system and the slime mold is not yet clear, it is reasonable to consider that the effect of valences of cations on the electrical double layer at the surface of the plasmodium plays an indispensable role in the reception and discrimination of chemical substances.

Another similarity between the recognition threshold and colloidal phenomena can be seen in the discrimination of monovalent cations and anions. Fig. 9 shows the dependence of $\log C_{th}$ on the lyotropic number of anions. Lyotropic numbers of anions were taken from Voet, 1939; Tasaki et al. 1965, which were determined by the relative concentration required to cause the coagulation of agar or protein solutions. Results shown in Fig. 9 indicate that various species of anions with a common cation fall on a straight line with the respective cation when $\log C_{th}$ are plotted against the lyotropic number of anions, and the slopes of the straight lines depend on the species of cations with the following order: $H < Li < K < Na < Rb < Cs < NH_4$. This cation sequence has not been published as far as the authors are aware (Diamond and Wright, 1969).

Temperature Dependence of the Threshold Concentration

The threshold phenomena observed in the membrane potential and the tactic motive force imply that a discontinuous process, e.g., a transition in

membrane structure, is accompanied by the reception and taxis of the plasmodium. This implication can be confirmed by measuring the temperature dependence of the threshold in chemoreception. We have found that the threshold concentration of various chemicals decreased appreciably by lowering the surrounding temperature. Fig. 10 shows the dependence of $\log C_{th}$ for various substances on the reciprocal of the absolute temperature. The linear relationship between $\log C_{th}$ and $1/T$ was observed for all chemicals studied: e.g., galactose, inorganic salts, and c-AMP. Note that the slope of each straight line was almost the same for each notwithstanding the diversity of substances and of C_{th} . This fact implies that a similar change in

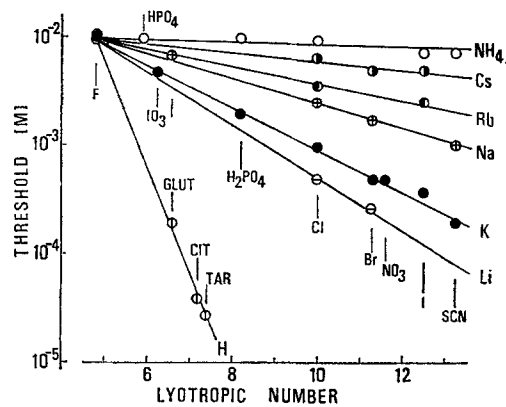


FIGURE 9. Relation between the threshold concentration and the lyotropic number of anions. Cation species are indicated in the figure at the right of respective lines.

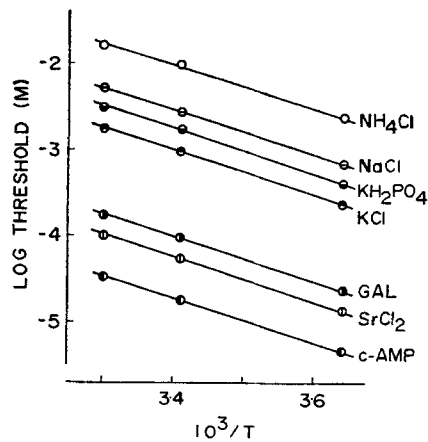


FIGURE 10. Temperature dependence of the threshold concentration. Logarithm of threshold is plotted against reciprocal of the absolute temperature, $1/T$. GAL indicates galactose.

the membrane structure is induced by the action of chemical substances at the threshold points. The apparent enthalpy changes were evaluated to be about 12 kcal/mol (exothermal) for all cases. This large difference in enthalpy change at the threshold affords additional evidence of the structural change of the membrane, although the underlying molecular nature of the change is not clear at present.

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