Effect of Sugars on Salt Reception in True Slime Mold *Physarum Polycephalum*

Physicochemical Interpretation of Interaction between Salt and Sugar Receptions

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Summary. Interaction between salt and sugar receptions in plasmodium of *Physarum polycephalum* was studied by using double-chamber method. Effect of sugars on salt reception was evaluated by measuring membrane potential and the motive force of tactic movement of the slime mold, where salt concentration in one compartment was increased successively with a fixed sugar concentration. Results are summarized as follows: (1) The presence of D-glucose, D-mannose, D-maltose, or sucrose in medium led to increase of the threshold concentration, C_{th}, for salts (chlorides and nitrates of Li, Na, K), whereas D-ribose decreased the threshold for salt reception. D-galactose showed no appreciable effect on C_{th} of every salt species examined. No change in C_{th} for salt reception was observed until concentration of sugars exceeded their respective thresholds. (2) Double logarithmic plots of C_{th} for salts against sugar concentration followed different straight lines for different cations, whose slopes being closely correlated with the effects of lyotropic number of anions in the absence of sugars. (3) Plots of log C_{th} against the reciprocal of the absolute temperature, $1/T$, gave linear relations, and the slopes of the straight line became small with increase of sugar concentration above their respective thresholds.

Experimental results obtained here suggest that the structure of water at the interface of cell membrane plays an indispensable role in the interaction between salt and sugar receptions.

The plasmodium of the true slime mold *Physarum polycephalum* exhibits positive or negative chemotaxis to various chemical substances. The plasmodium of the slime mold provides a suitable preparation for studying the molecular mechanism of chemoreception at receptor membrane level, because the plasmodium of the slime mold is a huge cell aggregate having neither boundary membrane of each cell nor cell wall. In a previous paper [10], we demonstrated that the motive force of chemotactic movement and the membrane potential of the slime mold changed simul-

taneously at a certain threshold for respective chemicals and that the thresholds for chemoreception to inorganic salts were closely related to the instability of colloidal systems. Namely, the threshold concentration, C_{th}, for polyvalent cations obeys the Schulze-Hardy rule and the plot of log C_{th} against the lyotropic number of anions falls on a straight line for a given species of monovalent cation.

In the present paper, we will report that the salt reception of the slime mold is greatly affected by the presence of sugars, and that the interaction between salt and sugar receptions may be analyzed in terms of the lyotropic number of anions. The important role of the water structure at the membrane surface will be discussed in connection with chemoreception.

Materials and Methods

The slime mold *Physarum polycephalum* used in the present study was kindly furnished by Prof. N. Kamiya at Osaka University, and cultured by the method employed by Camp [3]. The measurements of the membrane potential and the motive force of tactic movement were carried out by the double chamber method proposed by Kamiya [6, 7], the details of which were described in the previous paper [10]. Two pieces of plasmodium 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 glass slide so as to make the two compartments air-tight and insulated electrically, except for the plasmodial strand. The effects of the presence of sugar against the threshold of salt reception were examined as follows; aqueous solution of a given concentration of sugar was placed in both compartments and various concentrations of salts containing the same concentration of sugar was then flowed through only one compartment. The difference in membrane potential and motive force of taxis were measured successively by increasing the concentration of added salts. The difference in membrane potential was monitored by a beam of a synchroscope (Nihon Kohden Co., Model VC8) through a high input impedance dc preamplifier (Nihon Kohden Co., Type MZ4).

All chemicals used were analytical grade and used as delivered. Water used as solvent was distilled twice in glass vessels. Values of pH of the solutions were 5.6 ± 0.1 for all cases examined. Experiments were performed at room temperature, 20 ± 1 °C, except the experiments studying the temperature effect.

Results and Discussion

The effect of sugars (D-glucose, D-galactose, D-ribose, D-mannose, D-maltose and sucrose) on reception of salts (Cl- and NO_3-salts of K, Na, Li and NH_4) was examined by measuring the membrane potential and chemotactic motive force of the slime mold. The upper trace in Fig. 1 shows typical examples of changes in the membrane potential, $\Delta\varphi$, as a function of KCl concentration, [KCl], where concentration
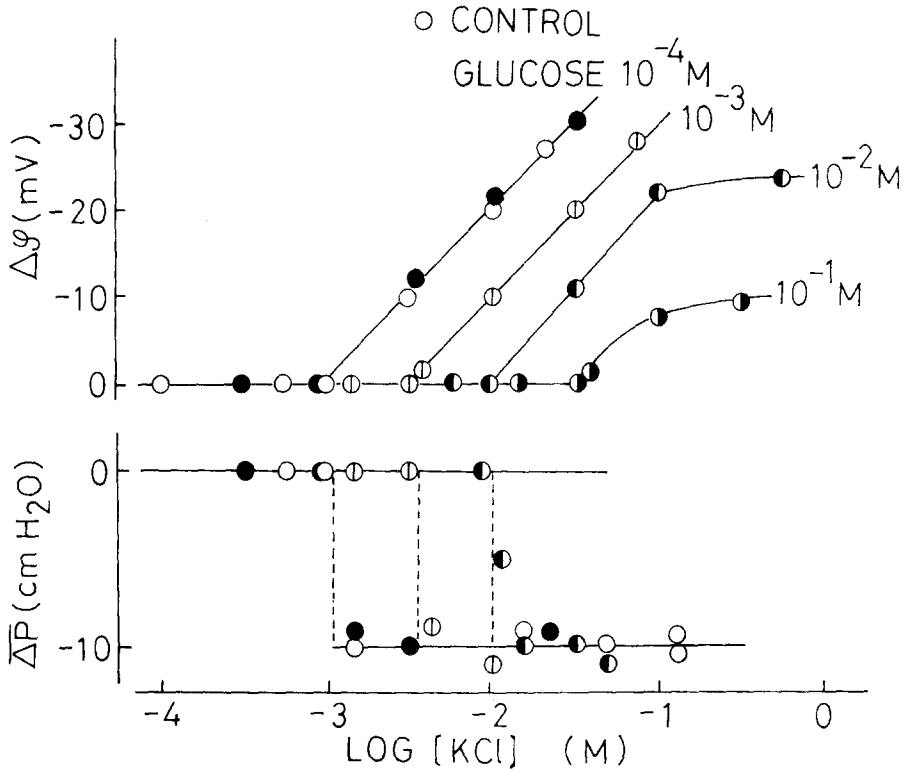
Fig. 1. Changes in membrane potential, $\Delta\varphi$, and chemotactic motive force, $\overline{\Delta P}$, as a function of KCl concentration, [KCl], in the presence of various concentrations of glucose. The concentration of glucose in media is shown in the figure at the right of their respective lines

of coexisting glucose is denoted in the figure. Glucose brought about no effect on salt reception below its threshold concentration, 10^{-4} M. The presence of glucose above 10^{-4} M increased successively the threshold of KCl reception in the slime mold as the glucose concentration increased. Shift of C_{th} for salts in the presence of glucose can also be observed in chemotactic response. The lower half of Fig. 1 shows chemotactic motive force, $\overline{\Delta P}$, as a function of KCl concentration. Experimental conditions were the same as those in the upper half of Fig. 1. As the concentration of glucose in media increased, C_{th} for KCl determined from $\overline{\Delta P}$ shifted to higher concentration. Here $\overline{\Delta P}$ could not be measured in 10^{-1} M glucose media, because an addition of KCl in this medium caused a gelation of protoplasm in the slime mold and hence the movement of plasmodium was greatly prevented. Comparison of upper and lower traces in Fig. 1 shows that C_{th} for KCl reception determined from
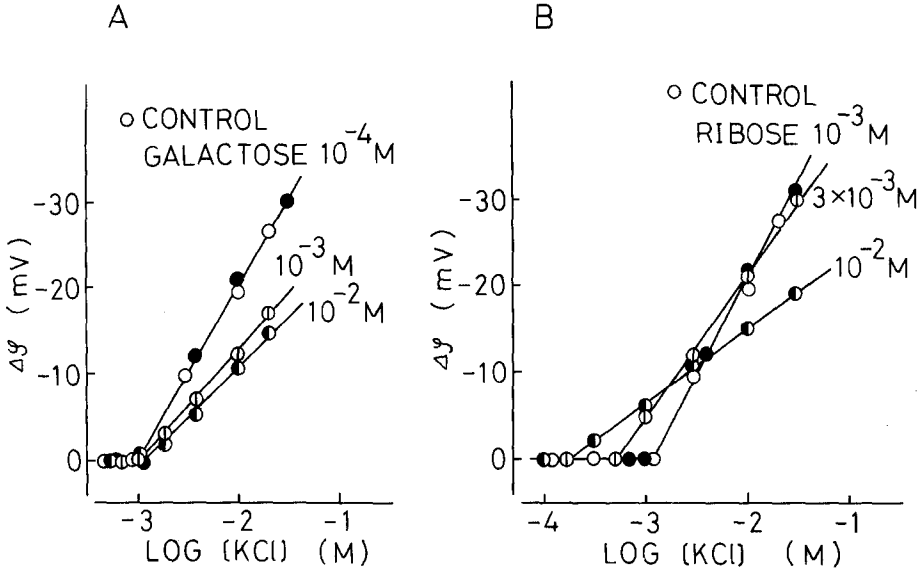
Fig. 2. Changes in membrane potential, $\Delta\varphi$, as a function of KCl concentration in the presence of various concentration of glucose (A) and of ribose (B). The concentrations of sugars in media are indicated in the figure at the right of their respective lines

$\Delta\varphi$ response agreed well with that from $\overline{\Delta P}$ measurement. This was pointed out previously [10].

Contrary to the case of glucose, galactose led to no appreciable change in KCl threshold even when [GAL] exceeded its threshold concentration, i.e., 10^{-4} M, whereas ribose led to a decrease in the threshold of KCl reception as ribose concentration exceeded its threshold, 10^{-3} M. These are illustrated in Figs. 2A and 2B, respectively. Data shows in Figs. 1 and 2 indicate that application of sugars to the slime mold affects the slope in $\Delta\varphi$ vs. log [KCl] relations as well as the thresholds for KCl. The slopes, however, changed in a complicate manner and we have not succeeded in finding out any simple relationship in $\Delta\varphi$ vs. log [salt] plots. Therefore, we will confine our discussion to the sugar effect on the thresholds of salt receptions.

Fig. 3A shows changes in threshold concentration, C_{th}, for reception of LiCl, KCl, NaCl and NH_4Cl as a function of glucose concentration in media. Note that C_{th} for salts was not affected until the glucose concentration reached to the threshold of glucose, 10^{-4} M, and that above the threshold of glucose log C_{th} changed linearly with log [GLU] with different slopes for different cation species. Similar relations hold with various sugars both for attractants (glucose, maltose, galactose,

mannose) or repellents (sucrose, ribose). Corresponding to the data given in Fig. 2, typical examples are presented in Figs. 3 B and C, where C_{th} for salts are plotted as a function of galactose or ribose concentration in medium, respectively. As seen in these figures, C_{th} for NH_4Cl reception did not change by the presence of all sugars examined, and the presence of galactose in media caused no effect on C_{th} for all salt species. The latter fact does not always mean that salt reception is not interfered by galactose. In fact, C_{th} for salts in the presence of galactose depended appreciably on temperature, as will be discussed in a later section. In Fig. 3 C, changes in membrane potential for LiCl became scant and could not be measured accurately in the presence of ribose above its threshold concentration, and so the corresponding data for ribose-LiCl system do not appear in the figure.

Relationship between Effects of Sugars and of Anion Species on Salt Reception

As shown in the previous paper [10], the linear relationship between log C_{th} and the lyotropic number of anion, N, holds for a given species of monovalent cation. Relevant data are reproduced in Fig. 4 for better understanding of the subsequent analysis in this paper. The slopes of the straight line in log C_{th} vs. log [GLU] shown in Fig. 3 A are decreased in the order, Li^+, K^+, Na^+ and NH_4^+. The slopes of the straight lines in log C_{th} vs. N shown in Fig. 4 are also decreased in the same sequence. This observation suggests that there exists a close correlation between these two linear relationships. In Figs. 5 and 6, the slopes of the straight lines in Fig. 3, i.e., $\partial \log C_{th}/\partial \log [sug]$, are plotted against those in Fig. 4, i.e., $\partial \log C_{th}/\partial N$. Figs. 5 and 6 illustrate the cases of chlorides and nitrates of NH_4, Na, K and Li, respectively. Results show that there is linear relationship between these two slopes irrespective of attractants or repellents. Namely, effects of sugars on salt reception, at least on the threshold, are interpreted in terms of lyotropic number of anions. This result indicates that the thresholds of a monovalent cations are changed greatly by the species of sugars added. Comparison of Figs. 5 and 6 indicates that effects of sugars on salt reception depend also on the anion species involved: for example, influence of maltose is about two times larger than that of glucose for chlorides. This difference, however, becomes negligible for nitrates.

The effects of sugars on the thresholds for salts of polyvalent cations were also examined, but no appreciable change was observed. Application
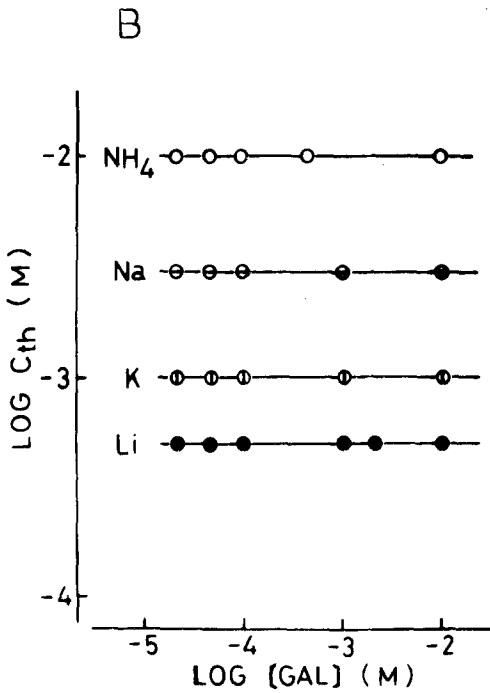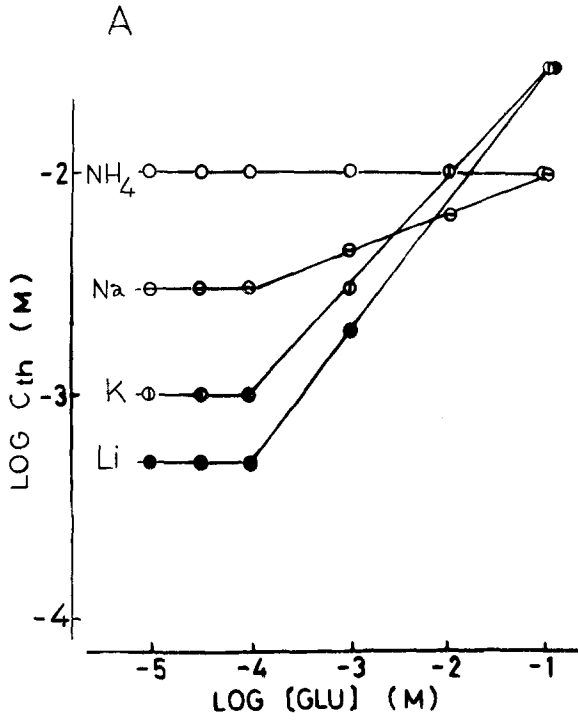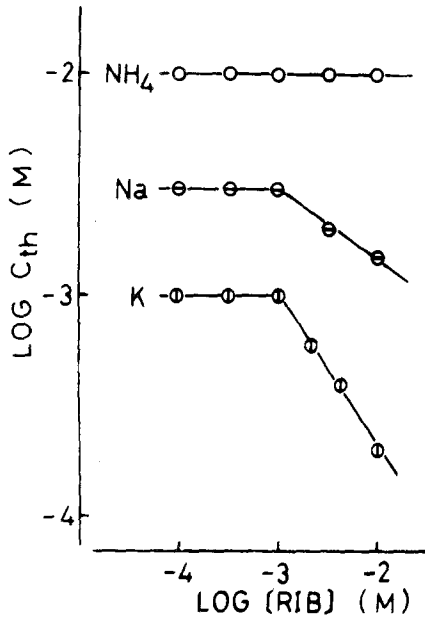
Fig. 3 *A* and *B*

C

Fig. 3. Relations between the threshold concentration, C_{th}, of LiCl, KCl, NaCl, NH$_4$Cl and glucose (A), galactose (B) and ribose (C) concentrations in media. Cation species are indicated at the left of their respective lines. C_{th} of LiCl in ribose solution could not be measured accurately, and hence was omitted in (C)
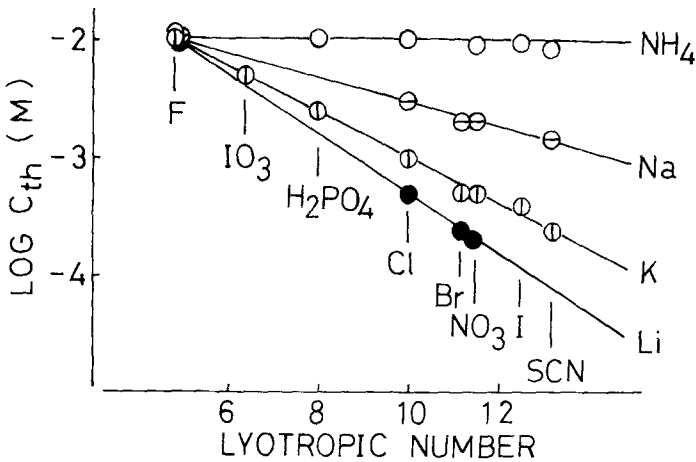

Fig. 4. Relation between the threshold concentration and the lyotropic number of anions, N. Cation species are given in the figure at the right of their respective lines (after Ueda et al., 1975 [1])
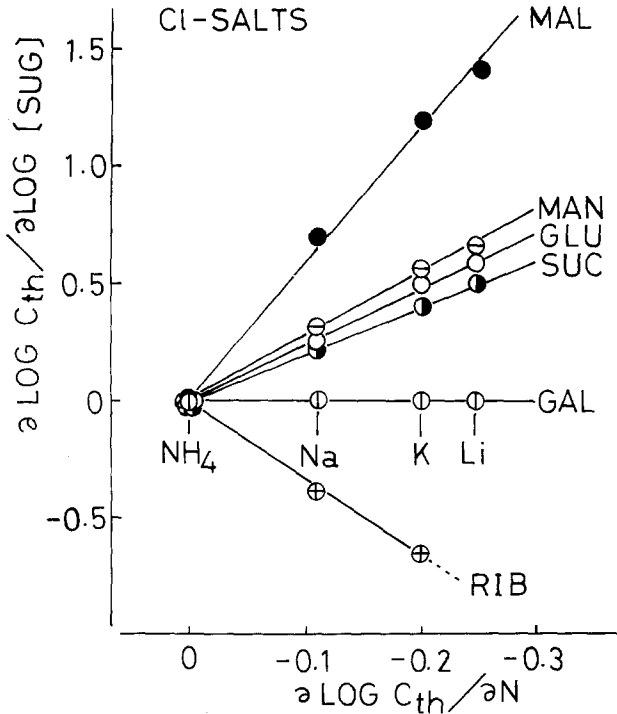
Fig. 5. Linear relations between $\partial \log C_{th}/\partial \log [SUG]$ and $\partial \log C_{th}/\partial N$ for chloride salts of NH_4^+, Na^+, K^+ and Li^+. The sugar species in media are shown in the figure

of glucose, for example, to the slime mold brought about no change in the thresholds for $CaCl_2$, $MgCl_2$, $LaCl_3$ and $Th(NO_3)_4$. This result is consistent with the above analysis, because the thresholds for salts of polyvalent cations are not affected by the difference in the anion species used [10].

The lyotropic number of anions, which is determined by coagulation or flocculation of lyophilic colloids such as protein solutions or agar sols, is known to represent the effectiveness of anions as the structure-breaker of water [5, 8]. The breaking power of water structure increases with the lyotropic number of anions, N. Fig 4 implies that the threshold of the slime mold to various salts decreased with increase of N for a fixed monovalent cation species. The thresholds for Li-salts were affected most greatly, while those of NH_4-salts were not changed appreciably by anion species used, and the order of cations was $Li > K > Na > NH_4$. The similar sequence of cations can be observed in Figs. 3A and 3C, where the threshold of the slime mold for Li-salts are changed most largely by the presence of glucose or ribose and those of NH_4-salts
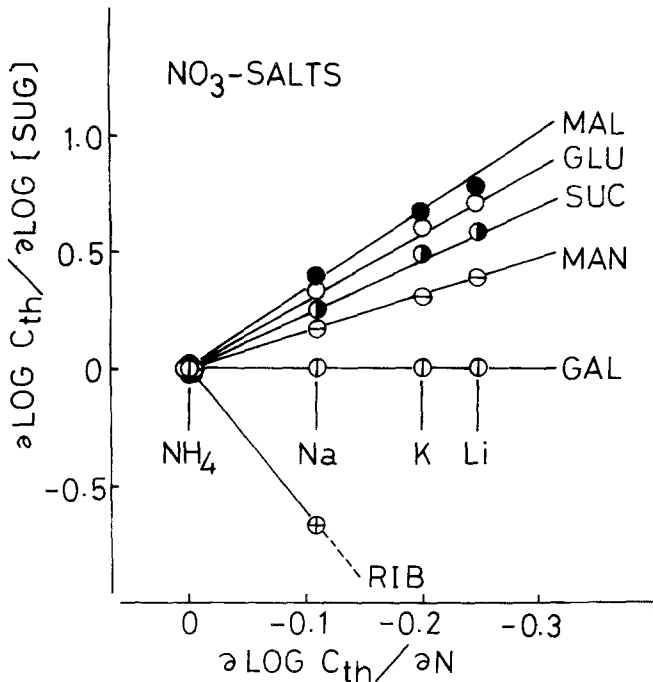
Fig. 6. Linear relations between $\partial \log C_{th}/\partial \log$ [SUG] and $\partial \log C_{th}/\partial N$ for nitrates of NH_4^+, Na^+, K^+ and Li^+. C_{th} for KNO_3 and $LiNO_3$ could not be measured clearly in ribose solution

are negligibly affected. The other sugars affect salt reception more or less in the similar manner as seen in Fig. 3. Recent physicochemical studies on the interaction of sugar and water have revealed that some sugars (e.g., sucrose, glucose, mannose) act as a structure "former" of water, whereas others (e.g., ribose) behave as "breaker"[1] [5, 8]. Considering these facts together with the results shown in Figs. 5 and 6, we may infer that the receptions of chemicals in the slime mold is closely correlated with the structure of water surrounding the receptor membrane. At present, the molecular mechanism underlying the interaction between the sugar and salt receptions is not clear to us, but the results obtained here seems to imply that the threshold of recognition of the slime mold to chemicals is related with each other through the effectiveness of forming and breaking of water structure of the chemical stimuli applied. It may be important to point out that only ribose decreases the threshold of salt reception. Note that both ribose and salts act as the structure breakers of water, whereas the other sugars studied here are structure formers.

1 H.Uedaira (1975), *personal communication.*

Effect of Temperature on C_{th} of Salts under the Presence of Sugars

In the previous paper [10], we showed that the threshold of chemoreception in the slime mold decreased with lowering of the surrounding temperature and that the plots of log C_{th} against the reciprocal of the absolute temperature, $1/T$, gave straight lines with a common slope from which the apparent enthalpy change was calculated to give -12 kcal/mole. This fact held irrespective of kinds of chemical stimuli, i.e., attractants or repellents, and electrolytes or nonelectrolytes. In the present study, the dependence of C_{th} for salt reception on temperature was examined in the presence of sugars. Fig. 7 shows the temperature dependence of the thresholds for KCl and $CaCl_2$ reception in the presence of glucose in various concentrations. Note that the linear relation between log C_{th} and $1/T$ holds even in glucose solution, and that the slope of the straight lines decreases with increase of glucose concentration. The latter fact implies that the apparent enthalpy change accompanying salt reception decreases with increase of sugar concentration. No change in C_{th} with temperature was observed below the threshold concentration of sugars. Other combinations of sugars and salts gave essentially the similar results so far as the slopes of the straight lines in log C_{th} vs. $1/T$ plots are concerned. As seen in the figure, C_{th} itself changed in a complicate manner depending on the salt species used. For example, C_{th} for KCl rose with glucose concentration at every temperature studied. Contrary to this, C_{th} for $CaCl_2$ at 5 °C (see the data at 3.6×10^{-3} of the abscissa in Fig. 7) increased as the concentration of glucose increased, whereas that at 30 °C (see the data at 3.3×10^{-3} of the abscissa) decreased when the glucose concentration increased. Notwithstanding the complicate behavior of the absolute value of C_{th} for salts, slopes of the plots shown in Fig. 7 changed systematically. The apparent enthalpy change, ΔH_{app}, is calculated from the slope of log C_{th} vs. $1/T$ plots. This determination of ΔH_{app} values may be justified by the following discussion. Response of the slime mold to chemical stimuli is represented by Eq. (1) (see Figs. 1 and 2);

$$\Delta \varphi = \alpha \log (C/C_{th}) \text{ for } C > C_{th} \qquad (1)$$

where α is a constant independent of concentration of chemical stimuli. Empirical relation given by Eq. (1) is expanded in powers of $\Delta C \equiv (C - C_{th})$ to give [9];

$$\Delta \varphi = 2\alpha K \Delta C/(1 + K \Delta C) + O(\Delta C)^2 \qquad (2)$$
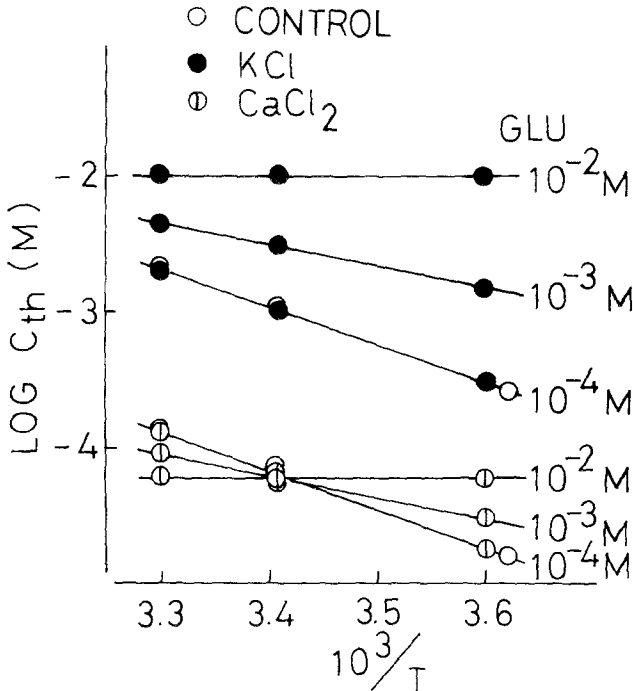
Fig. 7. Temperature dependence of the threshold concentration, C_{th}, for KCl and CaCl$_2$ in variety of glucose concentration in media. Logarithm of C_{th} is plotted against reciprocal of the absolute temperature, $1/T$. Glucose concentrations are indicated in the figure at the right of each line. C_{th} for KCl and CaCl$_2$ in pure water are indicated by o

where $K=1/2C_{th}$. The first term is compared to the Langmuir adsorption isotherm which is referred to as the Beidler taste equation in the field of chemoreceptions [2]. The constant K and hence C_{th}, thus corresponds to the equilibrium constant of adsorption of the chemicals on the membrane surface. Therefore, the following relation is derived by using the Van't Hoff equation:

$$\frac{\partial (\ln C_{th})}{\partial (1/T)} = -\frac{\partial \ln K}{\partial (1/T)} = \frac{\Delta H_{app}}{R}. \qquad (3)$$

Eq. (3) is used to determine the apparent enthalpy change at the threshold. ΔH_{app} thus obtained for KCl thresholds are plotted against the logarithm of sugar concentration in Fig. 8. ΔH_{app} decreases with increase of sugar concentration, following a straight line for each sugar species when the concentration of sugar exceeded its threshold. ΔH_{app} approaches zero when the concentration of sugars exceeds more than two orders of magnitude above the threshold and stays at zero level even in the more concen-
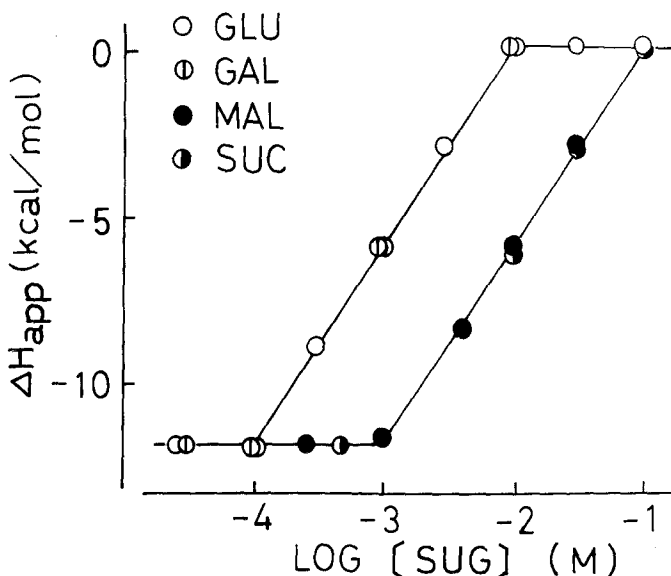
Fig. 8. The apparent enthalpy change ΔH_{app} at the threshold of KCl as a function of sugar concentration in media. Sugar species used are glucose, o; galactose, ⊕; maltose, •; and sucrose, ◐

trated region of sugar solutions. Essentially the same results are obtained for other combinations of sugars and salts including divalent cations. Note that galactose which led to no appreciable change in C_{th} for any kinds of salts (see Figs. 3 B, 5 and 6), gave the same temperature effects as those with maltose, glucose and sucrose as seen in Fig. 8. We may notice in Fig. 8 that sucrose higher than 10^{-3} M interferes the salt reception. This implies that the threshold of sucrose determined from interference of sugar and salt receptions is 10^{-3} M, which is contrast to the threshold of sucrose reported in the previous paper, i.e., 3×10^{-2} M, determined from the chemotactic movement [10]. The reason for this discrepancy between these two thresholds for sucrose is not clear to us at present, but suggests that the information sensed at the membrane does not always transmit to the motile systems in the slime mold. Further study is necessary.

Alteration of Cation Selectivity Caused by Change in Medium

It is believed that the sequence of cation selectivity both in biological and in nonbiological membrane systems is primarily determined by the character of receptor sites constituting the membrane [4]. Also it is well

known that the ability of mammalia to respond to various salts is different from one species of animals to another [2]. For example, the rodents respond well to NaCl and LiCl as compared with KCl, whereas the opposite is true with carnivores. NH_4Cl is a good stimulant for both mammalia. We have to reconsider the notion that the specificity for cation species is determined solely by the species of organism, or by the specific site bearing on the membrane. Fig. $3A$ shows that the slime mold recognizes cation species in a different order with increase of glucose concentration in media, e.g., the sequence of cations is $Li < K < Na < NH_4$ in glucose concentration lower than 10^{-4} M, $Na < Li < K < NH_4$ in 10^{-2} M glucose, but becomes $K < Li < Na < NH_4$ in 10^{-1} M glucose solution. Further studies will appear in a subsequent article.

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