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ELECTROSTATIC CONTROL BY LIPIDS UPON THE MEMBRANE-BOUND ($Na^+ + K^+$)-ATPase

II. THE INFLUENCE OF SURFACE POTENTIAL UPON THE ACTIVATING ION EQUILIBRIA

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Electrostatic influences upon the enzymatic activity of the ($Na^+ + K^+$)-ATPase from ox brain (EC 3.6.1.3) have been studied. (1) The characteristics of the temperature dependence of the activity – the slopes and inflection temperature, T_i, of the Arrhenius plots – have been shown to depend on the total concentration, but not on the specific properties of added monovalent ions. (2) The enzymatic activity has been shown to be subject simultaneously to unspecific and specific influences of alkali-metal ions or NH_4^+. Ion-specific effects result from different binding constants of complexation between activating ions and enzyme. These stability constants are affected by the formation of an electrical double layer at the membrane surface. With increasing electrostatic screening, the complex formation is destabilized and, as a consequence, the enzymatic activity decreases. (3) This interaction between ion binding and surface electrostatics enables the enzyme to adapt its activity to the actual ionic conditions. This gives rise to a complex net dependence of the enzymatic activity upon the concentrations of activating ions. Such dependencies are analyzed, and an 'activity surface' has been constructed which represents the enzymatic activity as a function of simultaneously varying concentrations of sodium and potassium. The shape of this activity surface is determined by the relations between ion concentrations, surface potential and the resulting stability of the complexation between the activating ions and the enzyme. By means of three-dimensional representation it is demonstrated that the adaptability of the stability constants is of great importance with respect to the maintenance of the optimal ionic concentrations within the living cell. Therefore, by means of the surrounding membrane, the ATPase is provided with a quality, in addition to its substrate specificity and catalytic ability, which is necessary for its function as a transport enzyme.

Introduction

This paper is concerned with the question if, and in which way, the enzymatic function of the ($Na^+ + K^+$)-ATPase from ox brain (EC 3.6.1.3) depends upon influences from the surrounding membrane. Since the ATPase is tightly embedded in the membrane, it seems reasonable to assume a functional interaction beyond the structural one. This hypothesis may be substantiated in more detail by the following points. (1) Important constituents of the membrane which surrounds the enzyme are phospholipids [1,2], which bear negative charges at pH values around 7; some experimental results even allow for the assumption that negatively charged lipids are enriched around the enzyme [3]. (2) For the activation of the ATPase, monovalent ions – especially Na^+ and K^+ – are required. If these ions approach the charged membrane surface, an electrostatic interaction between the ions and the surface charges will occur. Phenomena of this kind are well known, and theories

describing such interactions have been developed. In this paper, the Gouy-Chapman theory of the electric double layer [4] will be applied. (3) By the influence of ions upon the lipid surface, the structural properties of the membrane may be changed. More importantly, the surface charge or the electrostatic surface potential will be altered. These alterations depend only on the concentration and the charge of the ions, but not on the ion species, since they are electrostatic in nature.

If the function of the ATPase is really linked to the properties of the membrane, or at least of its surface, then it may be expected that those alterations will also be reflected in the behaviour of the enzyme. In a previous paper [5] such a correlation has been demonstrated between surface electrostatics and the determining parameters (slopes and break points) of the Arrhenius plots of the ATPase activity in the presence of Na^+ and K^+. In this paper, analogous measurements in the presence of monovalent ions other than Na^+ and K^+ are reported. They serve to prove the assumption that surface electrostatics affect the activation parameters independent of the ion species. In contrast to this, the enzymatic activity at given temperature and ion concentration depends distinctly on the ion species. This is due to specific binding between the activating (monovalent) ions and the enzyme. Since, however, the binding sites on the enzyme must – at least during the uptake of ions – be located near the surface, they are also exposed to the electrostatic conditions existing there. This would enable an influence of the surface electrostatics on the complexation between ions and the protein such that strong ionic screening of the membrane charges would reduce the stability of binding of ions to the enzyme. Under this assumption, the specific, activating binding of ions to the protein will be modified by the surface charges, which per se are unspecific but depend on the ion concentration. As a consequence, the ion-specific activation of the enzyme must be expected to be superimposed by a merely concentration-dependent effect. In this paper, activity measurements of the $(Na^+ + K^+)$-ATPase are discussed in the light of the model outlined above.

Materials and Methods

The chemicals used, the preparation and the assay methods were the same as given previously [5].

The activity measurements in Figs. 4–6 were taken at 30°C, those in Fig. 3a at 25°C and in Fig. 3b at 29°C; however, the temperature was always well above T_i.

Throughout this paper the term 'activity' has been used, though the numerical values are arbitrary in that sense that they refer directly to the absorbance measurements, but have not been related to a total protein or enzyme concentration. Such a procedure has been preferred, since the total protein is a sum of various protein compounds. To avoid difficulties, constant portions of one batch have been used throughout all measurements in this work. Therefore, the simultaneous use of reaction velocities and activities, and of complex concentrations and degrees of complexation, is permissible in this case.

Results and Discussion

The influence of different alkali ions, NH_4^+, and of combinations of these ions upon the characteristics of the Arrhenius plots of the ATPase reaction have been studied. These characteristics are the temperature, T_i, at which the slope of the Arrhenius plot changes, and the slopes themselves at $T < T_i$ and $T > T_i$, which are assigned as apparent energies of activation ($\Delta \log A^*/\Delta(1/T)$).

The points in Figs. 1 and 2 were read from arrhenius plots of the ATPase reaction in the presence of the various monovalent ion species. In the figures, T_i or ($\Delta \log A^*/\Delta(1/T)$), respectively, are plotted over the sum, c_+, of monovalent cations in the solution. The dependence of T_i on the total concentration, c_+, resembles that of the transition temperature of charged lipid membranes [6,7]. This is the same behaviour as shown previously [5] when only Na^+ and K^+ were used. Also, the apparent energies of activation, above as well as below T_i, depend on c_+, as demonstrated before in the presence of Na^+ and K^+ only. No specific influence of one particular kind of ion can be perceived, and it is the sum, c_+, rather than the ion species, which governs the dependence. (The

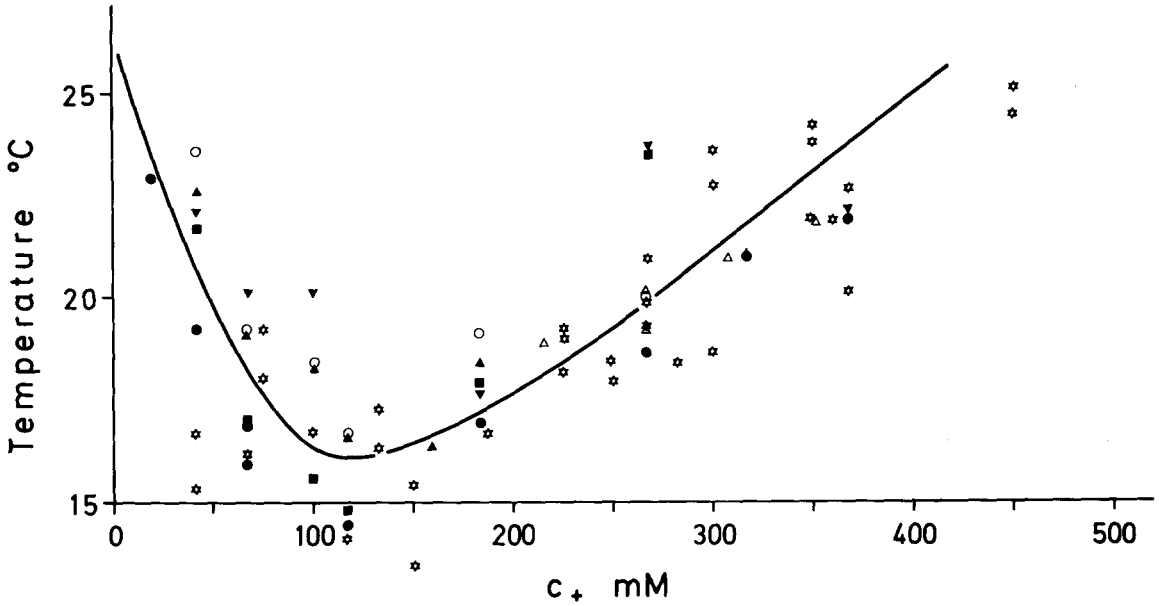


Fig. 1. 'Inflection' temperature, T_i, of Arrhenius plots versus the total concentration of monovalent ions. The different symbols refer to various combinations of the following ion species: $c_{Na^+} = 16.7$ nM, $c_{NH_4^+} = 16.7$ mM or $c_{Cs^+} = 16.7$ mM; c_{K^+} variable, $c_{Na^+} = 16.7$, 50, 100, 200 mM; $c_{NH_4^+}$ variable, $c_{Na^+} = 100$ mM. Conditions: pH 7.4 (Tris-HCl). 1.66 mM ATP; 1.95 mM $MgCl_2$.

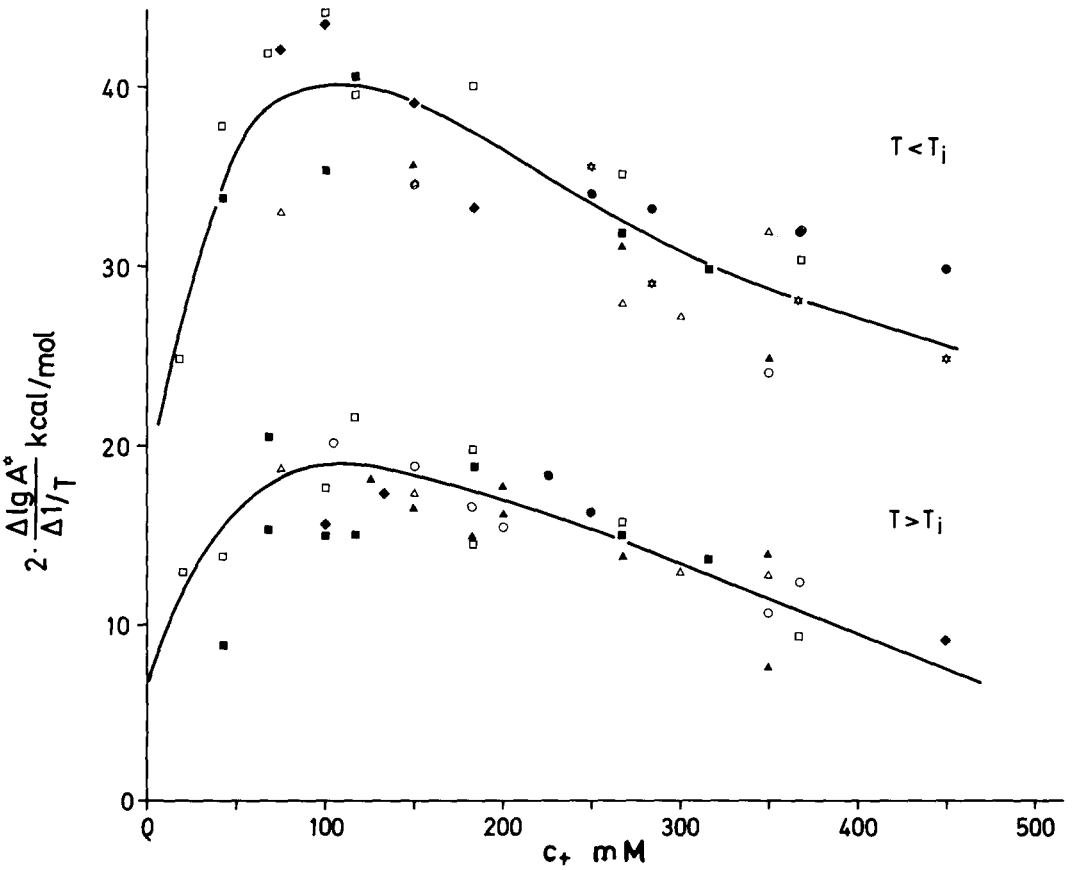


Fig. 2. Apparent energies of activation ($\Delta \log A^*/\Delta(1/T)$) at $T < T_i$ and $T > T_i$ taken from the Arrhenius plots in Fig. 1 versus the total concentration of monovalent ions. Conditions: see legend to Fig. 1.

relatively large scatter of points is due to the low activities in some cases, which reduce the precision of the analysis of the Arrhenius plots.) Therefore, it can be concluded that the results obtained with Na^+ and K^+ and the accompanying discussion [5] may be extended to all alkali ions and NH_4^+. For the activation parameters of the ATPase reaction, only the electrostatic conditions at the membrane surface, and no specific influences, are decisive.

In contrast to these parameters of the Arrhenius curves, the enzymatic activity evidently depends upon the ionic species present, though there also a phenomenological correlation with the unspecific electrostatic conditions exists [5].

The following discussion will deal with the activity and the ionic influences upon it in more quantitative terms. As is well established, the enzyme requires Na^+ and in addition one further alkali-metal ion species, preferentially K^+, to attain optimal activity [8,9]. The enzyme offers at least two different binding sites for complexation with those ions, and the activity depends upon the degree of complexation at both sites [8,10]. Since, however, these binding sites are surrounded by a charged surface, the formation of an electrical double layer at the surface must exert its influence upon the complexation. It has been demonstrated [6] that the formation of an electrical double layer weakens the binding of counter ions to a charged surface. The most thoroughly discussed example is the protonation of negatively charged lipid surfaces, where the surface pK_{diss} may be shifted by several units depending on the total ion concentration in the solution [7,11,12]. This is, however, a general phenomenon, which may render also the metal-ion-binding sites of the ATPase susceptible to the existence of a double layer.

Let K_1 and K_2 be the stability constants for complex formation at sites B1 and B2, and α_1 and α_2 the respective degrees of complexation at these sites, which will be defined as follows:

$$\alpha_i = \frac{c_{Bi}}{c^0_{Bi}} \tag{1}$$

$$K_i = \frac{\alpha_i}{(1-\alpha_i)c_i} \tag{2}$$

where c_i is the equilibrium concentration of free metal ions of the ith species, c_{Bi} the complex concentration at site Bi, and c^0_{Bi} the total concentration of site Bi.

In contrast to the occupation of the binding sites the double layer is formed unspecifically from all ion species with equal charge. As has been done previously [5], a simplified Gouy-Chapman equation will be applied to describe the surface screening:

$$e^{-\frac{F\Psi_0}{RT}} = \frac{(272\sigma)^2}{c_+} \equiv P \tag{3}$$

where Ψ_0 is the surface potential, σ the surface charge density (dim $\sigma = 1$ electron charge/Å^2) and c_+ designates the total concentration of monovalent ions.

Although specific adsorption of alkali ions to charged lipid surfaces has been reported [13], these phenomena may be ignored here. They become relevant only in special cases, which will be discussed in a forthcoming paper. Therefore, in this context the charge density σ in Eqn. 3 is to be considered as a constant, and consequently the screening factor, P, varies as $1/c_+$. Since this screening factor is responsible for the destabilization of the complex equilibria at the surface, the actual stability constant in the presence of a total ion concentration, c_+, is an apparent constant, $K_{i\,app}$, given by:

$$K_{i\,app} = P \cdot K_i = \frac{K_i'}{c_+} \tag{4}$$

(K_i' is equal to $K_i \times$ the constant portion of the potential factor). Therefore the degree of complexation, α_i, at the individual binding sites also becomes dependent on c_+, and as a consequence of the proportionality of the reaction velocity, v, to the product of the α_i values, the activity is also a function of c_+:

$$A \approx v \approx \alpha_1\alpha_2 = \frac{1}{\left(1+\frac{c_+}{K_1'c_1}\right)} \cdot \frac{1}{\left(1+\frac{c_+}{K_2'c_2}\right)} \tag{5}$$

Since the product term $c_+^2/(K_1'K_2'c_1c_2)$ in the denominator will always be small compared to the linear terms, an essentially hyperbolic dependence of A on c_+ is to be expected. This may be verified experimentally, and is demonstrated in Figs. 3a and 3b.

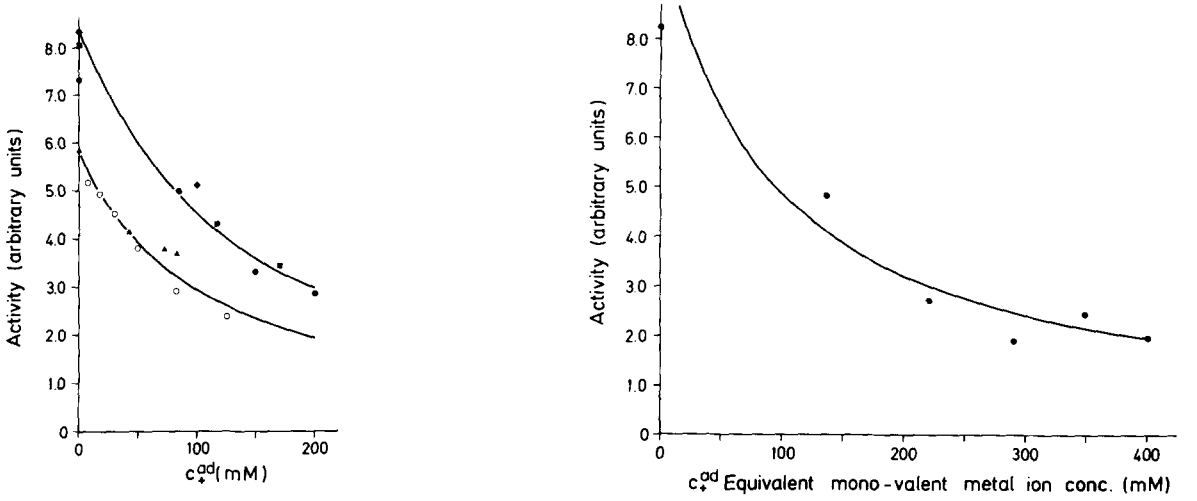


Fig. 3. Decrease in the enzymatic activity from levels determined by different Na^+ and K^+ concentrations by an increasing concentration of added inert ions. (a) added ions: lithium 30°C (full symbols), and tetrabutylammonium, 25°C (open circles); (b) added ion: strontium, 29°C; for the calculation of $c_{\pm}^{ad}$ see text. Conditions: see legend to Fig. 1.

In Fig. 3a the enzymatic activity starts at different, initially relatively high, levels defined by different Na^+ and K^+ concentrations. Upon addition of Li^+ or tetrabutylammonium ions the activity decreases. Since neither Li^+ nor tetrabutylammonium is able to activate the enzyme to any considerable extent, the main effect to be expected is the reduction of the degree of complexation of the activating ions, Na^+ and K^+, proportional to the potential effect, i.e., to $1/c_+$.

The data in Fig. 3b have been published previously [5]. Again the starting activity had been specified by constant Na^+ and K^+ concentrations, but in this case the added ion species was Sr^{2+}. Since Sr^{2+} is divalent, its potential effect is much stronger than that of a monovalent species. To make both figures commensurable, the concentration axis in Fig. 3b depicts, instead of the weighed-in Sr^{2+} concentration, the so-called 'equivalent monovalent concentration'. That means that the original concentration of divalent ions has been converted to a monovalent ion concentration, which gives rise to the same potential (for details see Refs. 5, 14). Again, a hyperbolic decrease of the activity with respect to the equivalent concentration is to be expected. Indeed, in both figures (3a, 3b) the experimental activities plotted against c_+ can be fitted well by $1/c_+$ hyperbolae, as predicted by Eqn. 5.

The measurements shown in Figs. 4a and 4b represent another approach to demonstrate the effect of potential upon the enzyme activity, but they bear, in addition, information about specific ion influences. The reaction velocity is measured as a function of varying concentrations of the activating ions. These experiments were designed in such a way that during one series the total ion sum and consequently the unspecific screening factor (cf., Eqn. 3) was kept constant. The concentrations of sodium and the co-activating ion species (K^+ or Rb^+), however, were varied complementarily from $c_{Na^+} = 0$, $c_{K^+,Rb^+} = c_{max}$ (150 mM or 300 mM) to $c_{Na^+} = c_{max}$, $c_{K^+,Rb^+} = 0$. Accordingly (cf., Eqn. 5), each of these curves represents the product of the titration curves of the binding sites B1 and B2 in the presence of the appropriate activator ion species under constant electrostatic conditions. Due to the constant potential effect, the complexation at both sites B1 and B2 is associated with a binding constant, K_{iapp}, during one whole series of experiments. When, however, the total concentration, c_+, is changed, both stability constants are shifted by a factor, P, according to the new value of the potential effect (see Eqn. 4).

In the experiments in Fig. 4a the potential has been varied by two different means. In curve B the total sodium plus potassium has been increased by

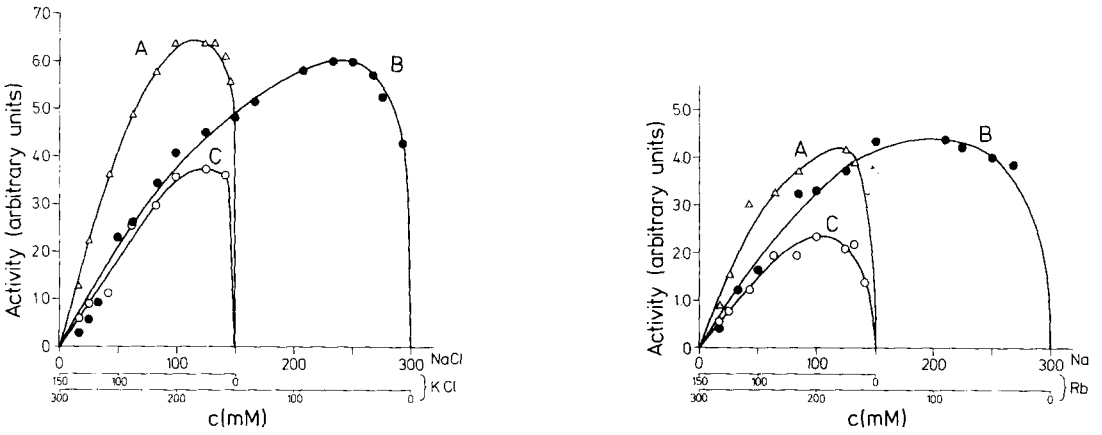


Fig. 4. Enzymatic activity at constant total concentrations of monovalent ions. (a) $c_{Na^+} + c_{K^+} = 150$ mM (A); $c_{Na^+} + c_{K^+} = 300$ mM (B); $c_{Na^+} + c_{K^+} = 150$ mM, $c_{Li^+} = 150$ mM (C). (b) $c_{Na^+} + c_{Rb^+} = 150$ mM (A); $c_{Na^+} + c_{Rb^+} = 300$ mM (B); $c_{Na^+} + c_{Rb^+} = 150$ mM, $c_{Li^+} = 150$ mM (C). Conditions: see legend to Fig. 1; $t = 30°C$.

a factor of 2 compared to A. In C, the sodium plus potassium is the same as in A, but the potential effect has been raised 2-fold by addition of the very weakly activating Li^+. Therefore, in both cases, the stability constants are affected by the same factor and, as expected, the concentration ratios at the maxima are unchanged. The value of the maximal velocity, however, is different in both cases, since in B the increase in the concentration of activating ions compensates for the decrease in the complex stability, whereas in C not enough ions are available to counteract this lack of stability. Therefore, the whole curve is depressed by a factor that depends on the concentrations. For this reason, for instance, values of ion concentrations at half-maximal velocity are, at best, of qualitative use as a measure of the stability. For the same reason, it may be misleading to normalize curves obtained under differing ionic conditions to the same maximum.

The experimental conditions in Fig. 4b are the same as in Fig. 4a with the only difference that Rb^+ has been used instead of K^+. Rb^+ is able to replace K^+ at binding site B2, though with a lower stability constant (see Fig. 6). Therefore, the activity curve results from the combination of the unchanged binding curve at B1 (sodium binding) and a weaker binding at B2 (rubidium binding). Due to the less steep binding curve at B2, the concentration ratio for the maximal velocity of the resultant is shifted to the left of the diagram compared to potassium binding at B2. Moreover, the initial slopes – particularly on the B2 side – are also less steep than in the former case, and thus all together the activities obtained are smaller than with potassium (Fig. 4a). With an increased potential effect and/or increased concentrations of the activating ions, the relative changes are equivalent to those discussed before.

One essential feature of this interpretation is that the surface potential has been taken into account. Furthermore, it is implicit in the above discussion that only activation, and no inhibition, by ions binding to sites B1 and B2 can occur. Differences in their ability to activate the enzyme are ascribed to their different stability constants only, which may be so weak that in effect no activity can be observed. This point of view is based essentially on the following considerations. If a binding site is able to form complexes with a series of ions with equal electron configuration, the resulting complexes will resemble each other with respect to their configuration, too. This must be assumed in particular in the case of alkali-metal ion complexes. Therefore, if one of the binding sites, B1 or B2, becomes occupied at all, then it should be activated, irrespective of the ion species. Thus the apparent 'absolute' requirement for Na^+ as well as the preference for K^+ as co-activators might be looked upon as a quantitative rather than

as a qualitative phenomenon.

This is reminiscent of ion selectivity phenomena known from a wide variety of ligands such as diverse kinds of glasses, polyethers, ion channels of nerves, and oligopeptides known as ion carriers [15–19]. The same concept for selective ion binding might be applied to the ATPase system. Though the chemical constitution of the ATPase binding sites is unknown, it is, nevertheless, conceivable that there are binding sites on the enzyme which are sterically and electrostatically adapted to the selection of Na^+ (B1) and K^+ (B2). It may even be that the selectivity of one of the binding sites is induced or improved by ATP binding or binding of the accompanying activator ion. But such considerations are speculative, and they exceed the scope of this study.

Some results in this paper strongly support the validity of the above-cited concept of selectivity also in the case of ATPase. If ion binding to the ATPase were not selective, the complex stability would be correlated monotonically with the ion radii, either in an ascending or descending sequence [19]. Thus Li^+ or Cs^+ would be the most potent activator, which clearly is ruled out by the present results (see Fig. 6). Or, if the maximal activity were obtained with ion complexation of medium stability, as could be the case if transport kinetics were decisive for activation by ions, again that ion species with the largest (or smallest) radius would bind most strongly, and at realistic concentrations the species with medium radius (and therefore medium binding stability) would have no

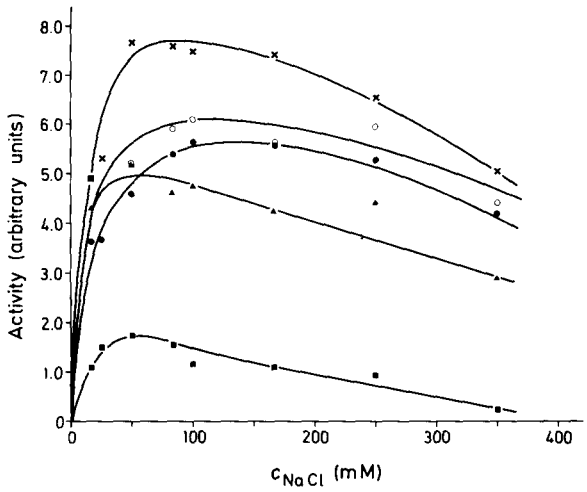
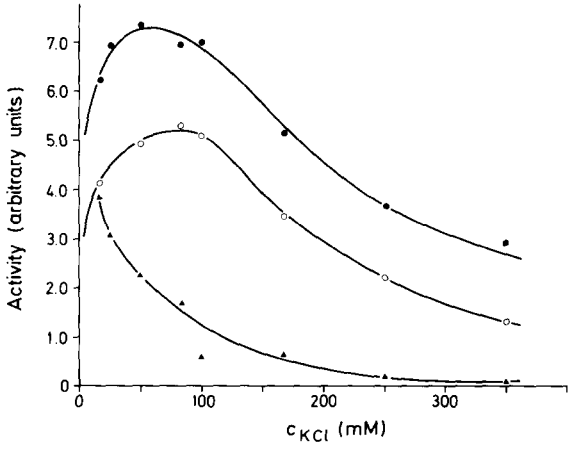


Fig. 6. Enzymatic activity in the presence of constant concentrations of alkali-metal ions or NH_4^+ (all 50 mM) versus a variable concentration of Na^+. (S.D. $\pm$ 0.5 activity units). Conditions: see legend to Fig. 1, $t = 30°C$. ■, Li^+; ▲, Cs^+; ●, Rb^+; ○, NH_4^+; ×, K^+ (chloride salts).



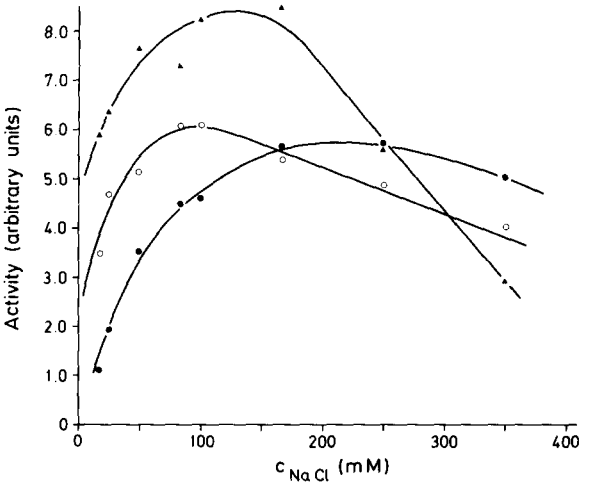


Fig. 5. Enzymatic activity in the presence of sodium and potassium ions versus the concentration of one of the activating ion species. (S.D. $\pm$ 0.5 activity units). (a) c_{Na^+} const. (16.7 (▲), 50 (○), 150 (●) mM), c_{K^+} variable; (b) c_{K^+} const. (16.7 (▲), 50 (○), 150 ((p0), c_{Na^+} = variable. Conditions: see legend to Fig. 1, $t = 30°C$.

chance. In such a situation the simultaneous requirement of two different ion species for maximal activity could hardly be explained.

In addition, the results in Fig. 3 also yield a proof in favour of selective binding, since otherwise one of the added ion species (Li^+ or tetrabutylammonium) would replace Na^+ or K^+ due to its extreme radii. Consequently, either Li^+ or tetrabutylammonium would destroy the activity, whereas in the presence of the other species the activity would remain unaffected. The results are not compatible with this.

The activity curves in Figs. 5a, 5b, and 6 were measured at constant concentrations of one of the activating ion species, i.e., Na^+ or K^+, while the second species was varied over a relatively wide range (16.7 mM to 350 mM). These experimental conditions are nearer to the natural situation than the activity measurements in Figs. 4a and 4b. The interpretation, however, becomes complicated insofar as the increase of the total ion concentration will increase the potential effect accordingly, which will reduce both stability constants. This causes a decrease in the concentration of complex species on the one hand, but on the other hand, the increase of the concentration of the variable species may counterbalance that loss at least partly at its binding site.

According to Eqn. 5, the activity in the presence of one constant and one variable activating ion species is proportional to:

$$\alpha_c\alpha_v = \frac{K'_c c_c}{(K'_c c_c + c_+)} \cdot \frac{K'_v c_v}{(K'_v c_v + c_+)} \tag{6}$$

(the indices c and v denote the constant and the variable species, respectively).

Each of the α_i values depends on the magnitude of $K'_i c_i$ relative to the total ion concentration, c_+. Thus if $K'_i c_i < c_+$:

$$\alpha_i = \frac{K'_i c_i}{c_+} \tag{7a}$$

and if $K'_i c_i > c_+$:

$$\alpha_i = 1 \tag{7b}$$

Therefore, depending on the values of K'_c and K'_v and on the choice of c_c and c_v, α_c and α_v may be of type 7a or 7b, independently. This implies that four different possibilities exist for the combination of α_c and α_v in Eqn. 6, three of which are of realistic interest, the fourth being the trivial case where $\alpha_c = \alpha_v = 1$.

Using appropriate expressions for α_c and α_v for calculating the activity according to Eqn. 6, the criteria for the shapes and the relative arrangement of the activity curves may be derived. From a short general discussion the detailed features of the individual curves presented in Figs. 5a, 5b and 6 will follow. Considering first the range of low concentration, c_v, where c_+ is determined essentially by c_c, it is evident that then α_c is constant in any case, either $\alpha_c = (K'_c c_c)/c_+ = K'_c$ or $\alpha_c = 1$, according to Eqns. 7a or 7b. Under these conditions for the product $\alpha_c\alpha_v$ a linear dependence on c_v will result, which is introduced by α_v. On the other hand, at high variable concentrations, c_v, α_v will become constant (either $\alpha_v = 1$ or $\alpha_v = (K'_v c_v)/c_+ = K'_v$) since then $c_+ \approx c_v$. Here, however, α_c is no longer necessarily constant, since increasing concentration, c_+, will progressively reduce the stability of the complex of the constant ion species. Thus, at a sufficiently high variable ion concentration, the product $\alpha_c\alpha_v$ will decrease as a function of $1/c_v$ due to the factor $\alpha_c = (K'_c c_c)/c_+ = (K'_c c_c)/c_v$. These two branches will merge at some intermediate c_v, resulting finally in the 'typical' activity curve with an (linearly) ascending part in the beginning, and a (hyperbolically) decending tail.

The position of the maximum, c_v^{max}, is determined by the constant concentration and the mutual relation of the stability constants. This may be verified by differentiation of Eqn. 6 with respect to the variable concentration:

$$c_v^{max} = c_c \sqrt{\frac{K'_c + 1}{K'_v + 1}} \tag{8}$$

From Eqn. 8 it follows immediately that if $K'_c > K'_v$, the maximum occurs at a variable concentration higher than the constant one, and if $K'_c < K'_v$ the opposite becomes true.

The relative order in which the curves are arranged with respect to each other, again results principally from Eqn. 6. At sufficiently high c_v values, the product $\alpha_c\alpha_v$ contains the factor c_c. That means that the greater the constant con-

centration, the higher will be the activity within the descending branches of the activity curves. At the beginning of the curves, i.e., at low c_v, the dependence of the product $\alpha_c\alpha_v$ on c_c is reversed. Therefore over this range, the highest activity will be obtained with the lowest constant concentration.

All of these theoretically derived features describing the activity curves are realized by the experimental results in Figs. 5a, 5b and 6, and may be verified by a comparison of the figures or by comparing the individual curves within one figure. As a result of such comparisons, it follows that K'_{K^+} must be greater than K'_{Na^+} and, furthermore, that $K'_{Na^+} < K'_{Rb^+} < K'_{NH_4^+} < K'_{K^+}$, and on the other hand $K'_{Li^+} < K'_{Cs^+} < K'_{Na^+}$.

Finally, two apparent irregularities should be mentioned. The first is the behaviour of the curves at low KCl concentration in Fig. 5a. This might be explained by the fact that in this case the limiting slopes of all curves are rather steep due to the high value of $K'_v = K'_{K^+}$, and that, furthermore, because of the relatively low stability of the constant species (Na^+ complex) the $1/c_v$ decrease begins at rather low concentrations. For these reasons the range over which c_v might be considered to be small becomes comparably narrow. In addition, especially at the low $c_c = c_{NaCl} = 16.7$ nM, contributions by ions other than Na^+ or K^+ may obscure the theoretical behaviour at the beginning of the curve.

The second point which deserves special explanation is the activity at $c_c = c_{KCl} = 150$ mM in Fig. 5b at high $c_v = c_{NaCl}$. In this case, obviously the constant concentration of 150 mM combined with the high stability constant of potassium binding is sufficient to hinder the variable concentration to become high enough to switch the condition $K'_c c_c = K'_{K^+} c_{K^+} > c_+ = c_{Na^+}$ to that of Eqn. 7a. Consequently, α_{K^+} will remain approximately constant even at high variable sodium concentrations, and therefore the activity, given by $\alpha_c\alpha_v$, remains nearly constant, in contrast to the decrease otherwise found.

Successful discussion and interpretation of activity curves on the basis of Eqns. 6, 7a and 7b and 8 is not restricted to the results demonstrated in this paper. The same holds true if these equations are applied to experimental results reported elsewhere in the literature (for instance, Ref. 10), and also with similar measurements in the presence of constant or variable Cs^+ concentrations instead of K^+ (results not shown).

A synopsis of a whole ensemble of activity curves may elucidate some interesting points. In Fig. 7 it has been attempted to demonstrate schematically an 'activity surface' when both the Na^+ and the K^+ concentrations are varied independently. This figure has been constructed on the basis of the experimental results in Figs. 5a and b. The results of Fig. 4a, however, are also implicit in it. These latter curves are diagonal intersections of the construction in Fig. 7, the baselines of which are the lines of equal total concentrations. Since $K_{Na^+app} < K_{K^+app}$ at given potential, an asymmetry is thereby introduced to the 'activity surface'.

The $(Na^+ + K^+)$-ATPase is looked upon as the transport enzyme for Na^+ and K^+ [8] which serves to maintain the proper intracellular ion composition and concentrations. The 'operating point' of the mammalian cell is at low sodium and relatively high potassium concentration, namely at approx. 10–20 mM Na^+ and approx. 150 mM K^+.

Changes of the ion concentration may occur, for instance, by diffusion or during the action potential in excitable cells. Neither the net increase in the Na^+ concentration nor the decrease in the K^+ concentration in these cases is very high, and therefore an optimally sensitive counteracting system is particularly important. In addition, for efficient restoration of the proper conditions it must

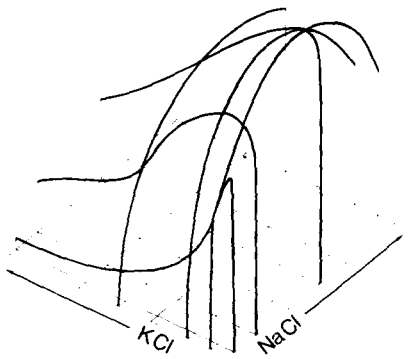


Fig. 7. Schematic diagram of the 'activity surface'. Vertical axis, enzymatic activity; horizontal plane, concentrations of the activating ion species Na^+ and K^+ as indicated.

be warranted that an increase in the Na^+ concentration and a decrease in K^+ will indeed push the enzymatic activity – and with it the transport rate – towards higher values. This implies that the optimal activity does not have to be identical to the maximal activity.

From an inspection of Fig. 7 or of Figs. 5a and b it becomes evident that both of the requirements stated above are realized by the ATPase. Starting from the intracellular concentration conditions, either a slight increase in the Na^+ concentration or a decrease in the K^+ concentration will result in a comparatively steep gradient of the ATPase activity. Moreover, the starting concentrations of Na^+ and K^+ are so far removed from those for maximal activity that the risk of passing to the side of decreasing activity is minimized. A comparison with Figs. 4b and 6 shows that less stably bound ions cannot fulfill these requirements.

From these considerations, it is evident that cooperation with the environment is of fundamental importance for the optimal functioning of the enzyme. The catalytic property to hydrolyze ATP, the substrate specificity towards ATP, and the ability to complex with ions may be attributed to the protein itself. Nevertheless, the protein alone must lack the versatility of the response of the enzymatic activity towards changing ionic conditions. This quality will be acquired only when the properties of the surrounding surface also come into play, namely when the potential effect can exert its influence upon the stability constants. The ability to adapt the stability constants to the actual conditions means a new functional degree of freedom for the enzyme, which expands its functional flexibility considerably.

The influence of the surroundings upon the enzyme therefore is essentially 2-fold:
(1) a structure mediated influence, which is expressed by the dependence on the electrolyte concentration of T_i, and
(2) a functional influence, which controls the cation binding ability of the enzyme.
Both aspects might be of more general importance, and they might be valid also in connection with other active membrane proteins.

Moreover, it may be suspected that in correspondence to the influence of the lipid matrix on the enzymatic function also the enzymatic processes act upon the state of the lipid portion. This might occur, for instance, by the appearance of reaction products or by the disappearance of reactants during the turnover. Therefore, at present it cannot be excluded that the situation discussed here is only part of a more complex control loop built up from the constituents of the membrane.

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