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Na^+ SITES OF THE ($Na^+ + K^+$)-DEPENDENT ATPase

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Summary

The concentration of NaCl for half-maximal activation of the ($Na^+ + K^+$)-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) the $K_{0.5}$ for Na^+, decreased as the concentration of KCl was reduced, as if K^+ competed with Na^+. Extrapolation to the absence of KCl gave a $K_{0.5}$ for Na^+ of 1.7 mM and a K_i for K^+ of 5.1 mM. The $K_{0.5}$ for Na^+ also decreased with the Mg · ATP concentration, and extrapolation to the absence of ATP gave a $K_{0.5}$ for Na^+ of 0.26 mM and a K_i for K^+ of 0.85 mM; the calculated K_i for Mg-ATP toward Na^+ and K^+ was 0.5—0.6 mM, in accord with an action of Mg · ATP through the low-affinity substrate sites of the enzyme. A plot of $-\log K_{0.5}$ for Na^+ against pH suggested that a group with a pK_a near 8.1 influenced activation by Na^+. The $K_{0.5}$ for KCl as an activator of the ATPase, through "β-sites" for K^+, varied with the NaCl concentration, with an extrapolated $K_{0.5}$ for K^+ of 0.11 mM and a K_i for Na^+ of 14 mM. However, the $K_{0.5}$ for KCl as an activator of the related K^+-dependent phosphatase reaction of the enzyme, through "α-sites" for K^+, was 2.0 mM; the K_i for NaCl as a competitor was 5.5 mM. These latter values also decreased with substrate concentration, and extrapolation to the absence of substrate gave a $K_{0.5}$ for K^+ of 1 mM and a K_i for Na^+ of 3.5 mM. The Na^+ sites and the α-sites for K^+ could also be distinguished in terms of the changes in their kinetic parameters in the presence of 10% dimethylsulfoxide or 0.1 mM tetraphenyl boron, although lowering the incubation temperature from 37 to 23°C generally reduced all values similarly. These results indicate that the Na^+ sites are distinguishable from both classes of K^+ sites; a formulation relating internal Na^+ sites with external α-sites for K^+, in a flip-flop dimeric transport model, is suggested.

Abbreviation: Me_2SO, dimethylsulfoxide.

Introduction

The $(Na^+ + K^+)$-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) is generally held to represent the enzymatic basis for the membrane sodium pump [1,2]. Among the consequences of this function is the presence on the enzyme of activating sites for Na^+ and K^+ that are spatially distinct: the Na^+ sites face the cell interior and the K^+ sites the extracellular fluid [3]. Mutual competition between Na^+ and K^+ occurs at each class of sites [4,5]. In addition, the transport process requires that there exist, at least transiently in the reaction cycle, discharge sites on the exterior for Na^+ and on the interior for K^+, and some proposals have linked the discharge sites for one cation to the activating sites for the other [1,2]. The enzyme also catalyzes a K^+-dependent phosphatase reaction [1,2], and activation is through K^+ sites that can be distinguished from those activating the ATPase reaction [6]. Again, Na^+ acts like a competitor toward K^+ [4]. Although earlier experiments localized these K^+ sites of the phosphatase to the exterior face [7,8], several current formulations [9,10] relate these sites to the interior Na^+ sites.

The experiments described here are concerned with the Na^+ sites activating the ATPase, particularly with regard to their possible relationship to sites through which K^+ influences activity, for the nature of the sites bears not only on the enzymology but also on the physiology of transport. Toward this end apparent affinities for Na^+ and K^+ have been estimated under various experimental conditions, including response to temperature, pH, inhibitors, and concentration of substrate.

Methods

The $(Na^+ + K^+)$-dependent ATPase was obtained from a rat brain microsomal preparation by treatment with deoxycholate and then NaI, as previously described [11].

$(Na^+ + K^+)$-dependent ATPase activity was measured in terms of the production of P_i, as previously described [11]. The standard medium contained 30 mM Tris/histidine · HCl (pH 7.8), 3 mM $MgCl_2$, 3 mM ATP (as the Tris salt), 90 mM NaCl, 10 mM KCl, and the enzyme preparation (0.1 mg protein/ml). Activity in the absence of Na^+ and K^+ was measured concurrently; such activity was only a few percent of the $(Na^+ + K^+)$-dependent ATPase activity [11], and was subtracted from the total activity in the presence of Na^+ and K^+ to give the $(Na^+ + K^+)$-dependent activity. This averaged 2.6 μmol P_i liberated per mg protein per min; although the specific activity of an enzyme preparation was quite constant, different preparations varied by as much as one-fourth. Consequently enzyme velocities are expressed relative to the $(Na^+ + K^+)$-dependent ATPase activity of a concurrent control incubation in the standard medium defined as 1.0.

K^+-dependent phosphatase activity was measured in terms of the production of *p*-nitrophenol after incubation with *p*-nitrophenyl phosphate, as previously described [12]. The standard medium contained 30 mM Tris/histidine · HCl (pH 7.8), 3 mM $MgCl_2$, 3 mM *p*-nitrophenyl phosphate (as the Tris salt), 20 mM KCl, and the enzyme preparation (0.1 mg protein/ml). Activity in the absence

of added KCl was measured concurrently; such activity was only a few percent of the K^+-dependent phosphatase activity under optimal conditions [12], and was subtracted from the total activity in the presence of KCl to give the K^+-dependent activity. This activity averaged 0.17 μmol nitrophenol liberated per mg protein per min. As with the ATPase, velocities are expressed relative to the K^+-dependent phosphatase activity of a concurrent control incubation in the standard medium defined as 1.0.

In experiments examining changes in enzymatic activity in response to variations in NaCl or KCl concentration no corrections for alterations in ionic strength were made: previous experiments [11,12] indicated that, within the range of concentrations used here, inclusion of choline chloride in the incubation media to maintain a constant ionic strength did not influence activity.

For both activities the incubation times were chosen, at 37 and 23° C, so that product formation increased linearly with time. The data presented represent averages of four or more separate experiments, each performed in duplicate.

Results

The concentration of Na^+ for half-maximal activation of the ($Na^+ + K^+$)-dependent ATPase, the $K_{0.5}$ for Na^+, decreased as the concentration of KCl was reduced (Fig. 1), as previously noted [4,10]. If this relationship reflects competition between Na^+ and K^+ for the Na^+ sites [4,5], then the $K_{0.5}$ for Na^+ in the absence of K^+ can be approached by extrapolation: in the absence of K^+ the $K_{0.5}$ for Na^+ at 37° C would be 1.74 mM (Fig. 2; Table I). Correspondingly, the K_i for K^+ as a competitor at these Na^+ sites would be 5.1 mM.

This analysis assumes, as a first approximation, a linear relationship between apparent $K_{0.5}$ and KCl concentration:

$$K_{0.5,app} = K_{0.5}\left(1 + \frac{[KCl]}{K_i}\right)$$

A more complex relationship, reflecting cooperative interactions between the sites [4,11], may exist:

$$K_{0.5,app} = K_{0.5}\left[1 + \left(\frac{[KCl]}{K_i}\right)^n\right]$$

In this latter case positive cooperativity ($n > 1$) would be represented in the plot (Fig. 2) by a curve concave upward; however, no such trend is apparent. Moreover, even if cooperativity did occur, the true $K_{0.5}$ would still be given by the extrapolation to the intercept on the ordinate, with the K_i still equivalent to the KCl concentration producing an apparent $K_{0.5}$ of twice the intercept. The plots, therefore, can provide a reasonable evaluation of the true $K_{0.5}$ and K_i, with or without cooperativity.

At 23° C similar relationships also were apparent (Fig. 1), and the extrapolated value of the $K_{0.5}$ for Na^+ was 1.23 mM, with a K_i for K^+ of 4.5 mM (Fig. 2; Table I).

The concentration of Mg · ATP also affected the apparent $K_{0.5}$ for Na^+ (Fig. 3). Mg · ATP influenced the K_i for K^+ as a competitor to Na^+, the K_i falling from 5.1 mM with 3 mM ATP to an extrapolated valued of 0.85 mM in the absence

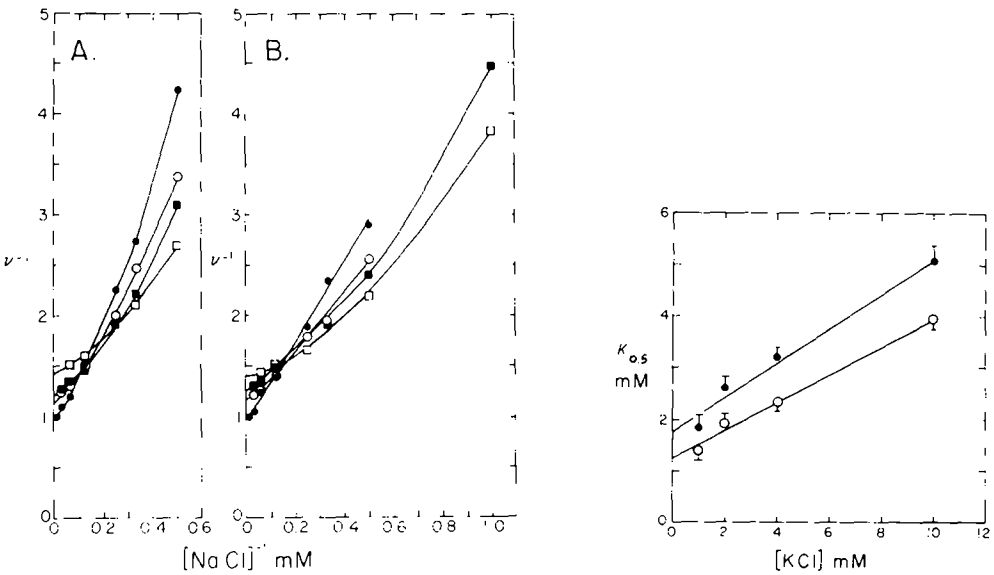

Fig. 1. Effect of NaCl on ($Na^+ + K^+$)-dependent ATPase activity. ATPase activity was measured at 37°C (panel A) or at 23°C (panel B) in media containing the concentration of NaCl indicated, 30 mM Tris/histidine · HCl (pH 7.8), 3 mM ATP, 3 mM $MgCl_2$, and the following concentrations of KCl: 10 mM (●); 4 mM (○); 2 mM (■); or 1 mM (□). Velocities are presented relative to that with 90 mM NaCl and 10 mM KCl, defined as 1.0; data presented are averages of four or more separate determinations, each performed in duplicate.

Fig. 2. Variation in apparent $K_{0.5}$ for Na^+ with KCl concentration. From the experiments shown in Fig. 1, at 37°C (●) and at 23°C (○), values of $K_{0.5}$ for Na^+ were obtained from Hill plots of the individual experiments, with straight lines fitted by the method of least squares [11]; the vertical bar represents one standard error of the mean. The straight lines in this graph were also fitted by the method of least squares; the intercept on the ordinate gives the extrapolated value for $K_{0.5}$ in the absence of KCl (Table I). The correlation coefficients were 0.98 (37°C) and 0.99 (23°C).

of ATP. The effect of Mg · ATP, however, was not limited to the K_i for K^+, but also was seen on the extrapolated value of the $K_{0.5}$ for Na^+ in the absence of KCl: the $K_{0.5}$ for Na^+ fell from 1.74 mM with 3 mM ATP to 0.26 mM in the absence of ATP (Table I). The calculated K_i for Mg · ATP to effect the changes was 0.5—0.6 mM. (In these experiments the $MgCl_2$ concentration was kept equal to

TABLE I

KINETIC PARAMETERS AT THE Na^+ SITES

$K_{0.5}$ values for Na^+, extrapolated to the absence of K^+, and the K_i for K^+ at these sites, are taken from Figs. 1, 2, 3, and 5.

Temperature	ATP concentration (mM)	Additions	$K_{0.5}$ for Na^+ (mM)	K_i for K^+ (mM)
37°C	3	none	1.74	5.1
	3	Me_2SO	0.96	3.7
	3	tetraphenyl boron	1.62	7.0
	0		0.26	0.85
23°C	3		1.23	4.5

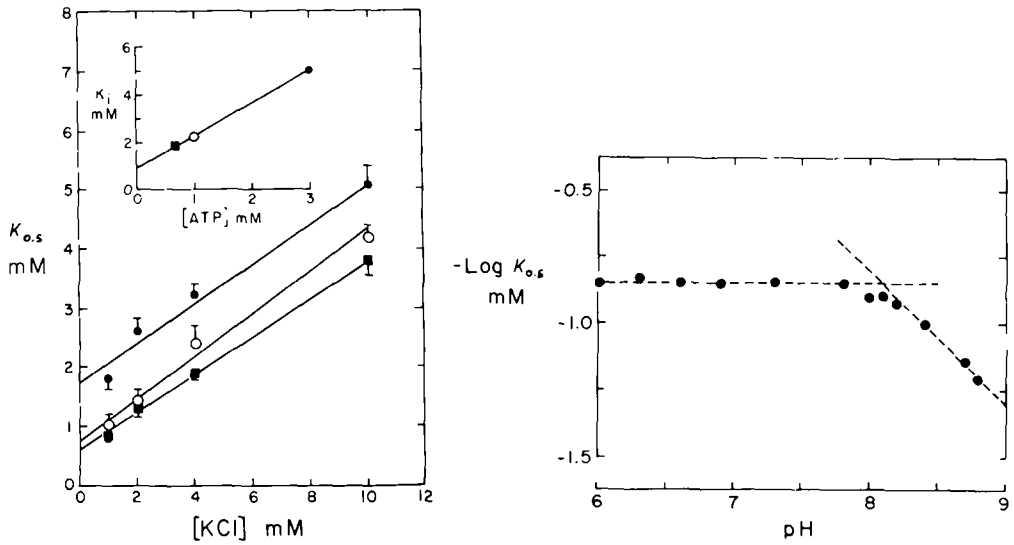

Fig. 3. Variation in apparent $K_{0.5}$ for Na^+ and K_i for K^+ with the ATP concentration. Experiments like those in Fig. 1 were performed in the presence of the following concentrations of ATP: 3 mM (●); 1 mM (○); and 0.7 mM (■). In all cases the $MgCl_2$ concentration was kept equal to the ATP concentration. The $K_{0.5}$ for Na^+, obtained as described in Fig. 2, is plotted against the KCl concentration for these concentrations of ATP, as in Fig. 2. In the inset, the calculated K_i for K^+ is plotted against the corresponding concentration of ATP. The straight lines were fitted by the method of least squares. The correlation coefficients were 0.98 (3 mM ATP), 0.99 (1 mM ATP), and 1.00 (0.7 mM ATP); for the insert the coefficient was 0.99.

Fig. 4. Variation in apparent $K_{0.5}$ for Na^+ with pH. The negative logarithm of the $K_{0.5}$ for Na^+, obtained as described in Fig. 2, is plotted against the measured pH of the incubation medium from experiments at 37°C with 3 mM ATP and 10 mM KCl. For pH values of 7.8 and below 30 mM histidine · HCl was adjusted to the desired pH with Tris; for pH 7.8 and above 30 mM Tris was adjusted with histidine · HCl. At pH 7.8 there was no difference in enzymatic response between incubations using the two buffers. The straight lines were fitted by eye.

the ATP concentration, so that the level of free Mg^{2+} was low in all circumstances [13].)

Variations in the pH of the incubation medium also affected the apparent $K_{0.5}$ for Na^+, and a plot of pH against $-\log K_{0.5}$ suggested that a group with a pK_a near 8.1 influenced the Na^+ sites (Fig. 4). By contrast, previous studies have shown that the $K_{0.5}$ for K^+ activation of the ATPase [6] and the K_m for Mg · ATP [14] do not vary over this pH range. The pH optimum for this enzyme preparation is broad, extending from pH 7.5 to 8.2 [14,15].

Me_2SO decreases the apparent $K_{0.5}$ for Na^+ [16] and examination of the extrapolated value for $K_{0.5}$ in the absence of K^+ (Fig. 5) indicated that 10% Me_2SO decreased both the $K_{0.5}$ for Na^+, to 0.96 mM, and the K_i for K^+, to 3.7 mM (Table I).

Post et al. [17] showed that 0.1 mM tetraphenyl boron, a concentration sufficiently low that the level of monovalent cations in the medium is not affected appreciably, influenced ATPase activity. This concentration of tetraphenyl boron inhibited ATPase activity slightly (Fig. 5), but decreased the apparent $K_{0.5}$ for Na^+ to 3.85 mM. The extrapolated $K_{0.5}$ in the absence of K^+ was, how-

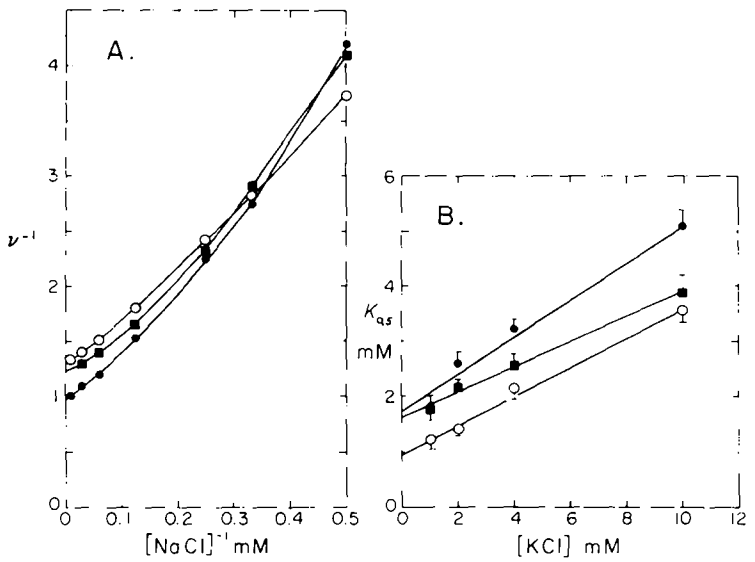

Fig. 5. Effect of Me_2SO and tetraphenyl boron on ($Na^+ + K^+$)-dependent ATPase activity. In panel A experiments like those in Fig. 1 are shown, from incubations at 37°C in the presence of 10 mM KCl (●), or in media containing in addition 10% (v/v) Me_2SO (○) or 0.1 mM tetraphenyl boron (■). In panel B the apparent $K_{0.5}$ for Na^+ from experiments with the concentrations of KCl shown are presented, as in Fig. 2. The straight lines extrapolating the $K_{0.5}$ to the absence of KCl were fitted by the method of least squares. The correlation coefficients were 0.98 (control) and 0.99 (Me_2SO and tetraphenyl boron).

ever, essentially unchanged at 1.62 mM, whereas the K_i for K^+ was increased to 7.0 mM (Table I).

To compare the characteristics of these Na^+ sites with the two classes of activating sites for K^+ [6] analogous experiments were undertaken. For the overall ATPase reaction, the $K_{0.5}$ for K^+ activation varied with the NaCl concentration (Fig. 6). Again, a linear extrapolation fitted the data (Fig. 7), permitting an

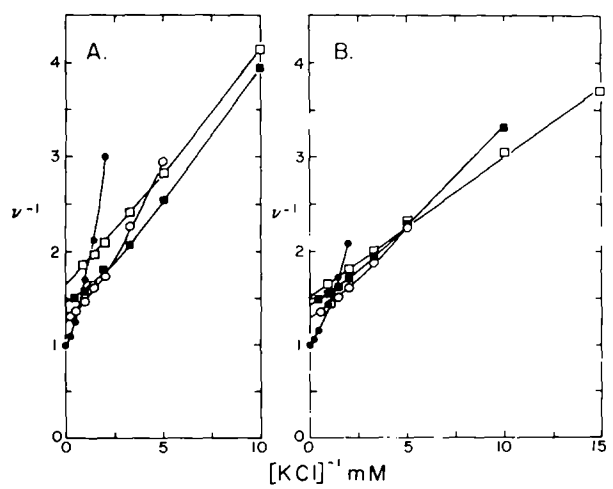

Fig. 6. Effect of KCl on ($Na^+ + K^+$)-dependent ATPase activity. ATPase activity was measured at 37°C (panel A) or at 23°C (panel B) in media containing the concentration of KCl indicated, 30 mM Tris/histidine · HCl (pH 7.8), 3 mM ATP, 3 mM $MgCl_2$, and the following concentrations of NaCl: 90 mM (●); 16 mM (○); 8 mM (■); or 4 mM (□). Data are presented as in Fig. 1.

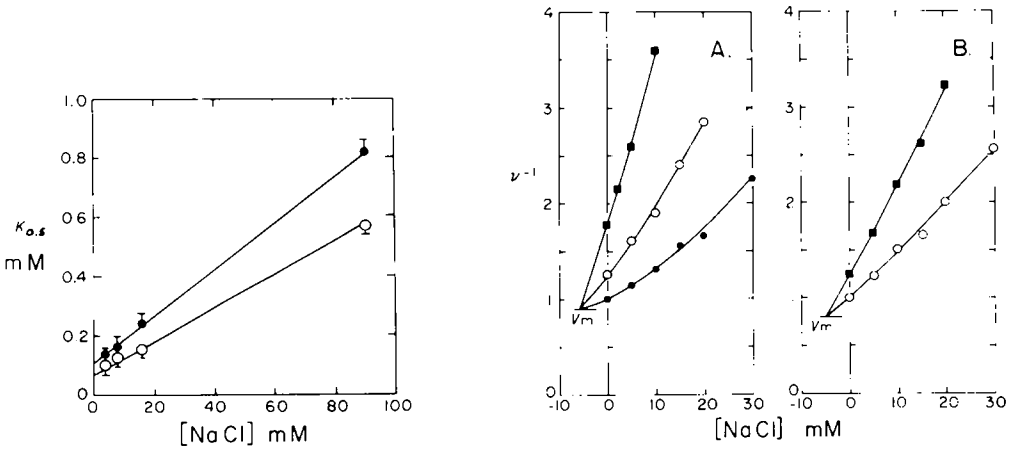

Fig. 7. Variation in apparent $K_{0.5}$ for K^+ with NaCl concentration. As in Fig. 2, values for $K_{0.5}$ from experiments at 37°C (●) and at 23°C (○) are plotted against the NaCl concentration in the medium. Straight lines were fitted by the method of least squares. The correlation coefficients were 1.00 for both.

Fig. 8. Effect of NaCl on K^+-dependent phosphatase activity. Phosphatase activity was measured at 37°C (panel A) or at 23°C (panel B) in media containing the concentration of NaCl indicated, 30 mM Tris/histidine · HCl (pH 7.8), 3 mM nitrophenyl phosphate, 3 mM $MgCl_2$, and the following concentrations of KCl: 10 mM (●); 4 mM (○); or 2 mM (■). Velocities are presented relative to that with 10 mM KCl without NaCl, defined as 1.0, and data are presented in the form of Dixon plots, with the maximal velocity indicated by the horizontal line.

estimate of 0.11 mM for the $K_{0.5}$ for K^+ in the absence of Na^+ (Table II); the calculated K_i for Na^+ was 13.8 mM. At 23°C (Figs. 6 and 7) the corresponding values were 0.077 mM for the $K_{0.5}$ and 14.3 mM for the K_i.

For the K^+-dependent phosphatase activity of the enzyme Na^+ is not required, and generally acts as an inhibitor [4,12]. The $K_{0.5}$ for K^+ in the absence of Na^+ (which can be measured directly in this case) was 1.96 mM, and the K_i for Na^+ was 5.5 mM (Fig. 8; Table II). At 23°C these values were 1.21 and 4.5 mM, respectively.

In contrast to the effects of Me_2SO on K^+-activation of the ATPase [16], the $K_{0.5}$ for K^+ in the phosphatase reaction was reduced in the presence of 10%

TABLE II

KINETIC PARAMETERS AT THE K^+ SITES

$K_{0.5}$ values for K^+ activation of the ATPase reaction, extrapolated to the absence of Na^+, and for K^+ activation of the phosphatase reaction, measured in the absence of Na^+, are presented together with the K_i values for Na^+.

Temperature	Additions	ATPase reaction: 'β-sites'		Phosphatase reaction: 'α-sites'	
		$K_{0.5}$ for K^+ (mM)	K_i for Na^+ (mM)	$K_{0.5}$ for K^+ (mM)	K_i for Na^+ (mM)
37°C	none	0.109	13.8	1.96	5.5
	Me_2SO			1.41	12.0
	tetraphenyl boron			5.92	8.5
23°C	none	0.077	14.3	1.21	4.5

Me_2SO, to 1.4 mM (Table II). On the other hand, the K_i for Na^+ was increased to 12 mM (Table II). Tetraphenyl boron, 0.1 mM, markedly increased the $K_{0.5}$ for K^+, to 5.9 mM (Table II), and also the K_i for Na^+, to 8.5 mM (Table II).

Previous experiments indicated that the $K_{0.5}$ for K^+ was increased as the concentration of substrate, nitrophenyl phosphate, was increased [12]; extrapolation to the absence of substrate gave a $K_{0.5}$ of 1 mM. The K_i for Na^+ also decreased somewhat with the concentration of nitrophenyl phosphate, with an extrapolated value of 3.5 mM in the absence of substrate.

Discussion

Characteristics of the Na^+ sites involved in activating the $(Na^+ + K^+)$-dependent ATPase were approached in terms of effects on the $K_{0.5}$ for Na^+. The measured $K_{0.5}$ varied with the KCl and ATP concentrations in the medium, and extrapolation to the absence of both gave a $K_{0.5}$ for Na^+ of 0.26 mM. The K_i for Mg · ATP (toward both Na^+ and K^+) was 0.5—0.6 mM, near a $K_{0.5}$ for Mg · ATP of this preparation [11,18,19].

This observation is consistent with recent formulations proposing two classes of substrate sites on the enzyme [18—21], high-affinity sites with a $K_{0.5}$ of about 1 μM and low-affinity sites with a $K_{0.5}$ of about 0.5 mM. The latter appear to serve in a modulating role [22,23], and at usual substrate concentrations may also act as catalytic sites [19]. The $K_{0.5}$ for Na^+, when extrapolated to the absence of ATP at the low-affinity sites, is near that recently reported by Foster and Ahmed [24] for enzyme phosphorylation, 0.29—0.41 mM, and also to the $K_{0.5}$ for Na^+ of the Na^+-dependent ATPase activity of this preparation, 0.4 mM (Robinson, J.D., unpublished observations); both enzyme phosphorylation and Na^+-dependent ATPase activity were measured at ATP concentrations below that required at the low-affinity substrate sites, and represent activities of the high-affinity substrate sites [19].

Foster and Ahmed [24] also described sites with far lower affinities for Na^+ (K_D = 24—40 mM) through which Na^+ activated phosphorylation, and Taniguchi and Post [25], in studies on enzyme phosphorylation by P_i, found stimulation with NaCl concentrations in the range 100—1000 mM. It is not known whether these very low-affinity sites are present on the exterior or interior faces of the cell membrane. Possibly phosphorylation is stimulated or stabilized in those experiments by Na^+ occupying its external low-affinity discharge sites. In any case, there is no indication of such activating sites in kinetic studies of $(Na^+ + K^+)$-dependent ATPase activity measured under usual experimental conditions.

The value found here for the $K_{0.5}$ for Na^+, 0.26 mM, is also near that observed for the sodium pump in red blood cells [5]; however, the K_i for K^+ is considerably smaller than in those studies. Moreover, the $K_{0.5}$ for Na^+ in red blood cells did not vary with ATP concentration [23], as was also shown in earlier enzyme studies [26]. The reason for these discrepancies is not apparent.

Because some transport proposals and enzyme models feature interconversion of Na^+ and K^+ sites or the identity of Na^+ sites with some class of K^+sites, further comparisons were pursued. As would be expected, the K^+ sites mediating enzyme dephosphorylation, termed "β-sites" for K^+ [6], differ markedly

from the Na^+ sites in $K_{0.5}$ (or K_i) for Na^+ and K^+. In this instance, both the $K_{0.5}$ for K^+ and K_i for Na^+ at the β-sites are in reasonable agreement with values for the external K^+ sites of the sodium pump in red blood cells [5], each differing from those values by a factor of two.

The K^+ sites activating the phosphatase reaction, termed "α-sites" for K^+ [6], share some similarities with the Na^+ sites, including affinities for Na^+ and K^+ in the same general concentration range. This is of particular interest because Skou [9] and Gache et al. [10] proposed that K^+ activation of the phosphatase reaction is at least in part through occupancy of the internal Na^+ sites. Post et al. [17] suggested a relationship between the activating sites for Na^+ and internal sites for K^+ with properties similar to the α-sites (e.g. in terms of affinity and antagonism to ATP binding), and Swann and Albers [27] described internal K^+ sites regulating the phosphatase reaction.

Perhaps the most striking similarity between the α-sites and Na^+ sites is the response to pH: for the α-sites a group with a pK_a near 8.1 has been implicated in altering the $K_{0.5}$ for K^+ [6], as is also seen here for the Na^+ sites. By contrast, plots of $-\log K_{0.5}$ for the β-sites were flat in this pH range [6]. But on closer examination differences between α-sites and Na^+ sites are more apparent than

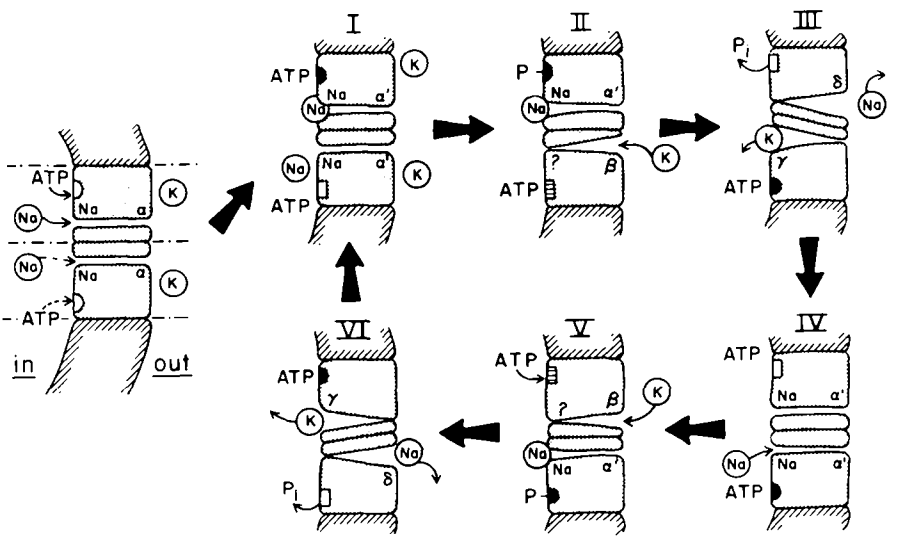

Fig. 9. Proposed transport model. The free enzyme, shown on the left, is composed of two catalytic units, each bearing a high-affinity substrate site and containing potential transport channels with Na^+ sites on the interior face and α-sites on the exterior face (since three Na^+ are transported for each ATP hydrolyzed [2] presumably three channels are present for each unit, although the drawing illustrates only one). In step I the addition of ATP to one subunit reduces the affinity of the substrate site on the other subunit, and also reduces the affinity for K^+ at the α-sites [14], $\alpha - \alpha'$. In step II, with Na^+ bound to the Na^+ sites, the enzyme is phosphorylated and β-sites appear [29] on the opposite subunit (under physiological conditions the high Na^+ concentration of the extracellular fluid would prevent occupancy of the α-sites by K^+ [28]). In step III dephosphorylation, resulting from K^+ occupying the β-sites, is accompanied by Na^+ and K^+ transport: a "flip-flop" translocation scheme [31,32] through "oscillating pores" [29,30] is shown. K^+ is released from transient discharge sites on the interior, γ [6], and Na^+ from transient discharge sites on the exterior, δ. The low-affinity substrate site, which had bound ATP at an earlier step, becomes the high-affinity site after dephosphorylation. Steps IV through VI then repeat I through III, but with the alternate subunits. The cycle continues without reappearance of the initial free enzyme as long as cation levels are sufficient to fill the Na^+ and β-sites and ATP levels to fill the low-affinity substrate site.

similarities. Apparent affinities do not coincide closely; this is not attributable merely to the different substrates used in ATPase and phosphatase assays, for after extrapolation to the absence of substrate the $K_{0.5}$ for Na^+ is 0.26 mM, as opposed to a K_i for Na^+ at the α-sites of 3.5 mM. In addition, the effects of Me_2SO and tetraphenyl boron on the $K_{0.5}$ and K_i for Na^+ differend between Na^+ sites and α-sites.

These discrepancies thus support previous formulations distinguishing between α-sites and Na^+ sites [6,28]. Moreover, the data are consistent with the earlier observations by Rega et al. [7,8] on the sidedness of the K^+-dependent phosphatase reaction in red blood cells: they found activation by external K^+, whereas internal K^+ alone or with external K^+ was ineffective.

Nevertheless, even with α-sites on one membrane face of the ATPase and activating Na^+ sites on the other, a direct relationship between the two may exist. Early models for transport proposed a cyclical reaction process in which internal sites for Na^+ were transformed to external K^+ sites, and then back again to internal Na^+ sites [1,2]. Evidence against such schemes included demonstrations of coexisting sites for Na^+ and K^+ [29,30]. This argument, however, may be reconciled with the earlier proposals in terms of a dimeric enzyme, one half of which has internal activating sites for sodium and the other half external activating β-sites [19]. Transport would be accomplished by a flip-flop mechanism, originally proposed by Repke and Schön [31] and Stein et al. [32], through ion-specific channels [12,29,30]. The similarities of the α-sites both to the Na^+ sites (e.g. response to pH) and to the β-sites (e.g. activation of hydrolysis) could thus reflect a cyclical interconversion between channels for Na^+ and for K^+. The α-sites might represent, during phases of the reaction cycle, the external aspect of the Na^+ channel on one monomer, as well as on the other monomer the precursor to the β-sites, which appear in the reaction sequence after enzyme phosphorylation [6,29,30]. A model consistent with these considerations is shown in Fig. 9.

Acknowledgements

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