Biochimica et Biophysica Acta, 397 (1975) 194–206

BBA 67535

INTERACTIONS BETWEEN K^+ AND ATP BINDING TO THE ($Na^+ + K^+$)-DEPENDENT ATPase

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(Received January 6th, 1975)

Summary

K^+ appears to decrease the affinity of the ($Na^+ + K^+$)-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) for its substrate, $Mg^{2+} \cdot ATP$, and $Mg^{2+} \cdot ATP$, in turn, appears to decrease the affinity of the enzyme for K^+. These antagonisms have been investigated in terms of a quantitative model defining the magnitude of the effects as well as identifying the class of K^+ sites on the enzyme involved. K^+ increased the apparent K_m for $Mg^{2+} \cdot ATP$, an effect that was antagonized competitively by Na^+. The data can be fitted to a model in which $Mg^{2+} \cdot ATP$ binding is prevented by occupancy of α-sites on the enzyme by K^+ (i.e. sites of moderate affinity for K^+ accessible on the "free" non-phosphorylated enzyme, in situ on the external membrane surface). By contrast, occupancy of these α-sites by Na^+ has no effect on $Mg^{2+} \cdot ATP$ binding to the enzyme. On the other hand, $Mg^{2+} \cdot ATP$ decreased the apparent affinity of the enzyme for K^+ at the α-sites, in terms of (i) the K_D for K^+ measured by K^+-accelerated inactivation of the enzyme by F^-, and (ii) the concentration of K^+ for half-maximal activation of the K^+-dependent phosphatase reaction (which reflects the terminal hydrolytic steps of the overall ATPase reaction). These data fit the same quantitative model. Although this formulation does not support schemes in which ATP binding effects the release of transported K^+ from discharge sites, it is consistent with observations that K^+ can inhibit the enzyme at low substrate concentrations, and that Li^+, which has poor efficacy when occupying these α-sites, can stimulate enzymatic activity at high K^+ concentrations by displacing the inhibitory K^+.

Introduction

Because the ($Na^+ + K^+$)-dependent ATPase (EC 3.6.1.3) apparently represents the enzymatic basis for the membrane sodium/potassium pump [1,2], considerable attention has centered on the activation of this enzyme by the cations that the pump transports [3]. Progress in defining the cation interac-

tions, however, has been slowed by the complexities of the reaction sequence and the multitude of cation effects. Recent studies [3,4] on enzyme activation have led to a formulation in terms of two distinct classes of sites for K^+. (i) α-sites of moderate affinity for K^+ are demonstrable on the "free" (non-phosphorylated) enzyme both in terms of K^+-dependent enzyme inactivation [5] and in terms of the K^+-dependent phosphatase activity associated with the enzyme which reflects the terminal hydrolytic steps of the overall ATPase activity [6]. (ii) β-sites of high affinity are demonstrable on the enzyme under conditions in which the enzyme is phosphorylated by nucleotides [3—6], including the overall ATPase reaction in which an acyl phosphate intermediate occurs [2].

This report is concerned with another facet of the interactions between K^+ and the enzyme, the relationship between nucleotide and K^+ binding sites. Early kinetic studies [7] showed that the apparent K_m for Mg^{2+} · ATP increased with the K^+ concentration. Subsequently, direct measurements of ATP binding to the enzyme [8,9] demonstrated that K^+ indeed increased the dissociation constant for ATP, an effect that, in one study [9], was antagonized competitively by Na^+. The experiments described here were undertaken to explore these interactions in terms of the distinguishable classes of sites available to K^+, and, in addition, to incorporate these observations into a quantitative model.

Methods and Materials

The (Na^+ + K^+)-dependent ATPase was obtained from a rat brain microsomal preparation by treatment with deoxycholate and then NaI, as previously described [7].

(Na^+ + K^+)-dependent ATPase activity was measured in terms of the production of P_i, as previously described [7]. The standard medium contained 30 mM histidine · HCl/Tris (pH 7.8), 3.5 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). Incubation was for 4—8 min at 37° C; activity was linear with time during these periods. Activity in the absence of Na^+ and K^+ ("Mg^{2+} · ATPase") was measured concurrently; such activity averaged only a few percent of the (Na^+ + K^+)-dependent ATPase activity [7], and was subtracted from the total activity in the presence of Na^+ and K^+ to give the (Na^+ + K^+)-dependent activity. Because of variations in the absolute activity of different enzyme preparations, 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 [6]. The standard medium contained 30 mM histidine · HCl/Tris (pH 7.8), 3 mM $MgCl_2$, 3 mM nitrophenyl phosphate (as the Tris salt), 10 mM KCl, and the enzyme preparation (0.1 mg protein/ml). Incubation was for 8—15 min at 37° C; activity in the absence of added KCl was measured concurrently; such activity averaged only a few percent of the K^+-dependent phosphatase activity under optimal conditions [6], and was subtracted from the total activity in the presence of KCl to give the K^+-dependent activity. 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.

The affinity for K^+ was also approached in terms of K^+-accelerated inactivation of the enzyme by F^- [3,4]. In these experiments the pseudo first-order rate constants for enzyme inactivation were determined as a function of K^+ concentration, by means of initial incubations at 37°C of enzyme (0.5 mg protein/ml), 30 mM histidine · HCl/Tris (pH 7.8), 0.5 mM $MgCl_2$, 1.5 mM LiF, and a range of KCl concentrations (plus other additions as specified). These inactivating incubations were terminated by adding four volumes of an ATPase incubation medium so that the final concentrations of reactants were those of the standard medium (above), and the residual activity was then measured during brief incubations (changes in activity during this assay incubation were negligible since the inactivation is essentially irreversible and the dilution of F^- and the added NaCl prevent further inactivation). The kinetic model is identical to that for the inactivation of this enzyme by Be^{2+} [5]: the pseudo first-order rate constants for inactivation, k'_{in}, can be treated analogously to initial velocities in enzyme kinetics, and thus plots of k'_{in} against KCl concentration in double reciprocal form permit estimation of the dissociation constant K_D.

The data presented are averages of four or more experiments performed in duplicate.

ATP and nitrophenyl phosphate were purchased from Sigma Chemical Co. as the sodium salts and converted to the Tris salts. All solutions were made in water redistilled from an all-glass still. Protein was measured by the biuret method, using bovine serum albumin as a standard.

Results and Discussion

Effects of K^+ on the K_m for Mg^{2+} · ATP

In these determinations of the K_m for substrate, $MgCl_2$ and ATP were varied together, with a constant molar excess of $MgCl_2$ (0.5 mM), since Mg^{2+} · ATP has been shown [10,11] to be the true substrate of the ($Na^+ + K^+$)-dependent ATPase. Essentially all the ATP is calculated to be in the form of the Mg^{2+} complex under these conditions [12].

As previously shown [7], the apparent K_m (K'_m) for Mg^{2+} · ATP increased with the K^+ concentration (Fig. 1). Moreover, the magnitude of the K^+ effect depended on the concentration of Na^+ (Fig. 1), although in all cases the "true" K_m, the extrapolated value in the absence of K^+, appeared to be about 0.3 mM.

To relate this apparent "competition" between K^+ and Mg^{2+}—ATP a model in which binding of K^+ and of Mg^{2+} · ATP to the enzyme are mutually exclusive seems plausible; in addition, since Na^+ decreased the effect of K^+ on K'_m (Fig. 1), competition between Na^+ and K^+ for the effector sites is also required:

$$\begin{array}{l} E \cdot K \overset{K^+}{\rightleftharpoons} E \overset{Mg^{2+}\cdot ATP}{\rightleftharpoons} E \cdot Mg^{2+} \cdot ATP \rightarrow \\ \qquad\quad \underset{Na^+}{\downarrow\uparrow} \\ \qquad\quad E \cdot Na \overset{Mg^{2+}\cdot ATP}{\rightleftharpoons} E \cdot Na \cdot Mg^{2+} \cdot ATP \end{array}$$

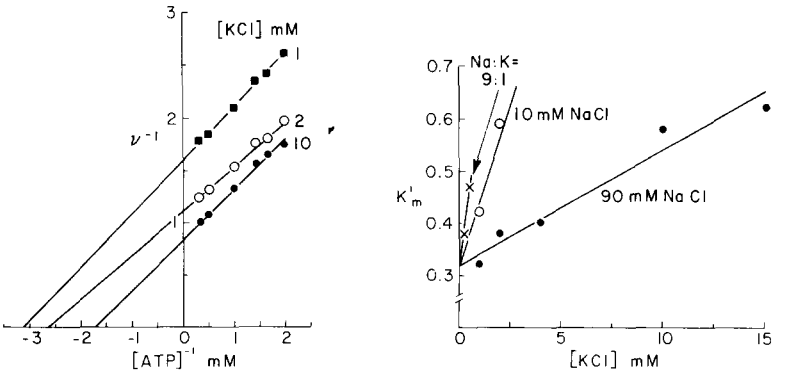


Fig. 1. Effect of K^+ on the apparent K_m for Mg^{2+} · ATP. In the left-hand panel experiments are shown in which the enzyme was incubated at pH 7.8 with the concentrations of ATP shown, $MgCl_2$ at a molar excess over ATP of 0.5 mM, 90 mM NaCl, and three concentrations of KCl: 10 mM (•), 2 mM (○), or 1 mM (■). Data are presented in double-reciprocal form. In the right-hand panel the apparent K_m for Mg^{2+} · ATP from such experiments is plotted against the KCl concentrations: with 90 mM NaCl (•), with 10 mM NaCl (○), and from experiments with a NaCl to KCl ratio of 9 : 1 (X).

The relative affinity of E and E · Na for Mg^{2+} · ATP would appear to be equivalent since the extrapolated value of K_m is the same at various Na^+ concentrations (Fig. 1). Thus these interactions (see Appendix) are expressed by:

$$K'_m = K_m \left\{ 1 + \frac{[K^+]}{K_1 \left(1 + \frac{[Na^+]}{K_2}\right)} \right\} \quad (1)$$

where K'_m is the observed K_m for Mg^{2+} · ATP, K_1 the dissociation constant for K^+ from the sites antagonizing Mg^{2+} · ATP binding, and K_2 the dissociation constant for Na^+ at these same sites.

Before evaluating K_1 and K_2 in terms of this relationship, the nature of the cation sites influencing Mg^{2+} · ATP binding may be considered. It would seem unlikely that occupancy of the β-sites by K^+ could influence Mg^{2+} · ATP binding, since Mg^{2+} · ATP binding occurs before the appearance in the reaction sequence of β-sites: the β-sites appear after phosphorylation of the enzyme by ATP [3—5]. Moreover, direct studies of ATP binding to the enzyme [8,9] under conditions in which enzyme phosphorylation by ATP would not occur (and hence β-sites could not be available) also demonstrated an antagonism between K^+ and ATP binding. Consequently, it would seem likely that the effect of K^+ is mediated through α-sites, available on the "free" enzyme. Thus values for K_1 and K_2 compatible with the affinities of K^+ and Na^+ for the α-sites (Table I) were tested in this formulation. As can be seen in Fig. 2, good agreement between observed and calculated values for the apparent K_m was obtained.

Although this relationship expresses a "competitive" interaction between Mg^{2+} · ATP at the substrate sites and K^+ at the α-sites, Fig. 1 (and Fig. 3) do not show simple competition between K^+ and Mg^{2+} · ATP: increasing K^+ concentrations increase the apparent V as well as K'_m . However, this effect on V is to be expected since K^+ also activates ATP hydrolysis through the β-sites [4].

TABLE I

KINETIC PARAMETERS

Values of the kinetic parameters used in Eqns 1—3 are listed, together with previously determined values for comparable parameters: for K_1, previous values for the K_D from K^+-accelerated inactivation experiments using F^- [4] or Be^{2+} [5], as well as values for $K_{0.5}$ for K^+ activation of the phosphatase reaction [4,6]; for K_2, the corresponding inhibitory constants for Na^+ at the α-sites. References are cited by the numbers in brackets.

Parameter	Value used (mM)	Previous values (mM)	
		From inactivation experiments	From phosphatase reaction
pH 8.6			
K_1 (K^+)	3.5		7.9 [4]
K_2 (Na^+)	21		21 [4]
pH 7.8			
K_1 (K^+)	1.1	1.1—1.4 [4, 5]	1.9 [6]
K_2 (Na^+)	6.0	5 —7 [4, 5]	6 [6]
K_m (Mg^{2+} · ATP)	0.32		
K_i (Mg^{2+} · ATP)	0.13		
K_m (nitrophenyl phosphate)	3.3		

This activating effect of K^+ at the β-sites, the usually considered action of K^+, increases with successively higher concentrations of KCl so that at infinite Mg^{2+} · ATP concentration (at the ordinate of the plots) the apparent V varies directly with the KCl concentrations. These diverse actions of K^+ necessitate the use of the model presented above to describe the actions at the α-sites on K'_m independently of actions at the β-sites on apparent V.

As a further test of this model the effect of pH on the K'_m for Mg^{2+} · ATP and the K^+-Mg^{2+} · ATP antagonism was examined. Although the apparent affinity for K^+ at the β-sites did not change over the pH range 6—9, the apparent

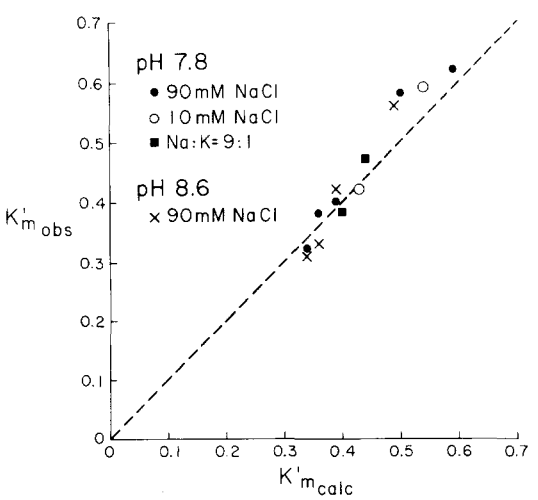


Fig. 2. Correspondence between the observed and calculated values of K'_m for Mg^{2+} · ATP. The observed K'_m values from Fig. 1 are plotted against values calculated from Eqn 1 and the parameters of Table I. In addition, values of K'_m from experiments at pH 8.6 (Fig. 3) are included. The dashed line indicates perfect correspondence.

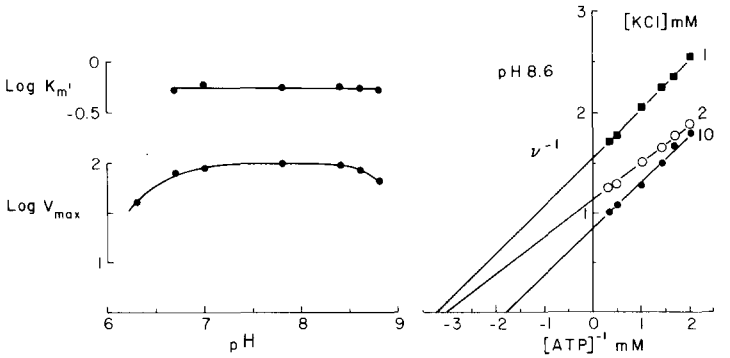


Fig. 3. Effect of pH on the ATPase reaction. In the right-hand panel the logarithm of the apparent K_m for Mg^{2+} · ATP and of the V are plotted against the measured pH of the incubation medium. For pH values from 7.8 downward, 30 mM histidine · HCl was adjusted to the desired pH with Tris; for values above 7.8, 30 mM Tris was adjusted with histidine · HCl. K'_m for Mg^{2+} · ATP and V (in terms of Mg^{2+} · ATP) were obtained by varying ATP and $MgCl_2$, as in Fig. 1, in the presence of 90 mM NaCl and 10 mM KCl. In the right-hand panel the effect of KCl on K'_m for Mg^{2+} · ATP is shown. Experiments were performed and the data are presented as in Fig. 1, except that the measured pH of the incubation media was 8.6.

affinity for K^+ at the α-sites decreased at basic pH, with an estimated pK_a of 8.1 for the modifying element [4]. Thus examination of the effects of K^+ at pH 8.6 should discriminate further between α- and β-sites.

The apparent K_m for ATP with 10 mM KCl did not vary with pH over the range 6.7—8.7, although V did (Fig. 3). Nevertheless, the apparent K_m did vary with K^+ concentration at pH 8.6 (Fig. 3) and when values for the dissociation constants for K^+ and Na^+ at pH 8.6 (Table I) were substituted in Eqn 1, in a range consistent with their previous determination [4], there was again good agreement between the calculated and observed values of the apparent K_m for Mg^{2+} · ATP (Fig. 2).

An alternative formulation might be considered in terms of K^+ and Na^+ occupying the Na^+ sites (i.e. those normally occupied by Na^+ to activate enzyme phosphorylation) and thereby influencing ATP binding. However, the values of K_1 and K_2 (Table I) that satisfy the model are incompatible with the respective affinities of Na^+ and K^+ for the Na^+ sites: at the Na^+ sites governing ATPase activity the K_D is about 2 mM [3,4], while the K_i for K^+ as a competitor at these sites is roughly 12 mM [3,4].

Post et al. [13] proposed that the effects of K^+ on ATP binding might be mediated through K^+ binding to the discharge sites of the pump mechanism (or to some occluded conformation in the translocation process). The ability of K^+ to decrease ATP binding in the "free" enzyme [8,9] argues against the necessary participation of K^+ at an intermediate occluded conformation, and the calculated affinities of K^+ and Na^+ (Table I) seem inappropriate for K^+-discharge sites were K^+ must be released into the high K^+/low Na^+ cytoplasm.

Effect of Mg^{2+} · ATP on the K_D for K^+ at the α-sites

The preceding formulation of the interaction between K^+ at the α-sites and Mg^{2+} · ATP binding implies that Mg^{2+} · ATP at the substrate sites should influence K^+ binding. That is, increasing concentrations of Mg^{2+} · ATP should increase the dissociation constant for K^+, K_D, at the α-sites. This K_D may be

evaluated in terms of the K^+-accelerated inactivation of the enzyme by F^-, as previously described [3,4]:

$$E \overset{K^+}{\rightleftharpoons} E \cdot K \overset{F^-}{\rightarrow} E_{in}$$

where E is the free enzyme, $E \cdot K$ the complex sensitive to inactivation by F^-, and E_{in} the inactivated enzyme.

In these experiments the enzyme is first incubated for various times with F^- and Mg^{2+}, in the absence and presence of K^+, and the residual (Na^+ + K^+)-dependent ATPase activity then measured in assay incubations [3,4]. The calculated pseudo first-order rate constants for inactivation, k'_{in}, are functions of the K^+ concentration, and double-reciprocal plots permit estimation of the K_D for K^+ from the sites of the enzyme-controlling inactivation (Fig. 4). These K^+ sites are classified as α-sites [4], corresponding to the K^+ sites activating the K^+-dependent phosphatase reaction in terms of specific criteria distinguishing them from the β-sites, such as affinity for K^+ and Na^+, relative efficacy of Li^+, and response to pH [4].

In these experiments the addition of ATP increased the apparent K_D for K^+, K'_D (Fig. 4). In accord with the effects of K^+ on the K'_m for $Mg^{2+} \cdot ATP$, these data should then be in quantitative agreement with the converse of the model described above:

$$E \cdot Mg^{2+} \cdot ATP \overset{Mg^{2+} \cdot ATP}{\rightleftharpoons} E \overset{K^+}{\rightleftharpoons} E \cdot K \overset{F^-}{\rightarrow} E_{in}$$

Therefore

$$K'_D = K_D \left(1 + \frac{[Mg^{2+} \cdot ATP]}{K_s}\right) \tag{2}$$

where K_s is the dissociation constant for $Mg^{2+} \cdot ATP$ from the enzyme.

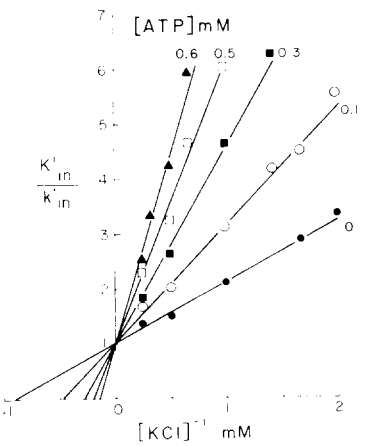


Fig. 4. Effect of ATP on the K^+-accelerated inactivation of the enzyme by F^-. In these experiments the pseudo first-order rate constant for inactivation by F^- was measured, as described under Methods and Materials, from incubations containing the concentrations of KCl shown, in the absence and presence of ATP: ●, none; ○, 0.1; ■, 0.3; □, 0.5; and ▲, 0.6 mM. An equimolar amount of $MgCl_2$ was added with the ATP. Data are presented in double-reciprocal form, with the k'_{in} normalized by division into K'_{in}, the maximal pseudo first-order rate constant at infinite KCl, for each ATP concentration.

Before fitting the data to this model it is necessary to consider a possible difference between (i) the K_m for Mg^{2+} · ATP, the Michaelis constant under steady-state conditions (as measured above: Fig. 1), and (ii) the true dissociation constant for the substrate, K_s. In single-substrate enzymatic reactions K_m is frequently greater than K_s because the rate constant for product formation from the enzyme-substrate complex, k_{+2}, may be of comparable magnitude to that for the breakdown of the complex, k_{-1}. Since $K_m = (k_{+2}) + (k_{-1})/k_{+1}$, whereas $K_s = k_{-1}/k_{+1}$, a considerable disparity can occur (for multi-substrate reactions the relationship between the observed K_m and K_s can be even more remote).

One approach to evaluating K_s for Mg^{2+} · ATP is through its action as a competitive inhibitor toward nitrophenyl phosphate, the substrate for the K^+-dependent phosphatase reaction: the K_i for a competitive inhibitor can be equated to the dissociation constant at the inhibitory site [14]. From such studies a K_i of 0.13 mM was obtained for Mg^{2+} · ATP (Fig. 5), about half of the value for K_m (Fig. 1).

Using this K_i value as the K_s for Mg^{2+} · ATP in Eqn 2, the correspondence between the observed and calculated values of K'_D may be examined, using the K_D for K^+ in the absence of Mg^{2+}-ATP, 1.1 mM, as the "true" K_D (Fig. 6, Table I): agreement is excellent.

Because higher concentrations of Mg^{2+} · ATP slow inactivation, the K'_D for K^+ could not be measured at ATP concentrations found in vivo, approx, 2 mM [15]. Nevertheless, from Eqn 2 and the values of Table I it can be calculated that the effective K'_D for K^+ would be increased only to 16 mM by 2 mM Mg^{2+} · ATP. This provides further evidence (cf. ref. 4) that these K^+ sites sensitive to Mg^{2+} · ATP binding do not correspond to K^+-discharge sites of the cation pump.

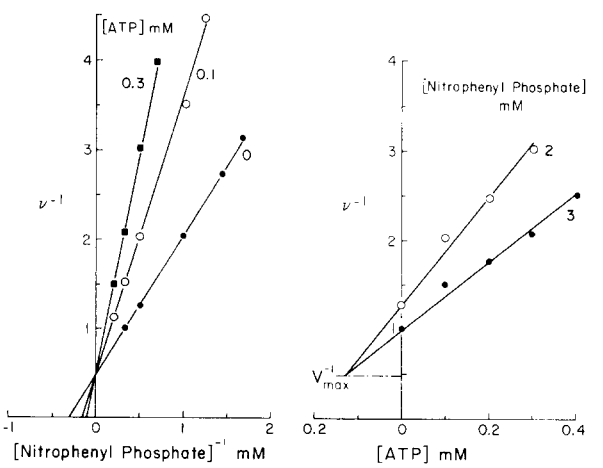


Fig. 5. Competition between ATP and nitrophenyl phosphate in the phosphatase reaction. K^+-dependent phosphatase activity was measured in the presence of 10 mM KCl, with the varied amounts of nitrophenyl phosphate and ATP shown. The $MgCl_2$ concentration equaled the sum of these two substances. In the left-hand panel experiments are shown in which the nitrophenyl phosphate concentration was varied at fixed levels of ATP; data are presented in double-reciprocal form. In the right-hand panel experiments are shown in which ATP is varied at fixed levels of nitrophenyl phosphate; data are presented in the form of a Dixon plot.

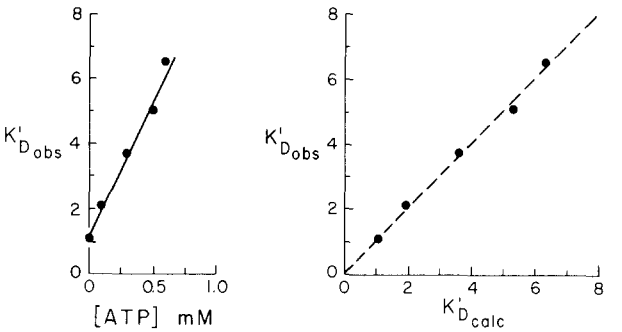


Fig. 6. Effect of ATP on the apparent K_D for K^+. In the left-hand panel the values of K'_D from Fig. 4 are plotted against the concentration of ATP added. In the right-hand panel these observed values of K'_D are compared with values of K'_D calculated from Eqn 2, using the parameters of Table I. The dashed line indicates perfect correspondence.

Effect of Mg^{2+} · ATP on the $K_{0.5}$ for K^+ of the phosphatase reaction

An alternative approach to evaluating the effects of Mg^{2+} · ATP on the apparent affinity for K^+ at the α-sites, in terms of the K^+-dependent phosphatase reaction, gave similar results (Fig. 7). The concentration of K^+ for half-maximal activation, $K_{0.5}$, increased as the Mg^{2+} · ATP concentration was increased. However, to evaluate these results quantitatively the preceding formulation must be modified since nitrophenyl phosphate, present in these ex-

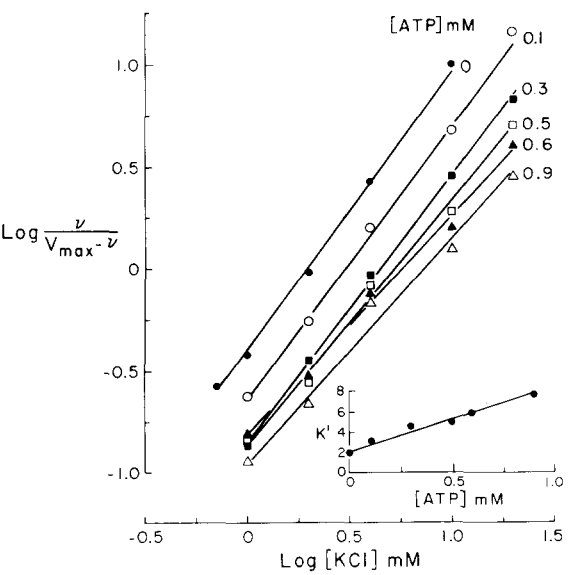


Fig. 7. Effect of ATP on the $K_{0.5}$ for K^+ of the phosphatase reaction. K^+-dependent phosphatase activity was measured in the presence of 3 mM nitrophenyl phosphate, the concentrations of KCl shown, in the absence of ATP (●) or with 0.1 (○), 0.3 (■), 0.5 (□), 0.6 (▲), or 0.9 (△) mM ATP. The concentration of $MgCl_2$ equalled the sum of the nitrophenyl phosphate plus ATP concentrations. Data are presented in the form of a Hill plot of the kinetic equation: $V/v = 1 + (K/S)^n$, so that K is equal to the concentration for half-maximal activation, $K_{0.5}$. In the inset, the apparent values of K are plotted against the ATP concentration.

periments as the substrate for the phosphate reaction, competes with $Mg^{2+} \cdot ATP$ (Fig. 5). Thus the interactions would be:

$$E \cdot Mg^{2+} \cdot ATP \overset{Mg^{2+} \cdot ATP}{\rightleftharpoons} E \overset{K^+, NPP}{\rightleftharpoons} E \cdot K \cdot NPP \rightarrow$$

where NPP represents nitrophenyl phosphate. The quantitative relationship is thus expressed:

$$K'_{0.5} = K_{0.5} \left\{ 1 + \frac{[Mg^{2+} \cdot ATP]}{K_i \left(1 + \frac{[NPP]}{K_S}\right)} \right\} \tag{3}$$

where K_S is here the dissociation constant for nitrophenyl phosphate at its substrate sites. Using the value of $K_{0.5}$ for K^+ in the absence of ATP, 1.95 mM, as the "true" $K_{0.5}$, the K_i for $Mg^{2+} \cdot ATP$ as before, and the K_m for nitrophenyl phosphate (Fig. 5) as K_S (Table I) fair agreement is found between observed and calculated values (Fig. 8). Better agreement can be obtained if a lower value for K_S, 2 mM, is substituted into Eqn 3 (Fig. 8), and this may be justified on the basis of considerations discussed above; i.e. K_S is often smaller than K_m. Another potential source of error lies in the possibility that nitrophenyl phosphate at the substrate sites might itself alter the affinity for K^+ at the α-sites [6].

Good general agreement with the model is achieved in accord with results from the inactivation experiments, but although the $K_{0.5}$ for K^+ in the phosphatase reaction and the K_D for K^+ from the inactivation experiments both represent α-sites on the enzyme, the observed values differ somewhat (Table I). This seeming discrepancy may be due (i) to competitive effects of Mg^{2+} toward K^+ [4,6], Mg^{2+} being present at different concentrations in the two types of experiments; (ii) to specific effects of nitrophenyl phosphate on the α-sites, as suggested above; and (iii) to the possibility that the kinetically determined

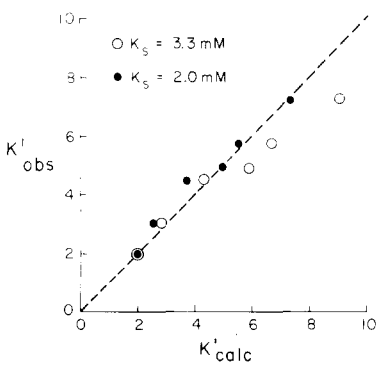


Fig. 8. Correspondence between the observed and calculated values of K'. Observed values of K', from Fig. 7, are plotted against the calculated values using Eqn 3 and the parameters of Table I, with the "true" K taken as 1.95 mM and the K_m for nitrophenyl phosphate, 3.3 mM, as the K_S (○). A smaller value assumed for K_S, 2 mM, gives a better correspondence (●).

$K_{0.5}$ may reflect other aspects of the overall catalytic process beyond enzyme-K^+ binding, just as K_m can differ from K_s.

Conclusions

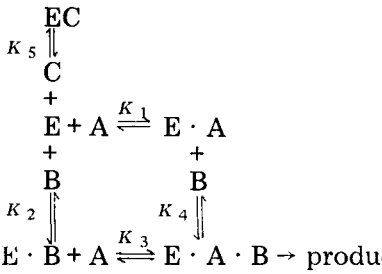
Occupancy of the α-sites on the (Na^+ + K^+)-dependent ATPase by K^+ is antagonized by Mg^{2+} · ATP, and Mg^{2+} · ATP occupancy of the substrate sites is antagonized by K^+ at the α-sites, in a mutually competitive fashion. Evaluations of these interactions in terms of K^+ effects on the observed K_m for Mg^{2+} · ATP and Mg^{2+} · ATP effects on the observed K_D for K^+ (measured through inactivation by F^-) and $K_{0.5}$ for K^+ (in terms of the phosphatase reaction) are in good quantitative agreement with this model. These data indicate that it is through occupancy of the α-sites on the enzyme that K^+ is able to decrease the binding of ATP to the enzyme, as observed by Nørby and Jensen [8] and Hegyvary and Post [9], and to inhibit the ATPase at low substrate concentrations, as observed by Neufeld and Levy [16] and Post et al. [13].

Post et al. [13] proposed that the antagonism seen between K^+ and ATP binding would reflect a similar antagonism between ATP and K^+ binding, and that such a relationship might represent a mechanism for effecting the discharge of transported K^+ by the membrane pump. Although the data presented here do show such an antagonism between ATP and K^+, the quantitative relationship is inconsistent with such a formulation: the level of Mg^{2+}· ATP found in vivo seems inadequate to alter the K_D sufficiently. Beyond this, the K^+ sites in question are identified here as α-sites, corresponding to those activating the K^+-dependent phosphatase reaction, and Rega et al. [17] have shown that the sites activating the reaction in situ in erythrocyte membranes are accessible from the external medium. By contrast, the K^+-discharge sites of the pump must face the cellular interior.

These experiments also suggest an explanation for the puzzling stimulation of the (Na^+ + K^+)-dependent ATPase activity by Li^+ reported in the presence of "saturating" concentrations of K^+ [18,19]. The efficacy of Li^+ at the α-sites (inhibitory to the ATPase) is quite low compared to K^+, whereas the efficacy at the activating β-sites is much more nearly equivalent to that of K^+ [4]. Since at high KCl concentrations significant occupancy of the α-sites will occur in vitro, inhibition can result from a decreased binding of Mg^{2+} · ATP to the substrate sites. Under such circumstances the addition of Li^+ as a competitor to K^+ can relieve this inhibition by virtue of its low efficacy at the α-sites. At the activating β-sites, where the efficacy of Li^+ more nearly approaches that of K^+ [4], the displacement of K^+ by Li^+ would have little effect on activation. The sum of the decreased inhibition at the α-sites and the nearly equivalent activation at the β-sites could then produce the stimulation reported with Li^+.

Appendix

The interactions between K^+, Na^+, and Mg^{2+} · ATP on the apparent K_m for Mg^{2+} · ATP, and between K^+, ATP, and nitrophenyl phosphate on the apparent $K_{0.5}$ for K^+, may be interpreted in terms of an enzyme model with two substrates, A and B, and a competitive inhibitor C:



such that the velocity $v = k\ [E \cdot A \cdot B]$ and $V = k[E]$. The relationship may be simplified by assuming that $K_1 = K_3$ and $K_2 = K_4$ (as seems to be the case at least with Na^+ and $Mg^{2+} \cdot$ ATP binding to the enzyme (Fig. 1)). Then in the absence of the competitor C:

$$\frac{V}{v} = 1 + \frac{K_1}{[A]} + \frac{K_2}{[B]} + \frac{K_1 K_2}{[A][B]} = \left(1 + \frac{K_1}{[A]}\right)\left(1 + \frac{K_2}{[B]}\right)$$

whereas in the presence of C:

$$\frac{V}{v} = 1 + \frac{K_1}{[A]} + \frac{K_2}{[B]} + \frac{K_1 K_2}{[A][B]} + \frac{K_1 K_2 [C]}{K_5 [A][B]}$$

To evaluate the response to changes in one substrate, A, at fixed levels of B and C this relationship can be rearranged:

$$\frac{V}{v} = \frac{[B] + K_2}{[B]} + \frac{K_1}{[A]}\left\{1 + \frac{K_2}{K_5}\left(\frac{K_5 + [C]}{[B]}\right)\right\}$$

Dividing by $[B] + K_2/[B]$ converts this expression to the form

$$1 + \frac{K_1}{[A]}\left\{1 + \frac{[C]}{K_5\ (1 + \frac{[B]}{K_2})}\right\}$$

in which the competition with C is intuitively apparent: K_1 is increased by a factor $(1 + [C]/K_5)$, as in simple competitive inhibition, while K_5 is also increased by the other species competing with C for the free enzyme, B, by the factor $(1 + [B]/K_2)$. Consequently, the observed K'_1 in the presence of the competitor C is related to the true K_1 by

$$K'_1 = K_1\left\{1 + \frac{[C]}{K_5\ (1 + \frac{[B]}{K_2})}\right\}$$

Acknowledgements

I wish to thank Miss Grace Marin for meticulous technical assistance. This work was supported by U.S. Public Health Service research grant NS-05430.

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