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Kinetic Studies on Na^+/K^+-ATP_{ase} and Inhibition of Na^+/K^+-ATP_{ase} by ATP

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Na^+/K^+-ATP_{ase} (EC 3.6.1.3) is an important membrane-bound enzyme. In this paper, kinetic studies on Na^+/K^+-ATP_{ase} were carried out under mimetic physiological conditions. By using microcalorimeter, a thermokinetic method was employed for the first time. Compared with other methods, it provided accurate measurements of not only thermodynamic data ($\Delta_r H_m$) but also the kinetic data (K_m and V_{max}). At 310.15 K and pH 7.4, the molar reaction enthalpy ($\Delta_r H_m$) was measured as $-40.514 \pm 0.9\,kJ\,mol^{-1}$. The Michaelis constant (K_m) was determined to be 0.479 ± 0.020 mM and consistent with literature data. The reliability of the thermokinetic method was further confirmed by colorimetric studies. Furthermore, a simple and reliable kinetic procedure was presented for ascertaining the true substrate for Na^+/K^+-ATP_{ase} and determining the effect of free ATP. Results showed that the MgATP complex was the real substrate with a K_m value of about 0.5 mM and free ATP was a competitive inhibitor with a K_i value of 0.253 mM.

Keywords: Na^+/K^+-ATP_{ase}; Microcalorimeter; Thermokinetic method; Competitive inhibitor; Kinetics

INTRODUCTION

Na^+/K^+-ATP_{ase} (ATP phosphohydrolase, EC 3.6.1.3) is an important membrane-bound enzyme found in most animal cells. It has been especially, convincingly identified with the sodium pump that transports Na^+ and K^+ across the cell membrane. Na^+/K^+-ATP_{ase} catalyzes the hydrolysis reaction of ATP to ADP and inorganic phosphate (P_i). In biosynthetic pathways, a thermodynamically unfavorable reaction is often coupled to the hydrolysis reaction. Because of the large negative standard free energy associated with ATP hydrolysis, this coupling shifts the overall equilibrium in such a manner that the desired product is obtained.

Kinetic studies on Na^+/K^+-ATP_{ase} have been widely carried out by using many classical methods such as spectrophotometry, $^{32}\gamma$-ATP isotopic determination and colorimetry.[1–6] Compared with these methods, the thermokinetic method has many advantages. It can provide *in-situ*, online, quasi-continuous, non-invasive and accurate measurements of not only thermodynamic data but also the kinetic data of the reaction under investigation. Furthermore, it has no special requirements for both the solvent properties and the spectral, electrochemical, or other properties of the reaction systems involved. Owing to these advantages, the thermokinetic method has received increasing attention from researchers in many fields. Since the absorption or production of heat is an intrinsic property of all enzyme-catalyzed reactions, the thermokinetic method has been extensively used in the study of enzymatic reactions.[7–12] However, thermokinetic studies on Na^+/K^+-ATP_{ase} have not been reported yet.

Although studies on Na^+/K^+-ATP_{ase} have been intensively carried out, there are still many fundamental questions unanswered. One of these is the precise identity of the true substrate: MgATP or ATP. This question has been studied for many years,[1–3] but no consistent conclusion has been made. Some researchers concluded that MgATP is the sole substrate and free ATP is a competitive inhibitor,[2,3] while others conclude that both MgATP and free ATP should be substrates.[1]

In this paper, kinetic studies on Na^+/K^+-ATP_{ase} were carried out under mimetic physiological conditions ($T = 310.15$ K, pH = 7.4). By microcalorimeter, a thermokinetic method was employed for

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the first time. Compared with classical methods, it can provide accurate measurements of not only thermodynamic data ($\Delta_r H_m$) but also the kinetic data (K_m and V_{max}). Its reliability was verified by the experimental results. Furthermore, a simple and reliable kinetic procedure as previously described[13,14] was presented for ascertaining the real substrate for Na^+/K^+-ATP_{ase} and determining the effect of free ATP. It was concluded that MgATP complex was the real substrate for Na^+/K^+-ATP_{ase} and ATP was a competitive inhibitor under mimetic physiological conditions.

MATERIALS AND METHODS

Materials

Na^+/K^+-ATP_{ase} (ATP phosphohydrolase, EC 3.6.1.3) from porcine cerebral cortex was purchased from Sigma Corporation. Protein concentration was determined by the method of Lowry *et al.*[15] using bovine serum albumin as standard. The enzyme activity at 310.15 K determined by the colorimetric method (see later) was expressed as micromoles P_i released per mg protein per min.

All ATP solutions used in experiments were diluted to the required concentrations with buffer solution (composition in millimolar: Tris–HCl, 100; NaCl, 100; KCl, 10; $MgCl_2$, equal to the concentration of ATP; pH 7.4).

Methods

Microcalorimetric Method

The calorimetric curves were obtained at 310.15 K using an LKB-2107 batch microcalorimeter system, which is the combination of a micro-batch reactor (BR) with a conduction calorimeter. Before each calorimetric experiment, all reagent solutions were diluted to the required concentrations with buffer solution.

In experiments, 100 μL Na^+/K^+-ATP_{ase} solution was injected into reaction cell I, and 4.00 mL Na_2ATP solution was injected into reaction cell II. In order to avoid the influence of the heat effect of diluting and mixing, etc., the same sample was added to reference cell except in the case of Na^+/K^+-ATP_{ase} addition. When the microcalorimetry system had been in thermal equilibrium and a steady baseline was obtained on the recorder, the reaction run was initiated by rotating the calorimeter 360° clockwise and counterclockwise, respectively, so as to fully mix the enzymes and substrate solutions, etc. The heat generated in the reaction process was detected in the form of thermal potential by thermoelectric piles, and the amplified output signal was recorded as the calorimetric curve by a LKB-2210 dual-pen integrating recorder.

In microcalorimetric experiments, all determinations were made in duplicate and a series of substrate concentrations were used.

Colorimetric Method

In order to verify the results obtained by microcalorimetry, the colorimetric method as a representative classical method was also used in our work. Furthermore, the effect of free ATP on Na^+/K^+-ATP_{ase} was studied by this method.

ATPase's activities were measured by determining the amount of released inorganic phosphate (P_i) based on the malachite green assay of Kallner[16] with modifications. All glassware was rinsed with 1 M HCl for several times, and then distilled water for at least four times to remove contaminating phosphates. Malachite green reagent was prepared by dissolving malachite green base (0.38 g/L) in 1.0 M HCl with 8 mM ammonium molybdate. In order to stabilize dye-phosphomolybdate complex, the detergent Tween 20 (0.75 g/L final concentration) was added to the above reagents. After stirring for 30 min, the solution was filtered and stored in the dark. Na^+/K^+-ATP_{ase} activities were assayed in Buffer (composition in millimolar: Tris–HCl, 100; NaCl, 100; KCl, 10; $MgCl_2$, unless especially indicated, equal to the concentration of ATP; pH 7.4). Each enzyme preparation was incubated in an appropriate assay buffer (0.5 mL final volume) at 310.15 K for about 20 min before the assay. At appropriate intervals (usually 5 min) after the addition of ATP (a series of final concentrations), sample aliquots (0.25 mL) were assayed for inorganic phosphate. The sample was added immediately into 3 mL malachite green reagent followed by 0.6 mL 24% sodium citrate after 1 min. Absorbance at 630 nm for the mixture was measured between 30 min and 2 h. Inorganic phosphate released by ATPase was calculated by subtracting the sample tube with zero-time incubation for each assay. A Standard Curve for inorganic phosphate (P_i) was obtained under the same experimental conditions by using KH_2PO_4 as standard.

In kinetic studies, initial velocities were estimated by selecting brief reaction periods over which P_i production was linear with time. By using this initial rate method, the fundamental kinetic parameters and the inhibition constant for ATP were determined by a Lineweaver–Burk plot analysis. When studying the effects of ATP, the total concentration of Mg^{2+} was fixed at three different levels (1, 3 and 5 mM) and the total concentration of ATP varied in a range of 0–12 mM. Determinations of the concentrations of MgATP and free ATP were made as described previously.[14]

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RESULTS

Microcalorimetric Studies on Na^+/K^+-ATP_{ase}

Measurements of the Kinetic Parameters by Analyzing Calorimetric Curves

When a thermal change of a chemical reaction takes place in a batch conduction calorimeter, the relation between input function Q and output function Δ should obey Tian's equation, so the following equations[7] can be obtained:

$$Q = K\cdot(A_t + \tau\cdot\Delta_t) \quad (1)$$

$$Q_\infty = K\cdot A \quad (2)$$

where τ is the time constant of the reaction system and K the proportionality constant, Q the heat liberated before time t, Q_∞ the total heat effect. As Figure 1 shows, Δ is the peak height at time t, A_t is the peak area up to time t, and A is the total area under the thermogram.

For a chemical reaction:

$$Q_\infty = n\Delta_r H_m \quad (3)$$

where $\Delta_r H_m$ stands for the molar reaction enthalpy and n is the number of moles of initial substrate. Then,

$$\Delta_r H_m = KA/n \quad (4)$$

K can be obtained from a calibration experiment.

Defining the substrate conversion ratio $\Phi_t = ([S]_0 - [S]_t)/[S]_0$, where $[S]_0$ and $[S]_t$ stand for the substrate concentration at the beginning and time t, then Φ_t can be described as:

$$\Phi_t = \frac{[S]_0 - [S]_t}{[S]_0} = \frac{Q}{Q_\infty} = \frac{K(\tau\cdot\Delta_t + A_t)}{KA} = \frac{\tau\cdot\Delta_t + A_t}{A} \quad (5)$$

where τ is a constant with time dimension. An electric calibration can be run after each experiment to determine this parameter. From the calorimetric curve of the calibration, a series of points behind the peak are chosen. For these points, the system is under a natural cooling procedure, so they should obey the equation: $\ln\Delta = \ln\Delta_0 - t/\tau$. By plotting $\ln\Delta$ against t, the parameter τ can be obtained from the slope of the linear curve. At the same time, the parameter K mentioned above can be determined through dividing the heat of calibration by area under the calibration curve.

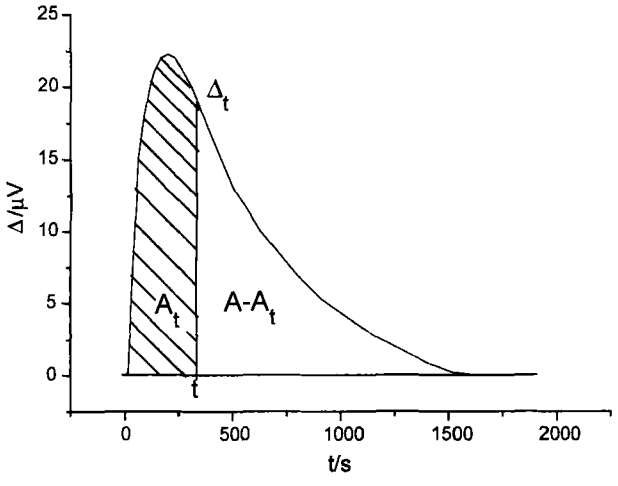

FIGURE 1 A typical microcalorimetric curve using LKB2107.

It was pointed out that when Mg^{2+} and ATP were varied together at a 1:1 molar ratio in a millimolar concentration range, the kinetics of Na^+/K^+-ATP_{ase} should obey a simple Michaelis–Menten equation:[3]

$$v = -\frac{d[S]}{dt} = \frac{V_m[S]}{K_m + [S]}. \quad (6)$$

For the above equation, can be readily proved that

$$\frac{\ln(1-\Phi_t)}{t} = \frac{[S]_0}{K_m}\cdot\frac{\Phi_t}{t} - \frac{V_m}{K_m} \quad (7)$$

where $[S]_0$ is the substrate concentration at the beginning, K_m is the Michaelis constant and V_{max} is the maximum velocity of an enzyme-catalyzed reaction.

Thus, choosing a series of points from calorimetric curves, plotting $\ln(1-\Phi_t)/t$ against Φ_t/t, from the slope and the intercept of the linear curve, the kinetic parameters can be obtained. Table I and Figure 2 show an example of the analysis of a calorimetric curve.

The results obtained under different substrate concentrations are listed in Table II. It can be seen that the data have good repeatability and self-consistency. At 310.15 K and pH 7.4, the molar reaction enthalpy ($\Delta_r H_m$) was determined as $-40.514 \pm 0.9\,kJ\,mol^{-1}$ and the Michaelis constant (K_m) as 0.479 ± 0.020 mM (consistent with literature figure of about 0.5 mM[3,4]). The value of V_{max} obtained was $0.681 \pm 0.026\,\mu mol\,P_i\,min^{-1}\,mg\,protein^{-1}$.

TABLE I An example of analyzing a microcalorimetric curve*

t (s)	Δ_t (mV)	A_t (V s)	Φ_t	$\Phi_t t^{-1}$ (10^{-3} s^{-1})	$\ln(1-\Phi_t)\,t^{-1}$ (10^{-3} s^{-1})
360	10.25	2.68	0.2803	0.779	−0.914
420	10.00	3.24	0.3206	0.763	−0.920
480	9.90	3.78	0.3606	0.751	−0.932
540	9.50	4.33	0.3990	0.739	−0.943
600	9.10	4.84	0.4344	0.724	−0.950
660	8.85	5.34	0.4702	0.712	−0.963
720	8.40	5.82	0.5030	0.699	−0.971

*Results: $K_m = 4.89\times10^{-4}$ mol L^{-1} = 0.489 mM, $V_{max} = 7.28\times10^{-7}$ mol L^{-1} l s^{-1} = 0.699 μmol P$_i$ min^{-1} mg protein^{-1}, correlation coefficient $R = 0.9945$, molar reaction enthalpy $\Delta_r H_m = -40.551$ kJ mol^{-1}. Other parameters: $\tau = 101.11$ s, $A = 13.26$ V s, $[S]_0 = 3.63\times10^{-4}$ mol L^{-1}, $[E]_t = 6.25\times10^{-2}$ mg protein mL^{-1}.

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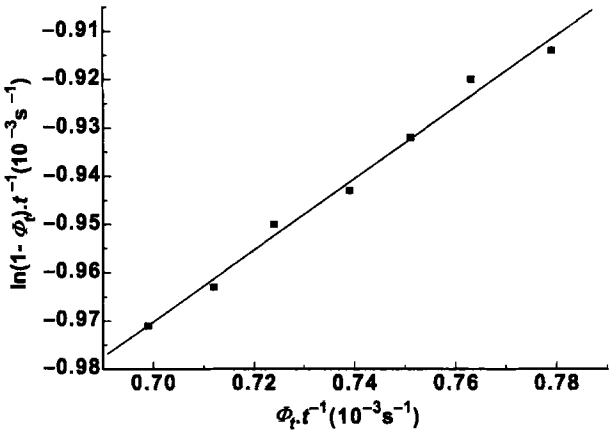

FIGURE 2 An example of a thermogram analysis. Related data are given in Table I. Reaction conditions are described in under methods.

Colorimetric Determination by Initial Rate Method

For a Michaelis–Menten equation

$$v_0 = \frac{V_{max}[S]_0}{K_m + [S]_0} \tag{8}$$

where v_0 and $[S]_0$ stand for the initial rate and the initial substrate concentration, K_m the Michaelis constant, V_{max} the maximum velocity. On inversion, the following equation is obtained:

$$\frac{1}{v_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]_0} \tag{9}$$

By plotting $1/v_0$ against $1/[S]_0$, the kinetic parameters K_m and V_{max} can be obtained from the slope and the intercept of the linear curve (Table III).

Results showed that K_m (0.464 mM) and V_{max} (0.716 μmol P_i min^{-1} mg protein^{-1}) are consistent with the values obtained by the thermokinetic method. The reliability of the thermokinetic method being used for kinetic studies on Na^+/K^+-ATP_{ase} was further confirmed.

Studies on the Effect of Free ATP on Na^+/K^+-ATP_{ase}

Effect of ATP on the Activity of Na^+/K^+-ATP_{ase}

Figure 3 shows that the velocity curve fails to conform to Michaelis–Menten kinetics when the total ATP concentration ($[ATP_t]$) was greater than the total Mg^{2+} concentration ($[Mg^{2+}]$). At each total Mg^{2+} concentration, ATPase activity rose to a maximum value and then declined. The peak was shifted to higher ATP concentrations with an increase in the total Mg^{2+} concentration. Peak activities occurred when $[ATP_t]$ equals $[Mg_t^{2+}]$. At this point, the concentration of the MgATP complex was highest. However, when $[ATP_t] > [Mg^{2+}]$, ATPase activity declined, which suggested an inhibitory effect of free ATP. The results showed that the activity depended upon, not only ATP, but also Mg^{2+} concentration. Thus, it can be presumed that Mg^{2+} plays a part in the MgATP chelate complex formation, which is the real substrate for Na^+/K^+-ATP_{ase}. The conclusion that Mg^{2+} is an indispensable ion for ATP_{ase} enzyme activities has been concluded from previous researches.[13,17–20]

Mechanism of Inhibition by Free ATP

The results in the section immediately above were examined by assuming that the MgATP complex is the real substrate and free ATP is an inhibitor. When the free ATP concentration ($[ATP_t]$) was relatively high, almost all of the Mg^{2+} was presented as MgATP and the free Mg^{2+} concentration ($[Mg_f^{2+}]$) was very low (Table IV), so that it may be omitted from the calculations. Thus, the free ATP inhibition can be studied alone without the Mg^{2+} effect. The effect of free ATP on Na^+/K^+-ATP_{ase} activity is shown in Figure 4. While the MgATP concentration ranged from 0.5 mM to 4.5 mM, the intersection of two straight lines on the $1/v$ axis (Figure 4) indicated that free ATP increased the K_m value of the enzyme for MgATP, but did not alter the V_{max}. This means free ATP acted as a competitive inhibitor. The inhibition

TABLE II Results obtained under different substrate concentrations in microcalorimetric studies*

10^3 $[S]_0$ (mol L^{-1})	10^3 K_m (mol L^{-1})	V_{max} (μmol P_i min^{-1} mg protein^{-1})	$\Delta_r H_m$ (kJ mol^{-1})	R
0.181	0.476	0.664	−39.960	0.9955
0.363	0.489	0.699	−40.551	0.9945
0.450	0.465	0.683	−40.032	0.9940
0.688	0.475	0.687	−41.245	0.9980
0.730	0.499	0.655	−39.883	0.9991
0.908	0.470	0.698	−41.415	0.9965
Average value	0.479 ± 0.020	0.681 ± 0.026	−40.514 ± 0.9	0.9963 ± 0.0028

*Other parameters: T = 310.15 K, pH = 7.4, $[E]_i = 6.25 \times 10^{-2}$ mg protein mL^{-1}.

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TABLE III Data for a double-reciprocal plot for kinetic parameters measured by the colorimetric method*

10^4 $[S]_0$ (mol L^{-1})	10^{-4} $[S]_0^{-1}$ (L mol^{-1})	v_0 (μmol P_i min^{-1} mg protein^{-1})	v_0^{-1} (min mg protein μmol^{-1} P_i)
0.91	1.099	0.115	8.70
1.36	0.735	0.167	5.99
1.81	0.552	0.214	4.67
2.27	0.441	0.229	4.37
3.63	0.275	0.297	3.37

*Results: $K_m = 4.64 \times 10^{-4}$ mol $L^{-1} = 0.464$ mM, $V_{max} = 0.716$ μmol P_i min^{-1} mg protein^{-1}, correlation coefficient $R = 0.9940$. Other parameters: $T = 310.15$ K, $[E]_t = 6.25 \times 10^{-2}$ mg protein mL^{-1}.

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could be described as follows:[21,22]

$$E + \mathrm{MgATP} \overset{K_s}{\rightleftharpoons} E\,\mathrm{MgATP} \xrightarrow{k_p} E + \text{products} \quad (10)$$

$$E + \mathrm{ATP} \overset{K_i}{\rightleftharpoons} E\,\mathrm{ATP} \quad (11)$$

where K_i is the dissociation constant for competitive inhibition by free ATP, K_s is the substrate dissociation constant and k_p is the rate constant for the breakdown of the complex into products. The rate equation for this model is:

$$v = \frac{V_{max}}{1 + (1 + [\mathrm{ATP}]/K_i)K_m/[\mathrm{MgATP}]} \quad (12)$$

where V_{max} is the highest rate of reaction and K_m is the Michaelis constant.

On inversion:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{\left(1 + \frac{[\mathrm{ATP}]}{K_i}\right)K_m}{V_{max}} \frac{1}{[\mathrm{MgATP}]}. \quad (13)$$

By plotting $1/v$ against $1/[\mathrm{MgATP}]$ (Figure 4), the kinetic parameters K_m, V_{max} and K_i can be obtained from the slope and intercept of the linear curves. The K_m, V_{max} and K_i were estimated as 0.500 mmol L^{-1}, 0.697 μmol P_i min^{-1} mg protein^{-1} and 0.253 mmol L^{-1}.

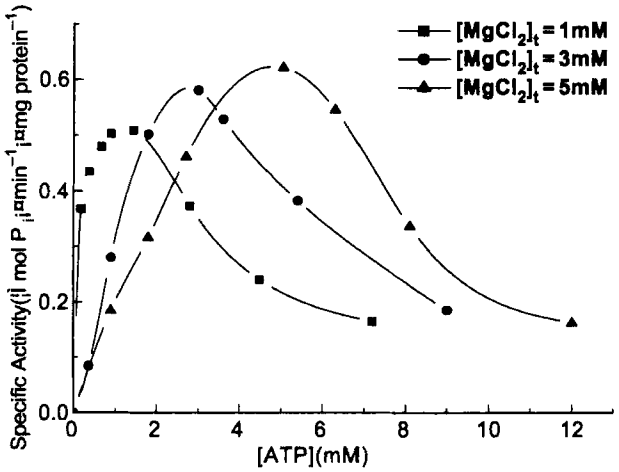

FIGURE 3 Effect of varying total ATP concentration on the activity of Na^+/K^+–ATP_{ase}. Reaction conditions are described under methods. The total Mg^{2+} concentration was fixed at 1, 3 and 5 mM.

ATPase Activity when [ATP_t] Equals [Mg_t^{2+}]

To determine whether the enzyme conforms to Michealis–Menten kinetics, Na^+/K^+-ATP_{ase} activity was determined when the total ATP concentration was equal to the Mg^{2+} concentration. Figure 5 shows that under this condition, Na^+/K^+-ATP_{ase} conforms to Michaelis–Menten kinetics well.

DISCUSSION AND CONCLUSION

Figures 3 and 4 show that under mimetic physiological conditions, MgATP was the actual substrate for Na^+/K^+-ATP_{ase} and free ATP was a potential inhibitor. Similar kinetic results have been reported for many Mg^{2+}-dependent ATPases.[22,23]

The interpretation of this kinetic data is difficult because of the equilibrium between the substrate, MgATP, and the potential inhibitors Mg^{2+} and ATP. This means that these three compounds cannot be varied independently. It can be concluded from our studies that free ATP was a classical competitive inhibitor. This result strongly suggests that free ATP competed for catalytic sites on the enzyme with the complex MgATP. In the catalytic sites, free ATP binds with the enzyme, forming an inactive enzyme–ATP complex, resulting in Na^+/K^+-ATP_{ase} inhibition.[1]

TABLE IV Apparent stability constants for MgATP (K_{MgATP} = $[\mathrm{MgATP}]/[Mg_f^{2+}][ATP_f]$), [MgATP], [$Mg_f^{2+}$], and [$ATP_f$] under different [$MgCl_2$] and [$Na_2ATP$]*

$[ATP_f]$ (mM)	$[ATP_t]$ (mM)	$[Mg_t^{2+}]$ (mM)	K_{MgATP} (M^{-1})	[MgATP] (mM)	$[Mg_f^{2+}]$ (mM)
2.0	2.5	0.5176	14236	0.5	0.0176
2.0	3.0	1.0356	14065	1.0	0.0356
2.0	3.5	1.5540	13896	1.5	0.0540
2.0	4.0	2.0728	13729	2.0	0.0728
2.0	4.5	2.5922	13565	2.5	0.0922
2.0	5.0	3.1119	13403	3.0	0.1119
5.0	5.5	0.5072	13823	0.5	0.0072
5.0	6.0	1.0146	13659	1.0	0.0146
5.0	6.5	1.5222	13497	1.5	0.0222
5.0	7.5	2.5379	13179	2.5	0.0379
5.0	8.5	3.5544	12870	3.5	0.0544
5.0	9.5	4.5716	12570	4.5	0.0716

*The date were calculated by the method described previously.[14]

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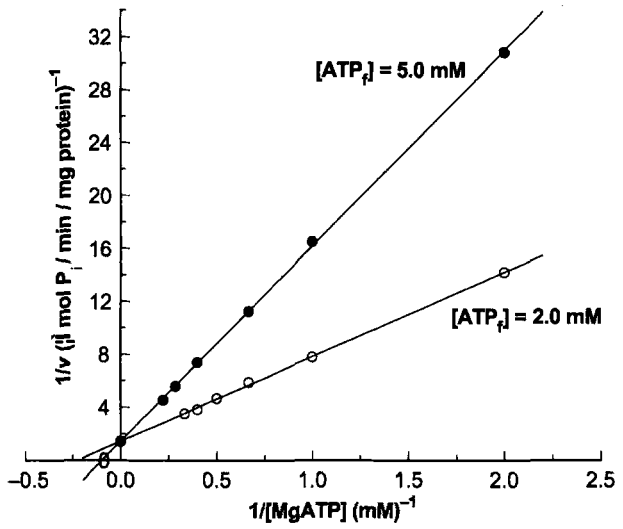

FIGURE 4 Lineweaver–Burk plots, $1/v$ vs. $1/[MgATP]$, showing the mechanism of inhibition of Na^+/K^+–ATP_{ase} by free ATP. The ATPase activity was determined at two fixed levels of free ATP: 2 and 5 mM. $[E]_t = 6.25 \times 10^{-2}$ mg protein mL^{-1}. The [MgATP] and $[ATP_f]$ were calculated by the method described in the literature.[14] The other reaction conditions were as described in under methods.

Kinetic studies on Na^+/K^+-ATP_{ase} using ATP concentrations in the range of those required for optimal hydrolytic activity (in the millimolar concentration range) reveal K_m values ranging from 0.1 mM to 1.0 mM.[5,24] Some kinetic studies, at which the levels of ATP (in millimolar) are near those found *in vivo*, indicated that MgATP should be the true substrate.[2,3,5] In this study, the conditions used are close to the physiological conditions *in vivo*. Results showed that the MgATP complex is the real substrate for Na^+/K^+-ATP_{ase} with a K_m value of about 0.5 mM, which is consistent with previous studies.[3,4] The K_m value is within the range mentioned above. The inhibition effect of free ATP has been studied.[1,2,4] A consistent conclusion made is that free ATP is a typical competitive inhibitor of Na^+/K^+-ATP_{ase}. The K_i value (0.253 mM) for free ATP obtained in this study is close to literature figures.[1,4]

It can be concluded from our results that ATP has two distinct effects on Na^+/K^+-ATP_{ase} activity. Firstly, when combined with Mg^{2+}, it is the substrate for the enzyme. Secondly, ATP behaves as a competitive inhibitor of the enzyme. Thus, free ATP appears to bind to the catalytic sites, although with lower affinity than MgATP. In addition, ATP is a strong chelator of Mg^{2+}. As described previously,[2,3] Mg^{2+} also has an effect on Na^+/K^+-ATP_{ase}. Therefore, any single experiment that shows an effect of free ATP can also be interpreted in terms of an effect of a far lower free Mg^{2+}. The physiological significance of ATP and Mg^{2+} is worthy of further researches.

In this work, the thermokinetic method as a reliable one was employed for the kinetic studies on Na^+/K^+-ATP_{ase} for the first time. Compared with classical methods, it can provide accurate measurements of not only thermodynamic data ($\Delta_r H_m$) but also the kinetic data (K_m and V_{max}). At 310.15 K and pH 7.4, the molar reaction enthalpy ($\Delta_r H_m$) was determined as -40.514 ± 0.9 kJ mol^{-1} and the Michaelis constant (K_m) as 0.479 ± 0.020 mM (consistent with the literature figure which is about 0.5 mM[3,4]). The value of V_{max} obtained was 0.681 ± 0.026 μmol P_i min^{-1} mg protein^{-1}. All of the data obtained had good repeatability and self-consistency. The reliability of the thermokinetic method was further confirmed by colorimetric studies. It was shown that microcalorimetry can be a promising tool in kinetic studies on Na^+/K^+-ATP_{ase}. Moreover, we presented a simple and credible kinetic procedure to confirm the MgATP complex as the real substrate for Na^+/K^+-ATP_{ase} with a K_m value of about 0.5 mM and free ATP as a competitive inhibitor with a K_i value of 0.253 mM.

Acknowledgements

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