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Molecular Mechanism of Mg-ATPase Activity

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Abstract Mg-ATPase is very important in living organisms. To better understand the molecular mechanism of Mg-ATPase activity, we applied the method of kinetic analysis of multi-sited enzyme systems; this is a suitable approach used for kinetic investigation of multi-sited enzyme systems. The study of Mg-ATPase has demonstrated: (1) It is a multi-sited enzyme system whose functional unit is minimum a dimmer; (2) Its substrate is MgATP, while free ATP and Mg^{2+} appear to be the enzyme modifiers with a dual effect; (3) The enzyme system for MgATP has at least three sites: i.e., the essential activator, full inhibitor, and partial effect modifiers sites; (4) Mg-ATPase carries out Mg^{2+} transport through the $1Mg^{2+}$:1ATP stochiometry. Based on the results of these analyses, the kinetic scheme for Mg-ATPase has been developed.

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Introduction

Magnesium plays a crucial role in a living organism's functioning: Mg^{2+} appears to be essential at every stage of protein synthesis; it is a cofactor of a variety of enzymes involved in energy metabolism, carbohydrate metabolism, and DNA synthesis (Szostak 2012; Cramer et al. 2001). Magnesium is essential for the formation of MgATP, so it is an element that is involved in the energy metabolism of the organism. In animal organisms Mg^{2+} is necessary for the muscular and skeletal systems to function, participates in regulation of the muscle–skeletal excitation, etc. The intracellular and extracellular concentrations of Mg^{2+}, similar to any other ion, differ from each other. The regulation of these differences is vital. It is known that one of the Mg^{2+} transporting systems is the Mg-ATPase, which is localized in the plasma membrane and sarcoplasma (Beeler et al. 1985; Veklich and Kosterin 2005) and belongs to the P-type ATPases (Axelsen and Palmgren 1998; Hakansson 2009). The molecular mechanism of its activity is still unknown. By applying the method of kinetic analysis of the multi-sited enzyme systems, we attempted to decipher it.

Materials and Methods

As a preparation, we obtained the synaptic membrane of albino rat brain through differentiated centrifugation in a sucrose gradient (De-Robertis and Rodriguez de lores Arnaiz 1969; Whittaker 1969). We used animals of both

sexes of 2 months old, weighing 200–250 g. The investigation object was whole brain. The protein concentration was measured using the Lowry method (Lowry and Rosenbrogh 1951). To measure the concentration of inorganic phosphorus, modified Fiske-Subbarow (1925) and Kazennov-Maslova (1980) methods were applied.

ATPase activity was judged by the amount of inorganic phosphorus per mg protein per hour released during ATP hydrolysis by the enzyme. Mg-ATPase activity was measured by this difference of inorganic phosphorus concentration in the medium with all conditions remaining the same, except for the presence or absence of Mg^{2+}. Thus, changes in ATPase activity were determined by changes induced directly by Mg^{2+}. For kinetic study of Mg^{2+} effect, we would add different concentrations of Mg^{2+} (MgATP concentration plus Mg^{2+} free concentration. Difference is only Mg^{2+} free concentration, MgATP concentration is the same in all samples) and compare them to each other.

MgATP, free ATP, and Mg^{2+} concentrations were calculated by taking into account the MgATP complex dissociation constant ($K = 0.0603$ mM) (Iacimirsky and Gvasdovskaya 1972).

To analyze function of Mg-ATPase, we have applied the method of kinetic analysis of multi-sited enzyme systems, which has been described as the only method used for kinetic investigation of multi-sited enzyme systems (Kometiani 2007). This kinetic method of complex geometric shape curves was used to establish a kinetic scheme for the enzyme system. Multi-sited enzyme reaction velocity is expressed by the following analytical formula:

$$V = e_0 \frac{x^n \sum_{i=0}^{p} \alpha_i x^i}{\sum_{i=0}^{S} \beta_i x^i}; \quad S = n + m + p,$$

where α_i and β_i are sum of product of individual velocity coefficients and constant ligand concentrations; x is a variable ligand concentration; e_0 is the enzyme overall concentration. n, m, and p are power parameters and positive values. n is number of sites allotted for the essential activators, m is number of full inhibitors' sites, while p is number of sites for the partial-effect modifiers. In order to define n, m, and p parameters, an appropriate computer program (Kometiani 2007) was used.

After determining the power parameters n, m, and p, a theoretical scheme was created that follows the "minimal model" principle, which in turn allowed creation of an analytical formula. The principle of the "minimal model" was to select the minimal number of enzyme forms and reaction steps and interdependence between them which ensures the coincidence of geometrical forms of theoretical and experimental curves at any ligand concentration. The method of geometric shape analysis of kinetic curves allows establishment of the geometrical form of a theoretical curve. Subsequently, the theoretical and corresponding experimental curves are compared. Exactly, the comparison and likeness of geometrical forms of the experimental and theoretical curves are the only criteria which estimate the coincidence of a "minimal model" and a molecular mechanism of enzyme activity.

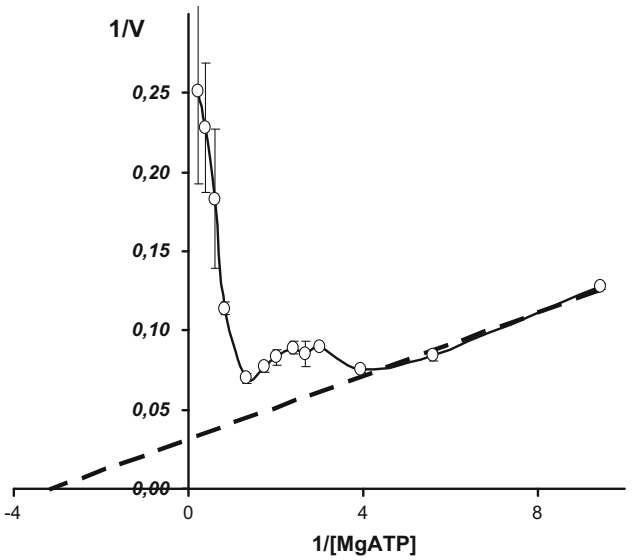


Fig. 1 Dependence of the Mg-ATPase activity on the MgATP concentration on double inverse values. $[Mg_f^{2+}] = [ATP_f]$. (*Dotted line* shows asymptote of the given function)

Results

To plot the Mg-ATPase kinetic scheme, it is necessary to define the substrate for its enzyme system, type, and number of modifiers and their interaction; functional unit of oligomeric system, etc. For this, it is required to perform the analysis of Mg-ATPase reaction velocity dependence upon concentration of minimum three ligands: Mg^{2+} (M), MgATP complex (S), and free ATP (A).

Mg^{2+} and ATP form MgATP complex. As a result of this, it is impossible to express the dependence of Mg-ATPase activity upon the substrate and modifiers' concentrations as one variable function. This makes kinetic analysis hard. Therefore, in the experiment the concentrations of the mentioned ligands were chosen so, that the enzyme reaction velocity was actually function versus one variable.

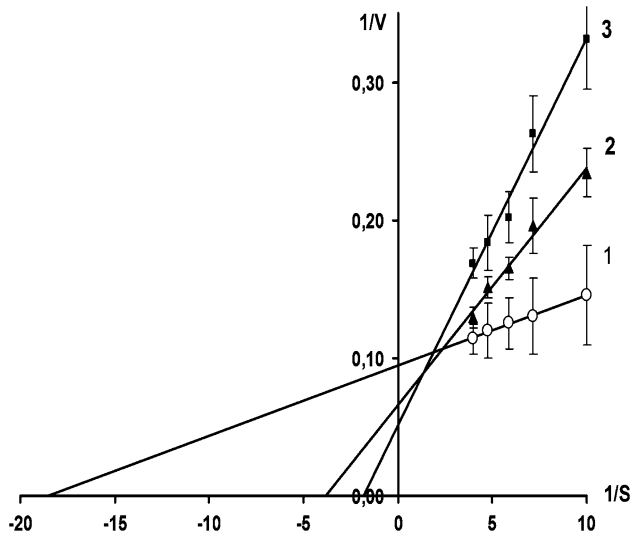


Fig. 2 Dependence of the Mg-ATPase activity on the MgATP (S) concentration (on double inverse values) at different fixed concentrations of Mg^{2+}: *Curve 1*—$[Mg_f^{2+}] = 0.25$ mM; *Curve 2*—$[Mg_f^{2+}] = 0.5$ mM; *Curve 3*—$[Mg_f^{2+}] = 4.0$ mM; Regression coefficients: *Curve 1*—$a = 0.0946 \pm 0.0014$; $b = 0.0051 \pm 0.0002$; *Curve 2*—$a = 0.0602 \pm 0.0087$; $b = 0.0172 \pm 0.0013$; *Curve 3*—$a = 0.0509 \pm 0.0157$; $b = 0.0281 \pm 0.0023$

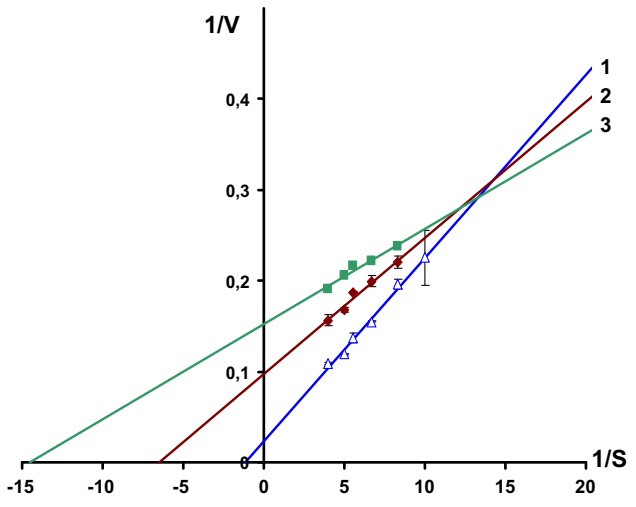


Fig. 3 Dependence of the Mg-ATPase activity on the MgATP (S) concentration (on double inverse values), at different fixed concentrations of ATP_f: *Curve 1*—$[ATP_f] = 0.1$ mM; *Curve 2*—$[ATP_f] = 0.5$ mM; *Curve 3*—$[ATP_f] = 1$ mM; Regression coefficients: *Curve 1*—$a = 0.0240 \pm 0.0110$; $b = 0.0200 \pm 0.0020$; *Curve 2*—$a = 0.0940 \pm 0.0290$; $b = 0.0150 \pm 0.0050$; *Curve 3*—$a = 0.1500 \pm 0.0260$; $b = 0.0110 \pm 0.0040$

Figure 1 represents Mg-ATPase activity dependence upon MgATP concentration on double inverse values when $[Mg_f^{2+}] = [ATP_f]$. MgATP concentration varied within 0.106–4.38 mM range. This dependence has a complex geometric shape. At low MgATP concentration, there is activation of enzyme system, and at its high concentration there is inhibition. At the same time, at high meaning of argument $\frac{1}{V} = f\left(\frac{1}{MgATP}\right)$ function has an asymptote, while at moderate quantities of argument, it has three turning and two inflection points. $\frac{1}{V} = f\left(\frac{1}{MgATP}\right)$ function rectlinearity, at MgATP low concentrations, indicates that MgATP is a essential activator for the enzyme system.

Figure 2 shows, in the double inverse values, dependence of the Mg-ATPase activity on the MgATP concentration at Mg^{2+} different fixed concentrations. The MgATP concentration was selected in such a way (0.1–0.25 mM) that the dependence was linear. Notably, along with rise of the fixed concentrations of Mg^{2+}, the slope increases, while the intercepts decrease. The lines' intercrossing occurs in the Quarter I of the coordinate system.

Figure 3 represents, in the double inverse values, dependence of the Mg-ATPase activity on the MgATP concentration, at different fixed concentrations of the ATP_f. The MgATP concentration varies within the 0.1–0.25 mM range, during which the linear dependence is noted. While increasing ATP_f fixed concentrations, the slopes of the lines decrease, while intercepts of the lines increase, and

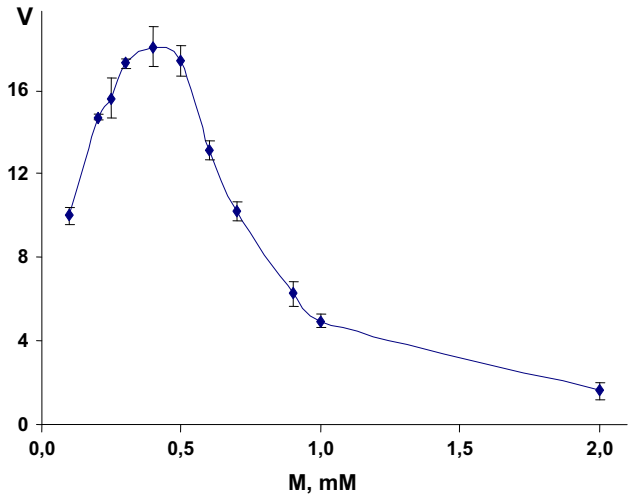


Fig. 4 Dependence of the Mg-ATPase activity on the Mg^{2+} concentration (M), at a fixed high MgATP concentration: [MgATP] = const = 1.2 mM

lines cross quadrant I of the coordinate system. Intersection of $\frac{1}{V} = f\left(\frac{1}{MgATP}\right)$ lines occurs in the first quadrant (Figs. 2, 3). This result, according to the method of kinetic analysis of multi-sited enzyme systems, is a necessary and sufficient condition to come to a conclusion that MgATP and Mg^{2+}, as well as MgATP and ATP binding, are released with the enzyme and mediated by a consecutive mechanism: first bind MgATP, then Mg^{2+} or ATP (Kometiani 2007).

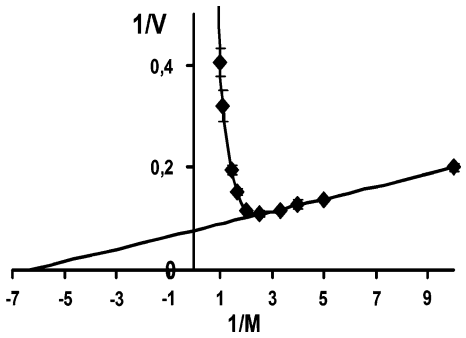


Fig. 5 Dependence of the Mg-ATPase activity on the Mg^{2+} (M) concentration (on double inverse values), at MgATP fixed concentration: [MgATP] = const = 1.2 mM (*Solid line* shows the asomptote of the given function)

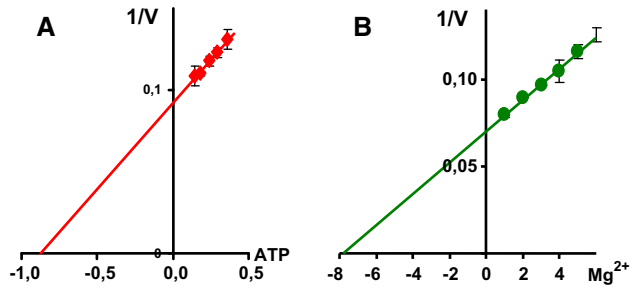


Fig. 6 Dependence of the Mg-ATPase activity (inverted values) on ATP_f (**a**), and Mg^{2+} (**b**) concentrations, at fixed concentration of the MgATP. [MgATP] = const = 1.2 mM. Regression coefficients: **a**—$a = 0.0924 \pm 0.0017$; $b = 0.1060 \pm 0.0068$; **b**—$a = 0.0702 \pm 0.0012$; $b = 0.0045 \pm 0.0002$

Figure 4 demonstrates the dependence of Mg-ATPase activity on Mg^{2+} concentration at a fixed high concentration of MgATP. At low Mg^{2+} concentrations, the enzyme system is activated, while at high Mg^{2+} concentrations, it is inhibited and $V = f(Mg^{2+})$ dependence has a bell-shaped curve. This Mg^{2+} concentration curve of Mg-ATPase activity is a necessary, but insufficient, condition to maintain Mg-ATPase transport of Mg^{2+}. When ion transport occurs, ATPase affinity for this ion should be high; when the ion transport is completed, its affinity decays, which is kinetically reflected by activity enhancement at ion low concentrations, and by the reduction of activity at high concentration.

Figure 5 represents, in the double inverse values, the dependence of the Mg-ATPase activity on Mg^{2+} concentrations, at fixed concentrations of MgATP. The concentration of the Mg^{2+} varied within the 0.1–1 mM range. Evidently, at low Mg^{2+} concentrations, it is activated; at high Mg^{2+} concentrations, inhibited; and in activation areas $\frac{1}{V} = f\left(\frac{1}{Mg^{2+}}\right)$ has an asymptote. At very low concentrations of Mg^{2+}, $\frac{1}{V} = f\left(\frac{1}{Mg^{2+}}\right)$ function is linear,

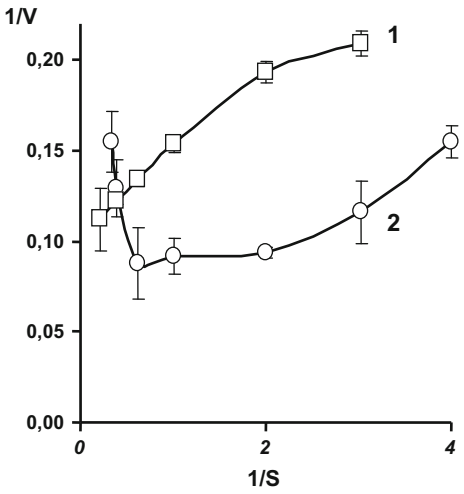


Fig. 7 Dependence of the Mg-ATPase activity on the MgATP (S) concentration, (on double inverse values), at different fixed concentrations of Mg^{2+} *Curve 1*—$[Mg^{2+}] = 4$ mM; *Curve 2*—$[Mg^{2+}] = 0.25$ mM

meaning that the number of essential activator sites for Mg^{2+} is $n = 1$ (Kometiani 2007).

Figure 6 shows dependence of Mg-ATPase activity (inverted values) on ATP_f (Fig. 6a), and Mg^{2+} (Fig. 6b) concentrations at fixed concentrations of the MgATP. The high concentrations of the Mg^{2+} (2–6 mM) were studied, while the ATP_f concentration varied within the 0.14–0.36 mM range. In both cases (Fig. 6a, b) a linear relationship was found. At Mg^{2+} (Fig. 6b) and ATP (Fig. 6a) high concentrations, linearity of $\frac{1}{V} = f\left(Mg^{2+}\right)$ and $\frac{1}{V} = f(\mathrm{ATP})$ functions indicates that full inhibitor number of sites assigned for Mg^{2+} and ATP is $m = 1$ (Kometiani 2007).

Figure 7 shows the dependence of Mg-ATPase activity on MgATP concentrations at different fixed concentrations of Mg^{2+} (double inverse values). At low fixed concentrations of Mg^{2+}, the curve has a complex geometric shape. With high values of the argument, there is activation; and with low values, there is inhibition. At high fixed concentrations of Mg^{2+}, the curve has a simple concave shape.

Discussion

While studying the molecular mechanism of Mg-ATPase activity, the main obstacle is multi-sitedness of the enzyme system and a complex geometric shape of kinetic curves. Because of this, it does not obey the classical Michaelis–Menten kinetics analysis and the formula of transformation utilized for the single-sited enzyme systems, which does not allow linearization of the function. The methods for kinetic analysis of multi-sited enzyme systems, developed

by us (Kometiani 2007), enable kinetic study of multi-sited enzyme systems including Mg-ATPase and to determine the molecular mechanism of the latter.

Rectilinearity of $\frac{1}{V} = f\left(\frac{1}{\text{MgATP}}\right)$ function (Fig. 1) at high quantities of argument (low concentrations of MgATP) indicates number of sites assigned for MgATP as an essential activator n = 1 [12]. Considering that the free ATP represents an inhibitor of the enzyme system (Figs. 3, 6a), $\frac{1}{V} = f\left(\frac{1}{\text{MgATP}}\right)$ function rectilinearity, at MgATP low concentrations (Fig. 1), as already noted, is a necessary and sufficient condition for saying that MgATP is a substrate for the enzymic system. It should be noted that at high MgATP concentrations, the enzymic system is subjected to inhibition. Under these conditions on $\frac{1}{V} = f\left(\frac{1}{\text{MgATP}}\right)$ curve with medium quantities of argument, the existence of 3 turning and 2 inflection points indicates that number of areas allotted to MgATP as a partial effect modifier is $p \geq 2$ (Kometiani 2007). Thus, the enzyme system has for MgATP the essential activator, full inhibitor and partial effect modifiers' sites. So, as in the case of other P-type known ATPases, particularly in the case of Na,K-ATPase, per one α subunit of the enzyme, there is one site of substrate bond (Robinson and Flashner 1979) and its functional unit is minimum a dimmer; therefore, Mg-ATPase can be supposed to be a multi-sited enzyme system, whose functional unit, proceeding from the "minimal model principle", is minimum a dimmer, with two identical subunits. This is designed in the kinetic scheme as OE_1O. At low concentrations, MgATP (S) is an activator for the enzyme system, whereas at high concentrations, it is an inhibitor. That is why the kinetic scheme would inevitably involve OE_1S activatory and SE_1S inhibitory forms. Since in the case of P-type ATPases a phosphorylated intermediate is formed, the scheme would inevitably contain OE_2P form, while the existence of the site for the partial effect modifiers is accounted for by catalytically active SE_2P form.

We have also studied Mg-ATPase activity dependence on low concentrations of MgATP at different fixed concentrations of Mg^{2+} (Fig. 2) and ATP_f (Fig. 3). Values of regression and correlation coefficients for each function are given in Table 1.

Upon increase of fixed concentrations of Mg^{2+} (Fig. 2) and ATP (Fig. 3), different features of $\frac{1}{V} = f\left(\frac{1}{\text{MgATP}}\right)$ such as lines slope, intercept, and intersection in first quadrant of coordinate system, indicate that for Mg-ATPase, ATP, and Mg^{2+} are modifiers with a dual effect (Kometiani 2007). To clarify whether Mg^{2+} induced activation (at substrate high concentration ([MgATP] ≥ 0.5 mM, Fig. 2) is due to the availability of binding the Mg^{2+} to the substrate site, we studied dependence of Mg-ATPase activity upon MgATP concentrations at different fixed concentrations of Mg^{2+} (Fig. 7). As seen from the figure, double inverse values, at fixed low concentration of Mg^{2+}, there is a complex shape curve and there is a strongly pronounced inhibition so as at Mg^{2+} fixed high concentration—yet the curve has a simple concave shape (at this time ATP_f concentration is so low (0.005–0.05 mM) compared to that of MgATP that excludes binding in the substrate site and that setting up of MgATP complex is ruled out). All these point that ME_2P (Mg^{2+}-bound form of phosphoenzyme) have the ability of catalysis. In the Mg-ATPase kinetic scheme, one should necessarily incorporate $OE_1O \leftrightarrow OE_1A \leftrightarrow OE_1AM \rightarrow OE_2P$ branching too.

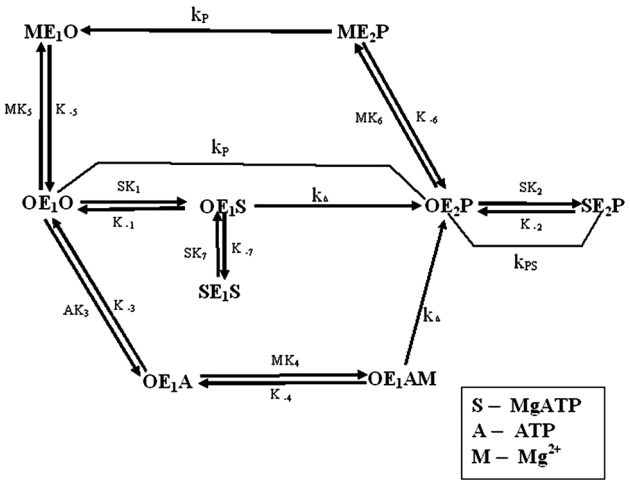
During the exploration of the transport system, the most convenient way to define ion transport is to use isotope and make a visual observation on the process of its accumulation on the other side of the membrane. However, there is a kinetic proof that the given enzyme system is responsible for ion transport (Kometiani and Nozadze 2007). The bell-shaped curve of Mg^{2+} concentration dependence of

Table 1 Values of regression and correlation coefficients for different functions

Figures	Functions	Fixed concentration of the ligands	Regression coefficients $1/V = a + bt$	
			a	*b*
2	$1/V$ = f(1/MgATP)	$[Mg^{2+}]$ = const = 0.25 mM	0,0946 ± 0,0014	0,0051 ± 0,0002
		$[Mg^{2+}]$ = const = 0.5 mM	0,0602 ± 0,0087	0,0172 ± 0,0013
		$[Mg^{2+}]$ = const = 4 mM	0,0509 ± 0,0157	0,0281 ± 0,0023
3	$1/V$ = f(1/MgATP)	$[ATP_f]$ = const = 0.1 mM	0,0240 ± 0,0110	0,0200 ± 0,0020
		$[ATP_f]$ = const = 0.5 mM	0,0940 ± 0,0290	0,0150 ± 0,0050
		$[ATP_f]$ = const = 1 mM	0,1500 ± 0,0260	0,0110 ± 0,0040
6	*A*—$1/V$ = f(ATP_f)	[MgATP] = const = 1.2 mM	0,0924 ± 0,0017	0,1060 ± 0,0068
	B—$1/V$ = f(Mg^{2+})	[MgATP] = const = 1.2 mM	0,0702 ± 0,0012	0,0045 ± 0,0002

Mg-ATPase activity (Fig. 4) that has already been noted is a necessary, but insufficient, condition to maintain that Mg-ATPase performs Mg^{2+} transport. Moreover, it is clear that during ion transport, the number of enzyme bound and released ions should be similar, i.e., the number of sites assigned for essential activators (n) and for full inhibitors (m) should coincide. The bell-shaped curve reflecting Mg^{2+} concentration dependence of enzyme activity (Fig. 4) and equality of the number of essential activator and full inhibitor sites assigned for Mg^{2+}, $n = m = 1$ (Figs. 5, 6b), as being already stated, constitute that it is necessary but an insufficient condition. Based on this evidence, suggestion was made that Mg-ATPase carries out Mg^{2+} transport at 1 Mg^{2+}:1ATP stoichiometry. It should be pointed out that the enzyme inevitably has a phosphorylated intermediate to which Mg^{2+} is associated (ME_2P). A "minimal model" of Mg-ATPase has been developed which approximates the genuine scheme of the enzyme.

The Mg-ATPase minimal model will look like



Thus, study of Mg-ATPase demonstrated that it is a multi-sited enzyme system, whose functional unit is minimum a dimmer. Its substrate is MgATP, while free ATP and Mg^{2+} appear to be the enzyme modifiers. It should be pointed out that the enzyme inevitably has a phosphorylated intermediate to which Mg^{2+} is associated. A "minimal model" has been developed for Mg-ATPase, which approximates the genuine scheme of the enzyme.

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