Enzyme Kinetics and the Activation Energy of Mg-ATPase in Cardiac Sarcolemma: ADP as an Alternative Substrate

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Abstract. Increasing concentrations of Mg within a range between 0.1–5.0 mmol/l step-by-step activated the Mg-dependent ATPase and ADPase in rat heart sarcolemma. Both Mg-dependent activities were influenced by NaN_3 in a similar way. Also, activation of both enzymes by their substrates, ADP and ATP, were affected by NaN_3 in a similar mode. It appears that both enzyme activities are secured by the same system which is capable of ADP hydrolysis during ATP insufficiency. In the absence of NaN_3 the enzyme revealed higher affinity to ATP than to ADP. The activation energy was lower for ATP hydrolysis. The above findings indicate that at non limiting concentiations of Mg^{2+} the enzyme is favoring ATP.

Key words: Mg-ATPase — Activation energy — Cardiac sarcolemma

Introduction

The cardiac sarcolemma plays a central role in cardiac excitation, conduction, excitation-contraction coupling, and regulation of myocardial contractility. An important role in the regulation of these processes, via maintaining intracellular concentrations of Na^+, K^+ and Ca^{2+}, is ascribed to sarcolemmal Na-pump and Ca-pump. Both systems use for the active transport of ions energy derived from ATP hydrolysis which, in the case of (Na,K)-ATPase and also (Ca,Mg)-ATPase, is dependent on the presence of Mg^{2+} (McNamara et al. 1974b; Fedelešová et al. 1976; Morcos 1981). However, in cardiac sarcolemma an ATPase was also described stimulated by Mg ions only (McNamara et al. 1974a; Fedelešová et al. 1976). Structure-function studies of the latter enzyme (Mg-ATPase) showed that binding of magnesium to the sarcolemma induced profound changes in the protein conformation. The energy for conformational changes was provided by the hydration energy of Mg ions, and no additional energy supply was needed (Vrbjar et al. 1985). Addition of ATP to the enzyme in Mg-stabilized conformation was followed by hydrolysis of ATP. This suggests that the concentrations of Mg and ATP in the

compartment surrounding the Mg-ATPase is critical The concentration of ATP in the cardiac tissue is strongly dependent on the physiological status of the heart For example, ischemia induces serious alterations of energy metabolism resulting in progressive reduction in ATP (Reimer et al 1981) Our recent ivestigations revealed that in rat hearts subjected to global ischemia for 15–60 min there was a significant decrease of sarcolemmal Mg ATPase activity The decrease in V_{max} value of the Mg ATPase was accompanied by a profound increase in activation energy (Vrbjar et al 1995) The present paper deals with the enzyme kinetics and the activation energy of the reaction in conditions when ATP stock is exhausted and ADP is only available to Mg-ATPase

Materials and Methods

Hearts from male Wistar albino rats (180 220 g) were used in all experiments Sarcolemmal membrane fraction was isolated using hypotonic shock (McNamara 1974b) combined with NaI treatment (Vrbjar et al 1984) that replaced the original LiBr treatment This modification resulted in higher specific activity of Na K ATPase the generally accepted marker enzyme for the sarcolemma A check of the purity of the obtained sarcolemmal membrane fraction from the point of view of ATPases indicated that there virtually was no significant contamination (less than 3%) of the sarcolemmal fraction by mitochondrial membranes sarcoplasmic reticulum or myofibrils (Vrbjar et al 1984)

Estimation of Mg dependent diphosphohydrolase (ADPase) and ATPase activities

Activation of Mg-dependent ADPase and ATPase by increasing concentrations of magnesium ions within a range of 0 1 5 0 mmol/l was estimated by incubating 50 μg membrane proteins in 1 ml medium containing 50 mmol/l Tris pH 7 4 at 37 °C After 10 min of preincubation in the presence of Mg^{2+} the reaction was started by addition of a constant amount of the substrate (final concentration 1 mmol/l ADP or ATP respectively) The reaction lasted 10 min and it was stopped by 1 ml of 0 73 mol/l ice cold trichloroacetic acid The Mg-ADPase or Mg-ATPase activity was calculated as the difference between the amounts of P formed from the substrate in the presence and in the absence of Mg^{2+} The inorganic phosphorus liberated was estimated by the method of Taussky and Shorr (1953) Protein concentration was determined according to Lowry et al (1951)

Activation of Mg dependent ADPase and ATPase by increasing concentrations of ADP or ATP within a range of 0 1 4 0 mmol/l was estimated in conditions similar to those used for activation by magnesium After 10 min of preincubation in the presence of a constant Mg concentration (5 mmol/l) the reaction was started by addition of the substrate The reaction time was 10 min

In both types of experiments the effect of sodium azide on the enzyme activation was studied in the presence of 10 mmol/l NaN_3 In the concentration applied NaN_3 is accepted to act as an inhibitor of ADPases (Hamlyn and Senior 1983 Turi and Torok 1985)

The activation energies of Mg ATPase and Mg-ADPase were evaluated by the Arrhenius plot of temperature dependence measured in a range of 15 40 °C in medium containing 50 mmol/l Tris pH 7 4 5 mmol/l $MgCl_2$ The concentration of the substrates (ATP or ADP) was kept constant at 4 mmol/l

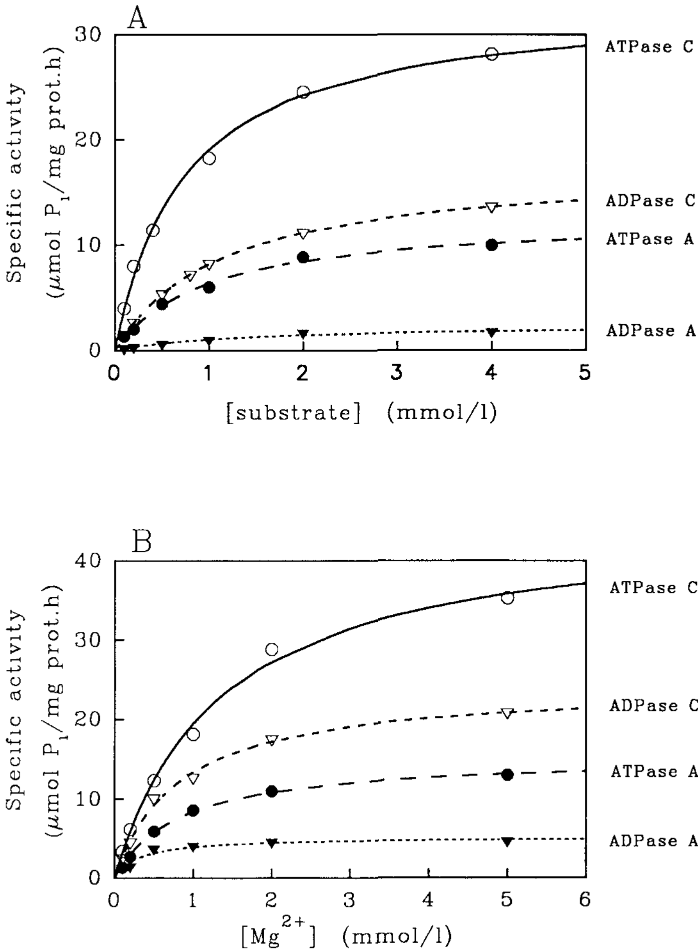

Figure 1. Activation of Mg-ATPase and Mg-ADPase by the cofactor and by the substrate in the absence (*C*) or presence of 10 mmol/l NaN_3 (*A*). Actual data of representative measurements. Panel *A*: Activation by the substrate, ATP or ADP. Panel *B*: Activation by the cofactor Mg^{2+}.

Results

Increasing concentrations of magnesium step-by-step activated the Mg-dependent diphosphohydrolase and Mg-dependent ATPase. At all investigated concentrations of Mg^{2+} the ATPase activity was higher than the ADPase activity (Fig. 1*A*). An analysis of the data according to Michaelis-Menten equation revealed a significantly lower value of K_a for Mg^{2+} ions and also a lower V_{max} value for the ADPase

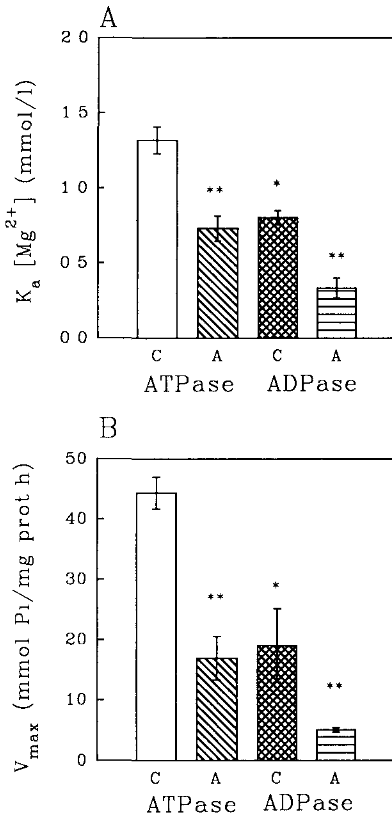

Figure 2. Kinetic parameters of Mg-ATPase and Mg-ADPase upon the activation by Mg^{2+} in the absence (C) or presence of 10 mmol/l NaN_3 (A). Results are means ± S.E.M of 5 measurements, each performed in triplicate. Statistical significance: * – $p < 0.01$ (ADP control vs. ATPase control), ** – $p < 0.01$ (NaN_3-treated groups vs. respective controls). Panel A: K_a values for cofactor Mg^{2+}. Panel B: V_{max} values.

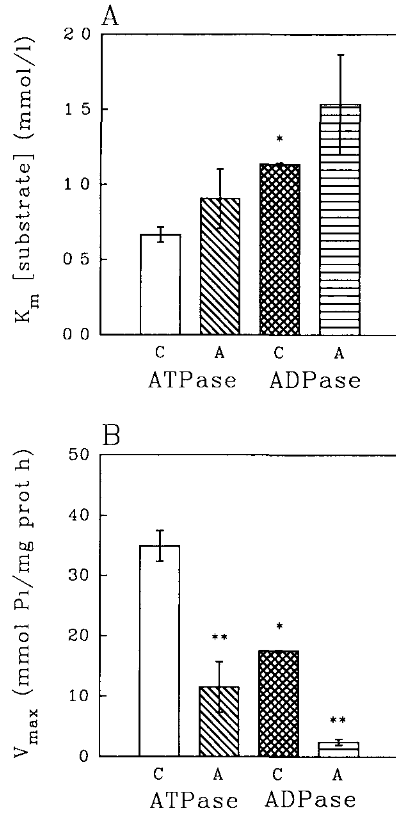

Figure 3. Kinetic parameters of Mg--ATPase and Mg-ADPase upon the activation by the substrate in the absence (C) or presence of 10 mmol/l NaN_3 (A). Results are means ± S.E.M of 5 measurements, each performed in triplicate. Statistical significance: * – $p < 0.01$ (ADP control vs. ATPase control), ** – $p < 0.01$ (NaN_3-treated groups vs. respective controls). Panel A· K_m values. Panel B: V_{max} values.

activity (Fig. 2A, B). Both enzyme activities investigated were markedly inhibited by NaN_3 (Fig. 1A). In both cases, sodium azide markedly decreased the V_{max} for Mg-activation together with an increase of the respective K_a values for Mg^{2+} (Fig. 2A, B). All NaN_3-induced changes of parameters characterizing the enzyme activation by magnesium were statistically significant.

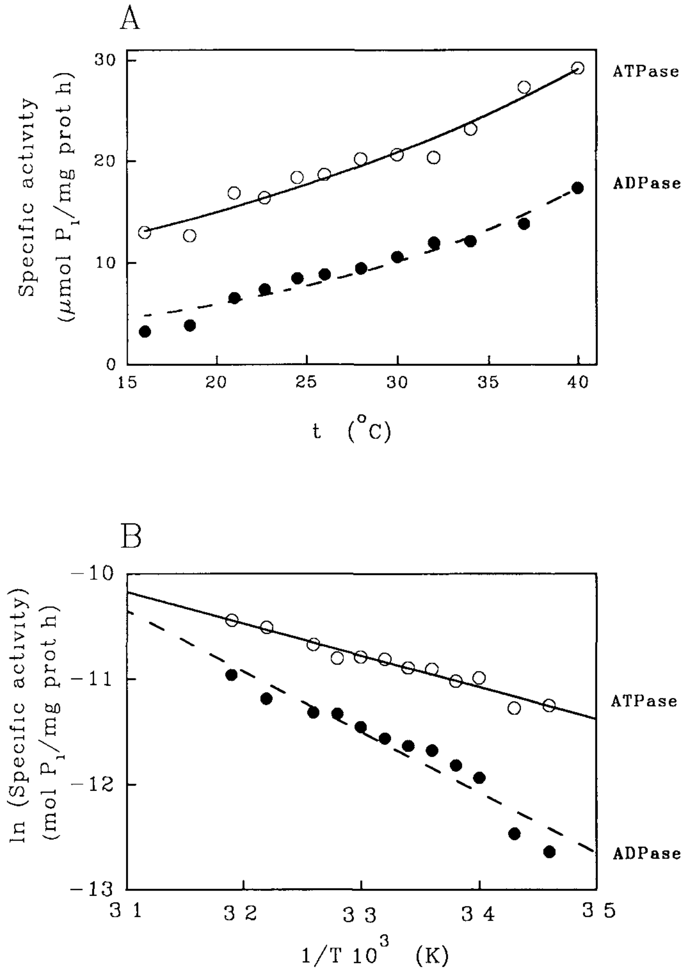

Figure 4. Panel *A*: Temperature dependences of Mg-ATPase and Mg-ADPase activities ın cardiac sarcolemma. Representative measurements. Panel *B*: Mg-ATPase and Mg-ADPase activities vs. temperature; Aııhenius plot.

When keeping magnesium concentration constant (5 mmol/l), an increasing the concentration of the substrates (ADP or ATP) gradually activated diphosphohydrolase and ATPase, respectively. At all substrate concentrations used the ADPase activity was lower (Fig. 1*A*), and it was characterized by a significantly lower V_{max} and a significantly higher K_m value (Fig. 3*A*, *B*). Activation of both enzymes was influenced by NaN_3 in the same manner (Fig. 3). The value of V_{max} for substrate at a fixed concentration of the ligand Mg^{2+} was markedly diminished

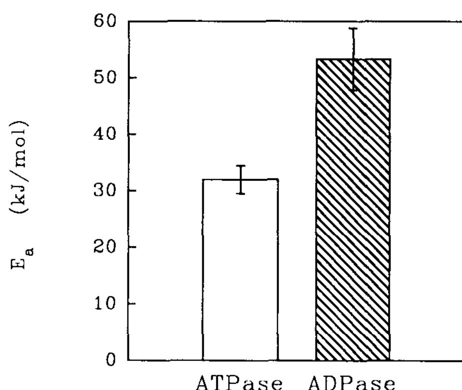

Figure 5. Activation energies of Mg-ATPase and Mg-ADPase in cardiac sarcolemma. Results are means ± S.E.M of 5 measurements, each performed in triplicate. Statistical significance: * - $p < 0.01$.

in the presence of sodium azide for both studied enzyme activities (Fig. 3*B*). The corresponding values of K_m for the substrate remained effectively unchanged when NaN_3 was applied (Fig. 3*A*).

Measurements of the temperature dependence of Mg-ATPase and Mg-ADPase activities showed that they were increasing step-by-step with lower values for Mg-ADPase at all temperatures investigated (Fig. 4*A*). Evaluation of the data by Arrhenius plot yielded straight lines with the slope being steeper for Mg-ADPase (Fig. 4*B*), indicating a higher activation energy for ADP than for ATP hydrolysis (Fig. 5).

Discussion

Although much information has already been accumulated about the activity, localization and specific properties of the Mg-ATPase and also of the Mg-ADPase of cardiac sarcolemma (McNamara et al. 1974a; Fedelešová et al. 1976; Flashner and Robinson 1979; Morcos 1981) the real role of these enzymes in the cell membrane is still not yet completely understood. As concerns the Mg-ATPase, this enzyme is believed to be involved in diverse processes occurring in cardiac sarcolemma such as: i.) Magnesium-dependent modulation of the secondary structure of sarcolemmal proteins, a process probably associated with both, the excitation contraction coupling as well as the transsarcolemmal ionic movements (Vrbjar et al. 1984, 1985); ii.) The activation and function of the sodium pump (Robinson 1985); iii.) The activation and function of the Ca^{2+}/Mg^{2+}-ATPase (Zhao and Dhalla 1991). Also, it was proposed that the role of ADPases in cardiac sarcolemma may be to substitute for malfunctioning ATPases. A need for such a substitution may occur when in the vicinity of the cell membrane, the relative availability of ADP exceeds that of the ATP (Vrbjar and Ziegelhöffer 1987). Since Mg-ATPase was shown to be an ecto-enzyme (Hamlyn and Senior 1983) it is reasonable to assume the same

about the Mg-ADPase too. Hence, both enzymes may fulfill the same physiological function, although they would act in different conditions.

In our sarcolemmal membrane preparation exhibiting Mg-ATPase activity was observed upon Mg-dependent diphosphohydrolase activity substituting ATP with ADP. With the aim to show that these enzyme activities are served by two different systems or by one system which is capable of also hydrolysing ADP at ATP insufficiency, we used NaN_3. Sodium azide applied at millimolar concentrations has been accepted to be an inhibitor of the diphosphohydrolase enzyme (Hamlyn and Senior 1983; Turi and Török 1985). The effect of NaN_3 on the Mg-ATPase is strongly concentration-dependent. At 5 mmol/l concentration it did not affect the Mg-ATPase activity in cardiac sarcolemma (Zhao and Dhalla 1991): when used at 10 mmol/l concentration in the present experiments the Mg-ATPase activity was significantly inhibited (Fig. 1).

Owing to minor contamination by mitochondrial membranes of the used sarcolemmal membrane fraction (deep below 3%), any interferring effect of NaN_3 on mitochondrial ATPase could be neglected. Experimental data from Fig. 3 show similar NaN_3-induced decrease of the V_{max} value for the substrate at the activation of the investigated enzymes by ADP or ATP. Binding sites for NaN_3 are different from those for both substrates as it was suggested by the effectively unchanged K_m value for the substrate (both ADP and ATP). Besides the Mg-ATP complex magnesium can also bind to ATPases alone. Such an effect was documented for the (Na,K)-ATPase (Flashner and Robinson 1979: Vrbjar et al. 1984) and also for the Mg-ATPase (Vrbjar et al. 1985). Therefore we also studied the Mg-induced activation of both investigated enzymes at a constant concentration (4 mmol/l) of their respective substrates. The K_a for the Mg-ADPase reaction was lower than the same constant for Mg-ATPase reaction (Fig. 2*A*) but the V_{max} was significantly higher for Mg-ATPase. Mg^{2+}-induced activation of both enzymes was inhibited by NaN_3 in an uncompetitive way as revealed from the decreased of K_a values with a simultaneous decrease of V_{max} (Fig. 2*A*, *B*). It is suggesting that in the sequence of events leading to inhibition, both enzymes first bind Mg^{2+}, then NaN_3. Summarizing the similarity of NaN_3-induced inhibition of Mg-ATPase and Mg-ADPase it appears that both enzyme activities are served by the same system which is capable of ADP hydrolysis during ATP insufficiency. Thus, under specific conditions characterized by lower ATP production or higher ATP utilization in the cardiac cell the sarcolemmal Mg-ATPase might be able to utilize ADP as an alternative substrate when ATP is deficient in the vicinity of the enzyme. However, in the absence of NaN_3 at the same concentrations of the substrate and Mg^{2+} the Mg-ATPase activity is higher than that of the Mg-ADPase. This can be explained by a lower K_m value for ATP (Fig. 3*A*) and a lower activation energy for ATP hydrolysis (Fig. 5). The affinity of the substrate-binding site of the enzyme is almost twice as high for ATP as for ADP. This implies that in the simultaneous presence of both substrates

the enzyme would prefer ATP In addition the energy barrier of hydrolysis by the investigated enzyme is significantly lower for ATP as revealed from a lower value of E_a (Fig 5) Recent available data about the specific properties and kinetic behavior of the heart sarcolemmal Mg-ADPase seem to indicate that the latter enzyme might represent an entity different from the Mg ATPase (Dhalla and Zhao 1988) Nevertheless a final decision in this respect may be probably made by using the techniques of molecular biology

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