Magnesium-dependent conformational changes of membrane proteins are related to the Mg^{2+}-dependent ATPase activity in cardiac sarcolemma

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Magnesium-induced enzymatic and structural changes of membrane-bound proteins in rat heart sarcolemma have been investigated. In the absence of ATP, increasing concentrations of magnesium within the range 0.1–10.0 mM gradually lowered the α-helix content of sarcolemmal proteins. The same magnesium concentrations stepwise activated the Mg^{2+}-dependent ATPase in the presence of ATP. Mathematical and graphical analysis of the data yielded a quantitative relationship between magnesium-induced stimulation of the Mg^{2+}-dependent ATPase activity and diminution of the α-helix content of membrane proteins in cardiac sarcolemma.

Protein structure *Mg^{2+}-ATPase* *Cardiac sarcolemma* *Structure-function relationship*

1. INTRODUCTION

Heart sarcolemma possesses several ATPases, which are dependent on different cations. One of these is the Mg^{2+}-dependent ATPase which is tightly bound to the sarcolemmal membrane. Proteins released from the sarcolemmal membranes by trypsin treatment exhibited Ca^{2+}-dependent ATPase activity but no Mg^{2+}-dependent ATPase activity [1]. Application of ionic or non-ionic detergents, such as deoxycholate or Lubrol, markedly decreased the Mg^{2+}-dependent ATPase activity of sarcolemmal membranes [2]. This was attributed to disruption of some specific protein-protein and/or protein-lipid interactions in the membrane. Here, as before [3], relatively moderately treated sarcolemmal membranes were used to study the relationship between the secondary structure of membrane-bound proteins and their Mg^{2+}-dependent ATPase activity at various concentrations of Mg^{2+}.

2. MATERIALS AND METHODS

Male Wistar rats (180–220 g) were killed by decapitation. Hearts were quickly removed and the sarcolemmal fraction isolated by hypotonic shock [4], combined with mild NaI treatment [3].

The protein concentration was determined according to [5]. Activation of Mg^{2+}-dependent ATPase by increasing concentrations of Mg^{2+} within the range 0.1–10 mM was estimated by incubating 100 μg membrane proteins in 1 ml medium containing 50 mM Tris, pH 7.4 at 37°C. After 5 min of preincubation in the presence of magnesium, the reaction was started by addition of ATP (final concentration 4 mM), and terminated by 1 ml of 0.73 M ice-cold trichloroacetic acid. The amount of P_i liberated during 10 min was determined as in [6]. The secondary structure of sarcolemmal proteins was studied by evaluating circular dichroism (CD) spectra of membrane vesicles recorded in the range 210–240 nm. The respective

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spectra were measured by means of a Jasco 40 C dichrograph calibrated with d_{10}-camphorsulfonic acid. CD spectra obtained with integrated sarcolemmal vesicles were corrected for the protein content by the computer-assisted method of Soós and Fajszi described in [3]. The α-helix content of membrane proteins was determined from corrected spectra as in [7].

3. RESULTS

Isolated sarcolemmal membrane fractions were routinely checked for purity by electron microscopy, and also by determining marker enzyme activities. The specific activity of Mg^{2+}-ATPase in the presence of 5 mM $MgCl_2$ was 25.11 ± 1.23 μmol P_i/mg protein per h with a sensitivity to oligomycin (5 μg/ml below) 1%. The (Mg^{2+} + Ca^{2+})-ATPase activity established in the presence of 2 mM $MgCl_2$ and 0.1 mM $CaCl_2$ was only 0.35 ± 0.20 μmol P_i/mg protein per h and was completely inhibited by 2 μm orthovanadate. From the point of view of ATPases, this practically excluded any significant contamination of the sarcolemmal fraction by mitochondrial membranes, sarcoplasmic reticulum or by myofibrils. The activity of (Na^+ + K^+)-ATPase, a sarcolemmal marker enzyme, was determined in the presence of 5, 10 and 100 mM $MgCl_2$, KCl and NaCl, respectively, and amounted to 12.51 ± 0.31 μmol P_i/mg protein per h.

Activation of the Mg^{2+}-dependent ATPase by increasing concentrations of its metallic cofactor magnesium keeping ATP constant at a saturation concentration of 4 mM yielded the following kinetic parameters V_{max}^{Mg} = 29.5 μmol P_i/mg protein per h and K_m^{Mg} 0.84 mM (fig.1).

The relationship between the activating concentrations of Mg^{2+} and the specific activity of membrane-bound Mg^{2+}-dependent ATPase can be described by the reciprocal form of the Michaelis-Menten equation:

$$\frac{1}{V} = \frac{1}{V_{max}^{Mg}} + \frac{K_m^{Mg}}{V_{max}^{Mg}\,[Mg^{2+}]} \qquad (1)$$

where V represents the actual velocity of the enzyme reaction. The same increasing concentrations of magnesium as applied to the activation of Mg^{2+}-ATPase induced a gradual diminution of the

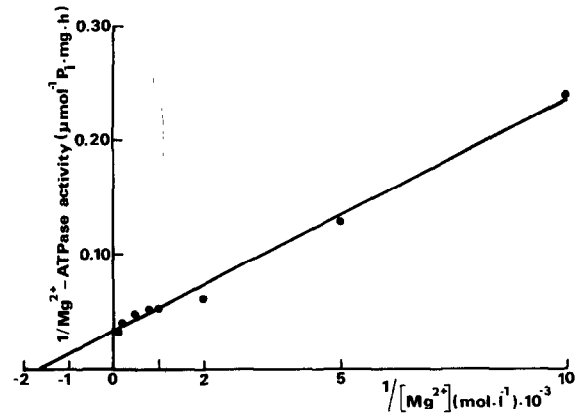

Fig.1. Lineweaver-Burk analysis of the magnesium-induced activation of sarcolemmal Mg^{2+}-dependent ATPase. Each experimental point represents the mean of 6–8 different measurements. The straight line obtained by linear regression is characterized by a correlation coefficient r = 0.996 at $p<0.01$.

α-helix content in sarcolemmal proteins in the absence of ATP (fig.2). This effect may be characterized by the following equation:

$$\alpha_H = a - b\log[Mg^{2+}] \qquad (2)$$

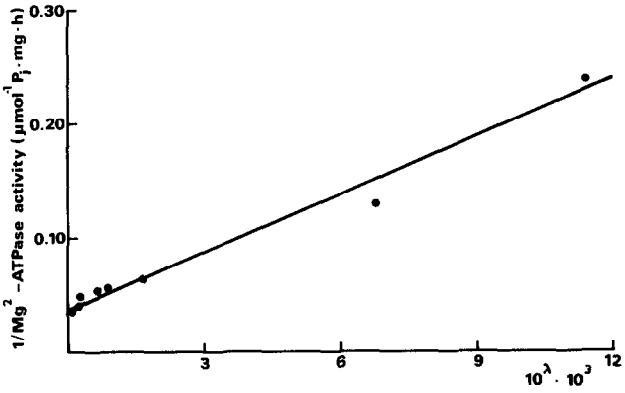

Fig.2. Magnesium-induced changes in the secondary structure of sarcolemmal proteins. The basal value of the α-helix content in sarcolemmal proteins (value taken as 1) established in Tris buffer (pH 7.4) in the absence of Mg^{2+} was 79.6%. Each experimental point represents the mean of 6–8 different measurements. The straight line obtained by linear regression (r = 0.981 at $p<0.01$) is characterized by the equation a_H = 0.779 − 0.044log[Mg^{2+}], where a_H represents the α-helix content of sarcolemmal proteins.

where α_H represents the α-helix content of sarcolemmal proteins, a denotes the α-helix content of sarcolemmal proteins at 1 M Mg^{2+} and b is the slope of the straight line. Combining eqns 1 and 2 a quantitative relationship between the magnesium-modulated changes in the α-helical organization of membrane proteins and the respective changes in the Mg^{2+}-ATPase activity of the sarcolemma was demonstrated (fig.3, eqn 2):

$$\frac{1}{V} = \frac{1}{V_{max}^{Mg}} + \frac{K_m^{Mg}}{V_{max}^{Mg} \cdot 10^{-\lambda}} \quad (3)$$

where $\lambda = (\alpha_H - a/b)$ is derived from eqn 2, i.e. $Mg^{2+} = 10^{-\lambda}$.

4. DISCUSSION

Estimation of the α-helix content in proteins provides, according to Siegel et al. [7], the most authentic information about their secondary structure. Recording CD spectra at 210–240 nm makes possible a reliable determination of the α-helix content in soluble proteins, since within this wavelength region the Cotton effect of the α-helical form markedly differs from those of β and unordered forms [8]. Nevertheless, in membrane-bound proteins a further special correction is required, in addition to the computer-assisted evaluation of CD spectra, to eliminate the influence of light scattering and absorption flattening effects. Using this approach several membrane proteins have already been studied exhibiting both structural similarity [9] and differences [10,11] in the non-solubilized and solubilized states, respectively. The CD method proved to be sensitive enough to detect selective changes also in relative crude preparations. Based on α-helicity measurements very similar structural changes were recorded during conversion of the E_1Na to E_2K form in highly purified renal ($Na^+ + K^+$)-ATPase [10] and the same enzyme in partially purified heart sarcolemma [3]. Moreover, these results corresponded well with those obtained by a fluorescence technique [12]. The observed quantitative relationship between Mg^{2+}-induced modulation of both the Mg^{2+}-dependent ATPase activity and α-helix content of proteins in cardiac sarcolemma (fig.3) indicates, as in the case of ($Na^+ + K^+$)-ATPase, that the interaction of enzyme with ATP is preceded by specific cation-induced conformation changes of particular sarcolemmal proteins. These changes seem to be necessary for the manifestation of Mg^{2+}-dependent ATPase activity. It should be noted that the Mg^{2+}-induced conformation changes may also concern membrane proteins different from the Mg^{2+}-dependent ATPase. Consequently, only a part of the decrease in α-helix content may be relevant to the Mg^{2+}-dependent ATPase molecule. Nevertheless, the statistical significance of the observed structure-activity relationship enables us to conclude that in cardiac sarcolemma the Mg^{2+}-induced modulation of the secondary structure of membrane protein is highly regulatory to Mg^{2+}-dependent ATPase activity.

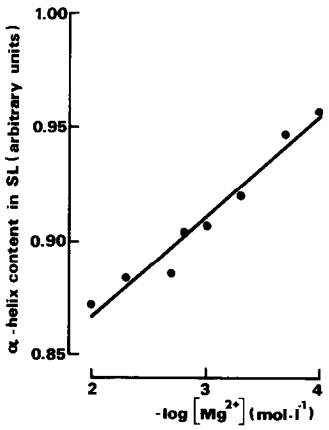

Fig.3. Relationship between the Mg^{2+}-dependent ATPase activity and the secondary structure of proteins in cardiac sarcolemma. The straight line was obtained using the data shown in figs 1 and 2 ($r = 0.990$ at $p < 0.01$). 10^{λ} is the reciprocal value of $[Mg^{2+}]$ (eqn 2).

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