Quantitative Relationship Between the Protein Secondary Structure in Cardiac Sarcolemma and the Activity of the Membrane-bound Ca^{2+}-ATPase

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Abstract. In the absence of ATP, increasing concentrations of calcium within a range between 0.1—8.0 mmol . l^{-1} gradually lowered the α-helix content of proteins in rat heart sarcolemma requiring no energy supply. In the presence of ATP, similar concentrations of calcium stepwise activated the sarcolemmal low-affinity Ca^{2+}-ATPase. A mathematical analysis of the data obtained revealed a quantitative relationship between calcium-induced stimulation of the Ca^{2+}-ATPase activity and a diminution of the α-helix contents of membrane proteins in cardiac sarcolemma. The cooperation between changes in protein conformation and energy consumption in relation to the supposed role of low-affinity Ca^{2+}-ATPase in gating the calcium channel are discussed.

Key words: Low-affinity Ca^{2+}-ATPase — Protein conformation — Calcium channel — Heart sarcolemma — Circular dichroism

Introduction

It is widely accepted that channel-mediated influx of calcium in the cardiac cell is controlled metabolically by an ATP-dependent mechanism participating in gating of the slow calcium channel (Sperelakis and Schneider 1976). The role of the molecular basis for such a mechanism has been ascribed to sarcolemmal ATP-ase with a low affinity to calcium as the activity of this enzyme was shown to respond to all the main impulses regulating calcium movements through the calcium channel (Dhalla et al. 1977). An unavoidable presumption for the latter notion is that certain phases of gating of calcium channel and of calcium-induced ATP splitting will be associated with similar specific conformational changes of a particular

Presented at the 2nd International Symposium on Ionic Channels and Membrane Receptors, 21—26 May 1984, Smolenice, Czechoslovakia

portion of membrane proteins. The present study deals with the investigation of a quantitative relationship between calcium-induced conformational changes in sarcolemmal proteins and the corresponding calcium-induced activation of sarcolemmal ATPase.

Materials and Methods

Isolated rat heart membrane preparation enriched in sarcolemma was obtained by the method of hypothonic shock (McNamara et al. 1974a) combined with NaI treatment (Kostka et al. 1981).

Protein concentration was determined according to Lowry et al. (1951). Activation of Ca^{2+}-ATPase by increasing concentrations of Ca^{2+} within the range of 0.1—8.0 mmol . l^{-1} was estimated by incubating 100 μg membrane protein in 1 ml medium containing 50 mmol . l^{-1} TRIS, pH = 7.4 at 37 °C. After 5 min of preincubation in the presence of calcium, the reaction was started by the addition of ATP (final concentration 4 mmol . l^{-1}) and it was stopped by 1 ml of 0.73 mol . l^{-1} ice-cold trichloroacetic acid. The amount of P_i liberated during 10 min reaction was determined by the method of Taussky and Shorr (1953).

The secondary structure of proteins in sarcolemmal membranes was studied by circular dichroism (CD). Spectra were recorded within a range between 210 and 240 nm in the absence or in the presence of various Ca^{2+} concentrations by means of a Jasco 40 C dichrograph. The equipment was calibrated with d-10-camphorsulfonic acid. CD spectra obtained with integrated sarcolemmal vesicles were corrected for the protein content applying the computer-assisted method of Soós and Fajszi as it has been described in our previous paper (Vrbjar et al. 1984).

The α-helix content of sarcolemmal proteins was determined from corrected spectra according to Siegel et al. (1980).

Results and Discussion

The isolated sarcolemmal membrane fraction was routinely investigated on purity by electrone microscopy as well as by marker enzymes. The following enzyme activities observed in the presence of 50 mmol . l^{-1} TRIS pH = 7.4, were found to be typical for the preparation: Specific activity of $(Na^+ + K^+)$-ATPase determined in the presence of 5; 10; and 100 mmol . l^{-1} of $MgCl_2$, KCl and NaCl, respectively was 12.34 ± 0.54 μmol P_i . mg^{-1} protein . h^{-1}. Specific activity of Mg^{2+}-ATPase assessed in the presence of 5 mmol . l^{-1} $MgCl_2$ was $22.10 \pm 0{,}61$ μmol P_i . mg^{-1} protein . h^{-1} with a sensitivity to oligomycin (5 μg/ml) below one per cent. The $(Mg^{2+} + Ca^{2+})$-ATPase activity measured in the presence of 2 mmol . l^{-1} $MgCl_2$ and 0.1 mmol . l^{-1} $CaCl_2$ was only 0.35 ± 0.20 μmol P_i . mg^{-1} protein . h^{-1} and it could be totally inhibited by 2 μmol . l^{-1} of orthovanadate. From the point of view of ATPase, this suggested that there practically was no significant contamination of the sarcolemmal fraction by mitochondrial membranes, sarcoplasmic reticulum or myofibrils.

The activation of the low-affinity Ca^{2+}-ATPase by its cofactor, calcium at 4 mmol . l^{-1} ATP (the saturation concentration) was investigated using the

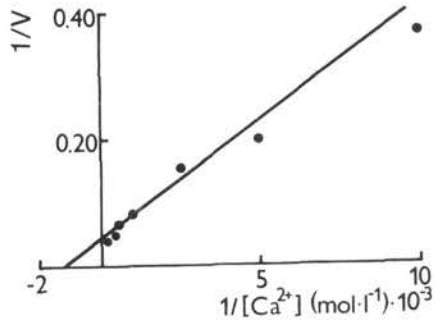

Fig. 1. The Lineweaver-Burk analysis of calcium induced-activation of sarcolemmal low-affinity Ca^{2+}-ATPase. Ordinate: Reciprocal values of Ca^{2+}—ATPase activity ($\mu mol^{-1} P_i$ mg.h). Each experimental point represents the mean of 6—8 different measurements. The straight line obtained by linear regression is characterized by a correlation coefficient, $r = 0.978$ at $p < 0.01$.

Lineweaver-Burk plot (Fig. 1), a V_{max}^{Ca} value of 24.40 μmol P_i . mg^{-1} protein . h^{-1} and K_M^{Ca} value of 0.84 mmol . l^{-1} (the Michaelis-Menten constant for the enzyme activation by calcium) were found. The relationship between the specific activity of membrane bound Ca^{2+}-ATPase and activating concentrations of calcium may be described by the reciprocal form of the Michaelis-Menten equation:

$$\frac{1}{V} = \frac{1}{V_{max}^{Ca}} + \frac{K_M^{Ca}}{V_{max}^{Ca}\,[Ca^{2+}]} \tag{1}$$

where V represents the actual velocity of the enzyme reaction.

Various concentrations of calcium induced typical conformation changes of sarcolemmal proteins as reflected in circular dichroism spectra measured in the range of 210—240 nm (Fig. 2). The α-helix contents of sarcolemmal membrane proteins were determined from these spectra. Increasing concentrations of calcium, as applied in enzyme activation studies induced a gradual diminution of the α-helix content in sarcolemmal proteins in the absence of ATP (Fig. 3). The relationship between the secondary structure of sarcolemmal proteins and calcium concentration could be characterized by the equation:

$$\alpha_H = a - b \,.\, \log [Ca^{2+}] \tag{2}$$

where α_H represents the α-helix content of sarcolemmal proteins; a represents the α-helix content of sarcolemmal proteins at 1 mol . l^{-1} Ca^{2+}; and b is the slope of the straight line.

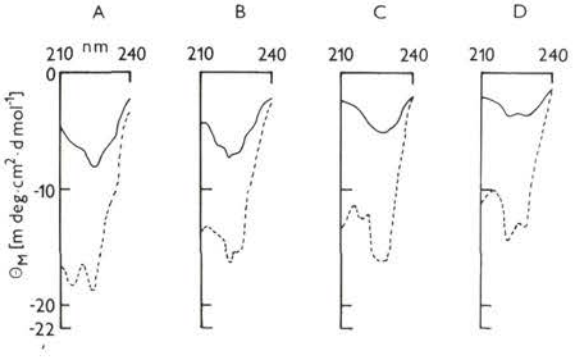

Fig. 2. Sarcolemmal protein circular dichroism spectra in the absence and in the presence of various concentrations of calcium. Solid lines: spectra obtained with non-solubilized, integrated membrane vesicles. Dashed lines: spectra corrected for protein contents. The presented curves are representative tracings from typical measurements. *A*: calcium free sarcolemma (SL); *B*: SL + 0.1 mmol . l^{-1} $CaCl_2$; *C*: SL + 0.2 mmol . l^{-1} $CaCl_2$; *D*: SL + 0.4 mmol . l^{-1} $CaCl_2$.

Combining equations (1) and (2) gives:

$$\frac{1}{V} = \frac{1}{V_{max}^{Ca}} + \frac{K_{Ca}}{V_{max}^{Ca} \cdot 10^{-\lambda}} \tag{3}$$

where $\lambda = \frac{\alpha_H - a}{b}$ is derived from equation (2) i. e., $[Ca^{2+}] = 10^{-\lambda}$.

The observed quantitative relationship between Ca^{2+}-induced modulation of both the Ca^{2+}-ATPase activity and the secondary structure of membrane proteins (Fig. 4) indicates that ATP splitting is preceeded by specific conformation changes of particular sarcolemmal proteins, these changes being required for the Ca^{2+}-ATPase activity to become manifested. It should be noted that the specific conformation changes evidently do not require any additional supply of chemical or electrical energy. No doubt that the observed Ca-induced changes in the protein secondary structure also concern membrane proteins other than the low-affinity Ca^{2+}-ATPase. Consequently, only a part of the decrease in α-helix content may be relevant to the Ca^{2+}-ATPase molecule. Nevertheless, the statistical significance of the observed structure-activity relationship enables to conclude that in cardiac sarcolemma Ca-induced modulation of the secondary structure of membrane proteins is highly regulatory to Ca^{2+}-ATPase activity. The above conclusion is strongly supported by recently reported, specific cationic ligand-induced changes in α-helicity of membrane proteins related to the conformation shifts from E_1 to E_2 state of $(Na^+ + K^+)$-ATPase in the membrane-bound form (Gresalfi and Wallace 1984; Vrbjar et al. 1984).

It was found earlier that Ca^{2+}-ATPase is inhibited by divalent cations, which

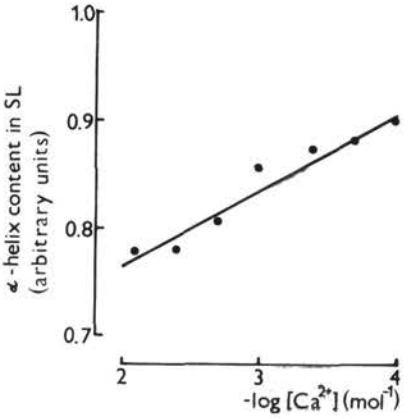

Fig. 3. Semilogarithmic plot of calcium-induced changes in the secondary structure of sarcolemmal proteins. The basal value of the α-helix content in sarcolemmal proteins (the value taken for 1) established in TRIS buffer (pH = 7.4) in the absence of Ca^{2+} was 79.6 per cent. Each experimental point represents the mean of 6—8 different measurements. The straight line obtained by linear regression ($r = 0.973$ at $p < 0.01$) is characterized by equation $\alpha_H = 0.623 + 0.071 \log Ca^{2+}$, where α_H represents the α-helix content of sarcolemmal proteins.

depress calcium influx in cardiac muscle (McNamara et al. 1974b). Moreover, electrical or hormonal impulses operating the Ca-channel also induce an increased activity of the sarcolemmal low-affinity Ca^{2+}-ATPase (Ziegelhöffer and Dhalla 1979). Based on these data as well as on our finding that the calcium induced change in protein conformation is a process, requiring no energy supply it would be reasonable to suppose that chemical energy liberated during the subsequent Ca^{2+}-ATPase reaction may rather be utilized for closing than for opening of the calcium-channel (Sperelakis and Schneider 1976; Dhalla et al. 1977; Ziegelhöffer et al. 1984) thus returning the respective sarcolemmal proteins into the original conformation state.

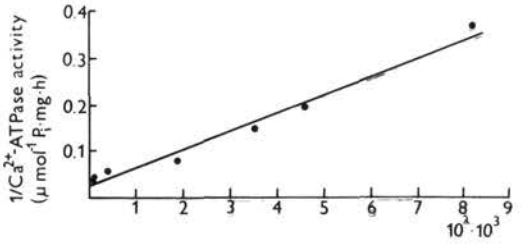

Fig. 4. Relationship between the Ca^{2+}-ATPase activity and the secondary structure of proteins in cardiac sarcolemma. The straight line was obtained using the data shown in Figs. 1 and 3, ($r = 0.990$ at $p < 0.01$). 10^{λ} is the reciprocal value of $[Ca^{2+}]$ (Eq. 2).

Acknowledgements. This work was in part supported by the Hungarian Academy of Sciences during N. V.'s participation in International Training Course organized by the Biological Research Centre of H. A. S. in Szeged. We thank Mrs. E. Havránková for the excellent technical assistance and Mrs. M. Hýbelová for preparing the manuscript.

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Received June 26, 1984/Accepted January 10, 1985