Short communication

Interaction of ATP with the Active Sites of ATPases in Heart Sarcolemma. Role of the Hydroxylic Group in Position Two on the Ribose Moiety

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It has been well demonstrated that the binding of ATP to the active site of $(Na^+ + K^+)$-ATPase requires the recognition and/or binding of both the terminal γ-phosphate group and the amino group in position 6 on the purine moiety. The former interacts with an aspartic group (Jørgensen 1982) and the latter with an essential sulfhydryl group (Patzelt-Wenczler and Schoner 1981; Ziegelhöffer et al. 1983) present in the active site of the enzyme. The participation of the ribose moiety of ATP in the recognition of the latter and its binding to the active site of $(Na^+ + K^+)$-ATPase is, however, still poorly understood. The aim of the present study was to investigate the role of the hydroxylic group in position 2 on the ribose component of ATP and compare the findings with those obtained in measuring Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in isolated rat heart sarcolemma.

The isolation of the membrane fraction enriched in sarcolemma, its characterization concerning the purity, ouabain sensitivity, integrity and orientation of the vesicles as well as the estimation of the specific activities of sarcolemmal ATPases and their kinetic properties were described in our earlier papers (Ziegelhöffer et al. 1983; Vrbjar et al. 1984; Vrbjar et al. 1985; Breier et al. 1986).

A comparison of stimulation of the sarcolemmal $(Na^+ + K^+)$-ATPase activity by increasing concentrations of ATP with the hydroxylic group in position 2 on the ribose moiety present or absent (deoxy-ATP) revealed that deoxy-ATP was hydrolyzed at a lower rate at all concentrations of the substrate used (Fig. 1). The respective K_m values for ATP and deoxy-ATP were 1.02 ± 0.04 and 0.29 ± 0.01 mmol . l^{-1} and those of V_{max} 21.02 ± 0.98 and 7.10 ± 0.35 μmol P_i . mg^{-1} of membrane protein . h^{-1}.

If the number of active collisions between the enzyme and its substrate represents the dominating principle in the control of the reaction velocity, the relatively small differences in hydrolysis rates at low ATP and deoxy-ATP concentrations would indicate that the OH group in position 2 on the ribose moiety is of only minor significance for the affinity of substrate binding to the active site of $(Na^+ + K^+)$-ATPase. On the other hand, the hydroxylic group on the ribose seems to participate considerably in reaction steps following the recognition and binding of ATP. This assumption is well supported by the observation that in absence of the OH group on the ATP-ribose moiety, the reaction rate of the $(Na^+ + K^+)$-ATPase was slowed down significantly at high substracte concentrations, i.e. deoxy-ATP was hydrolyzed at a decreased turnover.

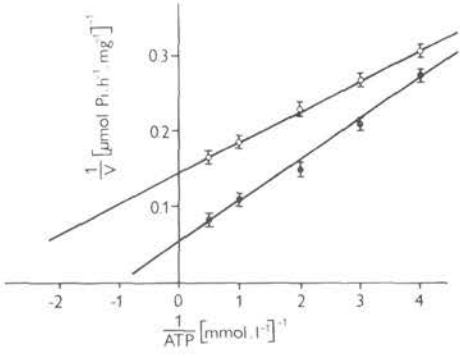

Fig. 1. Lineweaver-Burk plot of stimulation of $(Na^+ + K^+)$-ATPase with increasing concentrations of ATP and deoxy-ATP. Results are means ± S.E.M. from 9 separate measurements. Straight lines were obtained by linear regression, the correlation coefficients complying with the level of probability $p > 0.01$. ● — ATP; ○ — deoxy-ATP. The reaction medium contained (in mmol.l^{-1}) 100 NaCl, 10 KCl, and $MgCl_2$ in 50 mmol.l^{-1} imidazol-HCl buffer. ATP, deoxy-ATP and imidazol were purchased from Sigma Ltd. (USA), all other chemicals from Lachema Ltd. (Czechoslovakia), and were of analytical grade.

As compared to ATP, the lower rate of hydrolysis of deoxy-ATP may be attributed exclusively to different molecule of deoxy-ATP, since both ATP and deoxy-ATP were products of the same quality, prepared by phosphorylation of adenosine and deoxy adenosine respectively, by Sigma Ltd. (USA). However, essentially the same results were obtained with deoxy-ATP and ATP purchased from Boehringer Ltd. (FRG) (not shown).

The method used for the measurement of ATP hydrolysis at pH 7.0 (Ziegelhöffer et al. 1983; Breier et al. 1986) practically eliminates the effects of any other known contaminants or modulating influences which may be introduced with both ATP preparations (Michell and Taylor 1982; Searle et al. 1983).

In contrast to the results obtained with $(Na^+ + K^+)$-ATPase, both Ca^{2+}-ATPase and Mg^{2+}-ATPase hydrolyzed ATP and deoxy-ATP with similar velocities at any concentrations used (Fig. 2). The values of K_m for Mg^{2+}-ATPase and

Ca^{2+}-ATPase were 0.68 ± 0.03 and 0.62 ± 0.03 μmol $P_i \cdot mg^{-1}$ of membrane protein $\cdot h^{-1}$. Hence, it is reasonable to assume that the structure of the ATP binding sites of Ca^{2+}-ATPase and Mg^{2+}-ATPase differs also in this respect from that of $(Na^+ + K^+)$-ATPase.

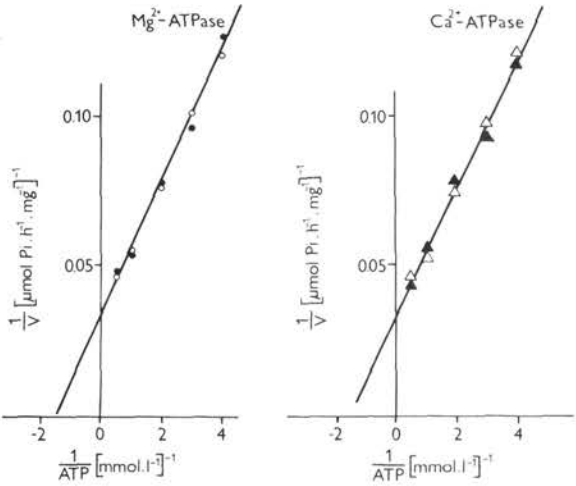

Fig. 2. Lineweaver-Burk plot of stimulation of Mg^{2+}-ATPase and Ca^{2+}-ATPase with increasing concentrations of ATP and deoxy-ATP. Results are means from 9 separate measurements. Straight lines were obtained by linear regression, respective correlation coefficients being complying with the level of probability $p > 0.01$. ●, ▲ — ATP; ○, △ — deoxy-ATP. The reaction medium for Mg^{2+}-ATPase contained 2 mmol $\cdot l^{-1}$ $MgCl_2$, for Ca^{2+}-ATPase 4 mmol $\cdot l^{-1}$ $CaCl_2$, both in 50 mmol $\cdot l^{-1}$ imidazol-HCl buffer. The origin and quality of the chemicals used is indicated in Fig. 1.

References

Breier A., Monošíková R., Ziegelhöffer A., Džurba A. (1986): Heart sarcolemmal $(Na^+ + K^+)$-ATPase has an essential amino group in the potassium binding site on the enzyme molecule. Gen. Physiol. Biophys. **5,** 537—544

Jørgensen P. L. (1982): Mechanism of the Na^+, K^+ pump; Protein structure and conformations of the pure $(Na^+ + K^+)$-ATPase. Biochim. Biophys. Acta **694,** 27—68

Michell A. R., Taylor E. A. (1982): The optimum pH of renal adenosine triphosphatase in rats: Influence of vanadate, noradrenaline and potassium. Enzyme **28,** 309—316

Patzelt-Wenczler R., Schoner W. (1981): Evidence for two different reactive sulfhydryl groups in the ATP-binding sites of $(Na^+ + K^+)$-ATPase. Eur. J. Biochem. **114,** 79—87

Searle B. M., Higashimo H., Khalil F., Bogden J. D., Tokushige A., Tamura H., Kino M., Aviv A. (1983): Vanadate effect on the Na, K-ATPase and the Na-K pump in in vitro-grown rat vascular smooth muscle cells. Circ. Res. **53,** 186—191

Vrbjar N., Soós J., Ziegelhöffer A. (1984): Secondary structure of heart sarcolemmal proteins during interaction with metallic cofactors of $(Na^+ + K^+)$-ATPase. Gen. Physiol. Biophys. **3,** 317—325

Vrbjar N., Ziegelhöffer A., Breier A., Soós J., Džurba A., Monošiková R. (1985): Quantitative relationship between the protein secondary structure in cardiac sarcolemma and the activity of the membrane-bound Ca^{2+}-ATPase. Gen. Physiol. Biophys. **4**, 411–416

Ziegelhöffer A., Breier A., Džurba A., Vrbjar N. (1983): Selective and reversible inhibition of heart sarcolemmal ($Na^+ + K^+$)-ATPase by *p*-bromophenyl isothiocyanate. Evidence for a sulfhydryl group in the ATP-binding site of the enzyme. Gen. Physiol. Biophys. **2**, 447–456

Final version accepted October 14, 1986