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ACTIVATION ENERGY OF SKELETAL MUSCLE SARCOLEMMAL Na^+, K^+-ADENOSINE TRIPHOSPHATASE*

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SUMMARY

Arrhenius plots of rabbit skeletal muscle sarcolemmal Na^+,K^+-ATPase contain no temperature breaks. The apparent activation energy (22.8 kcal/mole in the presence of 1 mM $MgCl_2$ or 15.9 kcal/mole in the presence of 3 mM $MgCl_2$) does not depend on the Na^+/K^+ ratio in the incubation medium, but decreases in the presence of anserine (instead of Tris buffer).

INTRODUCTION

Recent communications [1–3] suggest that the ATP hydrolysis catalyzed by the Na^+- plus K^+- stimulated and Mg^{2+}- requiring adenosine triphosphatase (Na^+, K^+-ATPase) consists of the several steps. First of them is Na^+-dependent phosphorylation of the enzyme resulting to the formation of a high-energy phosphoenzyme. There is also K^+-dependent and ouabain-sensitive step of dephosphorylation of it. Besides of these the special Mg^{2+}-dependent step should exist because of the necessity of high Mg^{2+} concentration for the whole reaction of ATP hydrolysis by Na^+, K^+-ATPase. It is unclear which of the steps of the overall process of ATP hydrolysis is rate-limiting. To identify the rate-limiting step, the measurement of the apparent activation energy may be employed as a possible approach. In our attempt to elucidate some aspects of the reaction mechanism of the enzyme we studied the temperature dependence of skeletal muscle sarcolemmal Na^+, K^+-ATPase under different incubation conditions.

MATERIALS AND METHODS

ATP (Na-salt) was purchased from Reanal (Hungary) and converted to tris- or anserine-forms by a passage through a Dowex 50 cm×4 cm (400 mesh) column. NaCl, KCl were recrystallized from glass-distilled water, containing 0.5 mM

Abbreviation: EGTA, ethyleneglycoltetraacetic acid.

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EDTA. Chromatographically pure anserine was obtained as described recently [4]. All other reagents were of analytical grade.

Sarcolemmal membrane fragments were isolated from limb muscles of adult rabbits by a previously described procedure [5]. The total ATPase activity was assayed in a medium, containing 80 mM NaCl, 60 mM KCl (optimal concentrations of monovalent cations for Na^+, K^+-ATPase, ref. 5), 1 mM ATP, 1 or 3 mM $MgCl_2$, 0.2 mM ethyleneglycoltetraacetic acid (EGTA), 10 mM or 16.7 mM Tris–HCl or 10 mM anserine (pH 7.4) in a final volume of 1 ml. Mg^{2+}-ATPase was assayed in a similar medium, containing 1 mM ouabain additionally or 140 mM KCl (in the absence of NaCl). The difference between total ATPase and Mg^{2+}-ATPase was taken to be Na^+, K^+-ATPase. The reaction was started by the addition of 20–40 mg of the sarcolemmal membrane protein and terminated by the addition of 2 ml ice-cold trichloroacetic acid (7.5 %). In all experiments the ATP added was cleaved not more than by 10 %. The ATPase reaction was determined by measuring the amount of inorganic phosphate liberated during incubation. Inorganic phosphate was determined by the method of Weil-Malherbe and Green [6] and the values obtained were corrected for non-enzymatic ATP hydrolysis. Protein was measured by the method of Lowry et al. [7].

The rate of ATP hydrolysis in the presence of 100 μg of the sarcolemmal membrane protein was linear for 60 min at temperatures below 15 °C, 25 min at 25 °C and 10 min at 37 °C. Therefore, the incubation time varied from 30 min at 5 °C to 5 min at 37 °C. As the pH of buffers was dependent on temperature [8], the incubation medium was adjusted to the optimal value: pH 7.4 (see ref. 5) at all temperatures studied within the range of 5-37 °C. The deviations of pH during incubation in all of the experiments were not more than 0.1 (7.4 ± 0.05).

RESULTS

Na^+, K^+-ATPase activity was found to be more temperature dependent than that of Mg^{2+}-ATPase. Contrary to the observations of some authors [9–11], in our experiments the Arrhenius plots of Na^+, K^+-ATPase consisted of a single straight line within the temperature range of 5–37 °C (Fig. 1). The absence of breaks on the Arrhenius plots of the enzyme system is reminiscent of the earlier observations of rat brain microsomal Na^+, K^+-ATPase by Hexum et al. [12].

It is of interest to mention that when the incubation medium was adjusted to pH 7.4 at room temperature and no further corrections were made for the shifts in pH of the buffer with the alterations of incubation temperature, a break was observed on the Arrhenius plots of the Na^+, K^+-ATPase around 20 °C (Fig. 2). In that case the apparent activation energies (19 kcal/mole above 20 °C and 32 kcal/mole below 20 °C) were similar to the values obtained from the Arrhenius plots of Na^+, K^+-ATPase containing breaks [9–11]. However, in order to limit the number of temperature-dependent parameters in the system, pH of the incubation medium was usually controlled as described in Methods.

It is well established now that the Na^+, K^+-ATPase activity depends on the ratio of Na^+ and K^+ concentrations in the incubation medium. The apparent activation energy, however, did not depend on whether the incubation medium contained Na^+ and K^+ in the ratios of 80/60, 120/20 or 20/120 mM (Table I). Therefore, it may

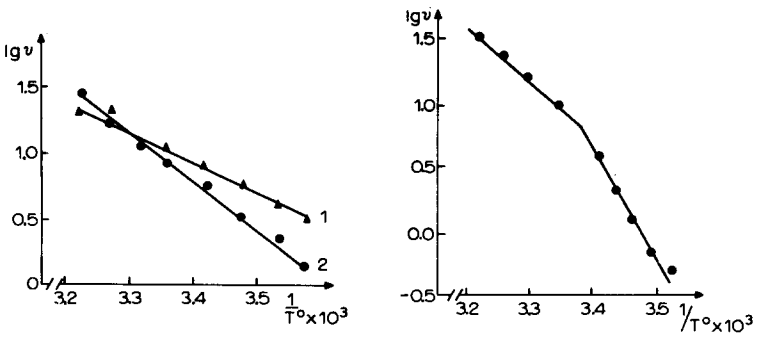

Fig. 1. Arrhenius plots of Mg^{2+}-ATPase (1) and Na^+, K^+-ATPase (2); incubation media adjusted to pH 7.4 at all temperatures used. Incubation conditions: 3 mM $MgCl_2$, 1 mM ATP, 80 mM NaCl, 60 mM KCl, 0.2 mM EGTA, 10 mM Tris–HCl.

Fig. 2. Arrhenius plot of Na^+, K^+-ATPase; incubation medium adjusted to pH 7.4 at room temperature only. Incubation conditions: 1 mM $MgCl_2$, 1 mM ATP, 80 mM NaCl, 60 mM KCl, 0.2 mM EGTA, 40 mM Tris–HCl.

TABLE I

APPARENT ACTIVATION ENERGY OF SARCOLEMMAL Na^+, K^+-ATPase

Lack of dependence on the ratio of monovalent cation concentration (data of four experiments from two enzyme preparations). Incubation conditions: 3 mM $MgCl_2$, 1 mM ATP, 16.7 mM Tris–HCl, 0.2 mM EGTA. pH 7.4.

NaCl/KCl (mM/mM)	Apparent activation energy, E_a* (kcal/mole)
120/ 20	13.5±0.5
80/ 60	12.0±0.2
20/120	14.0±0.2

* Differences between E_a values observed are statistically insignificant.

be inferred that the stimulation of the Na^+, K^+-ATPase by Na^+ and K^+ reflects the amount of catalytically active molecules of the enzyme. It should be noted at the same time that the decrease in the Na^+, K^+-ATPase activity due to long-term storage of enzyme does not affect the apparent activation energy values.

Fig. 3 shows that the activity of the Na^+, K^+-ATPase is enhanced by an increase in the concentration of free Mg^{2+} in the incubation medium (see also ref. 1), whereas Mg^{2+}-ATPase remains practically constant with a rise in Mg^{2+} concentration. Simultaneously with the stimulation of the Na^+, K^+-ATPase activity by Mg^{2+}, the apparent activation energy decreases from 22.8 kcal/mole in a medium, containing 1 mM ATP and 1 mM $MgCl_2$, to 15.9 kcal/mole in a medium, containing 1 mM ATP and 3 mM $MgCl_2$ (Table II).

It was reported previously [13] that anserine and carnosine, specific dipeptides of skeletal muscles, stimulate the Na^+, K^+-ATPase; however, the mechanism of their action remained obscure. To study this question we compared the apparent activation energy of the Na^+, K^+-ATPase, measured in anserine, with that, measured in Tris-

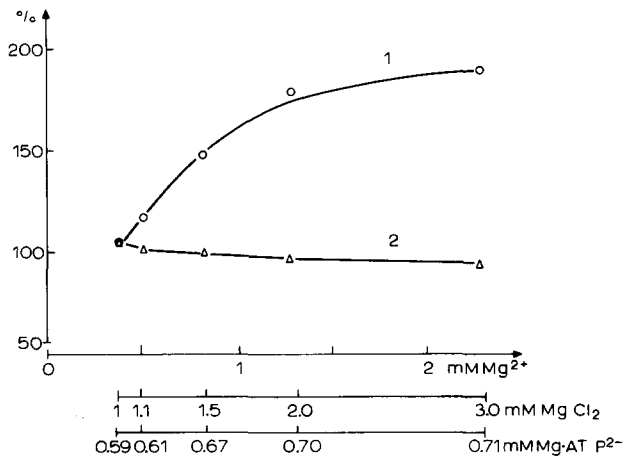

Fig. 3. Effect of Mg^{2+} on the activities of Na^+, K^+-ATPase and Mg^{2+}-ATPase. Incubation conditions: 1 mM ATP, 80 mM NaCl, 60 mM KCl, 0.2 mM EGTA ($\pm$1 mM ouabain), 16.7 mM Tris–HCl; pH 7.4, temperature 37 °C. Concentration of Mg · ATP complex and free Mg^{2+} concentrations were calculated from $MgCl_2$ added, using the relevant dissociation constants [17]. ATPase activities in the presence of 1 mM ATP and 1 mM $MgCl_2$ were taken for 100 %.

TABLE II

EFFECT OF BUFFERS AND Mg^{2+} ON THE APPARENT ACTIVATION ENERGY OF SARCOLEMMAL Na^+, K^+-ATPase

In the brackets the numbers of experiments are given. Incubation conditions: 80 mM NaCl, 60 mM KCl, 1 mM ATP, 0.2 mM EGTA. pH 7.4

Buffer		Apparent activation energy, E_a (kcal/mole)*	
Type	Amount (mM)	1 mM $MgCl_2$	3 mM $MgCl_2$
Tris–HCl	10.0	a. 22.8$\pm$1.2 (9)	d. 15.9$\pm$1.3 (12)
	16.7	b. 16.7$\pm$2.1 (2)	e. 12.0$\pm$0.2 (4)
Anserine	10.0	c. 10.9$\pm$2.5 (4)	f. 7.2$\pm$0.6 (6)

* The next E_a values differed statistically significant ($P < 0.001$): a. and d., a. and c., d. and f.

buffer (Table II). It was found that the activation energy of the ATPase in 10 mM anserine was about 11 kcal/mole. The decrease in E_a value may be caused by difference in buffer capacities of anserine and Tris (at pH 7.4 anserine is a much stronger buffer than Tris). It was found however that increase of capacity of Tris buffer caused some fall in activation energy value (from 22.8 to 16.7 kcal/mole for 10 mM and 16.7 mM Tris buffer, respectively), but no additional change of E_a was observed at further enhancement of buffer capacity (up to 30 mM of Tris).

Whereas the apparent activation energy of the Na^+, K^+-ATPase depends on the components of the incubation medium, that of Mg^{2+}-ATPase is non-sensitive to the nature and concentration of buffers used, as well as to the concentration of Mg^{2+}, and is about 10.2–12.8 kcal/mole (Table III). At the same time the apparent activation energy of Mg^{2+}-ATPase measured in an ouabain-containing medium is the same as in the medium, where Na^+ is replaced by K^+ (results not shown).

TABLE III

APPARENT ACTIVATION ENERGY OF Mg^{2+}-ATPase

The number of experiments is given into the brackets. Differences between values observed are statistically insignificant. Incubation conditions: 1 mM ATP, 140 mM KCl, 0.2 mM EGTA, pH 7.4.

Buffer		$MgCl_2$ (mM)	Apparent activation energy, E_a (kcal/mole)
Type	Amount (mM)		
Tris–HCl	10.0	1	10.3±1.3 (9)
		3	10.2±1.5 (12)
	16.7	1	10.7±1.2 (2)
		3	12.8±0.2 (4)
Anserine	10.0	1	12.8±2.3 (4)
		3	12.7±0.7 (6)

DISCUSSION

Na^+, K^+-ATPase from brain, kidney and other tissues have been shown to possess Arrhenius plots with breaks around the critical temperature, above and below which the enzyme has different activation energy [9–11]. One of the conditions for the occurrence of temperature breaks is the existence of at least two independent processes, predominantly occurring below and above the critical temperature respectively [14]. In agreement with this concept Charnock and Opit [15] suggested that the break observed on the Arrhenius plots of the Na^+, K^+-ATPase can be ascribed to the shift in the rate-limiting reaction (phosphorylation, dephosphorylation of the enzyme, for example) from one step to another. Fig. 2 shows the Arrhenius plot of the Na^+, K^+-ATPase with a break, which may be due to uncontrolled shifts in pH of the incubation medium. Moreover, when the incubation medium is adjusted to the same pH at all temperatures studied, the break on the Arrhenius plots disappears (Fig. 1). This would imply that within the temperature range used (i.e. 5–37 °C) the rate-limiting reaction of this enzyme remains the same.

The dependence of the apparent activation energy on the concentration of Mg^{2+} gives some insight into the nature of the rate-limiting step of the overall process of ATP hydrolysis. This decreasing is more pronounced with Tris, than anserine. The Mg^{2+} effect on the activation energy (see Table II) is accompanied by its stimulation of the ATPase activity (see Fig. 3) and cannot be ascribed to increasing of ionic strength or osmolarity. When concentration of $MgCl_2$ is rised from 1 to 3 mM, ionic strength and osmolarity of medium are changed from 0.179 to 0.185 and from 340 mosM to 346 mosM, respectively, but concentration of free Mg^{2+} increases up to 600 % approximately (see Fig. 3) and activation energy of Na^+, K^+-ATPase decreases markedly (Table II). Thus, it may be concluded that rate-limiting step of the reaction is Mg^{2+}-dependent.

At the same time, increasing the Tris–HCl concentration from 10 mM to 16.7 mM changes the ionic strength and osmolarity of the medium by 6–7 % only, but buffer capacity of the solution increases 1.5 times. This gives pronounced decreasing of activation energy (from 22.8 to 16.7 kcal/mole). It means, that rate of proton neutralization is important for Na^+, K^+-dependent activity. It is interesting to note,

that the introduction of anserine into the incubation medium also leads to a decrease in the apparent activation energy, even when the buffer capacity of anserine is the same as that of Tris buffer. It is probable that anserine acts specifically on the rate-limiting step of the reaction.

According to the Arrhenius equation the decrease of E_a value 1.5–1.8 times has to accelerate the reaction rate 5–7-fold. Mg^{2+} and anserine effectively decrease the E_a value (see Tables I and II), but increase the ATPase reaction about 1.5–2.5-fold only. Thus we may conclude that their effects are not confined to the rate-limiting step only.

Since the Na^+, K^+-ATPase activity depends on the concentration ratio of Na^+ to K^+, but its apparent activation energy does not, it is concluded that the role of monovalent cations consists only in the determination of the amount of actively functioning Na^+, K^+-ATPase molecules. This is in agreement with the fact that the reactivation of deoxycholate-treated preparation of Na^+, K^+-ATPase requires Na^+ and K^+ [16].

The effects described are connected with the Na^+, K^+-dependent enzyme system, but not with Mg^{2+}-ATPase (see Table III). This confirms the specificity of phenomena observed.

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