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THE ROLE OF ALKALI CATION IN FORMATION AND DECOMPOSITION OF MYOSIN · ATP COMPLEX

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Summary

The dependece of kinetic constants K_m, V (k_2) and k_1 of myosin-ATPase on the species and concentration of alkali cations and on temperature was investigated.

The value of V varies with the ionic radius of different alkali cations. The curve has a maximum at 1.33 Å at the ionic radius of potassium.

The detailed analysis of the cation dependence of the kinetics of the ATPase reaction shows that both formation and decomposition of the ES complex are affected by the cation present.

Introduction

It has been shown [1—5] that the steady-state reaction of myosin with ATP follows the Michaelis kinetics. The presence of cations is essential for the reaction, the effective substrate of myosin being Me · ATP complex [6]. The rate constant of the formation and decomposition of myosin · ATP complex have been determined by Lymn and Taylor [5] in the presence and absence of divalent cations. While relatively abundant data are available on the influence of divalent cations on reaction mechanism [5—7, 11—18] the role of different alkali cations is not yet fully clarified [3—10,13,16,19].

In the present paper the results of a study about the role of different alkali cations in the formation and decomposition of ES complex are reported.

Materials and Methods

The preparation of myosin was carried out as described by Portzehl et al. [20], and purified by ultracentrifugation for 1 h at 105 000 × *g*.

The protein concentration was determined by the biuret method of Gornall et al. [21]. Only fresh myosin, not older than 3 days, was used in the experiments.

For the calculations presented in this paper, the molecular weight of myosin was assumed to be $5 \cdot 10^5$.

All analytically pure reagents were dissolved in deionized distilled water. The ATP was a REANAL product. The [^{32}P] ATP labelled at γ-phosphate had been made by Glinn and Chappel's [22] enzymatical method and separated on DEAE-Sephadex A-25 column.

The ^{32}P content of purified [^{32}P] ATP counted by using Cherenkow radiation in Nuclear Chicago J 24 liquid scintillation counter and the nucleotide content was measured at 257 nm and identified by means of Randerath's chromatographic method [23].

The [^{32}P] ATP was devoid of other nucleotides, its specific activity was 1 mol/2 Ci, the impurity of inorganic $^{32}P_i$ being less than 0.5%. Only fresh [^{32}P] ATP (not older than 3 days) was used and stored at −20° C. The [^{32}P]-ATP was diluted with distilled water or, if necessary, mixed with unlabelled ATP.

ATPase activity of myosin was measured by a modification of Bárány's method [13]. The test solution contained 6—20 μg/ml myosin, 9 mM EDTA, 40 mM Tris · HCl buffer (pH 8) in general 0.3 M chlorides of alkali cations (or 0.1—0.7 M when studying salt effect) $5 \cdot 10^{-7}$—$1 \cdot 10^{-3}$ M [^{32}P] ATP. The measurements were carried out generally at 20° C (or at 0, 10 and 37° C when studying temperature dependence) using samples of 8 ml, with incubation times (1—10 min) chosen so as to obtain a decomposition of ATP less than 20%. Incubation was terminated by the addition of 1/13 volume of 0.88 M H_2SO_4. After mixing, 1.4 ml of charcoal suspension was added (composition: 1 g Norrit charcoal +90 ml distilled water +10 ml ethanol containing 1% octanol) in ice bath and mixed again. Some minutes later the samples were filtrated on Schleicher and Shuell AG Nr 589^3 filter paper. The radioactivity of $^{32}P_i$ liberated in the filtrate was measured as above. Knowing the impulses counted we could determine the number of $^{32}P_i$ mol liberated by means of the known concentration and impulse of [^{32}P] ATP. Thus the specific activity of myosin is given in mol P_i liberated per mol of myosin per s. Comparing this method with the traditional Fiske-SubbaRow method, a difference of ±5% was found.

The values of K_m and V were determined according to Lineweaver-Burk, Eadie, and Hofstee plots simultaneously.

Each plot was obtained from data for six substrate concentrations, all the values of V and K_m being averages of data from at least three runs with different myosin preparations.

Results

As it is known [1—5], in absence of divalent cations, the mechanism of ATP splitting by myosin can be described by a simple two-step Michaelis mechanism:

$$M + ATP \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} M \cdot ATP \xrightarrow{k_2} M + ADP + P_i \qquad (1)$$

In case if $k_{-1} \ll k_2$, the relation between k_1 and k_2 can be expressed by the equation $K_m = k_2/k_1$ where K_m is the Michaelis constant, and $k_2 = V/E_t$, and if

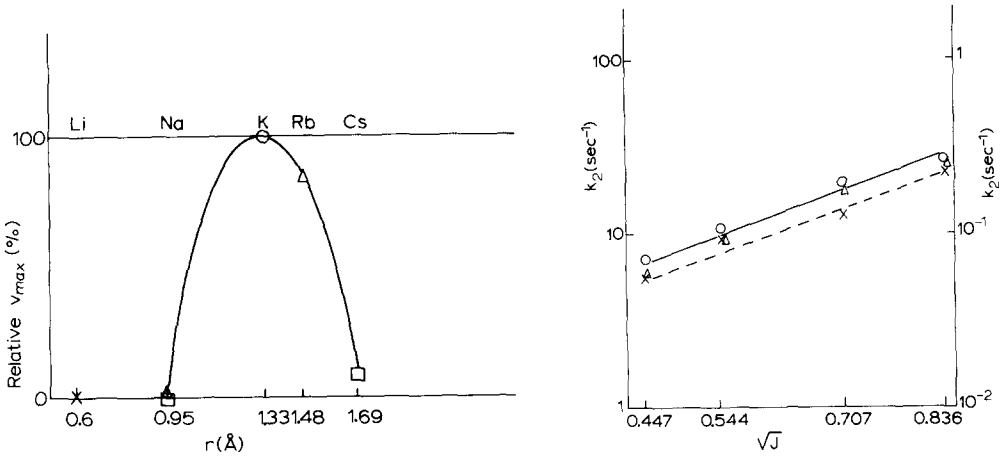

Fig. 1. Variation of relative V (taking 1.74 μM P_i/mg myosin per min, obtained in presence of 0.3 M KCl as 100%) with ionic radius in alkali cation-activated myosin-ATPase. The ATPase activity was measured in the presence of 40 mM Tris buffer (pH 8) and 9 mM EDTA. For details of the ATPase measurement, evaluation of V see Materials and Methods.

Fig. 2. Change of log k_2 in the function of square root of concentration of monovalent cations. The left hand ordinate refers to ○, KCl and △, RbCl; the right hand one to X, LiCl. The lines were calculated with regression analysis. The square scattering of the regression estimation ($s^2_{y.x}$) and the number of independent measurements (n) forming the basis of each line are the following: KCl: $s^2_{y.x} = 0.0301$, $n = 216$; RbCl: $s^2_{y.x} = 0.00195$, $n = 216$; LiCl; $s^2_{y.x} = 0.0051$, $n = 216$.

V is calculated per mol enzyme, numerically $k_2 = V$. In this case $k_1 = V/K_m \cdot E_t$ as it was observed on the K^+-activated myosin-ATPase also by Lymn and Taylor [5]. If $k_{-1} \gg k_2$ as with Na^+-activated ATPase then $K_m = K_s$.

The K_m and V values of myosin-ATPase activated by different alkali cations, were determined at 0.3 ionic strength. Plotting the V values versus ionic radius of alkali cations and taking the V obtained in presence of K^+ as 100%, the curve shown in Fig. 1 was obtained. The dependence of V on ionic radius in case of divalent cations is similar to the one found with the alkali cations [24], only Na^+ behaves in an aberrant way. While in the presence of Li^+ relatively well measurable values ($K_m = 5.82 \cdot 10^{-6}$ M and $V = 9.5 \cdot 10^{-2}$ M P_i/mol myosin per s) can be obtained at a smaller ionic radius, in the presence of Na^+, a K_m value smaller than 10^{-6} M must be assumed because of its immeasurability in our conditions. It was shown by Seidel [4], by Kelemen and Mühlrád [3] and by Mandelkow and Mandelkow [6] that in the presence of Na^+ myosin · ATP complex is formed but no hydrolysis takes place because the value of k_{-1} is relatively very high (0.2—0.35 s^{-1}).

The dependence of the kinetic constant of the reaction on the salt concentration was investigated. The logarithms of k_2 (constants of ES complex decomposition) in the function of the square root of different concentration of LiCl, RbCl, KCl are shown in Fig. 2. In spite of the fact that in the presence of Rb^+ and K^+ the values of k_2 are two orders of magnitude higher than in that of Li^+, the curves of log k_2 vs $\sqrt{I}$ are straight lines with similar slopes in all three cases. This means that the change of the kinetic constant of the reaction depends only on the ionic strength and is apparently independent of the quality of the investigated cations. The effect of different cations on the decomposition of the complex is analogous to a primary salt effect.

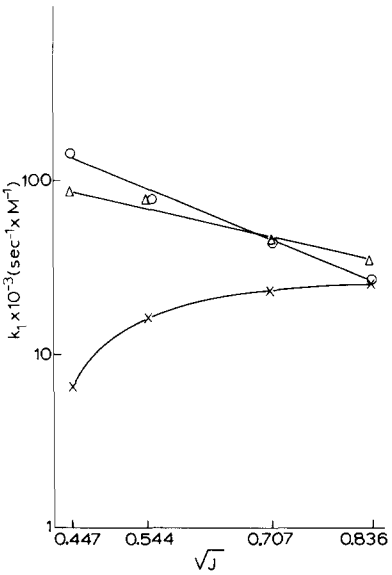

Fig. 3. Change of log k_1 in the function of square root of concentration of ○, KCl; △, RbCl; and X, LiCl. For evaluation of k_1 see text and Fig. 2. KCl: $s^2_{y.x} = 0.0005$, $n = 216$; RbCl: $s^2_{y.x} = 0.0046$, $n = 216$; LiCl: $n = 216$.

A similar plot for the constant of ES complex formation, k_1 gives three different lines as shown in Fig. 3. In the presence of both K^+ and Rb^+ the curves are straight lines with different slopes. In the case of Li^+ on the other hand, we obtained quite a different type of curve.

This indicates that the change of k_1 with the ionic strength depends on the nature of cations too.

A comparison of changes of k_2 and k_1 with the ionic strength suggests that the formation of ES complex depends on the quality of cations in a much more complicated way than its decay.

The dependence of rate constants on temperature in the presence of different monovalent cations was also investigated.

This dependence is described by the Arrhenius law:

$$k = A \cdot e^{\Delta H^{\ddagger}/RT} \tag{2}$$

where A = preexponential factor, $\Delta H^{\ddagger}$ = activation enthalpy.

In Fig. 4, logarithm of k_2 measured in the presence of 0.3 M Li^+, Cs^+ and K^+ is plotted against the reciprocal of absolute temperature. The value of activation enthalpy was calculated from the slope of the straight lines obtained (Fig. 4). From Eqn 2 the preexponential factor can be expressed by:

$$\log A = \log k_2 + \frac{\Delta H^{\ddagger}}{2.303R} \cdot \frac{1}{T} \tag{3}$$

Knowing the preexponential factor, the activation entropy can be calculated on the basis of the theory of absolute reaction rates:

$$k_2 = \kappa \frac{kT}{h} e^{\Delta S^{\ddagger}/R} \cdot e^{-\Delta H^{\ddagger}/RT} \tag{4}$$

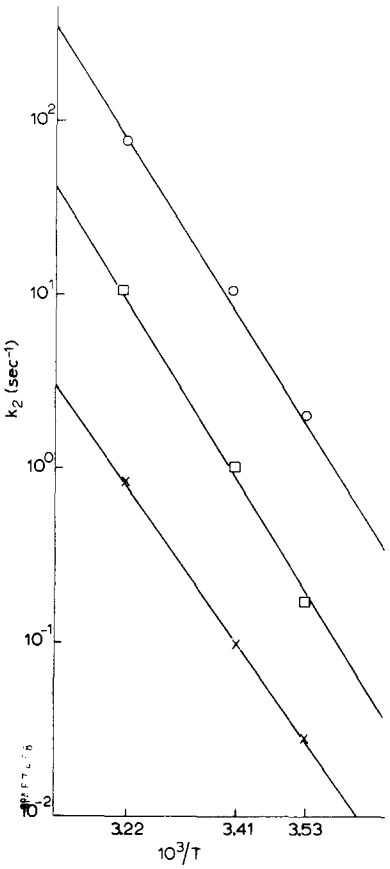

Fig. 4. Dependence of log k_2 on reciprocal of absolute temperature in case of myosin-ATPase activated by ×, 0.3 M LiCl, □, 0.3 CsCl and ○, 0.3 M KCl. For details of the measurements and evaluation see text and Fig. 2. LiCl: $s^2_{\bar{y}x} = 0.184$, $n = 162$; CsCl: $s^2_{\bar{y}x} = 0.211$, $n = 162$; KCl: $s^2_{\bar{y}x} = 0.109$, $n = 162$.

$$\kappa \simeq 1, \frac{k}{h} = 2.084 \cdot 10^{10} \text{ deg}^{-1} \cdot \text{s}^{-1}$$

From the Eqns 3 and 4:

$$\Delta S^{\ddagger} = 2.303 \cdot R \log \frac{A}{2.08 \cdot 10^{10} \cdot T} \tag{5}$$

The values of $\Delta H_2^{\ddagger}$ and $\Delta S_2^{\ddagger}$ calculated this way are shown in Table I.

It can be seen from Table I that the activation energy of the decomposition of ES complex is similar, i.e. apparently independent of the material quality of the metal; at the same time, the preexponential factor and $\Delta S_2^{\ddagger}$ differ in the presence of Li^+, Cs^+ and K^+.

Since the activation energies are similar and the value of rate constant k_2 is greater in the presence of K^+ than in that of Li^+ and Cs^+, the difference in rate must be due to the difference in the values of the preexponential factor and $\Delta S_2^{\ddagger}$.

TABLE I

DATA DERIVED FROM DEPENDENCE OF k_2 CONSTANT ON TEMPERATURE

For evaluation see text.

Ion	$\Delta H_2^{\ddagger}$ kcal · mol^{-1}	$\Delta S_2^{\ddagger}$ cal · mol^{-1} · degree^{-1}
Li^+	21.8	11.2
Cs^+	23.9	23.0
K^+	24.0	27.5

If the value of the rate constant is higher at the same activation energy, it means that the decomposition of ES is faster (because $\Delta S_2^{\ddagger}$ is greater) suggesting that in the presence of K^+ the ES complex has a looser structure than in that of Li^+, and Cs^+.

Fig. 5 is an analogous Arrhenius plot for k_1, the constant of formation of ES complex.

It can be seen that the constant of the formation of ES complex (k_1) is greater in the presence of potassium than lithium and cesium. The rate of complex formation is determined by both the activation energy and entropy, as in any chemical reaction (Table II). Therefore, $\Delta S_1^{\ddagger}$ in itself does not give any information about the rate of ES complex formation as at its decay, where the $\Delta H_2^{\ddagger}$ values are all cations practically identical. It is interesting that the value of $\Delta S_1^{\ddagger}$ in the presence of Cs^+ is negative.

Discussion

In addition to myosin there are several enzymes (e.g. pyruvate phospho-

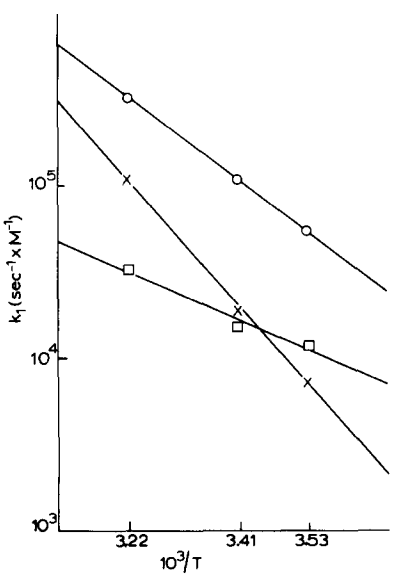

Fig. 5. Dependence of log k_1 on reciprocal of absolute temperature in case of myosin-ATPase activated by X, 0.3 M LiCl; □, 0.3 M CsCl and ○, 0.3 KCl. For details of the measurements and evaluation see text and Fig. 2. LiCl: $s^2_{y.x} = 0.300$, $n = 162$; CsCl: $s^2_{y.x} = 0.125$, $n = 162$; KCl: $s^2_{y.x} = 0.221$, $n = 162$.

TABLE II

DATA DERIVED FROM DEPENDENCE OF k_1 CONSTANT ON TEMPERATURE

For evaluation see text.

Ion	$\Delta H_1^{\ddagger}$ kcal · mol^{-1}	$\Delta S_1^{\ddagger}$ cal · mol^{-1} · degree^{-1}
Li^+	17.1	19.51
Cs^+	6.7	−16.4
K^+	11.6	4.2

kinase) where K^+, NH_4^+ and Rb^+ are activators, while Na^+ is inhibitory. A possible explanation of this observation was suggested by Melchior [25]. The structure of the Me · ATP complex consisting of one molecule of ATP and one alkali ion would vary with the size of the metal ion. Small metal ions (with small ionic radius as Na^+) would be completely surrounded by the polyphosphate chain of ATP, intermediate sized metal ions would rest in a "shallow cup" formed by the polyphosphate chain, and complexes with large metal ions of greater ionic radius than K^+ and Rb^+ would have an open configuration. It was pointed out that such differences in the shape of the ATP molecule could profoundly influence enzyme-catalyzed reactions. It is to be noted, however, that, besides this, the hydration of the alkali cations must also be considered simultaneously (since the mechanism of the influence of Li^+ on the reaction is different from that of Na^+ as a result of their difference in hydration) and the effect of monovalent cations on the conformation of myosin [26].

We suppose that the suitable conformation of Me · ATP for the active site of myosin is a "shallow cup" form (at 1.33 Å ionic radius of K^+ and 1.48 Å ionic radius of Rb^+) because the rate of reaction is the greatest in this case.

Lowenstein [27] studied the role of alkali cations in the transphosphorylation reaction. The rate of the reaction was greatest in the presence of K^+ and smallest in the presence of Na^+. Although we cannot make a comparison between the two reaction mechanisms, their starting step i.e. the interaction between the alkali cation and ATP is the same.

The reaction of Me · ATP with myosin can produce an entropy increase. From the dependence of the reaction rate of Mg^{2+}-ATPase, on temperature, Yoshimura et al. [28] have determined both the activation enthalpy and entropy. The value of $\Delta H_2^{\ddagger} = 20.0$ kcal · mol^{-1}, $\Delta S_2^{\ddagger} = 41.0$ cal · mol^{-1} · degree^{-1} (taking the molecular weight of myosin $4 \cdot 10^5$ and measuring the ATPase activity of myosin in the presence of 2 M KCl (sic), 10 mM $MgCl_2$ at pH 8.3). At a lower pH (7.0) the value obtained by Ouellet et al. [1] $\Delta H_2^{\ddagger} = 12.4$ kcal · mol^{-1}, $\Delta S_2^{\ddagger} = -8$ cal · mol^{-1} · degree^{-1} taking molecular weight of myosin $2 \cdot 10^7$. But as it is known, the presence of Mg^{2+} inhibits the myosin-ATPase activity and changes its mechanism so that the proper value of the alkali cation-activated ATPase can be measured in the presence of EDTA. That is why it cannot by compared with our result.

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