BBA 45686

EFFECTS OF ALKALI METAL IONS ON THE Mg^{2+}-ACTIVATED ATPase ACTIVITY OF RECONSTITUTED ACTOMYOSIN

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(Received March 1st, 1968)

SUMMARY

The Mg^{2+}-activated ATPase activity of reconstituted actomyosin at high ionic strengths (about 0.10 M KCl) is stimulated when Na^+ is substituted for K^+. This phenomenon does not appear to reflect the non-specific structure-disrupting effects seen with higher concentrations of these alkali metal ions because inhibition of actomyosin ATPase followed the order $Cs^+ > Li^+ > Rb^+ = K^+ > Na^+$. Alkali metal sensitivity was similar in the case of actomyosins reconstituted from tropomyosin-free actin preparations and from actin preparations that included the tropomyosin-containing protein complex that sensitizes actomyosin to Ca^{2+}. Modification of the myosin, effected by blocking some of the more reactive SH groups, abolished the sensitivity of the subsequently reconstituted actomyosin to substitution of K^+ by Na^+. Sensitivity to replacement of K^+ by Na^+ was seen only during the clearing phase at higher ionic strengths, and no significant differences between the effects of these alkali metal ions were seen at lower ionic strengths or after superprecipitation. These different actions of the alkali metal ions thus appear to be upon the association of actin and myosin in the presence of Mg^{2+}.

INTRODUCTION

The greater inhibitory effect of Na^+ than K^+ on the ATPase activity of myosin (EC 3.6.1.3) (ref. 1) appears to reflect differences between the non-specific structure-disrupting actions of these alkali metal cations. The cations of the HOFMEISTER series demonstrate a close parallelism between their abilities to denature a wide variety of macromolecules[2–4] and their inhibitory effects on myosin ATPase activity[5]. It was surprising, therefore, to find that at moderately high ionic strengths, the Mg^{2+}-activated ATPase activities of Ca^{2+}-sensitive reconstituted cardiac and skeletal actomyosins were higher in the presence of Na^+ than in equimolar amounts of K^+ (ref. 6). Further investigation of this phenomenon has demonstrated that these actions of the alkali metal ions on the Mg^{2+}-activated actomyosin ATPase activity

Abbreviations: Ac: tropomyosin-free actin; Aw: actin containing the tropomyosin complex that sensitizes reconstituted actomyosin to Ca^{2+}.

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do not reflect their non-specific structure-disrupting actions. Instead, the higher ATPase activity in the presence of Na^+ than of K^+, which may be of significance to the mechanisms which control the contractile force of the intact heart[7], appears to reflect a greater ability of K^+ than Na^+ to dissociate actin and native myosin.

EXPERIMENTAL PROCEDURE

The contractile proteins were prepared from rabbit back and leg muscles by methods described previously[6]. A few studies were carried out with the proteins extracted from canine ventricular myocardium. Myosin was prepared by a modification of Szent-Györgyi's method[8]. In view of the previous finding that the differential alkali metal sensitivity was lost when the myosin was allowed to age[6], dithiothreitol (0.1 mg/ml) was added to the water used to precipitate the myosin from solutions of high ionic strength. This reagent was removed during the final dialysis of the myosin. Tropomyosin-free actin (Ac) was prepared by extraction of acetone-dried muscle powder at 0°, followed by polymerization in 0.6 mM $MgCl_2$ (ref. 9). Actin containing the tropomyosin complex that sensitizes reconstituted actomyosin to Ca^{2+} (Aw) was extracted from the acetone-dried powder at 35° and polymerized in 0.1 mM $MgCl_2$ and 0.05 M KCl (ref. 9). Actomyosins were reconstituted in the manner described previously[10]. The weight ratios of myosin to actin were 4:1 when Ac was used and 3:1 when Aw was used. *N*-Ethylmaleimide-treated myosin was prepared by allowing 0.08 μmole of *N*-ethylmaleimide to react with 2 mg of myosin for 2 h at 25°.

ATPase activities were determined by measuring the liberation of P_i (ref. 11), and expressed in terms of the myosin content of the various systems. In some experiments ATPase activities were determined in the pH-stat (Radiometer Inc.) by the methods previously described[10]. All reaction mixtures contained some KCl, which was introduced along with the myosin. Because of the lability of the differential alkali metal sensitivity of actomyosin (see below), we did not employ additional preparative steps to remove this salt.

All chemicals used were reagent grade. NaCl and KCl were obtained from the Mallinckrodt Chemical Works. In some experiments the results were confirmed with NaCl and KCl obtained from Fisher Chemical Company. Disodium ATP, obtained from the Sigma Chemical Company, was generally used, but some studies were carried out with Tris–ATP prepared by passage of the disodium ATP through Dowex 50 columns, followed by neutralization with Tris. Distilled water was passed through two deionizing columns prior to use.

RESULTS

Studies of the Mg^{2+}-activated actomyosin ATPase confirmed the previous finding[6] that at alkali metal concentrations approximating 0.1 M, enzymatic activity was higher in the presence of Na^+ than in equimolar concentrations of K^+ (Table I). These differences, while small, were significant. On the other hand, the ATPase activities at this ionic strength were low, only 3–4 times those measured in the case of the myosin alone[6,9]. The latter ranged between 0.009 and 0.012 μmole P_i/min per mg.

Examination of enzymatic activity at lower ionic strengths, where activation of myosin ATPase by actin was more complete, failed to demonstrate differences

TABLE I

Na^+–K^+ SENSITIVITY OF ACTOMYOSIN ATPASE ACTIVITY AT HIGH AND LOW IONIC STRENGTH

Reactions were carried out at 25° with, per ml, 0.50 mg myosin + 0.125 mg tropomyosin-free actin in 0.1 mM $CaCl_2$, 1.0 mM MgATP and 20 mM Tris–acetate.

Salt	*ATPase activity* (*μmole P_i/min per mg*)	
0.050 M KCl	0.262 ± 0.010*	**
0.015 M KCl + 0.035 M NaCl	0.263 ± 0.012*	
0.100 M KCl	0.036 ± 0.004*	***
0.015 M KCl + 0.085 M NaCl	0.049 ± 0.009*	

* Standard deviation.
** $t = 0.185$, $n = 17$, $P > 0.80$.
*** $t = 3.85$, $n = 14$, $P < 0.01$.

between solutions containing Na^+ and K^+ (Table I). Although the total amount of Na^+ or K^+ added to give a final alkali metal ion concentration of 0.05 M was often less than was added in the experiments in which the final alkali metal concentration was 0.10 M, other experiments at higher ionic strengths have shown stimulation of ATPase activity when as little as 0.01 M K^+ is replaced by Na^+ (ref. 6). The failure to demonstrate a stimulatory action of Na^+ at the lower ionic strength thus represents a significant deviation from the effects of replacement of K^+ by Na^+ at the higher ionic strength.

The stimulatory effect of replacement of Na^+ by K^+ on the Mg^{2+}-activated actomyosin ATPase activity at high ionic strength was most prominent during the initial slow phase of the reaction (Fig. 1). This low rate of ATP hydrolysis occurred during the clearing phase of actomyosin which is seen at higher ionic strengths[10,12], whereas the more rapid liberation of P_i at the end of the reaction took place during and after superprecipitation. Analysis of the ATPase activity after superprecipitation at higher ionic strengths failed to show stimulation when K^+ was replaced by Na^+,

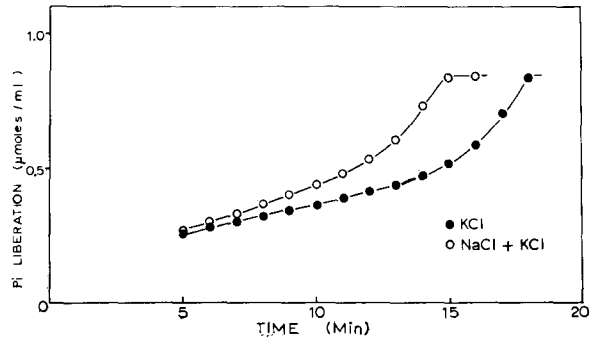

Fig. 1. Time course of P_i liberation by actomyosin, made from 0.50 mg/ml myosin and 0.167 mg/ml Aw, in 0.13 M KCl (●) and 0.12 M NaCl + 0.01 M KCl (○). Reactions were carried out in 1.0 mM MgATP at pH 6.8. These curves, which were calculated from pH-stat tracings, are representative of the results obtained in four duplicate pairs of reactions.

but these analyses were less precise than the determination of initial ATPase activity so that a slight stimulation by Na^+ cannot be excluded.

Examination of the actions of other alkali metal cations provides further evidence for the lack of parallelism between their non-specific structure-disrupting effects and their actions upon actomyosin ATPase activity. The ATPase activity in K^+ generally was higher than in Li^+ and Cs^+, and similar to that in Rb^+ (Table II).

TABLE II

EFFECTS OF ALKALI METAL CATIONS ON THE Mg^{2+}-ACTIVATED ATPASE ACTIVITY OF RECONSTITUTED ACTOMYOSIN

Each salt was added to a final concentration of 0.06 M in a solution containing, per ml, 0.625 mg actomyosin (0.50 mg myosin + 0.125 mg tropomyosin-free actin), 0.03 M KCl, 1.0 mM MgATP, 0.1 mM $CaCl_2$ and 10 mM histidine at pH 6.8.

Salt	*ATPase activity* (*μmole P_i/min per mg myosin*)
LiCl	0.090
NaCl	0.106
KCl	0.098
RbCl	0.096
CsCl	0.092

The possibility that the tropomyosin-containing protein complex which sensitizes actomyosin to Ca^{2+} played a role in these effects of the alkali metal ions could be examined by comparing the ATPase activities of AcM and AwM. The relative ATPase activites in the presence of LiCl, NaCl and KCl were similar for both kinds of actomyosin (Table III).

These differences between the effects of alkali metal ions on actomyosin ATPase activity were not seen when aged myosin preparations were used[6]. Virtually no differences could be elicited 2–3 days after preparation of the myosins in the absence of dithiothreitol. Protection of myosin sulfhydryl groups by addition of dithiothreitol during the purification (see EXPERIMENTAL PROCEDURE) greatly retarded the loss of this

TABLE III

EFFECTS OF LiCl, NaCl AND KCl ON THE Mg^{2+}-ACTIVATED ATPASE ACTIVITIES OF RECONSTITUTED ACTOMYOSINS MADE WITH TROPOMYOSIN-FREE ACTIN (AcM) AND ACTIN CONTAINING THE Ca^{2+}-SENSITIZING TROPOMYOSIN COMPLEX (AwM)

All reactions were carried out at 25° in 1 mM MgATP, 0.1 mM $CaCl_2$, 0.045 M KCl and 10 mM histidine at pH 6.8. The total concentration of alkali metal salts was 0.100 M. Myosin was present at a concentration of 0.50 mg/ml. Ac was added to give a concentration of 0.125 mg/ml, and Aw to give a concentration of 0.167 mg/ml.

Salt	*ATPase activity* (*μmoleP_i/min per mg myosin*)	
	AcM	*AwM*
0.055 M LiCl	0.0412	0.0492
0.055 M NaCl	0.0520	0.0588
0.055 M KCl	0.0441	0.0510

differential alkali metal sensitivity. The importance of myosin sulfhydryl groups for the exhibition of this phenomenon was clearly demonstrated by comparing the effects of Li^+, Na^+, and K^+ on native and *N*-ethylmaleimide-treated myosin. Alkylation of some of the more accessible myosin sulfhydryl groups completely abolished the stimulatory effect of Na^+ (Fig. 2). Similarly, this alkali metal sensitivity was lost

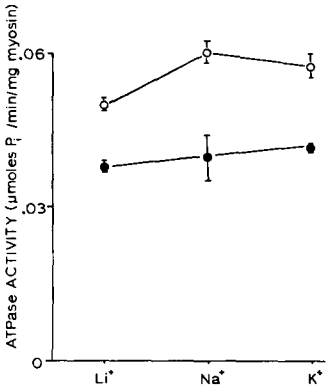

Fig. 2. Alkali metal sensitivities of reconstituted actomyosins made with native actomyosin, reconstituted from 0.500 mg/ml myosin and 0.125 mg/ml tropomyosin-free actin (○) and actomyosin made from the same concentrations of *N*-ethylmaleimide-treated myosin and actin (●). Reactions were carried out in 1.0 mM MgATP, 0.1 mM $CaCl_2$ and 10 mM histidine at pH 6.8. KCl was present at a concentration of 0.06 M in each reaction mixture. LiCl, NaCl or additional KCl was added in a final concentration of 0.03 M. *N*-Ethylmaleimide-treated myosin was prepared by allowing 0.08 μmole *N*-ethylmaleimide to react with 2 mg myosin for 2 h at 25°. The vertical bars represent one S.D. of the mean of 3 determinations.

when myosin was allowed to react overnight with 2 moles of *p*-mercuribenzoate per 500 000 g of myosin. The progressive reduction in the ATPase activity of actomyosin made with *N*-ethylmaleimide-treated myosin in solutions containing K^+, Na^+ or Li^+ follows the order expected on the basis of the structure-disrupting effects noted previously in the case of myosin alone[5], although the differences observed with the relatively low salt concentrations employed in the present experiments are quite small.

DISCUSSION

Effects of NaCl and KCl on the Mg^{2+}-activated ATPase activity of natural actomyosin (myosin B) similar to those demonstrated in the present study have been reported by BOWEN AND GERSHFELD[13]. These investigators found that, in 1 mM $MgCl_2$, 0.10 M KCl inhibited actomyosin ATPase to a level less than half that seen in the absence of alkali metal salts, whereas a slightly higher concentration of NaCl caused enhancement of actomyosin ATPase activity relative to a similar control. TONOMURA AND YOSHIMURA[14], on the other hand, found that the substrate inhibition of the myosin B ATPase activity was greater in the presence of Na^+ than K^+, but they were unable to demonstrate changes in ATPase activity when KCl was replaced by LiCl, NH_4Cl or choline chloride.

The possibility that the effects described in the present study, especially the enhancement of actomyosin ATPase activity when Na^+ and K^+ are present together, reflect the presence of a microsomal contaminant must be seriously considered because

a ($Na^+ + K^+$)-activated enzyme system can be isolated from skeletal muscle[15,16]. However, this possibility appears to be excluded because the actins were prepared from an acetone-dried muscle powder and the myosin preparations were subjected to prolonged high-speed ultracentrifugation prior to use in these experiments. Furthermore, the stimulatory effect of Na^+ on ATPase activity is accompanied by acceleration of superprecipitation[6]. The present phenomena thus represent effects of the alkali metal ions on the contractile proteins themselves.

Experiments with the reconstituted actomyosin made with tropomyosin-free actin (AcM) demonstrated that the tropomyosin-containing protein complex which sensitizes actomyosin to Ca^{2+} plays no significant role in these effects of alkali metal ions (Table III). Because the ATPase activity of this reconstituted actomyosin is unaffected by reduction in the activity of Ca^{2+}, it appears unlikely that these actions of the alkali metal ions are due to competition for a labile actomyosin-bound Ca^{2+}.

High concentrations of the alkali metal ions are well-known to disrupt the organized structures of a variety of macromolecules[3–5]. Although the mechanism by which this is brought about remains uncertain[3,4,17–19], there is general agreement that the effectiveness of these ions in disrupting macromolecular structure follows the order $Li^+ > Na^+ > K^+ > Cs^+$. In the case of several protein enzymes, including myosin, disruption of structure has been found to be associated with reduced enzymatic activity, and this loss of enzymatic activity can provide an index to structural disruption more sensitive to that detectable by physical methods[5]. Reduction of the Mg^{2+}-activated actomyosin ATPase activity during the initial clearing phase generally followed the order of $Cs^+ > Li^+ > Rb^+ = K^+ > Na^+$, whereas the order followed for the inhibition of myosin ATPase activity has been found to be $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$ (ref. 5). That this discrepancy reflects the operation of two distinct mechanisms is further demonstrated by the finding that modification of the myosin molecule by sulfhydryl reagents increases the sensitivity of myosin alone to the structure-disrupting effects of the alkali metal ions[5], whereas the present effects on actomyosin are absent when *N*-ethylmaleimide-modified myosin is used instead of native myosin (Table III). The present differences are thus not likely to be a manifestation of different non-specific structure-disrupting activities of the alkali metal cations.

Failure to demonstrate an effect of replacement of K^+ by Na^+ at low ionic strengths, where activation of myosin ATPase activity by actin is maximal (Table I), indicates that these alkali metal ions have their effects on the reactions leading to association of actin and myosin, rather than upon the actomyosin complex itself. This view is supported by the absence of a significant effect of alkali metal ion replacement on the ATPase activity of actomyosin after superprecipitation at higher ionic strengths (Fig. 1), and the inhibition of the Ca^{2+}-activated actomyosin ATPase activity by replacement of higher concentrations of K^+ with Na^+ (ref. 20). The present phenomenon thus appears to represent different effects of the various alkali metal ions during the evolution of superprecipitation, possibly during the period of increased viscosity which accompanies activation of myosin ATPase activity prior to superprecipitation[21]. It is of interest that the inhibitory effect of tropomyosin upon the Mg^{2+}-activated actomyosin ATPase activity, like this differential alkali metal sensitivity, occurs during a phase in the course of superprecipitation which follows activation of ATPase activity but precedes the increased turbidity[10]. Furthermore, like

the differential alkali metal sensitivity, this effect of tropomyosin is abolished by aging of the myosin preparations or prior treatment of myosin with *p*-mercuribenzoate. This early phase in the interaction of actin and myosin thus appears to show a unique sensitivity to a variety of agents, some of which may be of significance in the physiological control of contraction in both cardiac[7] and skeletal muscle[22].

ACKNOWLEDGEMENTS

I would like to acknowledge the technical assistance of Miss A. SANDERS and Miss B. R. COHEN.

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