DEPENDENCE OF ACTOMYOSIN ATPase ACTIVITY ON IONIC STRENGTH AND ITS MODIFICATION BY THIOL GROUP SUBSTITUTION

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1. Introduction

ATP plays a dual role in muscular contraction: on the one hand, it is the substrate of an enzymatic interaction between actin and myosin providing the energy for contraction; on the other hand, it is able to dissociate actomyosin into its constituents actin and myosin, thus performing relaxation. Which of the two influences prevails depends most sensitively on the ionic strength. If it remains constant as in the sarcoplasm other factors must decide whether contraction or relaxation is induced. This communication presents evidence that modification of SH-groups of myosin activates actomyosin ATPase by reducing its sensitivity against ionic strength and, in addition, diminishes, under special conditions, the influence of the tropomyosin–troponin system which confers Ca-sensitivity on the contractile system [1].

2. Methods and technical remarks

Three kinds of actomyosin are used: natural actomyosin (extracted as the complete complex as described in [2]) and so-called artificial actomyosin reconstituted from myosin and purified actin [2]. Tropomyosin-containing, artificial actomyosin is a highly Ca-sensitive preparation which contains the tropomyosin–troponin system. It is reconstituted from myosin and an actin preparation which is obtained by extraction of acetone dried actin powder at room temperature and subsequent precipitation with 40%-saturated $(NH_4)_2SO_4$. ATPase assay: If not otherwise stated: 0.03 M KCl, 0.02 M Tris-maleate buffer, pH 7.2, 2 mM $MgCl_2$, 2 mM ATP, room temperature. The enzyme is always actomyosin (about 0.1 mg/ml). SH-group modification was performed by treating either actomyosin or myosin (before mixing with actin) with DTNB or H_2O_2 (details in the legends of figures and tables). Ca-sensitivity means that the actomyosin ATPase is inhibited by chelating the free Ca^{2+} with EGTA. For other technical details see [2].

Abbreviations:
DTNB: 5,5-dithiobis (-nitrobenzoic acid)
EGTA: Ethyleneglycol-2-(2-aminoethyl)-tetraacetic acid)
Pi : Inorganic phosphate

3. Results

The experiments of table 1 are representative for the following statements: If the complete actomyosin is treated with SH-group modifiers the ATPase is activated and the Ca-sensitivity of those actomyosins which contain the tropomyosin–troponin system is reduced. SH-group modification, however, often fails to induce ATPase activtaion if only the myosin is treated: In the experiment of table 1 a difference of ATPase activity between control and actomyosin made from modified myosin can only be seen when the ATPase is measured at a higher ionic strength. At higher ionic strength only the ATPase of the control is inhibited. Hence, the modification of SH-groups results in a protection against the depressive effect of ionic strength and can thus the more easily be seen the more depressive the conditions are. Presumably a similar mechanism is involved in the elevation of the Mg-inhibited actomyosin ATPase by PCMB* [3].

* p-Chloromercuribenzoate

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Table 1
ATPase activity of actomyosin after treating either the complete actomyosin or only the myosin with SH-group modifiers.

ATPase activity (100 = 0.2 μmoles Pi/min per mg myosin)							
Artificial myosin, modified with H_2O_2		Tropomyosin containing artificial actomyosin, modified with DTNB		Myosin, modified with DTNB before mixing with actin			
				ATPase measured in 0.03 M KCl		ATPase measured in 0.07 M KCl	
C	M	C	M	C	M	C	M
120	262	150; 27	222; 107	115	135	25	112

C: ATPase activity (in %) of the control; M: ATPase activity after modification (if there are two values indicated, the second one refers to a EGTA containing ATPase assay). Conditions of modification: DTNB: 60 μM DTNB, 0.02 M tris-HCl buffer, pH 8.0, 0.6 M KCl, 20 min, 22°; H_2O_2: 0.02% H_2O_2, 0.01 M $NaHCO_3$, 0.6 M KCl, 20 min, 22°.

Fig. 1 shows how treatment with SH-group modifiers reduces the sensitivity of actomyosin ATPase against ionic strength. One can see that modification of actomyosin results in a stronger effect than modification of myosin alone.

Fig. 2 demonstrates another, more fundamental difference between both ways of modification: Even in those cases in which the actomyosin ATPase appears activated when measured at 0.03 M KCl, the Ca-sensitivity is not impaired when only the myosin was treated.

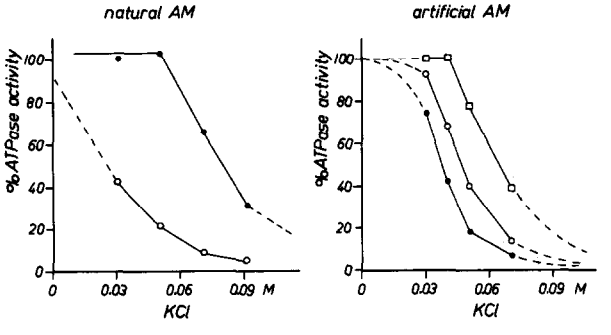

Fig. 1. Dependence of actomyosin ATPase activity on ionic strength. Abscissa: concentration of KCl in the ATPase assay, ordinate: ATPase activity in %. 100%: 0.45 μmoles Pi/min per mg protein. (Interrupted lines: supposed course of the curves.) Left: natural actomyosin (extracted from the muscle as the complete complex). o——o control; •——• treated with DTNB. Right: artificial actomyosin (reconstituted from the purified components actin and myosin). •——• control; o——o myosin treated with DTNB before mixing with actin; □——□ complete actomyosin treated with DTNB.

These different results produced by modifying actomyosin or myosin alone are contained in the experiments of table 2 too. When modification of natural actomyosin was performed under conditions promoting dissociation of actomyosin into its components as in solutions containing polyphosphates such as ATP or pyrophosphate plus Mg, the effect of SH-group modification (activation and reduction of Ca-sensitivity) was reduced indicating that there was no more influence of actin on the modification of myosin.

The presented results indicate that, at least so far as activation is concerned, the modified SH-groups belong to the myosin molecule. This is corroborated by the experiment of table 3 which shows that the

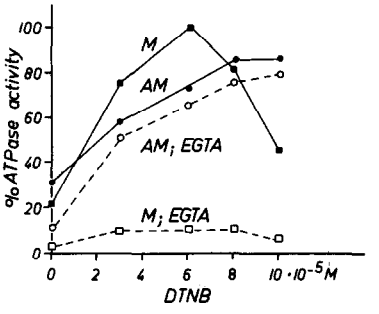

Fig. 2. Dependence of ATPase activity of artificial, tropomyosin containing actomyosin on concentration of DTNB in the modifying assay. M: myosin alone was treated before mixing with actin; AM: actin and myosin were first mixed and then treated together with DTNB. EGTA: ATPase measurements in the presence of 1 mM EGTA. 100%: 0.55 μmoles Pi/min per mg myosin.

Table 2
ATPase activity of natural actomyosin which was oxidized with H_2O_2 under actomyosin-dissociating conditions.

Treatment	Conditions of ATPase assay	ATPase activity 100 = 0.12 μmole Pi/min per mg protein
Control	−EGTA	100
	+EGTA	36
H_2O_2	−EGTA	269
	+EGTA	309
H_2O_2, PP	−EGTA	300
	+EGTA	264
H_2O_2, MgPP	−EGTA	135
	+EGTA	74
H_2O_2, MgATP	−EGTA	113
	+EGTA	53

PP: 1 mM Na-pyrophosphate pH 7.0; MgPP: 1 mM pyrophosphate plus 1 mM $MgCl_2$; MgATP: 1 mM ATP, 1 mM $MgCl_2$.

Table 3
Persistence of the modification effect of actomyosin in its myosin component.

	ATPase activity (100 = 0.2 μmole Pi/min per protein)			
	Control		Oxidized	
	−EGTA	+EGTA	−EGTA	+EGTA
Before centrifugation	100	34	321	250
After centrifugation				
Splitting assay: +actin	20	9	8	9
Splitting assay: −actin	73	8	195	93

Natural actomyosin was oxidized with H_2O_2 and then centrifuged at 150,000 *g* for 3 hr in 1 mM ATP, 1 mM $MgCl_2$, 0.6 M KCl, pH 7.0. The supernatant containing the separated myosin was mixed with tropomyosin-containing actin. All steps without adding H_2O_2 were equally performed as control.

activation effect and the reduced Ca-sensitivity is retained when the myosin of modified actomyosin is isolated and mixed with another actin.

The described results can only be obtained with actomyosin ATPase but not with ITPase.

4. Discussion

The above results demonstrate that treatment of actomyosin with reagents which can react with SH-groups influences the enzymatic behaviour of actomyosin in such a way that the dissociating and hence relaxing effect of ATP is suppressed. Myosin must therefore possess regulatory groups which are responsible for this effect. The state of these groups – or the conformation they determine – appears to change sometimes spontaneously during the course of preparation. This explains the somtimes considerable differences in the ATPase activity of different actomyosin preparations which have been also observed by Muir, Weber and Olson [4].

It is not yet clear if activation and reduction of Ca-sensitivity depend on modification of the same groups or if these events are only parallel effects. Both events are functionally connected insofar as they both affect the balanced state between relaxation and contraction promoting effects of ATP. In any case both phenomena are different in that Ca-sensitivity, as was already reported in [5], can only be reduced after treatment of the complete actomyosin and not after treatment of myosin alone, thus reflecting a different conformation of myosin in the myosin complex.

Acknowledgement

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