DEPENDENCE OF ACTOMYOSIN NTPase ACTIVITY ON IONIC STRENGTH AND ITS MODIFICATION BY α-ACTININ

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Received 1 July 1971

1. Introduction

In the foregoing communication [1] evidence has been presented which shows that the enzymatic properties of actomyosin are influenced by modification of SH-groups of myosin. The present communication draws attention to the fact that analogous results as those obtained by SH-group modification can be demonstrated by treating actomyosin with the muscle protein α-actinin. This protein was first described in [2–4]. It promotes gelation of actin and superprecipitation of actomyosin and was recently shown to activate actomyosin ATPase [5]. Its functional role is until now unclear although Ebashi and Ebashi [3] have already mentioned that it may counteract the dissociating influence of ATP on actomyosin. The common view is now that it is a constituent of the Z-line of the myofibril.

2. Material and methods

α-actinin (subsequently called actinin) is prepared essentially according to [6] (extraction from myofibrils at low ionic strength, pH 8.5, and subsequent precipitation with $(NH_4)_2SO_4$) and used without further purification. The fraction between 0 and 25% saturation was used. It contained still denatured actin; it had no influence on myosin alone. For other procedures see [1].

Abbreviations:
DTNB : 5,5-dithiobis-(-nitrobenzoic acid).
EGTA : Ethyleneglycol-2-(2-aminoethyl)-tetraacetic acid.
NTP : Nucleoside triphosphate.

3. Results

Fig. 1 shows that actinin protects actomyosin in quite the same way as does SH-group modification against the dissociating influence of ionic strength. (This substantiates the statement of [6] that the relative activating effect of actinin is more pronounced at higher ionic strength.)

It was recently demonstrated [7] that actinin activates only the ATPase but not the ITPase of actomyosin. The right part of fig. 1 compares the nucleotide specificity of both activating the actomyosin by SH-group modification and by actinin demonstrating that in either case only the ATPase is activated. As a result, the formerly similar activities of ATPase and ITPase become now different. The table corroborates the assumption that activation by actinin as well as activation by SH-group modification acts via the same mechanism. Activation can only be seen if actomyosin is in a "suboptimal" state. If it is already "elevated" as after treatment with actinin, DTNB has no further effect: the same activation level is approached by both treatments.

The discussed results show that actinin is able to suppress relaxation promoting influences on actomyosin. This is substantiated in fig. 2 in which the ATPase activity of artificial actomyosin is plotted against the concentration of total Mg. The pattern of the control reflects a rather "relaxed" state as can

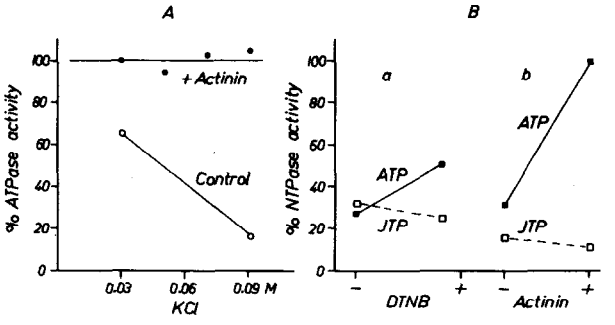

Fig. 1. Modification of NTPase activity of actomyosin by DTNB and α-actinin. A: protection of ATPase of artificial actomyosin (reconstituted from actin and myosin) against the depressive action of ionic strength by α-actinin. o——o control, •——• α-actinin present. B: comparison of ATPase and ITPase of artificial actomyosin in the presence and absence of modifier. a: modified with DTNB, b: α-actinin in the NTPase assay. 100% = 0.45 μmole Pi/min × mg myosin. NTPase assays: KCl: if not otherwise stated 30 mM; Tris-maleate buffer: 20 mM; pH 7.2; $MgCl_2$: 2 mM for ATPase measurements, 10 mM for ITPase measurements, ATP: 2 mM; actomyosin: ca. 0.1 mg/ml; α-actinin (if any): 0.3 mg/ml. (Actin and myosin were premixed before adding α-actinin.) DTNB treatment: 60 μM DTNB, 0.6 M KCl, 0.02 M Tris-HCl buffer, pH 8.0, 20 min, 22°.

Table 1
Activation of ATPase of artificial actomyosin by DTNB and/or α-actinin.

ATPase activity (100 = 0.6 μmole Pi/min × mg myosin)			
Control	Actinin	DTNB	Actinin DTNB
33	100	65	91

Both kinds of activation produce the same degree of activation. 100%: 0.6 μmole Pi/min × mg myosin.

often be seen in preparations of artificial actomyosin (the ATPase activity first declines with increasing Mg before reaching a rather low peak). In the presence of actinin the picture is that of a "normal" Mg-activation: Mg activates the ATPase until an equimolar ratio of Mg and ATP is reached.

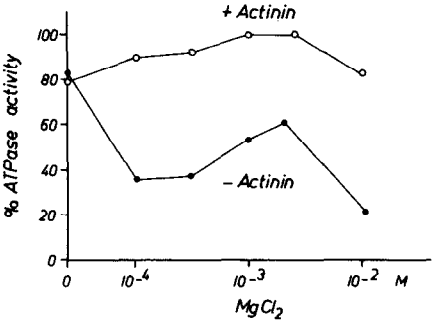

Fig. 2. Dependence of ATPase activity of artificial actomyosin on concentration of total Mg. ATP: 2 mM. 100%: 0.45 μmole Pi/min × mg myosin.

4. Discussion

The fact that actinin can stabilize actomyosin ATPase against the dissociating action of ATP seems to be not accidental but seems to reflect its physiological function. This explains why myofibrils which naturally contain actinin are less sensitive against ionic strength than is artificial actomyosin. Actinin is thus an antagonist of the tropomyosin-troponin system which in contrast makes actomyosin extremely sensitive for the dissociating action of ATP. The function of this system is not impaired by actinin.

This function of actinin further implies that it is not only part of the Z-line but must be localized in such a way that it can interfere with the actin-myosin interaction. The nucleotide specificity can be explained by the fact that ITP is not so effective in dissociating actin and myosin. Accordingly, the ITPase of actomyosin is not influenced by the variation of ionic strength between $\mu = 0.1$ and 0.2 [8, 9]. Hence, changes in the sensitivity against ionic strength will not be reflected by the ITPase.

It is a matter of further investigation whether actinin acts by modifying SH-groups as SH-reagents do or if it exerts a more direct influence.

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