## SHORT COMMUNICATION

BBA 43260

# Nucleotide and cation specificity of the α-actinin-induced increase in actomyosin nucleoside triphosphate phosphohydrolase activity*

α-Actinin was discovered in myofibrillar protein extracts because it accelerated the rate of turbidity development of reconstituted actomyosin suspensions[1-3]. EBASHI AND EBASHI[1] therefore suggested that α-actinin may have an important physiological role in strengthening or promoting the actin–myosin interaction. Shortly after the discovery of α-actinin, MARUYAMA[4] reported that α-actinin also increased the $Mg^{2+}$-modified ATPase activity of actomyosin suspensions, although large amounts of the α-actinin preparations then available were required to cause this increase. Since the $Mg^{2+}$-modified ATPase activity is specifically associated with the contractile process in muscle[5], the finding of MARUYAMA[4] supported the suggestion of EBASHI AND EBASHI[1] that α-actinin strengthens the actin–myosin interaction. In their extensive reexamination of the findings of EBASHI AND EBASHI[1], SERAYDARIAN *et al.*[6] and BRISKEY *et al.*[7,8] confirmed the earlier findings that α-actinin increased the rate of turbidity development and ATPase activity of reconstituted actomyosin suspensions, but also noted that α-actinin caused extensive cross-linking of actin polymers, an effect mentioned earlier by MARUYAMA AND EBASHI[2]. The cross-linking ability of α-actinin suggested that α-actinin may be located in the Z-disk, for only here does cross-linking of actin filament occur *in vivo*. Subsequently, GOLL *et al.*[9,10] and MASAKI *et al.*[11] reported two independent lines of evidence that α-actinin is indeed located in or next to the Z-line. As a result of these findings, it is presently uncertain whether the role of α-actinin in muscle is purely a structural one, serving to connect actin filaments across the Z-line, or whether the effects of α-actinin on the $Mg^{2+}$-modified ATPase activity and turbidity response presage a more direct role for α-actinin in the contractile process[10,12]. We have now found that the ability of α-actinin to increase the nucleoside triphosphate phosphohydrolase (NTPase) activity of reconstituted actomyosin suspensions depends on both the kind of nucleoside triphosphate present and the activating cation used. Our results support the conclusion that α-actinin has a direct effect on the actin–myosin interaction.

The procedures for preparation of α-actinin-free actin, crude α-actinin, and reconstituted actomyosin (2 parts myosin to 1 part actin by weight) from rabbit muscle have been described[13]. Purified α-actinin was made by double chromatography on DEAE-cellulose columns according to ROBSON *et al.*[12]. The NTPase and turbidity assays were conducted as described by ARAKAWA *et al.*[13]. The results of the NTPase assays (Table I) show that, when $Mg^{2+}$ is the principal modifier, α-actinin causes an increase in the NTPase activity of reconstituted actomyosin suspensions if ATP and

Abbreviation: NTPase, nucleoside triphosphate phosphohydrolase.

* Journal paper No. J-6468 of the Iowa Agriculture and Home Economics Experiment Station, Project 1796.

TABLE I

EFFECT OF DIFFERENT NUCLEOTIDES AND ACTIVATING CATIONS ON THE α-ACTININ-INDUCED INCREASE IN ACTOMYOSIN NTPase ACTIVITY

Conditions of assay: 0.20 mg reconstituted actomyosin per ml, 100 mM KCl, 20 mM Tris–acetate, pH 7.0, 1 mM substrate (ATP, CTP, GTP or ITP), 25.0°, purified α-actinin indicated as percent of actomyosin present. $Mg^{2+}$ indicates that 1 mM $MgCl_2$ *plus* 0.05 mM $CaCl_2$ were the activating cations; $Ca^{2+}$ indicates that 1 mM $CaCl_2$ was the activating cation. Results are expressed as μmoles $P_i$ per mg actomyosin per min.

| *Activating cation* | *Substrate* | *Percent α-actinin added:* | | | | |
|---|---|---|---|---|---|---|
| | | *0* | *5* | *10* | *20* | *30* |
| $Mg^{2+}$ | ATP | 0.151 | 0.186 | 0.192 | 0.281 | 0.286 |
| | CTP | 0.174 | 0.296 | 0.326 | 0.326 | 0.339 |
| | GTP | 0.106 | 0.109 | 0.101 | 0.107 | 0.104 |
| | ITP | 0.188 | 0.208 | 0.201 | 0.213 | 0.227 |
| $Ca^{2+}$ | ATP | 0.470 | 0.456 | 0.406 | 0.414 | 0.401 |
| | CTP | 0.127 | 0.124 | 0.127 | 0.127 | 0.129 |
| | GTP | 0.007 | 0.006 | 0.007 | 0.006 | 0.007 |
| | ITP | 0.018 | 0.020 | 0.018 | 0.018 | 0.018 |

CTP are the substrates but not if ITP and GTP are the substrates. If $Ca^{2+}$ is the principal modifier and no $Mg^{2+}$ is added, α-actinin will not cause any increase in the actomyosin NTPase activity, regardless of whether ATP, CTP, GTP or ITP is the substrate. It has been reported before[6,10] that α-actinin does not increase the $Ca^{2+}$-modified ATPase activity of reconstituted actomyosin suspensions. We have obtained results similar to those shown in Table II at KCl concentrations of 50 or 150 mM. Furthermore, turbidity tests (not shown here) show that α-actinin will accelerate the rate of turbidity development of actomyosin suspensions if ATP or CTP are the substrates and $Mg^{2+}$ is the principal modifier. However, α-actinin did not cause any consistent increase in rate of turbidity development in experiments where GTP or ITP were the substrate. When $Ca^{2+}$ is the principal modifier, it is impossible to elicit a clear turbidity response from reconstituted actomyosin suspensions, so turbidity tests cannot be done under conditions where $Ca^{2+}$ is the principal modifier.

Table II shows that α-actinin has no effect on either the $Mg^{2+}$-modified or the $Ca^{2+}$-modified ATPase activities of myosin. These results and those in Table I suggest

TABLE II

EFFECT OF α-ACTININ ON MYOSIN ATPase ACTIVITY

Conditions of assay: 0.20 mg myosin per ml, 1 mM ATP, 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, 20 mM Tris–acetate, pH 7.0, 25°; α-actinin indicated as percent of myosin present. Results are expressed as μmoles $P_i$ per mg myosin per min.

| *KCl concn. (mM)* | *Percent α-actinin added:* | |
|---|---|---|
| | *0* | *20* |
| 50 | 0.046 | 0.045 |
| 125 | 0.014 | 0.014 |

the hypothesis that the ability of α-actinin to increase the NTPase activity of myosin is specific for a system that contains actin, $Mg^{2+}$, and a nucleoside triphosphate with an amino group at the 6-position of the base.

Actin and $Mg^{2+}$ are obligatory requirements for contraction, and SZENT-GYORGYI AND PRIOR[14] have shown that both superprecipitation and exchange of the actin nucleoside are much slower when GTP or ITP are the energy sources than when ATP or CTP are the energy sources. Moreover, HASSELBACH[15] has shown that ATP or CTP are more effective in producing contraction of glycerol-extracted fibers than GTP or ITP, and WEBER[16] has found that ATP is more effective in producing either contraction or relaxation in myofibrillar suspensions than GTP or ITP. Consequently, our findings concerning the effects of nucleotide and activating cation on the α-actinin-induced increase of actomyosin NTPase activity suggest that α-actinin affects those processes that are coupled to *in vivo* contraction and relaxation and that α-actinin therefore has a direct role in the molecular transduction processes accompanying contraction.

Supported in part by Grant No. AM 12654 from the National Institutes of Health, Grant No. 60-G-23 from the Iowa Heart Association, and a grant from the National Livestock and Meat Board. We thank Dr. R. M. Robson for assistance with some of the myosin ATPase data and Jean Fatka for devoted and expert technical assistance.

*Muscle Biology Group,*
*Departments of Animal Science and Biochemistry and Biophysics,*
*Iowa State University, Ames, Iowa 50010 (U.S.A.)*

JOANNE TEMPLE
DARREL E. GOLL

1 S. EBASHI AND F. EBASHI, *J. Biochem.*, 58 (1965) 7.
2 K. MARUYAMA AND S. EBASHI, *J. Biochem.*, 58 (1965) 13.
3 S. EBASHI AND K. MARUYAMA, *J. Biochem.*, 58 (1965) 20.
4 K. MARUYAMA, *J. Biochem.*, 59 (1966) 422.
5 W. F. H. M. MOMMAERTS, *Physiol. Rev.*, 49 (1969) 427.
6 K. SERAYDARIAN, E. J. BRISKEY AND W. F. H. M. MOMMAERTS, *Biochim. Biophys. Acta*, 133 (1967) 399.
7 E. J. BRISKEY, K. SERAYDARIAN AND W. F. H. M. MOMMAERTS, *Biochim. Biophys. Acta*, 133 (1967) 412.
8 E. J. BRISKEY, K. SERAYDARIAN AND W. F. H. M. MOMMAERTS, *Biochim. Biophys. Acta*, 133 (1967) 424.
9 D. E. GOLL, W. F. H. M. MOMMAERTS AND K. SERAYDARIAN, *Federation Proc.*, 26 (1967) 499.
10 D. E. GOLL, W. F. H. M. MOMMAERTS, M. K. REEDY AND K. SERAYDARIAN, *Biochim. Biophys. Acta*, 175 (1969) 174.
11 T. MASAKI, M. ENDO AND S. EBASHI, *J. Biochem.*, 62 (1967) 630.
12 R. M. ROBSON, D. E. GOLL, N. ARAKAWA AND M. H. STROMER, *Biochim. Biophys. Acta*, 200 (1970) 296.
13 N. ARAKAWA, R. M. ROBSON AND D. E. GOLL, *Biochim. Biophys. Acta*, 200 (1970) 284.
14 A. G. SZENT-GYORGYI AND G. PRIOR, *J. Mol. Biol.*, 15 (1966) 515.
15 W. HASSELBACH, *Biochim. Biophys. Acta*, 20 (1956) 355.
16 A. WEBER, *J. Gen. Physiol.*, 53 (1969) 781.

Received January 19th, 1970