# Dissociation of actomyosin complex by monovalent fragments of polyclonal antibodies to myosin

K.D. Mazander and U. Gröschel-Stewart*

*Institut für Zoologie, Technische Hochschule, Schnittspahnstraße 10, 6100 Darmstadt, FRG*

Received 17 December 1984; revised version received 28 January 1985

The monovalent fragments of antibodies specific for skeletal muscle myosin inhibit myosin ATPase activity and dissociate the actomyosin complex, as shown by analytical ultracentrifugation and viscosity measurements.

*Anti-myosin* *Monovalent fragment* *Actomyosin* *Dissociation* *ATPase inhibition*

## 1. INTRODUCTION

Polyclonal antibodies to native skeletal muscle myosin [7,12] and actomyosin [6] were shown to be predominantly directed to the biologically active part of the molecule, inhibiting enzymatic activity as well as contraction in isolated myofibrils [11]. The inhibitory action of myosin antibodies on myosin itself or its proteolytic fragments HMM and $S_1$ could be interpreted as direct interference at the head region, since antibodies to the inactive rod part [12], or antibodies to SDS-denatured myosin heavy chain (although reactive with native myosin) were non-inhibitory. The action of myosin antibodies on myofibrils or the actomyosin complex was more difficult to explain. We therefore performed the following in vitro studies with both bivalent and monovalent anti-myosin antibodies to check whether they simply attach to the actomyosin complex or whether they dissociate it.

## 2. MATERIALS AND METHODS

Native actomyosin was extracted from chicken muscle homogenates and purified by several precipitation-solution steps [6]. Myosin was prepared as in [7]. Precipitating antibodies to myosin that were ATPase-inhibiting were raised in pathogen-free rabbits by i.v. injection of 48 mg over a period of 5 to 14 weeks. IgG fractions were prepared from the hyperimmune sera and preimmune controls according to Harboe and Ingild [8] and were rendered protease-free by a passage through DEAE-Affigel-blue (Bio-Rad). Fab-fragments were obtained by papain digestion of the above IgGs following the procedure of Garvey et al. [5]. $Ca^{2+}$-activated myosin ATPase was assayed at 25°C in a final volume of 400 $\mu$l in 20 mM Tris, 10 mM $CaCl_2$, 245 nM NaCl, 2 mM ATP (pH 7.5). The assay was terminated by the addition of 10% (w/v) SDS and inorganic phosphate was determined according to Fiske and Subbarow [4]. Inhibition of $Ca^{2+}$-activated ATPase by bi- and monovalent antibodies was measured as above after 24 h pre-incubation of actomyosin (40 $\mu$g) with increasing amounts of antibodies (20–400 $\mu$g) at 4°C in a nitrogen atmosphere. Viscosity measurements in the absence and presence of monovalent myosin-specific or control IgG were performed in a final volume of 800 $\mu$l in a Cannon-Manning Semi-Micro viscosimeter.

Sedimentation behaviour of actomyosin,

* To whom correspondence should be addressed

This paper is dedicated to Professor C.H. Li, University of California, San Francisco, CA, USA

*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/85/$3.30 

myosin, Fab-fragments and the incubates of the immunoreaction was studied in a Spinco mode E ultracentrifuge equipped with Schlieren optics. Protein concentrations were determined by nesslerization [2].

## 3. RESULTS AND DISCUSSION

Although precipitating antibodies to myosin can be generated by injecting a total of less than one mg immunogen into a rabbit, ATPase-inhibiting antibodies were only obtained after prolonged immunization and/or with rather high doses of myosin. This regimen is presumably necessary to overcome the natural tolerance to the phylogenetically conserved catalytic site of enzymes, as postulated by Cinader [1]. Of the total IgG present in these antisera, 10–15% were specific myosin antibodies, as estimated by their ability to bind to immobilized myosin.

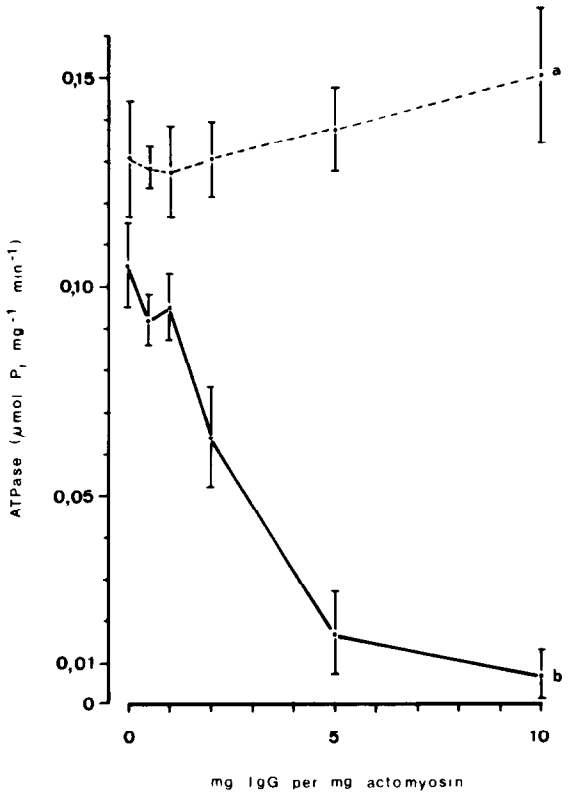


Fig.1. Inhibition of $Ca^{2+}$-activated actomyosin ATPase bivalent antibodies (IgG) to myosin (b). No inhibition is observed in the presence of control IgG (b). Points with vertical arrows represent means ± SE for 5 experiments.

While control IgG or Fab did not measurably influence the $Ca^{2+}$-activated ATPase of natural actomyosin (figs 1a and 2a), both the bi- and monovalent anti-myosins were inhibitory (figs 1b and 2b). The Fab-fragment was less active than the intact IgG, which can be explained by the reduced functional activity of the monovalent fragments [10]. The experiment showed, on the other hand, that the enzyme inhibition was not solely due to the formation of insoluble immunocomplexes. When incubates of anti-myosin Fab and actomyosin were diluted to an ionic strength of $\mu = 0.25$ they did not precipitate due to filamentogenesis, as in control experiments. Since free myosin will not precipitate under these conditions, we had reasons to assume that the specific anti-myosin Fab had dissociated the actomyosin complex. This assumption was indeed corroborated by viscosity and sedimentation behaviour studies.

The presence of control Fab did not alter the viscosity properties of actomyosin; addition of

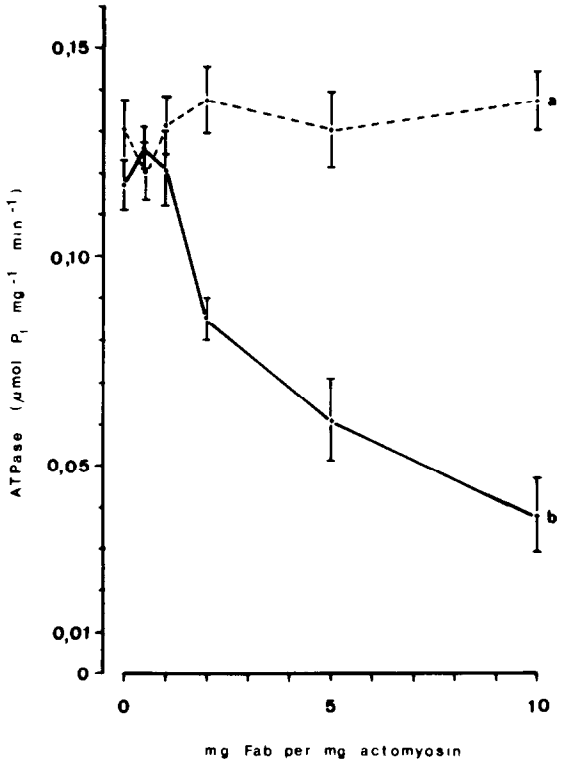


Fig.2. Inhibition of $Ca^{2+}$-activated actomyosin ATPase by monovalent anti-myosin Fab (b). Control Fab are non-inhibiting (a). Points with vertical arrows represent means ± SE for 5 experiments.

$Mg^{2+}$ and ATP reduced the viscosity (from $\eta_{rel}$ = 2.86 to $\eta_{rel}$ = 1.54) by dissociating it into its constituents actin and myosin. Since ATPase activity was unimpaired, viscosity returned to its initial value (or higher) both in the absence or presence of control Fab (fig.3a).

In contrast, incubates of actomyosin with anti-myosin Fab had a very low initial viscosity, and the addition of $Mg^{2+}$ and ATP did not cause any further decrease. Also, the viscosity did not increase even after prolonged incubation (fig.3b). These data clearly indicated that the specific anti-myosin Fab had dissociated the complex, and due to their binding at or near the catalytic site also prevented ATP hydrolysis and re-association of actin and myosin. Further evidence for the dissociation of the actomyosin complex was obtained by analytical ultracentrifugation. When a mixture of 4 mg/ml actomyosin and 24 mg/ml control Fab was allowed to sediment at 41000 rpm, two peaks were observed: actomyosin with an $s_{20}$ of about 20 and Fab with an $s_{20}$ of 2.2 (the sedimentation coefficient of Fab decreases from its reported value of 3.5 with increasing concentrations). In incubates of actomyosin with specific Fab, three components were noted: a minor one sedimenting with an $s_{20}$ of 18.2 (non-dissociated actomyosin), a major one with an $s_{20}$ of 9 (myosin-anti-myosin Fab complexes, as identified by sedimenting myosin-anti-myosin Fab complexes alone) and excess Fab with an $s_{20}$ of 2.2.

The experiments of Dancker and Hoffmann [3] have suggested a stability constant for the actomyosin complex of about $2 \times 10^6$ $M^{-1}$ and thus a high affinity of actin to myosin. Our above data give evidence that the affinity of the myosin antibodies – even in their less functional monovalent state – must be even higher in order to dissociate the complex.

Antibodies to native folded proteins seem to be mainly directed to mobile sites in the protein that can adopt multiple conformations [14]. Both myosin head ($S_1$) and neck (hinge/$S_2$) regions would fulfil this criterion [9]. Indeed, the majority of myosin antibodies react with the $S_1$ and $S_2$ fragments [13]. Antibodies to either site might be responsible for the dissociation of the actomyosin complex. ATPase inhibition seems to be mediated by anti-$S_1$ only, since anti-$S_2$ was shown to be non-inhibitory (unpublished).

## ACKNOWLEDGEMENTS

This study has been supported by the Deutsche Forschungsgemeinschaft (St 105/19, 21). We thank Professor L.M.G. Heilmeyer (Universität Bochum) for letting us perform the ultracentrifugation studies in his laboratory.

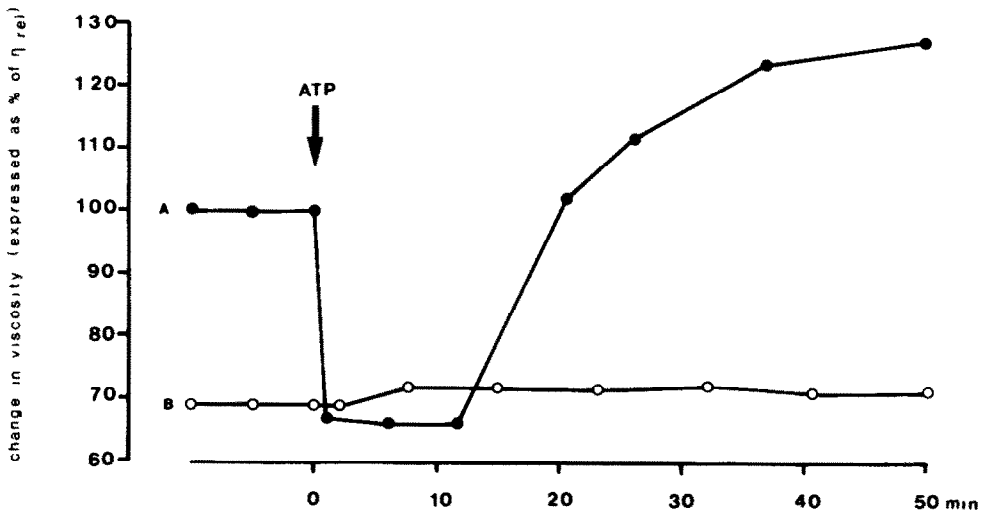


Fig.3. Initial viscosity of actomyosin incubated with a 6-fold excess (w/w) of control Fab fragments (A) or anti-myosin Fab fragments (B); and changes induced by the addition of ATP (arrow).

## REFERENCES

[1] Cinader, B. (1971) in: Regulation of the Antibody Response (Cinader, B. ed.) 2nd edn, pp.3–18, Thomas, Springfield.
[2] Cleghorn, R.A. and Jendrassik, L. (1934) Biochem. Z. 274, 189–193.
[3] Dancker, P. and Hoffmann, M. (1973) Z. Naturforsch. 28c, 401–411.
[4] Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400.
[5] Garvey, J.S., Cremer, N.E. and Sussdorf, D.H. (1977) in: Methods in Immunology, 3rd edn, pp.256–266, Benjamin, Reading, MA.
[6] Gröschel-Stewart, U. (1971) Biochim. Biophys. Acta 229, 322–334.
[7] Gröschel-Stewart, U., Schreiber, J., Mahlmeister, C. and Weber, K. (1976) Histochemistry 46, 229–236.
[8] Harboe, N. and Ingild, A. (1973) Scand. J. Immunol. 2, suppl.1, 161–164.
[9] Harrington, W.F. and Rodgers, M.E. (1984) Annu. Rev. Biochem. 53, 35–74.
[10] Hornick, C.L. and Karush, F. (1972) Immunochemistry 9, 325–340.
[11] Kominz, D.R. and Gröschel-Stewart, U. (1973) J. Mechanochem. Cell Motil. 2, 181–191.
[12] Lowey, S. and Steiner, L.A. (1972) J. Mol. Biol. 65, 111–126.
[13] Mestan, J. (1981) Diplomarbeit, FB 10, Techn. Hochschule Darmstadt.
[14] Tainer, J.A., Getzoff, E.D., Alexander, H., Houghten, R.A., Olson, J.A., Lerner, R.A. and Hendrickson, W.A. (1984) Nature 312, 127–134.