REGULATORY LIGHT CHAINS OF MYOSIN FROM THE OBLIQUELY-STRIATED BODY WALL MUSCLE OF *LUMBRICUS TERRESTRIS*

Jochen D'HAESE

Institut für Zoologie II, Universität Düsseldorf, Universitätsstr. 1, D-4000 Düsseldorf, FRG

Received 15 September 1980

1. Introduction

The myosin-linked Ca^{2+}-regulatory system of invertebrate muscle has been described in detail only for some molluscan muscles. In this muscle-type a regulatory light chain which is readily removed by EDTA was found to serve as the regulatory component [1,2]. So far, only for the myosin from scallop adductor [2,3] and clam foot muscles [4] such regulatory light chains have been found, whereas in other molluscan muscles a similar system could not be demonstrated [5,6]. In contrast to these molluscan muscles the majority of invertebrate muscles are doubly regulated by a myosin- and an actin-linked regulatory system [5,7]. Here the Ca^{2+}-regulation of obliquely striated muscle was analyzed with particular attention to the mode of regulation confered by the myosin component. It is shown that, as in molluscan muscles the myosin of obliquely striated muscle posseses a regulatory light chain that is extractable with EDTA. As with molluscan muscles the light chain may be reversibly removed and also functionally replaced by the P light chain of vertebrate smooth muscle.

2. Materials and methods

The earthworms *Lumbricus terrestris* were obtained from fishing-supply shops. After removal of the internal organs the body walls were rinsed and homogenized in wash solution containing 30 mM KCl, 1 mM $MgCl_2$, 0.05 mM PMSF, 20 mM 2-mercaptoethanol, and 20 mM Tris–maleate buffer (pH 6.5). The pellet obtained after centrifugation at 30 000 × *g* for 30 min was washed twice with the same solution and extracted with 0.5 M KCl, 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 50 mM Tris–maleate buffer (pH 7.4) for 45 min. The extract was centrifuged at 30 000 × *g* for 1 h and Tris–maleate buffer (pH 7.0) was then added slowly to give 0.125 M KCl final [KCl]. The precipitated actomyosin was collected by centrifugation and washed once with 0.125 M KCl in 30 mM Tris–maleate buffer (pH 7.0). Most of the extracted paramyosin remained in the supernatant. The actomyosin was resuspended in 0.5 M NaCl, 30 mM Tris–maleate buffer (pH 7.0) and insoluble material was removed by centrifugation at 30 000 × *g* for 1 h. Myosin-enriched actomyosin was obtained by ammonium sulfate precipitation of the supernatant at 40–70% saturation in the presence of 0.5 M NaCl and 10 mM MgATP. HMM and actin from rabbit skeletal muscle was prepared according to [8,9] and were kept in liquid nitrogen ready for use.

3. Results and discussion

The high ionic strength extract of the body wall muscles contained three predominant proteins: paramyosin, actin, and myosin. Most of the paramyosin is removed during the preparation of actomyosin. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) of the actomyosin preparation showed (fig.1a) the presence of myosin with two types of light chains of M_r 25 000 and 18 000, together with actin and the actin-linked regulatory components (A. Ditgens, J. D'H., in preparation).

The ATPase activity of the isolated earthworm actomyosin required the presence of Ca^{2+} and an 80–90% inhibition was found in the absence of Ca^{2+}. When the actomyosin was combined with various amounts of

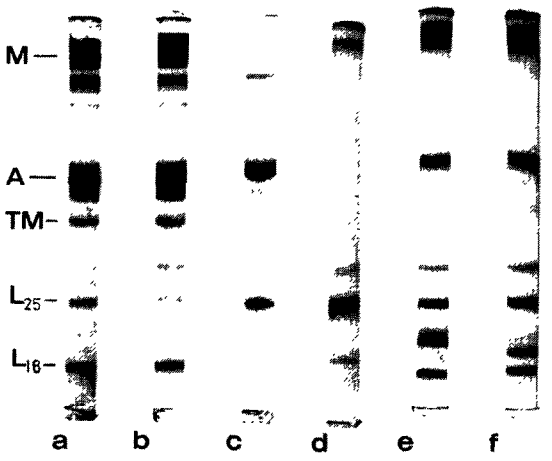

Fig.1. SDS–12% polyacrylamide amide gel electrophoresis of: (a) earthworm actomyosin; (b) EDTA-treated actomyosin; (c) the EDTA-extract; (d) the 25 000 M_r light chain fraction; (e) myosin-enriched actomyosin hybridized with the 20 000 M_r chicken gizzard myosin LC; (f) hybridized with the 19 000 M_r LC from rabbit skeletal muscle myosin. SDS–PAGE was performed according to [10]. Samples were incubated in the presence of 8 M urea and 5% 2-mercaptoethanol for about 5 min at 100°C. M, heavy chain of myosin; A, actin; TM, tropomyosin; L_{in}, light chain.

desensitized actin or HMM from rabbit skeletal muscle the Ca^{2+}-sensitivity of the ATPase activity was not decreased (table 1). This competition test indicated the presence of a double regulation as has been described also for the majority of invertebrate muscles [7]. A double regulation was also demonstrated using purified myosin and a thin filament fraction, respectively (A. Ditgens, J. D'H., in preparation). The treatment of actomyosin or myosin enriched fractions with 10 mM EDTA by the method used with scallop muscle [3] led to a complete loss of the myosin-linked Ca^{2+}-regulation without a change of the intrinsic actomyosin ATPase activity (table 2). The EDTA-treatment led to the extraction of predominately the 25 000 M_r light chain and variable amounts of actin and myosin impurities depending on the actin–myosin ratio of the actomyosin used (fig.1b,c). The light chain fraction obtained in the 50–80% ammonium sulfate saturation cut of this extract (fig.1d) was used, after dialysis against KCl-buffer solution, for the recombination experiments. Only ~1 mol of the 25 000 M_r light chain was readily released by EDTA; the remaining other part of the 25 000 M_r light chain which is apparently more strongly bound was nearly

Table 1
Double regulation of earthworm actomyosin as shown by the competitive actin- and myosin-binding assay with HMM and unregulated actin from rabbit skeletal muscle

	Mg-ATPase activity		Ca-sensitivity
	Ca	EGTA	
Actomyosin (0.4 mg)	78	10	0.87
+ actin (0.2 mg)	107	15	0.86
+ actin (0.4 mg)	131	16	0.88
+ HMM (0.2 mg)	225	38	0.83
+ HMM (0.4mg)	231	69	0.70

Data given are representative values from 4 expt. with different earthworm preparation. Protein was determined according to [11]. ATPase activity (nmol P_i . min^{-1} . mg actomyosin^{-1}) was determined in a medium containing 20 mM KCl, 1 mM $MgCl_2$, 30 mM Tris–maleate buffer (pH 7.0), 1 mM ATP, 0.1 mM $CaCl_2$ or 2 mM EGTA. After incubation for 10 min at 25°C the reaction was stopped with 5% trichloroacetic acid (final conc.) and liberated P_i was determined [12]. Ca^{2+}-sensitivity is expressed as 1–(ATPase activity at 2 mM EGTA/ATPase activity at 0.1 mM Ca^{2+})

Table 2
Recombination and hybridization of EDTA-desensitized earthworm actomyosin with different light chain fractions LC

	Mg-ATPase activity		Ca-sensitivity
	Ca	EGTA	
Actomyosin (untreated)	221	35	0.84
Actomyosin (desensitized)			
+ rabbit actin (1:1, w/w)	224	210	0.06
+ EDTA-extracted LC	189	49	0.74
+ DTNB-extracted LC	183	38	0.79
+ 20K LC chicken gizzard	199	83	0.58
+ 19K LC rabbit skeletal	207	153	0.26

Recombination and hybridization was performed in a solution containing 0.5 M NaCl, 2.5 mM $MgCl_2$, 30 mM Tris–maleate buffer (pH 7.0) for ~1 h. After subsequent dialysis against KCl–buffer solution (20 mM KCl, 30 mM Tris–maleate buffer (pH 7.0), 5 mM 2-mercaptoethanol) containing 2.5 mM $MgCl_2$ the precipitate was collected by centrifugation and washed twice with the same buffer. Conditions for ATPase activity (nmol P_i . min^{-1} . mg actomyosin^{-1}) were as described in table 1. Data given are representative values from at least 3 different preparations

completly removed when the EDTA-desensitized actomyosin was furthermore treated with DTNB, by the method used to release one particular class of light chain also from other myosins [3,13,14], led to an irreversible loss of the ATPase activity of the earthworm actomysin. Similar results were obtained with scallop muscle preparations: one of the two regulatory light chains are extracted with EDTA and both light chains were released only by a combined use of DTNB and EDTA [3] or at high temperature during EDTA-extraction [15]. The different extractability and the negatively co-operative rebinding of the regulatory light chains could be explained by the proposed 'head clumping model' [16].

Recombination experiments showed (table 2) that the removal of the 25 000 M_r light chain by EDTA was almost completely reversible and that its reincorporation (fig.1e) in desensitized actomysin fully restored the myosin-linked Ca^{2+}-sensitivity. It was thus clear that the 25 000 M_r subunit is the regulatory light chain of the earthworm muscle and as such is considerably larger than its 18 000 M_r counterpart in molluscan muscles. From the fact that DTNB-extracted light chains could also restore the Ca^{2+}-sensitivity it appeared that the 'EDTA' and 'DTNB-released' light chains are functionally identical. The P-light chains of 20 000 M_r from chicken gizzard myosin also recombined with EDTA-extracted actomyosin (fig.1) and restored Ca^{2+}-sensitivity. The P-light chains from rabbit skeletal muscle myosin were not as effective and the Ca^{2+}-sensitivity was only partially restored (fig.1, table 2).

If Ca^{2+} was used instead of Mg^{2+} in these recombination experiments a reconstitution of Ca^{2+}-sensitivity was not achieved. As EGTA was not effective in releasing the light chains, the existence of an attachment site of a light chain to the heavy chain that requires Mg^{2+} is assumed. The actin-binding region appears to be at some distance from this Mg^{2+}-binding site because ATP was not necessary for reconstitution.

Thus these results suggest a very similar mode of myosin-linked regulation in the obliquely striated muscle of the annelid *Lumbricus terrestris* to that of the scallop muscle, namely by direct binding of Ca^{2+}.

Acknowledgements

This study was supported by the Stiftung Volkswagenwerk. I wish to thank Miss R. Pilgram for expert technical assistence, Drs Small and Sobieszek for helpful discussion and for providing light chain fractions from smooth and skeletal muscle.

References

[1] Kendrick-Jones, J., Lehman, W. and Szent-Györgyi, A. G. (1970) J. Mol. Biol. 54, 313–326.
[2] Szent-Györgyi, A. G., Szentkiralyi, E. M. and Kendrick-Jones, J. (1973) J. Mol. Biol. 74, 179–203.
[3] Kendrick-Jones, J., Szentkiralyi, E. M. and Szent-Györgyi, A. G. (1976) J. Mol. Biol. 104, 747–775.
[4] Asada, T., Ashiba, G. and Watanabe, S. (1979) J. Biochem. 85, 1543–1546.
[5] Lehmann, W. (1976) Int. Rev. Cytol. 44, 55–92.
[6] Konno, K. (1978) J. Biochem. 84, 1431–1440.
[7] Lehmann, W. and Szent-Györgyi, A. G. (1975) J. Gen. Physiol. 66, 1–30.
[8] Lowey, S. and Cohen, C. (1962) J. Mol. Biol. 4, 293–308.
[9] Spudich, I. A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[10] Laemmli, U. K. (1970) Nature 227, 680–685.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Fiske, C. H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400.
[13] Weeds, A. G. (1969) Nature 223, 1362–1364.
[14] Gazith, J., Himmelfarb, S. and Harrington, W. F. (1970) J. Biol. Chem. 245, 15–22.
[15] Chantler, P. D. and Szent-Györgyi, A. G. (1980) J. Mol. Biol. 138, 473–492.
[16] Bagshaw, C. R. (1980) J. Muscle Res. Cell Motil. in press.