ELSEVIER

Biophysical Chemistry 61 (1996) 85–92

Biophysical Chemistry

Force production by chemically crosslinked myosin–actin crossbridges in rabbit skinned fibers in response to MgATP depletion

Yumiko Emoto [1], Katsuhisa Tawada [*]

Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812-81, Japan

Received 25 December 1995; revised 9 February 1996; accepted 19 February 1996

Abstract

In order to study the contractile property of myosin crossbridges attached to thin filaments, myosin heads were crosslinked to the filaments at their interface in single skinned rabbit psoas fibers with a zero-length chemical crosslinker, 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide (EDC). The results obtained show that a partially crosslinked single fiber produces a large rigor-like force when MgATP is depleted from the myofibrillar space. Such crosslinked fibers contain two types of crosslinked myosin heads: one with one of the two heads of the myosin molecule crosslinked to actin with the other head uncrosslinked; the other has both heads crosslinked to actin. The results of this work suggest that a crosslinked myosin head of the former type produces a much larger force than the latter type.

Keywords: Muscle contraction; Force production; Crossbridges; Myosin; Skinned fiber; Chemical crosslinking

1. Introduction

Muscle contraction results from the cyclic interaction of myosin heads with actin, coupled with actin-activated ATP hydrolysis [1–3]. It is widely believed that myosin crossbridges produce contractile force while their heads are attached to actin. Recent mechanical and X-ray experiments have provided evidence for fast crossbridge reattachment after a step length change in muscle [4,5]. Because of the cyclic nature of the interaction between myosin heads and actin consisting of their attachment and detachment [3], detailed analysis of the force producing process during myosin head attachment to actin has been difficult.

Mornet et al. [6] introduced a chemical crosslinking technique with a zero-length crosslinker, 1-(3-dimethylamino-propyl)-3-ethylcarbidiimide (EDC), to the study of the myosin–actin interaction without such an "attachment/detachment" problem. EDC crosslinks a myosin head to actin at their interface. EDC-crosslinked myosin–actin complexes have a superactive ATPase activity, which is close to V_{max} in the actin-activated ATPase reaction at physiological ionic strengths [6]. The high ATPase activity of the covalent complexes indicates that there are, if any, few side effects of the crosslinking

* Corresponding author. Fax/Tel: +81-92-642-2634; E-mail: ktawascb@mbox.nc.kyushu-u.ac.jp

[1] Present address: Department of Animal Science, Faculty of Agriculture, Kyushu University, Fukuoka 812-81, Japan.

PII S0301-4622(96)00024-5

reaction on myosin and actin. The complexes retain a superactive ATPase activity even at high ionic strength. For example, at 0.5 M KCl, where uncrosslinked myosin has no actin-activated ATPase activity [7].

In this present study the EDC crosslinking technique was applied to skinned fibers of rabbit psoas muscle which resulted in crosslinked myosin crossbridges in partially crosslinked single fibers producing a large rigor-like force when MgATP is depleted from the myofibrillar space. Application of sinusoidal analysis and caged-ATP techniques to partially crosslinked fibers were reported elsewhere [8,9]. During the preparation of this paper, we noticed an interesting paper by Bershitsky and Tsaturyan on a temperature jump-induced force development in partially crosslinked fibers in the presence of MgATP [10]. A preliminary account of the present study was reported at the third Muscle Energetics Conference [11].

2. Materials and methods

Skinned fibers were prepared by glycerinating rabbit psoas muscles in the relaxed state, as described previously [12].

Reagents such as EDC were purchased from Nakalai Tesque Co., Kyoto, Japan.

2.1. Solutions

Rigor solution: 80 mM KCl, 40 mM imidazole, 7.4 mM EDTA, pH 7.0 at 25°C. Relaxing solution: 80 mM KCl, 40 mM imidazole, 5 mM Na_2ATP, 2 mM $MgCl_2$, 5 mM EGTA, pH 7.0 at 25°C. Contracting solution: 80 mM KCl, 40 mM imidazole, 5 mM Na_2ATP, 2 mM $MgCl_2$, 9 mM EGTA, 9.1 mM $CaCl_2$, pH 7.0 at 25°C.

The above three solutions will be referred to as "low-salt" solutions, e.g. low-salt rigor solution. High-salt rigor, relaxing and contracting solutions contain 0.5 M KCl instead of 80 mM KCl, but the other solute concentrations and pH are the same as those of the corresponding low-salt solution.

The apparatus and general procedures for the crosslinking and the force and stiffness measurements were the same as previously described [12,13]. A short segment of a single skinned fiber, with one end of the segment tied to a force transducer and the other end tied to the tip of a servomotor arm, was crosslinked at 25°C in a low-salt rigor solution (see below) containing 8 mM EDC. The crosslinking reaction was terminated by rinsing the fiber segment with a solution containing 80 mM KCl, 40 mM imidazole (pH 7.0) and 0.2% (v/v) β-mercaptoethanol at 25°C [7], and the fiber was then rinsed with a fresh low-salt rigor solution before measuring the stiffness and the force production. The stiffness and force were measured at 15°C.

The fraction of myosin heads crosslinked to the thin filaments in muscle fibers was determined by comparing the fiber linear stiffness in a high-salt relaxing solution to that in a high-salt rigor solution [13].

3. Results

In this work, the extent of myosin head crosslinking to the thin filament in muscle was varied by controlling the EDC crosslinking time. As shown previously, the rod portion in myosin filaments is quickly crosslinked by EDC, so that the myosin thick filaments become insoluble even in the presence of 0.5 or 1 M KCl [13]. Skinned fibers after crosslinking with EDC at least for about 20 min under the condition of the present study thus retain their sarcomere structure in high-salt solutions such as those containing 0.5 or 1 M KCl. This enables us to measure the stiffness of crosslinked fibers in high-salt relaxing and rigor solutions. In high-salt relaxing solution, (i) all uncrosslinked myosin heads are completely detached from the thin filament and only crosslinked heads contribute to the stiffness, and (ii) the stiffness is proportional to the extent of myosin head crosslinking in muscle [13]. By comparing the linear stiffness of a partially crosslinked fiber in high-salt relaxing solution with that in high-salt rigor solution, we thus estimated the extent of myosin head crosslinking to the thin filament [13].

Fig. 1 shows the time course of the myosin head crosslinking to the thin filament in single fibers. The crosslinking is a single exponential process and the time course could be fitted by an equation: $100 \times [1 - \exp(-k \times \text{time})]$, where k was found to be 0.010

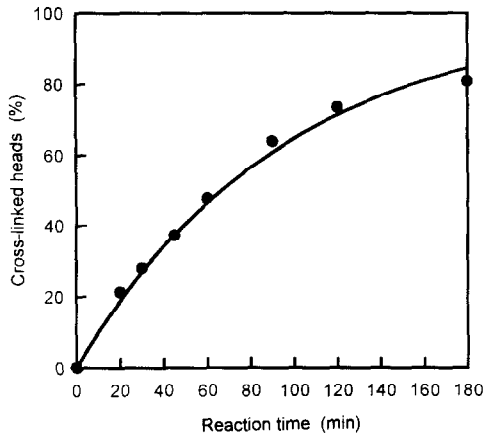

Fig. 1. Myosin head crosslinking to the thin filament in skinned muscle fibers as a function of reaction time. See Section 2 for the crosslinking conditions and the method for measuring the crosslinking extent. Each point in the figure shows an average of two to five different single fibers. The standard deviation of the mean is less than the size of the symbol mark. In total 26 different single fibers were used.

min^{-1}. This rate constant is close to the values obtained in our previous studies with skinned fibers [13], and also with heavy meromyosin/actin or myosin subfragment-1/actin systems in vitro at a molar ratio of the myosin head to the actin monomer corresponding to their ratio in muscle [7].

When a partially crosslinked fiber was transferred from a high-salt relaxing solution to a high-salt rigor solution, the fiber developed a force (Fig. 2). The force was reversibly lost when the fiber was transferred back to the relaxing solution. The force production (such as that shown by ΔF in Fig. 2) required pre-stretching of fibers in a high-salt relaxing solution before their transfer to a rigor solution, and depended upon the extent of the pre-stretching. There is an optimal pre-stretching force to obtain the maximum force production ($F5$ in Fig. 2). Beyond this pre-stretching level, a smaller force was produced ($F6$). However, if the pre-stretching force was reduced back to the optimal level, the same maximum force was again produced ($F7$). This reversibility suggests that a smaller force production beyond the optimum pre-stretching is not due to some irreversible damage in the fibers, which pre-stretching over an optimum level might cause.

When a fiber is transferred to a rigor solution from a relaxing solution, MgATP is removed from the fiber. We can thus consider that removal of MgATP from the myofibrillar space in partially

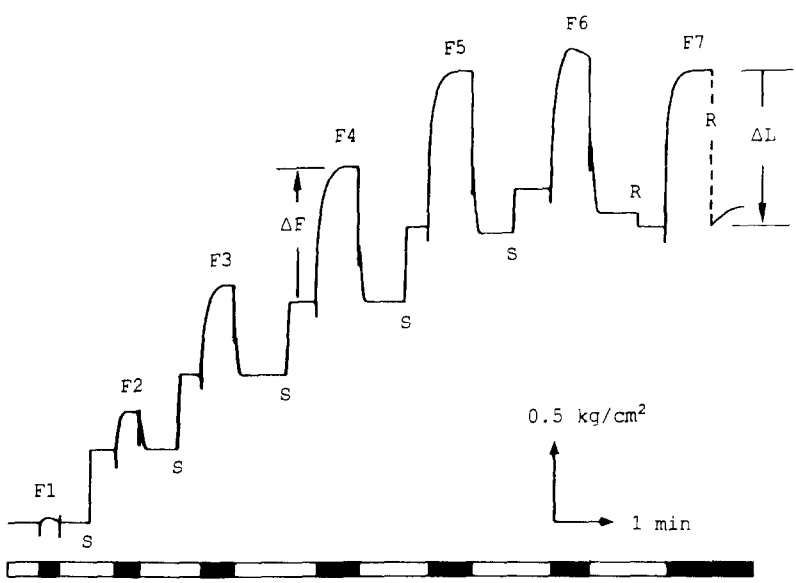

Fig. 2. Force production (as indicated by ΔF) by a partially crosslinked single fiber upon transfer from a high-salt relaxing solution into a high-salt rigor solution at 15°C. Open and filled rectangular boxes refer to relaxing and rigor solutions. S, stretched manually; R, released manually; ΔL, see the text. Muscle was stretched by 1.8% of its length to give the optimal pre-stretching force (at F5). The extent of myosin head crosslinking was 52%.

crosslinked skinned fibers causes the force production in the fibers. Removal of either Mg^{2+} or ATP alone from a relaxing solution induced the same amount of force as obtained when MgATP was removed. Use of MgADP or MgPPi instead of MgATP was not effective (data not shown). Therefore MgATP appears to be a prerequisite for the force production by partially crosslinked fibers in a high-salt (0.5 M KCl) solution. In the following, we studied the force production (ΔF) under various conditions.

The filled circles in Fig. 3 show the force production by partially crosslinked fibers upon transfer from a high-salt relaxing solution to a high-salt rigor solution at various levels of pre-stretching force. The left and right panels in Fig. 3 compare the force production in a single fiber with 19% myosin head crosslinked with that in another single fiber with 52% myosin head crosslinked. The optimal pre-stretching force required to obtain a maximum force production was larger in a fiber with a larger fraction of crosslinked myosin heads as was the maximum force production when the crosslinking extent was less than about 50% (see Fig. 5). Force produced by partially crosslinked fibers upon their transfer from a high-salt relaxing solution to a rigor solution at 0.5 M KCl was the same as that at 1 M KCl (data not shown), but the force produced upon transfer to a rigor solution at 80 mM KCl was slightly larger (open circles in Fig. 3) than that at 0.5 M KCl.

The difference between the maximum forces produced by partially crosslinked fibers in low-salt (80 mM KCl) and high-salt (0.5 M KCl) rigor solutions (difference between ○ and ● in Fig. 3) is shown as a function of the crosslinked heads (triangles in Fig. 4). This force difference almost linearly decreases with increasing the fraction of crosslinked heads. This linear decrease indicates that the binding of uncrosslinked heads to the thin filament in partially crosslinked fibers upon deletion of MgATP generates rigor force in a low-salt rigor solution.

Partially crosslinked fibers similarly produced force when they were transferred from a high-salt relaxing solution to a low-salt contracting solution (open squares in Fig. 3); but the fibers did not produce force when transferred to a high-salt contracting solution (data not shown). An optimal pre-stretching force exists for the maximum force production by partially crosslinked fibers in a low-salt contracting solution, as in the case for the force

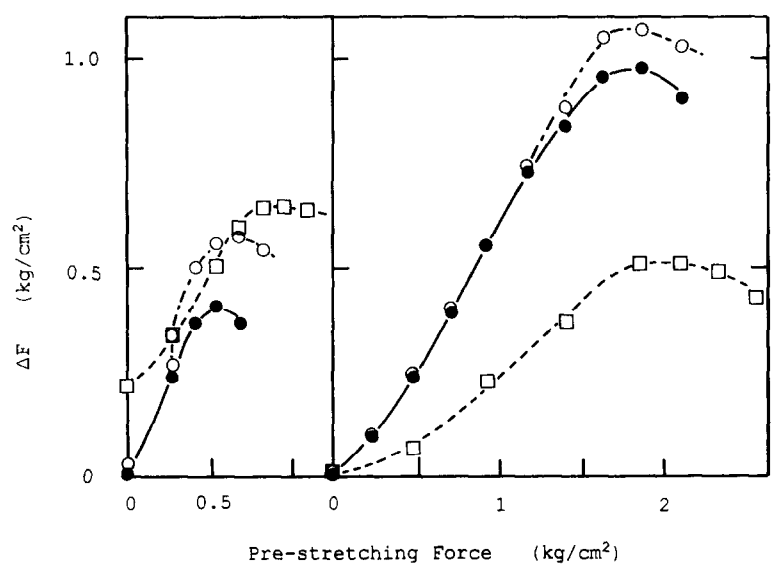

Fig. 3. Force produced by partially crosslinked single fibers upon transfer from a high-salt relaxing solution into a low-salt rigor solution (○), a high-salt rigor solution (●), and a low-salt contracting solution (□). No force was produced upon transfer into a high-salt contracting solution. Crosslinking extent of myosin heads to actin in a single fiber: 19% (left panel) and 52% (right panel). Other conditions are the same as those for Fig. 2.

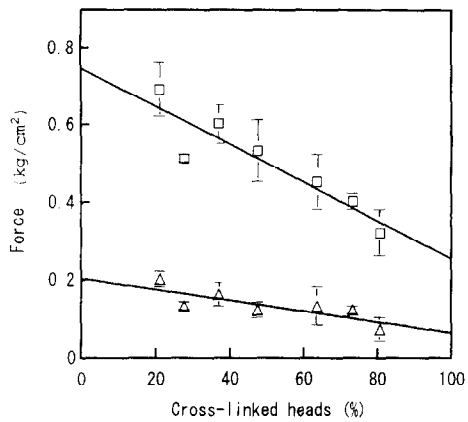

Fig. 4. Force produced by partially crosslinked single fibers upon transfer from a high-salt relaxing solution into a low-salt contracting solution (□), and the difference (△) between the maximum forces produced by the fibers upon transfer from a high-salt relaxing solution into a low-salt rigor solution and into a high-salt rigor solution such as those shown in Fig. 3, as a function of myosin head crosslinking extent. The data show mean ± sem (N = two to five different single fibers). Conditions are the same as those for Fig. 2. The filled lines are regression lines.

production in rigor solution. In contrast to the force production in rigor solution, however, the larger the crosslinking extent, the smaller the force production in the contracting solution (compare open squares in the left and right panels in Fig. 3).

The squares in Fig. 4 show the maximum force produced by partially crosslinked fibers in a low-salt contracting solution as a function of the crosslinked heads. There are two findings concerning this force production: (1) the force almost linearly decreases with increasing the fraction of crosslinked heads; (2) the force extrapolated back to 0% crosslinking is about 0.8 kg/cm^2, while the force extrapolated up to 100% crosslinking is about 0.25 kg/cm^2. The first finding indicates that the force production in a low-salt contracting solution is primarily caused by uncrosslinked myosin heads in partially crosslinked fibers but not by crosslinked heads. The force level extrapolated up to 100% crosslinking is not zero. This could be caused by possible tension development associated with the lattice expansion in skinned fibers when they are placed in an ATP solution of low-salt concentration from rigor [14], or by a possible small force generation by crosslinked myosin heads in a low-salt contracting solution.

Fig. 5 shows the maximum force produced by partially crosslinked fibers upon transfer from a high-salt relaxing solution to a high-salt rigor solution (such as *F*5 in Fig. 2) as a function of crosslinked heads (filled circles), together with the optimal pre-stretching force required to obtain the maximum force production (crosses). In contrast to the data in Fig. 4. the force dependence on the fraction of crosslinked heads is complex in Fig. 5. Here, the relationship is bell-shaped. Note that, nonetheless, the optimal pre-stretching force (crosses) linearly increases with the increase in the myosin head crosslinking.

Comparison of the bell-shaped curve in Fig. 5 with the descending linearity in Fig. 4 indicates that the force produced in partially crosslinked fibers in a

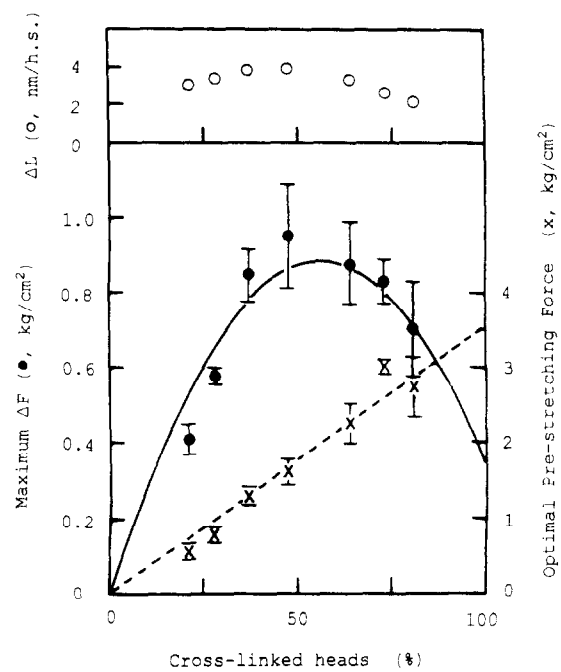

Fig. 5. Lower panel: maximum force (●) and optimal pre-stretching force (×) in a high-salt rigor solution as a function of myosin head crosslinking extent. Filled line, Eq. (1) given in Section 4; see the text. Broken line, linear regression line for the pre-stretching data. The data show mean ± sem (N = two to five different single fibers). Upper panel: Muscle length changes required to reduce the maximum force (such as ΔF of F5 in Fig. 2) to zero (see Fig. 2 for the ΔL definition) as a function of myosin head crosslinking extent. Standard error of mean is less than the size of symbols. The unit for ΔL is nm per half sarcomere. Conditions are the same as those for Fig. 2.

high-salt rigor solution is generated by myosin with at least one head (out of the two heads of a myosin molecule) crosslinked to actin. There are thus two possibilities for the force generation by such crosslinked myosin. One possibility is that the force is generated by crosslinked crossbridges of myosin, but not by uncrosslinked heads of myosin, upon MgATP depletion. The other possibility is that one uncrosslinked head of myosin with the other head crosslinked generates force by binding to actin, whereas the latter crosslinked head of the myosin does not generate force upon MgATP depletion at 0.5 M KCl. As will be described in the Section 4, the first possibility is much more likely.

After a partially crosslinked fiber developed a maximum force in a high-salt rigor solution, we measured the muscle length change required to reduce the developed maximum force (ΔF) to zero by releasing one end of the fiber segment. The length change will be referred to as ΔL, as indicated in Fig. 2. Values of ΔL were collected with many partially crosslinked fibers and the results are summarized in Fig. 5 (upper panel). The values of ΔL were between 2 and 4 nm per half sarcomere. This suggests that crossbridges are strained by 2 to 4 nm when they have generated maximum force in a high-salt rigor solution. These ΔL values are close to the strain present in crossbridge heads in isometrically stimulated intact muscle fibers [15].

4. Discussion

The main observation in this study is that skinned fibers in which myosin heads have been partially crosslinked to thin filaments with EDC, develop rigor-like force when MgATP is depleted at 0.5 or 1 M KCl. This force production is reversibly lost when MgATP is reintroduced into the myofibrillar space. As will be explained below, this force production is considered to be generated by crosslinked myosin heads, but not by uncrosslinked heads. This conclusion is consistent with that given by Bershitsky and Tsaturyan in their temperature-jump study of partially crosslinked skinned fibers in a high-salt MgATP solution [10]. The most interesting characteristics of the force production at 0.5 M KCl is its bell-shaped dependence on the extent of the myosin head crosslinking (closed circles in Fig. 5). Bershitsky and Tsaturyan [10] have reported a similar finding: force produced in a partially crosslinked muscle fiber induced by a temperature-jump in a high-salt MgATP solution becomes smaller when crosslinking extent increases more than about 50%.

4.1. Requirement of pre-stretching

Force production by partially crosslinked fibers required pre-stretching of the fibers (Fig. 2 and Fig. 3). Pre-stretching with larger force was required for a muscle fiber with a larger fraction of crosslinked myosin heads (Fig. 3 and Fig. 5). Such pre-stretching was necessary even for the active force production by uncrosslinked myosin heads in partially crosslinked fibers (open squares in Fig. 3). A possible explanation for this requirement is as follows. It is necessary to take the slack of a fiber out so that force produced by myosin crossbridges within the fiber can be detected at the fiber ends. Since the more crosslinks there are the stiffer the fiber, a larger force is necessary for taking the slack out of a fiber with a larger extent of crosslinking.

There is an optimal pre-stretching to obtain a maximum force production by a partially crosslinked fiber (Fig. 2 and Fig. 3). This suggests that over-strain in the actin filament, the myosin filament or the myosin crossbridge in muscle causes the force generation by myosin crossbridges to be smaller.

4.2. Relation to superactivated ATPase activity

EDC-crosslinked myosin subfragment-1 (S1)-actin covalent complexes retain superactivated ATPase activity at 0.5 M or 1 M KCl as well as at physiological ionic strengths [7]. In contrast, the ATPase activity of uncrosslinked myosin is not activated at all by actin at such high salt concentrations because these myosin heads are completely detached from actin in the presence of ATP at high salt concentrations [7].

There is a linear relationship between the ATPase activity at 0.5 M KCl by partially crosslinked S1-actin complexes and the extent of S1 crosslinking to actin [7]. It is linear up to 80% of S1 crosslinked to actin. In contrast, a bell-shaped relationship has been found between the superactivated ATPase activity at 0.5 M

KCl of partially crosslinked heavy meromyosin (HMM)-actin complexes and the extent of myosin head crosslinking to actin [7], which is similar to the bell-shaped relationship found here between the force production by partially crosslinked fibers at 0.5 M KCl and the extent of myosin head crosslinking.

The bell-shaped relationship of the superactive ATPase activity is not due to irreversible damages in HMM during prolonged crosslinking. The reason is that subsequent conversion of HMM to S1 in partially crosslinked HMM-actin complexes by chymotryptic digestion restored a linear relationship of the ATPase activity, which is similar to the linear relationship found with partially crosslinked S1-actin complexes [7]. A possible explanation for the bell-shaped relationship in the ATPase activity is to assume that some physical constraint is imposed on a myosin with two heads crosslinked to actin and that such constraint suppresses the enzymatic activity of the myosin. In other words, the ATPase activity of a crosslinked head in a myosin is lower when the other head in the myosin is crosslinked to actin than when the latter head is not crosslinked [7]. We may assume a similar situation for the force production by crosslinked myosin heads, considering a general belief that actin-activated ATPase activity of myosin is closely coupled with force production by myosin crossbridges.

4.3. Bell-shaped relationship of the force production in partially crosslinked muscle fibers at 0.5 M KCl

Based on the forgoing discussion, we assume the following for the explanation of the force production dependence on the crosslinking extent shown in Fig. 5:

1. In a skinned muscle fiber, EDC crosslinks two heads of a myosin molecule to actin independently [13]. The crosslinking extent is denoted by α (≤ 1).
2. A crosslinked myosin head generates a force whereas an uncrosslinked head generates no force.
3. A crosslinked head in a myosin molecule with both heads crosslinked to actin generates less force than a crosslinked head in a myosin molecule with the other head uncrosslinked. The ratio of the former force to the latter is denoted by p (≤ 1).

From the first assumption, the fraction of myosin molecules with two heads uncrosslinked is given by $(1-\alpha)^2$. The fraction of myosin molecules with one head crosslinked and the other head uncrosslinked is given by $2\alpha(1-\alpha)$. The fraction of myosin molecules with both heads crosslinked is given by α^2. From the second and third assumptions, therefore, the force (ΔF) produced in a partially crosslinked muscle fiber is given by

$$\Delta F = A\left[\alpha(1-\alpha) + p\alpha^2\right], \qquad (1)$$

where A is a proportionality constant. By fitting Eq. (1) to the data of closed circles in Fig. 5 with a non-linear regression (simplex) method [16], we obtained 3.15 kg/cm^2 for A and 0.11 for p. The fitted equation is shown by the filled curve in Fig. 5. This equation nicely fits the force data. The small value obtained for p means that when the two heads of a myosin molecule are crosslinked to actin one head in the molecule generates only about 10% force of that generated by a crosslinked head of a myosin molecule with the other head uncrosslinked. The value of 3.15 kg/cm^2 obtained for A in Eq. (1) is close to a maximum force which an uncrosslinked skinned muscle fiber from rabbit produces in a contracting solution at a physiological ionic strength. The good fit of the model based on the above assumptions with the data in Fig. 5 indicates that uncrosslinked myosin heads do not generate rigor force upon attachment to the filament after MgATP depletion at 0.5 M KCl. It should be pointed out that uncrosslinked myosin head in partially crosslinked fibers do generate rigor force upon MgATP depletion at 80 mM KCl as shown in Fig. 3 and Fig. 4.

Bershitsky and Tsaturyan [10] have proposed an explanation similar to that described above for their observation that a smaller force is produced in a partially crosslinked fiber by a temperature-jump in a high-salt MgATP solution when myosin head crosslinking exceeds about 50%.

As was pointed out in Section 3, there is another possible explanation for the bell-shaped curve in Fig. 5. This is that one uncrosslinked head of a myosin with the other head crosslinked generates force by binding to actin, whereas the latter crosslinked head of the myosin does not generate force, upon MgATP depletion at 0.5 M KCl. If this is assumed, the force

(ΔF) produced in a partially crosslinked fiber is given by

$$\Delta F = B[\alpha(1-\alpha)] \quad (2)$$

where B is a proportionality constant. ΔF in Eq. (2) is symmetrical for α around $\alpha = 1/2$, and $\Delta F = 0$ at $\alpha = 0$ and $\alpha = 1$. Eq. (2) is hence not in agreement with the force data (filled circles) in Fig. 5 (the fitted curve of Eq. (2) not shown) as well as Eq. (1) is. Moreover, this alternative explanation assumes the force generation by the uncrosslinked head of a myosin upon its binding to actin at 0.5 M KCl where the ATPase activity of uncrosslinked heads is not activated by actin [7]. This is against a general biochemical belief that the actin-activated ATPase activity is closely coupled with force generation by myosin crossbridges. Therefore, we conclude that this alternative explanation is not likely and that the explanation expressed by Eq. (1) is more likely.

4.4. Relation to other muscle studies

A good deal of biochemical knowledge about EDC crosslinking of myosin to actin is now available. A concise and comprehensive summary about this is given in a paper by Bershitsky and Tsaturyan [10]. Crosslinked myosin crossbridges in a high-salt MgATP solution produce both work during small-amplitude length oscillations [8] and tension transients immediately after small length changes are given [10], which are similar to the tension transients observed with intact muscle fibers by Huxley and Simmons [15]. Crosslinked crossbridges which have initially been stressed in rigor, "relax" upon binding of ATP with a rate similar to that of the ATP-induced dissociation of uncrosslinked myosin heads from thin filaments [9]. Kinetic properties of crosslinked crossbridges, which have been studied so far, are thus similar to the corresponding kinetic properties of uncrosslinked crossbridges in muscle. The results obtained with partially crosslinked fibers, taken together, indicate that the mechanism of the force-production by myosin crossbridge attached to actin does not involve concomitant translocation of the myosin head from one actin monomer to the other along the thin filament.

References

[1] H.E. Huxley, Science (Washington), 164 (1969) 1356.
[2] A.F. Huxley, J. Physiol. (London), 243 (1974) 1.
[3] E. Eisenberg and T.L. Hill, Science (Washington), 227 (1985) 999.
[4] V. Lombardi, G. Piazzesi and M. Lanari, Nature (London), 355 (1992) 638.
[5] M. Irving, V. Lombardi, M.A. Ferenczi and G. Piazzesi, Nature (London), 357 (1992) 156.
[6] D. Mornet, R. Bertrand, P. Pantel, E. Audamard and R. Kasab, Nature (London), 292 (1981) 301.
[7] Y.-P. Huang, M. Kimura and K. Tawada, J. Muscle Res. Cell Motil., 11 (1990) 313.
[8] K. Tawada and M. Kawai, Biophys. J. 57 (1990) 643.
[9] Y. Emoto, K. Horiuchi, K. Tawada and K. Yamada, Proc. Natl. Acad. Sci. USA, 92 (1995) 1461.
[10] S.Y. Bershitsky and A.K. Tsaturyan, Biophys. J., 69 (1995) 1011.
[11] K. Tawada, Y.-P., Huang and Y. Emoto, In Muscle Energetics, Alan R. Liss, New York, 1989, p. 37.
[12] K. Tawada and M. Kimura, Biophys. J., 45 (1984) 593.
[13] K. Tawada and M. Kimura, J. Muscle Res. Cell Motil., 7 (1986) 339.
[14] E. Rome, J. Mol. Biol., 65 (1972) 331.
[15] A.F. Huxley and R.M. Simmons, Nature (London), 233 (1971) 533.
[16] W.H. Press, B.P. Flannery, S.A. Teukolsky and W.T. Vettering, Numerical Recipes in Pascal, Cambridge University Press, Cambridge, 1989.