Vol. 200, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1994 Pages 59-64

# ACTIN MAY CONTRIBUTE TO THE POWER STROKE IN THE BINARY ACTOMYOSIN SYSTEM

Enrico Grazi, Ermes Magri, Christine Schwienbacher and Giorgio Trombetta

Istituto di Chimica Biologica, Università di Ferrara, Ferrara, Italy

Received February 21, 1994

At the physiological protein osmotic pressure, the angle formed between the long axis of the actin monomer and the pointed end of the filament axis is roughly 61° in F-actin and about 90° in the myosin subfragment 1 - decorated F-actin. This implies that, in the course of the contractile cycle, actin itself contributes, by about 4 nm, to the displacement of the actin filament toward the center of the sarcomer. 

The sliding filament model of muscle contraction, proposed independently by A.F. Huxley and R. Niedergerke (1) and by H. E. Huxley and J. Hanson (2), has recently received strong support by the demonstration that myosin head movements are synchronous with the elementary force-generating process (3). In this model actin plays a passive role and myosin is the motor unit. The role of myosin is indeed confirmed by the observation that MgATP binding and hydrolysis change the structure of myosin subfragment 1 (S1) so that one end of it moves at least 3.9 nm (4). We present evidence that, in the actomyosin binary system, actin itself may contribute to the power stroke. In fact, at the physiological protein osmotic pressure, the angle (alpha), formed between the long axis of the actin monomer and the pointed end of the filament axis, is roughly 61° in F-actin and about 90° in the myosin subfragment 1 -decorated F-actin. This implies that, in the course of the con-

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tractile cycle, actin itself contributes, by about 4 nm, to the displacement of the actin filament toward the center of the sarcomer.

## MATERIALS AND METHODS

G-actin and myosin subfragment 1 from rabbit muscle were prepared and their molar concentrations determined as previously described (5). Buffer solutions contained per 1000 g of water : KCl 0.1 mole; triethanolamine, 0.01 mole, $MgCl_2$, $NaN_3$, 2-mercaptoethanol, 2 mmoles each; ATP 0.2 mmole. ATP was omitted in the experiments with the S1-decorated F-actin rigor complex. pH was 7.45. The osmotic pressure associated with poly(ethyleneglycol) solutions (up to 5 g per 100 g of water) was measured by means of osmometers equipped with UH 100/25 Schleicher and Schuell membranes, Mr cutoff 25,000. At larger poly(ethyleneglycol) concentrations measurements were made directly with a pressure gauge. The osmotic pressure of protein systems was measured using a "secondary osmometer" : protein solutions (1 ml) were equilibrated by dialysis against buffer solutions (100 ml) supplemented with poly(ethyleneglycol) 40,000. Cellulose dialysis tubing (Mr cutoff 6000) was purchased from Medicell International. Protein concentration was determined as previously described (6).

## RESULTS AND DISCUSSION

F-actin solutions are concentrated by equilibration against increasing osmotic pressure, generated by increasing concentrations of poly(ethyleneglycol) 40,000. Beyond a given osmotic pressure ($10^5$ dynes/$cm^2$), bundles of hexagonally packed actin filaments are formed. Under these conditions the radius of the hydrated actin filament is easely related to the concentration of actin by the following formula (6) :

$$r^2 = \frac{(0.718 \times Mr \times m) + 1000 + 5}{m \times N \times 2.73\times10^{-7} \times 2\sqrt{3}} \qquad (i)$$

where 0.718 is the partial specific volume of F-actin, Mr is 42,000 daltons, the molecular mass of actin, m is the number of moles of actin per 1000 g of water; 1000 and 5 are, respectively, the volume (in ml) of water and salts; N is the Avogadro number

and $2.73 \times 10^{-7}$ cm is the axial repeat of the actin monomer along the genetic helix (7). As calculated from equatio (i), the diameter of the hydrated actin filament decreases from 9.0 at $10^5$ dynes/cm$^2$ to 6.8 nm at $9 \times 10^6$ dynes/cm$^2$ (6) and Fig. 1. These figures are not in disagreement with the figure of 9.5 nm, proposed by Holmes et al. (8) for actin filaments at the approximate actin concentration of 1.84 mmolal(9), i.e. at a protein osmotic pressure lower than $10^4$ dynes/cm$^2$ (Fig. 1). The decrease of the diameter of the actin filament is explained by the decrease of the angle (alpha), that, in the model of Holmes et al. (8), is roughly 90°. (The alternative explanation of a large decrease of the partial specific volume of the actin monomer is untenable, because of the relatively small pressure involved in our experiments). On these premises the value of

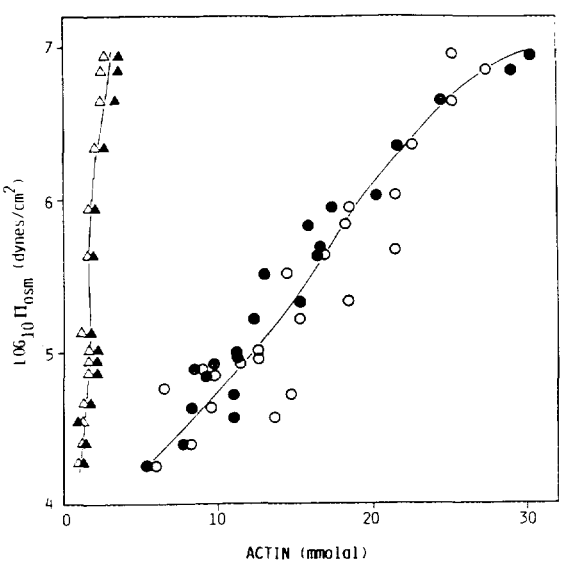


Fig. 1. Osmotic pressure of F-actin (O,●) and of the S1-decorated F-actin rigor complex (Δ,▲) solutions, as a function of actin concentration, measured by equilibration against poly(ethyleneglycol)40,000 solutions. The concentration of actin in the S1-decorated F-actin rigor complex is calculated on the basis of an equivalent weight of 152 kDa. Protein concentration calculated from the dry weight (filled symbols) and from the absorbance at 290 nm (empty symbols). Temperature is 22°C.

the angle (alpha), at different osmotic pressures, can be calculated by the following formula :

$$\frac{\text{diameter of the actin filament (nm)}}{9.5 \text{ nm}} = \frac{\text{sen (alpha)}}{\text{sen } 90°} \qquad \text{(ii)}$$

As an example, at $1.8 \times 10^5$ dynes/cm$^2$ (the protein osmotic pressure in frog muscle (10), the diameter of the hydrated actin filament is 8.34 nm (Fig. 1) and the angle (alpha) is 61.5°.

Equilibration of the S1-decorated F-actin rigor complex, against increasing concentrations of poly(ethyleneglycol) 40,000, reveals that the molality of the complex remains approximately constant (1.7 mmolal) at the protein osmotic pressures between $10^5$ and $10^6$ dynes/cm$^2$ (Fig. 1). In the same range of pressures, the diameter of the S1-decorated actin filament is 22.16 nm, as calculated from equation (i), for a Mr of 152,000 daltons.

Cryo electron microscopy and helical image processing of F-actin and of S1-decorated F-actin reveal no significant difference in the alignement of the actin monomers, these being arranged, in both filaments, with their long axes almost perpendicular to the filament axis (11). From the three dimensional map of S1-decorated F-actin it is estimated that the maximum filament diameter is about 22.3 nm (11). This value, obtained at a protein osmotic pressure of approximately $10^4$ dynes/cm$^2$, matches with the value of 22.16 nm obtained at osmotic pressures ranging between $10^5$ and $10^6$ dynes/cm$^2$ (Fig. 1). It appears, therefore, that, in the S1-decorated actin filament, the angle (alpha) is roughly 90°, also at osmotic pressures between $10^5$ and $10^6$ dynes/cm$^2$. These findings show that, at $1.8 \times 10^5$ dynes/cm$^2$ (the protein osmotic pressure in frog muscle (10), the angle (alpha) is 61.5° in F-actin and roughly 90° in the S1-decorated F-actin. Thus, the formation of the S1-decorated F-actin rigor complex, causes the sliding of the actin filament with respect to the myosin filament, the actin filament being pulled

toward the center of the sarcomer. The sliding of the actin filament is approximately measured by the expression :

Maximum radius of the S1-F-actin complex x (cos $alpha_F$ - cos $alpha_{FS}$)= sliding (iii)

where $alpha_F$ and $alpha_{FS}$ are the values of the angle (alpha) in F-actin and in S1-decorated F-actin, respectively. At $1.8x10^5$ dynes/$cm^2$, the sliding equals :

(cos 61.5° - cos 84°) x 11.5 nm = 4.15 nm

The contribution of both actin and the myosin head is illustrated in fig. 2. It is assumed that each active cross-bridge stro-

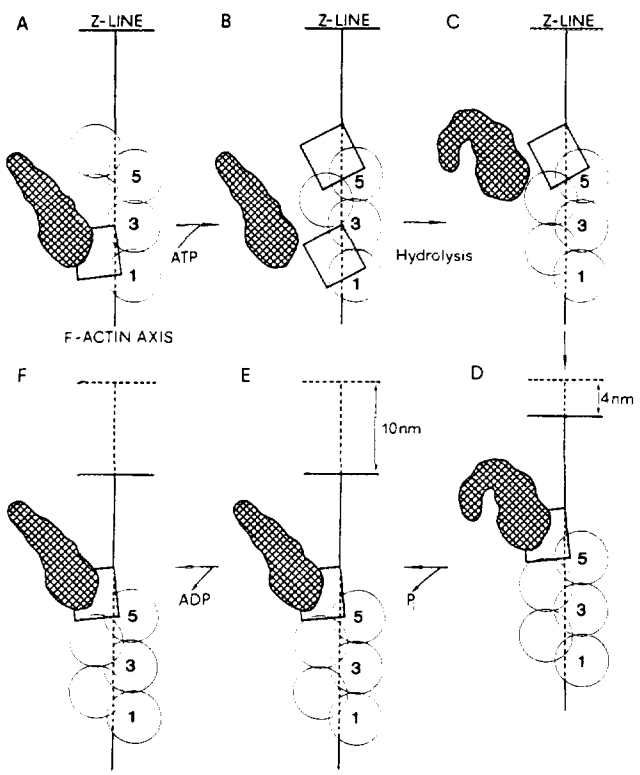


Fig. 2. The contractile cycle incorporating the proposed involvement of the myosin head and of the actin monomer. The actin monomers "2" and "6", involved in the interaction with the myosin head, are represented as rectangles, the other actin monomers as circles. The angle formed between the actin monomer and the pointed end of the filament axis is 84° in the rigor complex and 61.5° in F-actin.

ke involves an axial shift of 10 nm, the distance between an actin monomer and the next but one on the same strand (12). In the rigor complex formed between the myosin head and the actin monomer "2" the angle (alpha) is 84° (step A), when the complex is dissociated by MgATP the angle (alpha) becomes 61.5° (step B). Following the hydrolysis of MgATP, the distal part of the myosin head moves toward the proximal part (4) and gets into close proximity to the actin monomer "6" (step C). The following two steps D and E are represented separately only for sake of clarity. The formation of the activated complex and the following release of orthophosphate cause the displacement of the Z line, toward the center of the sarcomer, by 10 nm. A displacement of 4 nm is produced by the shift of the angle (alpha) from 61.5° to 84° (step D), the remaining shift is due to the conformational change of the myosin head (step E). Step F closes the contractile cycle with the formation of the rigor complex.

ACKNOWLEDGMENTS: This work was supported by the grants 92/40/05/15 and 92/60/05/035 of the Italian Ministero dell'Università e della Ricerca Scientifica and from the grant 93.04459.CT04 of the CNR.

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