Biophysical Chemistry 41 (1991) 237–251
Elsevier Science Publishers B.V., Amsterdam

BIOCHE 01590

The molecular basis of chemomechanical coupling in muscle and in other biological engines

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(Received 11 March 1990; accepted in revised form 27 March 1991)

Abstract

It is argued that the force driving muscular shortening (ψ) differs from that (ϕ) responsible for rigor tension generation. ψ is associated with ATP-induced dissociation of actomyosin (a.m.), whereas ϕ is due to an isomerization reaction of a.m., following the hydrolysis of ATP. Both forces are intimately coupled with appreciable changes in the structure of the hydration shell of a.m., mainly at the interface between the two proteins, which involve the release of stored energy. When an active muscle is allowed to shorten freely, ψ gives rise to a sliding distance (s.d.) Δl_1 which differs in character and in magnitude from the s.d. (Δl_2) observed when a muscle which had developed rigor tension isometrically is released. The maximal values of the two forces (ψ_0 and ϕ_0) as well as Δl_2 are calculated on the basis of experimental data. The forces and their corresponding s.d.'s are related through the standard free energies of the chemical reactions which are responsible for them. It is claimed that the same mechanochemical (m.c.) mechanisms operate also in all microtube-based locomotion and force-generation systems and, furthermore, that practically the same values of ψ_0, ϕ_0, Δl_1 and Δl_2 are shared by the two types of biological m.c. convertors.

Keywords: Chemomechanical coupling; Biological engines; Muscle dynamics

1. Introduction

A great effort has been made in order to elucidate the detailed mechanism of the interaction between myosin and actin in solution in the presence of MgATP [1]. The main purpose of that endeavor has been to find the step in which tension is developed when this reaction occurs in muscle (the so-called "power stroke"). Most of the investigators in the field believe that the force is generated by actomyosin (a.m.) complexes, with or without the decomposition product(s) of ATP. This force should be responsible for isometric tension. However, at this point it has been taken for granted that this is also the driving force for isotonic contraction. This sounds quite logical: after all, which other force could lead to shortening? If we want an isometrically contracting muscle to shorten, all that we have to do is to release it. The very use of the term "contraction" (or "contractile process") for both shortening and tension generation implies that they are both due to the same force i.e., the so-called "contractile force". The development of force by the a.m.

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complexes in a stimulated muscle held at a constant length must be due to some conformational change which is not allowed to proceed freely since the complexes form part of a continuous three-dimensional protein network. It has quite generally been assumed that if such a muscle is released under zero load, the complexes will be free to undergo the full transformation which will then lead to a translational movement of the actin filaments towards the center of the sarcomere while the myosin heads are firmly bound to actin. The most important finding of the originators of the sliding filament theory, A.F. and H.E. Huxley, was that, contrary to the belief which then prevailed, actin and myosin form two separate sets of filaments in relaxed muscles. From then on, muscular contraction could not anymore be considered to be the outcome of a conformational change similar to that of the continuous macromolecular network of a rubber band which had been stretched and then released. In spite of that, it has probably been psychologically difficult to extend the notion of separate filaments to a shortening muscle, and everybody has taken it for granted, without considering any other possibility, that the myosin heads must be firmly bound to actin in order that, and while, the actin filaments are translocated. Myosin was "permitted" to be detached from actin only for the sake of binding to another actin submit to be pulled later on. Nobody has ever thought that muscle might take advantage of the fact that it contains two separate sets of filaments and allow movement to take place while the two proteins are sliding past each other, the force responsible for movement having been generated by the very dissociation of complexes formed earlier by the two proteins. One could then reverse the argument and say that such a mechanism was the reason for the very existence of separate actin and myosin filaments rather then filaments made of actomyosin complexes as believed originally by Albert Szent-Györgyi and others.

The picture visualized by most scientists has been one in which the myosin heads undergo a rotation. With this view in mind, the maximum distance covered in an isotonic contraction by an actin filament following the breakdown of one ATP molecule (the "sliding distance", s.d.) should be close to the length of a myosin head i.e., about 20 nm. Unfortunately, despite many years of extensive work, no clue has been found for such a rotation [2]. Moreover, it has been shown that, for an un-loaded muscle, the s.d. seems to be larger than 100 nm, i.e., at least five, or maybe even seven, times larger than the anticipated translocation [3]. We are thus forced to conclude that one or more of the arguments or assumptions underlying the "swinging crossbridge" theory must be wrong.

It is clear that, for the s.d. to be that large, the myosin heads and the actin filaments cannot be firmly bound together when the s.d. is covered i.e., the a.m. complexes must be either fully dissociated or be rather loose [4]. However, dissociation of the force-generating complex must immediately bring about the abolishment of the "contractile" force which has so far been believed to be responsible not only for isometric tension generation but also for shortening. How could shortening take place without the "contractile" force? Since, nevertheless, movement occurs, and with it possibly also mechanical work performed, what are the driving force and the energy source for these processes? One may also ask the question: would it not be a waste to spend the free energy of hydrolysis of the "energy-rich" ATP molecules for the generation of a force which would subsequently be abolished prior to movement? And, finally, what makes the myosin heads and actin separate?

In the following, an attempt will be made to answer these questions on the basis of the idea that two different mechanical forces, associated with two different chemical reactions, are responsible for tension generation and for movement.

2. Analysis of the molecular mechanical forces and of movements in active and in rigor muscles

If, indeed, the dissociation of a.m. (after, or maybe before, the "contractile" force-generating step) is the origin of movement and work, then it must be also the source of the energy to be transformed into mechanical work or to be dissi-

pated as a sort of frictional heat in the case of an un-loaded shortening. What could cause the dissociation of the a.m. complex and give rise to the liberation of the free energy needed? Conventional chemical thinking would suggest, as the only possibility, that this is induced by ATP:

$$M\text{–}A + ATP \rightleftharpoons M\text{–}ATP + A \qquad (1)$$

where M and A represent, respectively, myosin and actin. This would be in line with the fact that this reaction involves a free energy drop which is larger than that accompanying any other stage in the enzymic cycle and is comparable to the full free energy of hydrolysis of ATP [5]. However, even though the values are similar, they are not related to each other, and the free energy of dissociation by ATP of a.m. is not part of the free energy of ATP hydrolysis in water; this is a reaction in which the proteins myosin and actin participate, in addition to ATP which is not hydrolyzed at all.

It is obvious that the performance of work requires an energy source. However, does the same apply also to the development of tension? Upon considering the various stages of the kinetic schemes which have been proposed for the ATP hydrolysis cycle, it has been hoped that the "power stroke" will be associated with a relatively large drop in free energy. Quite disappointingly, none of the steps involving a.m. complexes appeared to be such. This should not be surprising since the whole idea is mistaken: a mechanical force is not mechanical work and its value has, therefore, nothing to do with the magnitude of energy changes. Force and energy have different dimensions and, therefore, it does not make any sense to "compare them: a "small" (in our eyes) free energy change can lead to the development of a "large" mechanical force, and *vice versa*. In other words: true that the breakdown of ATP must precede isometric tension generation. However, what matters is the very breakdown and not the energy changes associated with it or with subsequent steps in the enzymic cycle. The purpose of splitting is to enable the formation of the proper complex between myosin and actin. Once binding has taken place, force can eventually be developed. The free energy change associated with the force-generating step should therefore not be considered as the energy source of the force. On the other hand, mechanical work and/or "friction" are energetic processes and their energy source appears to be the dissociation of a.m. by ATP, which by itself does not involve any splitting or hydrolysis of ATP.

How can the liberated free energy give rise to relative movement of the two proteins? A possibility might be that most of this energy is converted into kinetic energy of the myosin heads, thus enabling them to travel a distance of 100 nm or more along the conjugated actin filaments in an unloaded muscle (or less if the muscle is loaded). In other words: chemical energy is directly converted into mechanical energy. The velocity of movement of a myosin head past the corresponding actin filament must gradually decrease due to "viscous" resistance or, more precisely, to the mutual repulsion of the electrical double layers of the two negatively charged proteins (Debye–Hückel forces), to mutual repulsion of the hydration shells and to the electroendoosmotic effect associated with movement. The exact path traversed by the myosin heads should also be dictated by the dynamic interplay between these forces. In addition, a chemical factor should operate: the myosin-bound ATP must be hydrolyzed within a short time, giving rise to myosin-ADP-P_i and to its derivatives which can "chemically" bind to actin. Movement will then come to a standstill. The distance covered by the heads following the dissociation of their original complex with actin i.e., the sliding distance, would then be determined (a) by the initial value of the kinetic energy of the myosin heads, which is assumed to be close to the free energy change of the actomyosin-dissociation reaction; (b) by the electrical and hydration forces operating between the moving heads and the actin filaments, and (c) by the lifetime of the M–ATP complex and the affinity of its various decomposition products to actin. The movement of the myosin heads must, according to this picture, be smooth and with no fluctuations and the heads do not have to make "repetitive interactions" with actin while they split one ATP molecule. It is obvious the s.d. will decrease when an external load is applied and

apparently vanish under isometric conditions (see below). Since electrical and hydration forces are involved, the s.d. is entitled to change when the ionic strength or the temperature are changed or if water is partialy replaced by an organic solvent. No surprise, therefore, that whereas for different muscles under physiological conditions the maximal velocity of shortening, V_0, is proportional to the ATPase activity of the corresponding a.m. [6], the value of V_0 for a muscle at 20% ethylene glycol was found to be only 5% that in water, while the ATPase activity was diminished by only 40% [7]. In all probability, the high sensitivity of the value of the s.d. to the change in the medium reflects the most important role of the hydration shells in the generation of movement and this shall be elaborated on later. Since, by definition, the s.d. is equal to the distance covered per unit time by a gliding actin filament divided by the number of ATP molecules hydrolyzed for that purpose, we should have expected that a drop of 40% in the rate of ATP hydrolysis would lead to the same drop in V_0 if the s.d. was not affected. The diminution of V_0 by a factor of 20 then suggests that the value of the s.d. in 20% ethylene glycol is 1/12 that in water. Since a decrease in ionic strength causes a decrease in V_0, in spite of the increase in the ATPase activity, we can anticipate that the value of the s.d. must increase with increasing ionic strength.

Which forces hold the myosin heads in the close vicinity of actin while they are moving? It makes sense to believe that the binding is purely electrostatic and does not involve hydrogen-bond formation as in the "static" complex. In all probability, mono-valent counterions are displaced from the F-actin double helix by positively-charged amino-acid residues on the myosin head surface. The cations released [8] (K^+, Na^+, Mg^{2+}) are diluted and this should give rise to a temporary local entropy of dilution. However, the cations displaced from in front of the moving head must be replaced by others binding to the F-actin behind the head. The latter can thus be considered to be sliding over the actin on an isopotential surface. Hence, there is no thermodynamic barrier to the sliding [8]. It should be most interesting to analyze the effect on shortening of a possible displacement of Ca^{2+} ions (bound to actin (in thin filament-regulated) and to myosin (in myosin-regulated) muscles): it might give rise to a reciprocal relationship between Ca^{2+} and the m.c. proteins— Ca^{2+} causing movement by binding to the proteins, while movement affecting the very binding of Ca^{2+}. Indeed, temporary dissociation of the movement-inducing Ca^{2+} ions due to movement would be in line with the Le Chatelier principle of action and reaction according to which a system reacts to a change imposed from the outside by undergoing a process which will lead to the diminution of the perturbation. In conclusion: regulation in a.m. systems is a feedback process. Possible deactivation of a segment of a thin filament in the leading edge of a myosin head might, in principle, lead to a sort of stepwise or wavy shortening.

The relationship between a myosin head and a neighboring actin filament can thus assume two different forms: (a) site-specific rigor complex formation in the classical sense, in which a myosin head is bound to one (or maybe two) actin subunit(s) in an equilibrium reaction (a "static" complex") and (b) a "dynamic", non-equilibrium, complex in which the head moves continuously from one actin subunit to another until it is halted by forming an equilibrium complex with an actin subunit which is 100 nm or more away from the subunit with which it had formed such a complex before it was released by ATP. There is no reason to believe that the myosin heads should assume the same shape and/or conformation in the static and in the dynamic complexes.

From what has been said above it is clear that the value of the s.d. should be dictated by a combination of physical and chemical factors, none of which is directly related to the geometrical parameter named the length of the myosin head. There is, therefore, no reason whatsoever for the s.d. to be equal to about 20 nm (swinging crossbridge theory).

What is the molecular mechanism by which the dissociation of a.m., induced by ATP, causes the ejection of the myosin head possessing kinetic energy? It has recently been found [9] that when DNA molecules are brought close to each other a

repulsive force develops, the value of which is independent of ionic strength. This force has been ascribed to the compression of the hydration layers of the DNA molecules. Such a force should be operating also when actin and myosin form a "static" complex in which the binding of the two proteins by hydrogen bonds and by salt linkages must overcome the repulsive force between the hydration shells, thus leading to the compression of the latter. The compressive force should be transmitted to the protein backbones so that they, as well as the fused hydration shells, will store energy. This potential energy should be directly converted into kinetic energy of the two proteins the moment the bonds stabilizing the complex are broken, e.g. by ATP.

With this mechanism in mind, it is obvious that, if the myosin heads are incapable of moving freely, e.g. by forming part of a myosin filament or by being immobilized on a glass surface, the actin filaments with which they interact will undergo a translational movement past the myosin heads. The decompression induced by ATP might well lead also to a twist and stretch in the actin filaments. On *a priori* grounds, there is no reason to assume that conformational changes, rotation etc. should be limited to myosin only. The sliding of a myosin head past an actin filament discussed above is a relative movement and actin, just like myosin, is entitled to acquire potential (elastic) as well as kinetic energy when an a.m. complex is dissociated by ATP. In all probability, a major reason for the neglect of actin as an active partner for m.c. activity by practically everybody except Oosawa has been the repetitive employment of the term "crossbridges" as the sole generators of tension and of movement. Another reason has probably been the fact that myosin is considered as a fibrillar protein, contrary to the globular G-actin, and this entitled it to undergo helix-coil transitions which are characteristic of fiber-forming and "contractile" proteins such as collagen and keratin. Another reason could be the observation that the length of the thin-filaments-containing I-band region of striated muscles remains paractically constant during shortening.

The ejection of myosin heads must be associated with a "pushing" force which is equal and opposite to the compressive force. It is clear that there is no reason for the value of this force to be equal to that of the force developed in an isometrically contracting muscle by an a.m. complex after it had hydrolyzed an ATP molecule and, as intact a.m., underwent a force-generating change. Let us try to get an idea about its magnitude. For this purpose, we shall assume that the free energy drop (ΔG) which accompanies the dissociation reaction of a.m. (eq. 1) is practically fully converted into kinetic energy. Assuming also, as a first approximation, that the force driving the movement of the myosin heads decreases linearly with the distance covered, we may write

$$-\Delta G/N_A = -\Delta G^\circ/N_A = 0.5\ \psi_0 \Delta l_1 \qquad (2)$$

where ψ_0 and Δl_1 denote, respectively, the initial value of ψ and the step distance, and N_A represents Avogadro's number. With -7.0 kcal/mol (-29 kJ/mol) [5] for the standard free energy change ΔG° and $\Delta l_1 = 100$ nm, we obtain $\psi_0 = 1.0$ pN. Since the value of Δl_1 is probably larger than 100 (e.g. 140) nm [3], ψ_0 could be as small as 0.7 pN.

We may use the above value of ΔG° also for the evaluation of the initial velocity, v_0, of the myosin heads from

$$-\Delta G^0/N_A = \tfrac{1}{2} m v_0^2 \qquad (3)$$

where m is the mass of a myosin head. Hence, $v_0 = 2.3 \times 10^4$ cm/s (with 110 kD as the molecular weight m of the myosin head).

In order to maintain macroscopic shortening, the "quantum" process

$$\text{M–A} + \text{ATP} \rightleftharpoons \text{M–ATP} + \text{A} \rightleftharpoons \text{M–ADP} \cdot \text{P}_i + \text{A}$$
$$\rightleftharpoons \text{M–A} + \text{ADP} + \text{P}_i \qquad (4)$$

must be repeated several times. It is clear that the net reaction, which is the ultimate energy source, is the hydrolysis of ATP which precedes "static" complex formation between myosin and actin.

Had we started from a muscle in rigor rather than from a relaxed muscle then the a.m. complexes would have already existed, and shortening could have occurred (and work obtained) also following the addition of a non-hydrolyzable sub-

stance which is capable of dissociating a.m., or ATP under conditions at which its splitting by myosin is inhibited. Movement would have then occurred without any hydrolysis reaction, i.e., without the utilization of a "high-energy" substance as a high energy source due to the splitting of a P–O bond which should actually require energy.

The dissociation of a dynein-microtubule complex is also accompanied by a large drop in free energy. Since ATP hydrolysis at the active site of dynein occurs with only a small change in free energy it has been concluded that the major fraction of the energy from net hydrolysis occurs with the rebinding of the dynein head(s) to the microtubule, the step coupled to movement. Again, as in the case of a.m., it has been taken for granted, without any further thought, that movement requires the tight binding of the heads to the linear polymer (thin filament or microtubule).

If we allow an isometrically active muscle to consume all its ATP and creatine phosphate, rigor occurs. In this state, the muscle continuous exhibiting tension, the value of which is quire close to that of the active muscle [10]. The tension maintained by a muscle in rigor is due to the forces developed by the a.m. complexes after each of them had broken an ATP molecule for the last time. The rigor tension is equal to the number of myosin heads interacting with actin per half sarcomere of a unit cross-sectional area times the molecular "contractile" force, ϕ_0. If we release such a muscle, in which actin and myosin form a continuous network, it will shorten slightly [10], the driving force for this shortening being ϕ_0 (see Fig. 1A). The change in length cannot exceed the length of a myosin head. The translocation of all the thin filaments in half a sarcomere should be equal to the translocation of a single thin filament as a result of the conformational change of a single a.m. complex. This is nothing else but the sliding distance which has so far been considered to be the same for both actively shortening and rigor muscles. It is clear that the value of this s.d. is determined by factors which differ profoundly from those governing the shortening of active museles at maximal velocity.

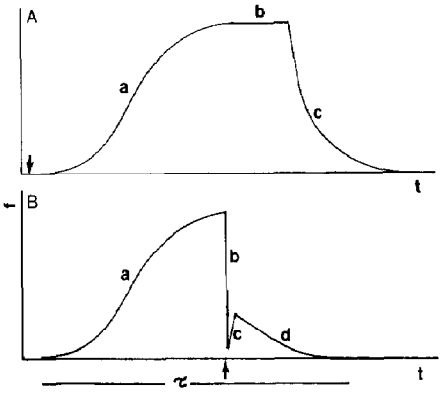

Fig. 1. (A) Time plot of the generation, maintainance and decline of the molecular force, f, in rigor. (a) Development of ϕ by an a.m. complex following the breakdown (marked by an arrow) of the last ATP molecule during an isometric contraction in which ATP is being depleted (ϕ_0 is the maximal value of ϕ); (b) maintained isometric rigor state; and (c) decline of rigor force following muscle release. (B) Time plot of the molecular force, f, in an isometric active state. (a) Generation of ϕ by an a.m. complex following the breakdown (marked by an arrow) of an ATP molecule; (b) drop of ϕ as a result of the dissociation of the complex by ATP (marked by an arrow); (c) "burst" of ψ, reaching the maximal value of ψ_0; and (d) Decline of ψ, associated with either limited or no movement of the myosin head past the thin filament. The areas under the plots of ϕ and ψ represent the molecular impulses β_1 and β_2, respectively.

Let us calculate this s.d. which we will designate Δl_2. According to White [10], the shortening necessary to return the tension in rigor muscle fibers to zero tension once they have contracted under isometric conditions is 0.7–1.5%. This variability in shortening is probably due to the possibility that some of the rigor complexes in a rigor muscle do not generate tension since their formation had not involved ATP breakdown. The s.d. associated with the tension- and the non-tension-generating complexes may, in principle, differ as they are the outcome of different conformational changes of different types of a.m. complexes taking place when the rigor muscle under tension is released. Assuming that any one of the values 0.7 and 1.5% might represent 100% of tension-generating a.m. complexes, we come out with two possible values for Δl_2:

$$\Delta l_2 = 0.7(\text{or } 1.5) \times 10^{-2} \times 10^3 = 7 \text{ or } 15 \text{ nm} \quad (5)$$

where 10^3 nm is the length of a thin filament. The second value is closer to the length of a myosin head (about 18 nm) and hence in better accord with the swinging crossbridge picture and we may, therefore, conclude that the value of 1.5% represents 100% of tension-generating a.m. complexes. This suggests that values lower than 1.5% indicate that part of the rigor complexes do not generate tension. Thus, the value of 0.7% might result from about 50% of the complexes being inactive.

Geeves and his collaborators [11], on the basis of pressure-relaxation studies of heavy meromyosin subfragment-1 (S1) and pyrene-labelled actin in solution, have proposed the model

$$M + A \rightleftharpoons (M\text{–}A)_1 \rightleftharpoons (M\text{–}A)_2 \qquad (6)$$

They have noticed that both isometric tension generation by muscle and the transition from a weakly $(A\text{–}M)_1$ – to a strongly $(A\text{–}M)_2$ – attached state in solution are inhibited by low temperature, high pressure, high ionic strength and the presence of ethylene glycol. This led them to the suggestion that tension development is associated with this reaction. One should, however, be aware of a fundamental difference between this reaction occurring in solution and the same process taking place in an isometrically "contracting" muscle: in solution the transition from state 1 to state 2 can occur freely, leading to a conformational change of the a.m. complexes and, presumably, to a relative movement of the myosin heads with respect to the actin filaments. However, in a muscle which is held at a constant length, the isomerization process is inhibited, and this is the origin of the tension which is observed. The drop in free energy which accompanies the transition cannot then take place and the a.m. complexes will store this energy in the form of potential chemical energy. This will be the situation also in rigor muscle fibers under tension. If such fibers are released, the equilibrium $(M\text{–}A)_1 \rightleftharpoons (M\text{–}A)_2$ will be shifted in favor of state 2, as it is in solution, and shortening will take place. The stored free energy will then be liberated, thus enabling the muscle to do mechanical work if a load smaller than the original isometric force is attached. Assuming that a large part of this energy is utilized for the performance of work when the load per a.m. complex assumes an optimal value somewhere between ϕ_0 and zero, we may write, as a first approximation,

$$-\Delta G^0/N_A \simeq \tfrac{1}{2}\phi_0 \Delta l_2 \qquad (7)$$

where ΔG° represents the standard free energy change of the isomerization reaction. The value of ΔG° can be calculated from the values given by Geeves and his associates [11] for the equilibrium constant of the isomerization reaction and it amounts to -3.26 kcal/mol at 20°C. Substituting the value of 18 nm for Δl_2 eq. (7) we then obtain

$$\phi_0 = 1.27 \text{ pN} \qquad (8)$$

What happens in an isometrically active muscle if an ATP molecule interacts with an a.m. complex after this had generated force? In this case, as in a shortening muscle, the complex will undergo dissociation which would immediately lead to the abolishment of the force ϕ_0. The second force, ψ_0 will now operate and eject the myosin head along the actin filament. The distance covered will be restricted due to the fact that the myosin filament, to which the head is linked via heavy meromyosin subfragment 2 (S2), is not free to move (assuming all the sarcomeres have the same length; otherwise some will shorten at the expense of others). The static complex formed when movement is terminated may then undergo many cycles of dissociation and association, accompanied by the abolishment of ϕ occurring concurrently with the development of ψ. The latter will be incapable of giving rise to the sliding of the head away from the actin subunit, in the vicinity of which it will stay until the end of the isometric stimulation. Does this ψ contribute to the isometric tension P_0 even though it operates while the heads are not firmly bound to actin? The answer is presumably yes since S1 i.e., isolated myosin heads, is mechano-chemically potent [12–22]. The kinetic energy of heads liberated by ATP must in the long run be transferred vectorially to the surrounding water molecules, thus creating a pressure gradient to be perceived as part of the isometric tension. ψ must gradually decay until the head forms a "static" complex

with the same actin subunit (see Fig. 1B). The average force, $\bar{f}$ developed by a myosin head will be given by

$$\bar{f} = \frac{1}{\tau}\left[\int \phi \, dt + \int \psi \, dt\right] \equiv \frac{1}{\tau}(\beta_1 + \beta_2) \equiv \frac{1}{\tau}\beta \equiv \beta v \quad (9)$$

where β_1 and β_2 denote the areas under the plot against time of ϕ and of ψ, respectively, τ is the combined lifetimes of the force-generating complexes and v is the molecular rate of ATP hydrolysis. β_2 and β_2 are the mechanical impulses due to ϕ and to ψ, respectively (cf. [23]).

It should be stressed that the average force $\bar{f}$ is not simply the sum of the ϕ and ψ but rather proportional to the sum of the time integrals of ϕ and ψ. The lifetimes of the static and the dynamic complexes also should determine the value of $\bar{f}$ and, therefore, of the isometric tension, P_0. Thus, there is no reason why the values of β_2 and of $\bar{f}$ should be the same at the beginning of tension development (i.e., when the myosin heads may slide over a limited distance) and under steady state isometric "contraction" (i.e., when a head must interact with the same actin monomer time and again). It is, therefore, not impossible that the isometric tension will not be proportional to the number of "active" crossbridges during isometric tension development or relaxation. This could account for the observation that while the longitudinal stiffness of single muscle fibres during steady tetanic "contraction" is proportional to the overlap length of thick and thin filaments (and to the isometric tension) [24], this is not so during force development and relaxation [25].

P_0, is thus given by

$$P_0 = x\beta v \equiv \dot{n}\beta = \dot{n}(\beta_1 + \beta_2) \equiv P_{0,1} + P_{0,2} \quad (10)$$

where x is the number of myosin heads in half a sarcomere of unit cross-sectional area. The isometric tension is thus composed of two tensions, $P_{0,1}$ and $P_{0,2}$. The first is due to the "elastic" force ϕ associated with the actomyosin static complex and can, therefore, be exhibited only when actin and myosin form a continuous network (as in striated muscle). It is fully responsible for rigor tension. The second one is the compressive force ψ which drives the "rocket" movement within an a.m. dynamic complex. $P_{0,2}$ does not, therefore, require a continuous protein network and can be demonstrated in systems in which myosin or its proteolytic products do not form filaments or other aggregates (e.g. myosin I). These setups obviously cannot give rise to $P_{0,1}$.

The value of $\bar{f}$ can be derived by dividing the maximal isometric rigor tension by the number of myosin heads in half a sarcomere of unit cross-sectional area. Unfortunately, to the author's knowledge, the values of these two parameters have never been determined for the same muscle fiber. Since the largest maximal isometric tension reported is 40 kN/cm^2 (cf. [23]), and as the concentration of myosin in vertebrate skeletal muscles is probably close to 120 μM [26], the value of $\bar{f}$ turns out to be approximately 2.4 pN whereas a similar procedure, employing other data, gave 4.6 pN [23]. The similarity of the experimental value of 2.4 pN for $\bar{f}$ and of the value of 1.27 pN derived for ϕ_0 on the basis of eq. (8) is in line with the observation that rigor tension does not vary much from tetanic tension for the same muscle length [10].

Kishino and Yanagida [27] have recently measured the force developed by a single actin filament upon interacting with isolated head fragments of myosin immobilized on a glass surface. From this they calculated the force generated by a single myosin head, assuming that only 33–50% of the heads are properly oriented, to be 0.4–0.6 pN. This was claimed to be comparable with the maximal force developed per head in an isometrically contracting muscle, as taken from a paper by Oosawa (1 pN). It is disappointing to find out, however, that Oosawa had never measured the tension per head (he just stated that the force generated per thin filament is of the order of $3 \cdot 10^{-5}$ dynes or 300 pN) nor did he refer to any other work. Considering the values presented above for $\bar{f}$, the value found by Kishino and Yanagida for the force developed by S1 *in vitro* could be an order of magnitude *smaller* than the force generated per myosin head *in vivo*. It does not make sense to compare the rigorously determined value for the force generated by S1 *in vitro*

with an estimated value of the force developed by a myosin head *in vivo*. It may well be that in living muscle the myosin heads contribute only part of the tension observed and the rest might, for instance, originate from an helix-coil transition of S2. The same kind of transition in collagen fibers immersed in a concentrated LiBr solution may lead to the generation of an isometric force which amounts to about 4 pN per collagen triple-helical chain which is practically equal to the value of 4.6 pN presented above for $\bar{f}$ (see [23], and also [28] for other references).

If the above is true, then the creation of a much more efficient tension generator could well be one of the reasons for striated muscles to employ filamentous myosin rather than free, isolated myosin molecules. Another reason is obviously the fact that the effective concentration of aggregated, and therefore immobilized, myosin is much higher than that of freely floating single myosin molecules [12]. The possible conclusion that the tension generated by the heads constitutes only part of the tension *in vivo* fits quite nicely with the claim made recently by Lovell et al. [29], that skinned muscle fibers preincubated by a polyclonal antibody directed against S2 were able to sustain only 7% of the active isometric force generated by control fibers whereas Yanagida's force of about 0.5 pN corresponds to 11% of the value of 4.6 pN for $\bar{f}$ presented above. Furthermore, in principle, there is no reason why the force developed *in vitro* should match the force per head *in vivo*. It is hard to believe that the properties (e.g. electrostatic potential) of the surface employed for immobilization have no effect whatsoever on the value of the force measured in view of the common knowledge that isometric tension depends strongly on ionic strength. This could have been checked by the authors by using several types of surfaces. Hence, the claim that the *in vitro* and the *in vivo* values are comparable may be misleading and, therefore, any conclusions which might be derived on the basis of this claim may not be justified.

The importance of the work of Kishino and Yanagida, and that of Spudich and his collaborators [30] who have employed a similar technique, originates from the fact that it has redrawn attention to the possibility that S1 may be capable of generating tension and movement even if it is not linked to the core of a myosin filament. Many years earlier it was claimed that free S1, as well as free heavy meromyosin (HMM), are capable of generating tension [12–14], causing the shortening of muscle preparations in which the native myosin had previously been fully and irreversibly inactivated [12,15–17] and, *in vitro*, giving rise to superprecipitation [18] and to the contraction of actin fibers [19], whereas HMM can also induce active streaming of solutions containing F-actin and MgATP [20,21] and the translocation of isolated actin filaments in the presence of natural tropomyosin and MgATP, as evidenced from laser light scattering measurements of acto-HMM solutions [22]. Obviously, in all these cases the myosin heads do not form part of myosin filaments but one can derive from these experiments a much more general and far reaching conclusion, viz. that the heads do not need to be attached to anything, neither to a glass nor a nitrocellulose surface, nor to a phospholipid vesicle nor to anything else, a conclusion which cannot be arrived at on the basis of the elegant observations of Spudich, Yanagida and their associates. Needless to say that the interpretation of their results should not differ from that of the classical experiments of A.F. and H.E. Huxley who found that muscle shortening is the outcome of the interaction between separate actin and myosin filaments. Had Yanagida, Spudich et al. not been able to observe movement of F-actin past immobilized myosin heads then their conclusion would have been that the region on the heads required for contacting actin had been damaged or bound to the surface in the most non-specific process of "glueing", and the fact that translocation can still be detected simply indicates that such unfortunate events had not occurred. True that these experiments suggest that S2 as such may not be obligatory for tension generation but this conclusion had already been derived on from experiments employing free S1 and HMM [12–22].

3. Discussion and application to other biological engines

The analysis presented above leads us to the conclusion that one should distinguish between two different sliding distances: (a) Δl_1, which is the distance covered by a myosin head following the dissociation of its complex with actin by an intact ATP molecule which, in a macroscopically shortening muscle, is the distance associated with the breakdown of one ATP molecule, and (b) Δl_2 which is the translocation of the center of mass of a myosin head relative to the actin filament with which it forms a rigor complex following the release of a muscle exhibiting rigor tension. There is, therefore, no sense in talking about a "discrepancy" between the value of Δl_1 obtained by Yanagida and his collaborators (larger than 100 nm) and the value of Δl_2 expected on the basis of the idea that myosin heads undergo a rotation (about 20 nm), the ratio $\Delta l_1 : \Delta l_2$ being larger than 5 or maybe even 7. This apparent discrepancy has so far caused confusion and embarrassment: 20 nm appeared to be too short for some people whereas 100–140 nm has been too long for others. However, both numbers are right and none of them is too large or too small because each of them corresponds to a different parameter: Δl_1 is due to a "rocket" force, ψ, whereas Δl_2 is associated with the "elastic" force ϕ.

It is important to stress that the shortening of active muscles is not characterized by the value of Δl_2 i.e., by the sliding distance which has so far been considered to be one of the pillars of the swinging crossbridge theory and which is a characteristic of rigor muscles only.

As we have seen, ϕ_0, the maximal value of ϕ, is 1.27 pN whereas ψ_0 equals 0.7–1.0 pN or less. It is interesting to note that the values of ϕ_0 and ψ_0 are not much different and one may wonder whether this fact has any significance. The generation of ϕ_0 should not present any conceptual difficulties concerning the mechanism: the process is not fundamentally different from the development of tension by a collagen fiber, fixed at both ends, when immersed in a concentrated LiBr solution [28]. The situation is different, however, in the case of ψ_0 which is more intricate. However, thinking in terms of this force enables us to explain the finding that free S1 and HMM can induce tension generation and shortening in muscle preparations, translocation of actin filaments and active transport of water [12–22].

It is the present author's belief that much confusion has originated from the fact that many people were not clear or consistent about the very definition of the sliding distance: the s.d. as originally presented by H.E. Huxley [31] referred to the distance covered when each of the myosin [molecules] had hydrolyzed one ATP molecule i.e., he talked in terms of a concerted action of all the myosin molecules in half sarcomere. This was intimately linked with the swinging crossbridge model according to which movement occurs when myosin is tightly bound to actin. On the other hand, the value derived by Yanagida and his collaborators applies to a single myosin head translocating an actin filament following the hydrolysis of an ATP molecule. A concerted action is to be anticipated for a muscle shortening following the development of rigor tension since the myosin heads are part of a continuous three-dimensional protein network. This is not the case for an active muscle where the operation of the heads is asynchronous. The shortening following the hydrolysis of one ATP molecule by each of the heads must be equal to the product of the s.d. (i.e., > 100 nm) times the number of heads interacting with a thin filament. This amounts to thousands of nm's. Needless to say that this sounds alarming to the followers of the swinging crossbridge theory who are then forced to claim that only an extremely small fraction of the myosin molecule is active — a belief which is not necessary and does not make much sense.

The mechanochemical reactivity of a muscle fiber is characterized by two physical parameters: the maximal isometric tension, P_0, and the maximal velocity of shortening, V_0. On the basis of what has been said above, we may now claim that, on the molecular level, muscular activity is determined by two forces: ϕ which is responsible for rigor tension, and ψ which drives the sliding of myosin heads past the thin filaments in a shortening muscle. Both molecular forces contribute P_0. The first force is due to an isomeriza-

tion reaction of a.m. whereas the second is the outcome of the dissociation of a.m. by ATP. The generation of ϕ is preceded by the breakdown of ATP while ψ is associated with intact ATP which is hydrolyzed only after movement has commenced. It seems as if research workers have felt obliged to first pay (by ATP hydrolysis) for the generation of force and for locomotion. This appears to be true for ϕ but not for ψ, in which case one may say: "move now, pay later". P_0 is equal to the product of the number of myosin heads in half sarcomere with a unit cross-sectional area times the sum of the molecular impulses β_1 and β_2 due to ϕ and to ψ, respectively, times the molecular turnover rate of ATP (v), whereas V_0 is proportional to the number of half sarcomeres lying along a unit length of the muscle and to the number of times myosin heads interact with a given thin filament per second. As is well known, the value of P_0 does not vary much from one muscle to another. Differences are probably due mostly to a variability in the concentration of myosin facing actin. On the other hand, V_0 may vary by a factor of up to 200; however, as mentioned above, the ratio of V_0 and the specific enzymic activity of a.m. obtained from the same muscles is practically constant [6], the proportionality constant being proportional to the s.d. Δl_1 [23]. We may, therefore, conclude that different muscles share the same values for $\beta v = (\beta_1 + \beta_2)v$ and for Δl_1, and hence probably also for ψ_0 (since the equilibrium constant for the ATP-induced dissociation of a.m. is the same for different a.m.'s [6]) and for the initial velocity, v_0, of the myosin heads in shortening unloaded muscles. Since the value of the "geometrical" parameter Δl_2 is determined by the length of the myosin head, and as it is difficult to imagine that the latter varies much for myosin heads in different muscles, it makes sense to believe that Δl_2 is also a universal constant. On the basis of eq. (7) we may also argue that the values of ϕ_0 and of the free energy change for the a.m. isomerization reaction (eq. 6) should not vary much upon comparing different striated muscles. Note that Δl_1 and Δl_2 are not independent parameters since their values are determined by the corresponding ΔG° and force (ϕ_0 and ψ_0) values.

The model suggested above for the mechanism of movement involves real sliding, on the molecular level down to a single myosin head, whereas the sliding filament model refers to the movement past each other of myosin and actin filaments which is a statement of fact and, therefore, should neither be considered as a model nor as a theory.

The generally held assumption that the same force, the "contractile force", is responsible for both tension generation and movement originated, at least partly, from the very use of the same term, "contractility", in both cases. Since it is difficult to imagine a force being developed without the existence of a continuous three-dimensional protein network, it has been also impossible to think of shortening taking place when the myosin and actin are not tightly bound together during the molecular shortening process. This applies not only to the swinging crossbridge theory but also to most other theories. As we have seen above, one has to distinguish between the force driving movement and the force operating in a muscle brought into rigor isometrically. Sometimes our way of thinking becomes servant to words and concepts we ourselves have coined. This author therefore, suggests that we refer to muscle shortening as "shortening" rather than as "contraction" and, furthermore, that we stop talking of "contractile proteins", which does not make any sense and is so misleading.

From what we have said (eq. 2) it is clear that there is a relationship between the values of ψ_0 and of the free energy change, ΔG^0, of the ψ_0-generating reaction (eq. 1) which involves ATP. This is not so for ϕ_0, the value of which is not related at all to a free energy drop associated with the breakdown of ATP or with the release of its decomposition products. If, indeed, the tension-generating event involves pure actomyosin alone then the free energy change leading to ϕ_0 is that of an isomerization reaction of a complex between myosin and actin only. True that ATP, ADP and P_i had been involved in previous stages of the enzymic cycle but none of them is present during the force producing event. The reaction ATP $\rightarrow$ ADP + P_i had already occurred, with both ATP and ADP + P_i being present in the medium,

$$M + ATP \rightleftharpoons M\text{–}ATP \overset{A}{\rightleftharpoons} M\text{–}A + P_i + ADP \quad (11)$$

The isomerization reaction is due to the existence of two isomers of actomyosin, 1 and 2. The role of ATP appears to be to help actomyosin form the no. 1 isomer which can then spontaneously isomerize into the no. 2 state. In summary: while (intact) ATP is directly involved in the generation of the force ψ_0, neither it nor its decomposition products are present during the development of ϕ_0 which has so far been considered to be the only mechanical force operating in active muscle, namely the "contractile force". We may, therefore claim that tension generation in an isometrically contracting muscle is not intimately coupled with the breakdown of ATP while the force responsible for movement is directly coupled with a reaction which involves intact ATP which is, however, not a real chemical reaction in which covalent bonds are either formed or broken. We should, therefore, be careful and not apply the term "mechanochemical coupling" to muscular "contraction". The term "mechanochemical transformation" may, however, be employed for the overall process

$$\mathrm{M{-}ATP + A \rightleftharpoons M{-}A + ADP + P_i + tension}$$

$$\overset{\mathrm{ATP}}{\rightleftharpoons} \mathrm{M{-}ATP + A + movement} \quad (12)$$

which is a real chemical reaction, the ultimate energy source for any mechanical work done being the hydrolysis energy of ATP.

The energy source for shortening in active muscle is the reaction $\mathrm{A{-}M + ATP \rightarrow M{-}ATP + A}$ ($\Delta G^\circ = -7.0$ kcal/mol) while that leading to the shortening of muscle in rigor is $(\mathrm{M{-}A})_1 \rightarrow (\mathrm{M{-}A})_2$; $\Delta G_2^\circ = -3.26$ kcal/mol. The value of Δl_1 for the interaction between free S1 and F-actin in solution under physiological conditions does not probably differ much from the above value for muscle. However, Δl_2 is defined only for rigor muscle since it is a measure of the quantum of contraction of a continuous three-dimensional protein network.

A common denominator of ψ_0 and ϕ_0 is that both are associated with stored energies: ψ_0 is related to the compressive force between the hydration shells of the proteins and, therefore, to the resulting stored energy. ϕ_0 is due to the fact that the isomerization reaction of the actomyosin complex, i.e., a transition which should lead to the shortening of the muscle, is prevented by holding the muscle at a constant length, thus keeping the complex at a higher free energy state, which means that the a.m. complexes store extra energy. If we release such a muscle, without letting any ATP molecule interfere (i.e., the muscle had developed rigor tension) it will shorten as the outcome of the transition of the a.m. complexes into their lower free energy state. In analogy to a shortening muscle, in which energy can be stored in both the hydration layers and in the protein backbones, we may argue that, in isometrically "contracting" muscle also, energy is stored in the hydration layers of the complexes just as in the protein backbones. If stored energy is the origin of ϕ, as it is of ψ, we may speculate that changes in the hydration shells, induced by changing temperature, ionic strength or hydrostatic pressure or by replacing part of the water in the medium by another solvent, may affect the isomerization reaction and, therefore, in parallel, the values of ϕ_0 and of P_0. This has, indeed, been found to be the case by Geeves and his collaborators [11] who suggested that it may be taken as evidence that the isomerization of the actomyosin complex, which is obtained after ADP and P_i are released is, indeed, responsible for tension generation. Thus, the "power stroke" is not a step in which chemical energy associated with the breakdown of ATP or with the release of its products is liberated but rather the isometrization of an a.m. complex occurring after the processes involving ATP and its products are over. Since S1 and F-actin in solution are not restricted, it makes sense to believe that the isomerization in Geeves et al.'s experiments involves the sliding of the S1 molecules along F-actin i.e., the actin subunits in $(\mathrm{M{-}A})_1$ and in $(\mathrm{M{-}A})_2$ in eq. (6) are not the same.

From the hydrostatic pressure-dependence of the equilibrium constant of the isomerization reaction, Geeves et al. have evaluated the volume change for the transition between the two acto-S1 states and found it to be 110 $\mathrm{cm^3 \cdot mol^{-1}}$. As indicated by these authors, a volume change of this size implies a substantial rearrangement of the hydration sphere around the acto-S1 complex

which probably involves mainly the contact area between the two proteins. In other words: the water at the interphase appears to undergo a sort of "melting" or some other phase transition. Since the transition from the weakly to the strongly attached state is inhibited by low temperature, high pressure, high ionic strength and the presence of ethylene glycol, and as these changes also cause a diminution of isometric tension in muscle fibers, we may speculate that tension generation associated with the molecular force ϕ is the outcome of the above mentioned phase transition of the hydration shell at the interface between the myosin heads and actin in muscle. This might be the answer to the statement made by Geeves et al. that the exact relationship between the isomerization reaction and the force-generating event remains open.

Both molecular mechanical forces ϕ and ψ thus appear to be intimately connected with changes in the hydration shell at the interface: phase transition in the case of ϕ and dissociation of the compressed shell when ψ is generated. This is of course related to the fact that two proteins are the building blocks of the muscle machine, as well as of all other biological engines. We may, therefore, conclude that changes in the hydration shells of the proteins (probably including also tropomyosin and troponin) are part and parcel of the molecular mechanism of muscular contraction. This means that there is no sense in considering only conformational changes of the protein backbone(s).

As mentioned above, the ratio of V_0 and the specific ATPase activity of a.m. obtained from the same muscle, is constant. According to Bárány [6], the value of the ratio is the same for both smooth and striated muscles. Since the organization of the a.m. systems is profoundly different in the two types of muscle, we may conclude that the value of Δl_1 is a universal molecular constant, characteristic of the interaction between a single myosin head and an actin filament and, therefore, independent of the type of organization of actin and myosin in any kind of cell.

Needless to say that all the arguments and conclusions of this model should apply also to other biological engines such as dynein (or kinesin) + microtubules which share with the actomyosin systems many characteristics. In particular, it is important to mention the fact that the value of the maximal force developed by a dynein head intereacting with a microtubule (0.7–3.0 pN) [32] is strikingly similar to that of the force generated by a myosin head in muscle (about 2.4 pN). This strongly suggests the possibility that the force-generating mechanisms are basically the same for all biological engines and, furthermore, that energy storage in the fused hydration layers of the enzymic protein (myosin, dynein, kinesin etc) and the vectoriality guarantying filament (thin filament, microtubule and maybe also some intermediate filaments) is actually the driving force for movement and for tension generation in all these systems. According to our model, this will originate from similar transitions of the hydration layers at the interface between the interacting proteins which is, in turn, probably associated with similar protein interfaces. Since ATP serves as the energy source also for the translocation of microtubules and as the free energy liberated when a dynein (kinesin, etc.) – tubulin complex is dissociated by ATP does not differ much from that generating ψ_0 in muscle [5,33], it makes sense to believe that the values of the sliding distances Δl_1 for microtubule-based locomotions also do not differ much from that of active muscle.

Since the ratio myosin : actin in non-muscle cells is much lower than in muscle cells (while the concentrations of actin are comparable) and as many ATP- and actin-binding proteins are present in both types of cells, it is tempting to speculate that these proteins, just like myosin, interact mechanochemically with actin filaments in non-muscle cells, probably utilizing the above-mentioned mechanism, thus giving rise to movement, and also tension-generation when the filaments are fixed. Other processes which may turn out to be also m.c. are the polymerizations of actin and of tubulin. This may not be due to the growing end of the chain hitting a surface (e.g. a plasma membrane) thus generating a resisting force but to the repulsion of hydration layers. Another possibility is that G–G bonds at the end(s) of, or inside, F-actin chains are dissociated

by the action of ATP, giving rise to the ejection of monomeric actin or to the mutual repulsion and movement of the resulting two actin segments just like the relative movement of myosin and actin in muscle. As is well known, ATP is not obligatory for the polymerization of actin and it is, therefore, not impossible that the utilization of ATP during actin (and tubulin) polymerization *in vivo* is necessary for polymerization to become a m.c. process.

If, indeed, the values of the most characteristic mechanical (i.e., ϕ_0 and ψ_0) and geometrical (i.e., Δl_1 and Δl_2) parameters of the various biological engines will turn out to be similar, despite the differences in their protein constituents, then we shall be able to conclude that the role of mechanochemical proteins is to determine the character of the fused hydration layers which by dissociating from each other or by undergoing a "phase transition" due to an isomerization process, can generate mechanical forces.

When a repressor molecule (a protein) slides along a DNA chain towards its target site, the operator the so-called "sliding length" i.e., the distance the repressor molecule can slide past DNA without dissociation into solution (which is a measure of the lifetime of the non-specific binding of the protein to DNA before it hits the operator to which it sticks specifically) is a function of ionic strength [8] just like the s.d. in muscle (as we have seen above). The sliding in this case has been defined as a diffusion of the repressor (to which a myosin head might be considered as analogous) while non-specifically bound, in a one-dimensional random walk along the DNA molecule. This reduces the dimensionality of the operator-search process, and could, under some conditions, lead to an accelerated rate of target (the operator) location [8]. Needles to say that if the DNA (and for that matter also the F-actin) length is smaller than the "sliding length" (or the "sliding distance") then the traveling protein molecule will be ejected from the linear macromolecule without being halted. Just as a repressor may slide by rotating around the DNA chain, an S-1 or myosin I (i.e., tailless myosin) molecule might slide by rotating around an actin filament. Another analogy between these two processes is that both might be considered as involving facilitated transport mechanisms. As has been proposed above, the transport of myosin along actin is driven by the energy released when a complex between the myosin head(s) and actin is dissociated by ATP. The "specific" actin subunit to which the head binds at the end of the process is determined by the energetics of the non-specific dynamic interaction between the head and the actin subunits along its path. In a similar fashion, in the case of the repressor–DNA interaction, specific binding will be favored when the binding constant is larger than that for non-specific binding i.e., the binding constant of the kinetic energy-possessing myosin head must be smaller than the equilibrium constant. In other words: ΔG° for the "dynamic" association should be smaller than the standard free energy change of the equilibrium reaction by the instantaneous value of the kinetic energy of the moving particle. One may wonder whether the double-helical structure of actin is linked with the energetics of the active transport of myosin heads guided by the actin active rails.

The fact that both F-actin and DNA are double helical is quite striking. These two macromolecular chains share an additional important structural feature: in F-actin, each of the G-actin subunits in each of strands carries an ADP (and during polymerization, temporarily, ATP) molecule. We know nothing about the steric relationship between an ADP molecule and nucleotide molecules on the adjacent strand. It is tempting to speculate that this spatial relationship changes when F-actin interacts with myosin and that this transition plays a major role in biological mechanochemical transformations, in the dynein (or kinesin)-micotubule as well as in the acto-myosin, system since all of them contain a nucleotide molecule bound to each of the subunits of the filamentous component. To be more specific, the angle between two (planar and quite large) neighboring ADP molecules belonging to different strands might continuously change during shortening and tension development due to minimal free energy requirements. If the nucleotide is rigidly bound to actin this might lead to twisting and/or stretching of the strands. One

of the states (maybe also in relaxed muscle) might be one in which the two planes are parallel to each other, or maybe co-planar as in DNA. The stability of chemical forces holding together the two nucleotide molecules might depend on the energy released when the myosin-bound ATP is hydrolyzed in conjunction with a F-actin filament. The spatial relationship between this ATP (and the ADP derived from it) and the actin-bound ADP is another exciting matter for consideration and speculation.

Acknowledgement

I wish to thank Dr. John Gergely and members of his laboratory for their hospitality and for helpful discussions during the period in which this work was carried out.

References

1 cf. A. Oplatka, Biophys. Chem. 32 (1988) 111.
2 cf. A. Ajtai, A.R. French and T.P. Burghardt, Biophys. J. 56 (1989) 535.
3 Y. Harada and T. Yanagida. In: Progress in clinical and biological research, eds. R.J. Paul, G. Elzinga and K. Yamada, Muscle energetics, vol. 315 (Alan R. Liss, New York, NY, 1989) p. 27.
4 A. Oplatka. In: Progress in clinical and biological research, eds. R.J. Paul, G. Elzinga and K. Yamada, Muscle energetics, vol. 315 (Alan R. Liss, New York, NY, 1989) p. 45.
5 T. Kodama, Physiol. Rev. 65 (1985) 467.
6 M. Bárány, J. Gen. Physiol. 50 (1967) 197.
7 T. Maruyama, K. Kometani and K. Yamada. In: Progress in clinical and biological research, eds. R.J. Paul, G. Elzinga and K. Yamada, Muscle energetics, vol. 315 (Alan R. Liss, New York, NY, 1989) p. 223.
8 O.G. Berg, R.B. Winter and P.H. von Hippel, Trends Biochem. Sci. 7 (1982) 52.
9 D.C. Rau, B. Lee and V.A. Parsegian, Proc. Natl. Acad. Sci. USA 81 (1984) 2621.
10 D.C.S. White, Physiol. J. (London) 208 (1970) 583.
11 J.H. Coats, A.H. Criddle and M.A. Geeves, Biochem. J. 232 (1985) 351.
12 J. Borejdo and A. Oplatka, Biochim. Biophys. Acta 440 (1976) 241.
13 R. Tirosh, N. Liron and A. Oplatka. In: Cross-bridge mechanism in muscle contraction, eds. H. Sugi and G.H. Pollack (University of Tokyo Press, Tokyo, 1979) p. 593.
14 J. Borejdo and A. Oplatka, Pflüg. Arch. 366 (1976) 177.
15 A. Oplatka, H. Gadasi, R. Tirosh, R. Lamed, A. Muhlrad and N. Liron, J. Mechanochem. Cell Motility 2 (1974) 295.
16 H. Gadasi, A. Oplatka, R. Lamed and A. Muhlrad, Biochem. Biophys. Res. Commun. 58 (1974) 913.
17 A. Oplatka, J. Borejdo, H. Gadasi, R. Tirosh, N. Liron and E. Reisler. In: Proteins of contractile systems, Proc. FEBS meetings, Vol. 31 (9th FEBS meeting, Budapest, 1974) (Akademiai Kiado, Budapest, 1975) p. 41.
18 A. Oplatka, J. Borejdo and H. Gadasi, Biochem. Biophys. Res. Commun. 66 (1975) 1323.
19 A. Oplatka, J. Borejdo and H. Gadasi, J. Cell Biol. 67 (1975) 317a.
20 A. Oplatka and R. Tirosh, Biochim. Biophys. Acta 305 (1973) 684.
21 R. Tirosh and A. Oplatka, J. Biochem. (Tokyo) 91 (1982) 1435.
22 R. Tirosh, W.Z. Low and A. Oplatka, Biochim. Biophys. Acta, 1037 (1990) 274.
23 A. Oplatka, J. Theor. Biol. 34 (1972) 379.
24 L.E. Ford, A.F. Huxley and R.M. Simmons, J. Physiol. 269 (1977) 441.
25 G. Cecchi, P.J. Griffiths and S. Taylor, in: Contractile Mechanism in Muscle, eds. G.H. Pollack and H. Sugi (Plenum Press, New York, NY, 1984) p. 641.
26 J. Squire, The structural basis of muscular contraction (Plenum, New York, NY, 1981).
27 A. Kishino and T. Yanagida, Nature 334 (1988) 74.
28 I.Z. Steinberg, A. Oplatka and A. Katchalsky, Nature 210 (1966) 568.
29 S. Lovell, T. Karr and W.F. Harrington, Proc. Natl. Acad. Sci. USA 85 (1988) 1849.
30 Y.Y. Toyoshima, S.J. Horn, E.M. McNally, K.R. Niebling, C. Toyoshima and J.A. Spudich, Nature 328 (1987) 536.
31 H.E. Huxley. In: The cell, Vol. 4, eds. J. Brachet and A.E. Mirsky (Academic Press, New York, NY, 1960) p. 365.
32 S. Kamimura and K. Takahashi, Nature 293 (1981) 566.
33 cf. M.E. Porter and K.A. Johnson, Annu. Rev. Cell Biol. 5 (1989) 119.