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Minireview

The role of water in the mechanism of muscular contraction

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Abstract Twenty-five years after its proposal, the swinging theory of muscular contraction, in which the majority of scientists in the field have blindly believed, has not yet been verified. Rapidly growing experimental evidence indicates that the myosin heads do not swing. It is time to look for an alternative mechanism. Data is presented indicating that water is liberated during tension development and the extent to which it is released appears to affect the degree of tension. Since water can move (because of acquired extra energy, involvement in hydration forces etc.), it might cause protein movement.

Key words: Actin; Hydration forces; Muscle contraction; Myosin; Swinging Crossbridge theory; Water

1. Introduction

The classical swinging cross-bridge (c.b.) theory, as well as most other theories of muscle contraction, have taken it *for granted* that contraction is the outcome of major *conformational changes* in one of the 'contractile' proteins, particularly myosin. Unfortunately, most vigorous efforts during the last quarter of this century have *failed* to confirm the most fundamental predictions of this theory, i.e. that muscle shortening is due to the rotation of the myosin heads (i.e., the enzymatically active part), *firmly* bound to the actin filaments, pushing the latter over a distance which should be close to the head's length [1,2]. It, therefore, seems legitimate to start adopting an entirely different approach that is *not* based on major conformational changes. Most surprisingly, a large variety of data has been presented in the literature, starting many decades ago, which suggest that water undergoes *structural* changes in active muscle and, moreover, that it is, just like the proteins, on the move. Just as the backbone of a protein molecule can undergo a *conformational* change, so can water be subjected to a transition from one state (e.g. bound to a protein) to another (e.g. free water). Both kinds of transformation can lead to changes in mechanical and in geometrical parameters. In the following sections, I will analyze the data on water and try to demonstrate that it plays an *active* role.

2. Indications for the release of bound water in active muscle

Various data suggest that part of the water which is bound to the contractile proteins, is liberated in active muscle:

Long ago, optical changes were observed in stimulated striated muscles. These have been described and interpreted by A.F. Huxley [3]. During an isometric twitch, there is a decrease in the amount of light scattered by the muscle, which roughly follows the time course of tension development. The strength of the birefringence falls during the twitch; the decrease is maximal when the muscle is near its rest length, i.e. when the degree of overlap of actin and myosin and the isometric tension (P_o) are maximal. Both effects have been ascribed to the transfer of water from the myofibrils into the sarcoplasm (i.e., the medium surrounding them) which leads to diminution of the difference in refractive index between the two phases. The finding that the lateral spacing between the thick and the thin filaments in the myofibrils decreases during isometric contraction (in skinned and in glycerinated muscles, as well as in intact fibres) supports this interpretation [4,5]. The spacing in permeabilized active muscles can be decreased also by the addition of a non-penetrating polymer, e.g. dextran, to the external medium [4,6]. This suggests the possibility that the thermodynamic activity of water inside the lattice increases during activation, e.g. due to the liberation of bound water.

Indeed, measurements of nuclear magnetic relaxation times of water suggested that when a skeletal frog muscle is stimulated isometrically, part of the bound water is liberated [7]. On the basis of the data given, 1.33×10^{-5} mol of water are liberated per cm^3 muscle. If we divide this by the concentration of myosin heads (about 2.4×10^{-7} mol/cm^3, cf. [8]), we get $n = 55$ water molecules *per head*. Of course, this does not mean that the water was, fully or partially, released by the heads (alone or in combination with actin).

The interaction between actin and heavy myosin (HMM) subfragment-1 (S-1) (i.e. isolated myosin heads), both in the presence and in the absence of ADP, appears to involve a step in which a weakly-attached complex undergoes isomerization into a strongly-attached state [9–11]. The authors noticed that both the equilibrium constant of this reaction in the absence of nucleotide [9] and tension generation in muscle fibers are affected similarly by low temperature, high ionic strength, hydrostatic pressure and the presence of an organic solvent, which strongly suggested that tension generation is associated with isomerization and it is the strongly-attached complexes which generate the contractile force. The reaction leads to a marked increase in volume ($\Delta V^\circ \cong 110$ cm^3/mol heads). Since the reaction is endothermic ($\Delta H^\circ = 21.9$ kcal/mol heads, calculated from [9]), it must be entropy-driven ($\Delta S^\circ > 0$). Increase in both molar volume and entropy is commonly largely ascribed to a change in the hydration shells of interacting proteins. Free water molecules have larger molar entropy and specific volume than bound water [12]. The value of $\Delta H^\circ/\Delta V^\circ = 21.9/$

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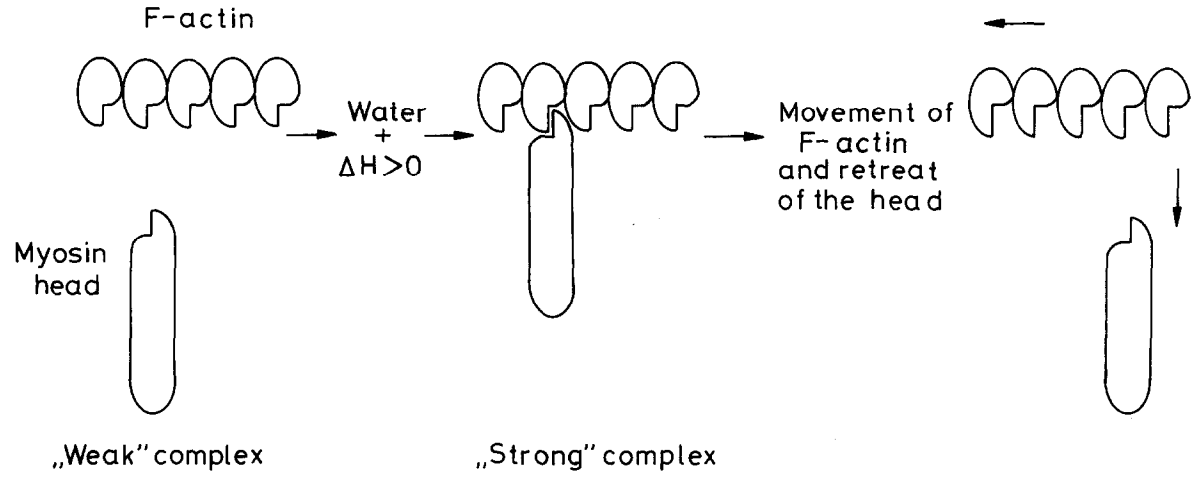

Fig. 1. Schematic representation of the model according to which the liberation of water generates energy which allows a close approach of a myosin head to actin thus creating a large repulsive force, whose component parallel to the actin filament causes its movement. The distance between the two proteins in the 'weak' complex has been largely exaggerated in order to emphasize the approach.

110 = 0.199 kcal/cm^3 is quite close to 0.181 kcal/cm^3 calculated for the rod-forming, entropy driven, polymerization of the tobacco mosaic virus protein (TMVP) [13], which suggests that a similar 'phase transition' occurs in the two cases. Water was found to be *released* in the case of TMVP and its amount, V, was measured. The ratio $\Delta V/V$ was 0.2. Taking this value to be approximately the same for the isomerization reaction we get $V = 550$ cm^3/mol heads, which corresponds to $n = 31$ water molecules per head. This is practically one half of the value derived above from the NMR data. The similarity between the two values for n suggests that in active muscle, as in solution, the liberated water originates from the hydration shells of actin and/or the myosin head(s) and that our comparison between acto-S-1 and TMVP was not unjustified. The difference in the values of n might be due, at least partly, to cooperativity between the two heads of the myosin molecules in muscle. One may wonder whether this difference is responsible for the fact that S-1 is less effective mechanochemically than the double headed HMM [14–15].

3. Force generation by the 'phase transition' of water

An indication for an intimate association between tension generation and liberation of water during the isomerization comes from an analysis of the observation [6] that the isometric tension of glycerinated muscle fibers exhibited a pronounced maximum (135% of control) when increasing concentrations of dextran were added to the medium. If, indeed, water is liberated during isomerization then the reaction should be written as

'weak' complex ↔ 'strong' complex + *water*. (1)

The addition of dextran induces an osmotic stress, i.e. the thermodynamic activity of water inside the lattice is decreased. The reaction should then be shifted to the right, i.e. more 'strong' complexes should be formed and P_o should increase, as was indeed observed. This finding may be taken as an additional indication for the release of water during the force-generating event.

From studies of the effects of pressure on equatorial X-ray fiber diffraction from skeletal muscle, Knight et al. [16] reported that the reduction in active tension under pressure seems *not* to be accompanied by c.b. detachment and concluded, in line with eq. **(1)**, that pressure increases the proportion of weakly-attached c.b.s.

Laser temperature-jump and length-jump experiments [17] recently suggested that tension generation occurs in a single endothermic (entropy driven) step in Huxley-Simmons phase 2. It was stated that two different types of structural changes in proteins can give rise to entropy-driven reactions capable of generating tension: the first is akin to protein self-assembly in which the order–disorder transition is the result of the exclusion of water from the interacting surfaces of the protein and the second involves the conversion of an organized structure of low entropy into a random coil (as in Harrington's mechanism [18] for muscular contraction).

Thus, both physical and biochemical studies suggest that water molecules are released in active muscle. It makes sense to believe that this water is liberated from the hydration shells of actin and/or the myosin head, most probably in the region of their contact.

The problem which arises at this point is whether the strongly-attached c.b.'s formed *after* the isomerization have a conformation which is under strain (like a stretched spring), demonstrated as force, or that the isomerization *process, while it occurs*, is responsible for the generation of force, e.g. that the release of water molecules and the accompanying increase in volume and in enthalpy are capable of producing a mechanical force. If for example one of the domains of the head gets inside one of the clefts of actin (or vice versa) [19] then supporters of the swinging c.b. theory would ascribe force generation to a translocation of the head domain from a patch of ionic residues to one with hydrophobic groups, thus attaining a new orientation which may or may not be the prelude for additional orientational states, leading somehow finally to the 45° rigor bond (while always being tightly attached to actin by 'bonds', i.e. hydrogen, hydrophobic and ionic bonds). (cf. [20]). In other words, the classical rotation of the *whole* heads would now be replaced by a sort of tilting of a domain. Unfortunately, the chemical modification (*extensive* methylation) which was necessary in order to enable the crystallization of S-1 (which was obligatory for the determination of its three-dimensional struc-

ture [19]) has profoundly affected both the actin-activated ATP-ase activity (down to about 10% that of the un-modified S-1 [21–22]) and the mechanical performance (no movement of F-actin on immobilized HMM in an in vitro motility assay [22]). This should not be surprising if we recall that, for example, the attachment of a probe to the SH_1 group of the head (for the purpose of following changes in orientation) can cause an appreciable diminution of both ATPase activity and tension (cf. [23]) and thus may cast doubts on the value of the tilt angle observed. Moreover, it is not at all clear how could the translocation of the head domain in *free* HMM to another patch in the actin filament enhance the translational motion of F-actin in solution in the presence of Mg-ATP which has recently been demonstrated [14,15]. Not to mention the development of a contractile force induced by S-1 (or HMM) plus Mg-ATP in inactivated muscle segments and the demonstration of active streaming of solutions containing actin, HMM and Mg-ATP reported much earlier [24,25]. In these experiments the heads are not connected to myosin filaments' shafts that, together with the actin filaments, are supposed to form a continuous three-dimensional network in which 'elastic elements' could be stretched thus giving rise to contractile force in muscle [26].

4. Some suggestions on mechanisms by which the liberation of water might generate tension

In view of the fact that the evidence disfavoring the swinging cross-bridge theory and the number of scientists who feel that an alternative hypothesis for contraction has to be developed is rapidly increasing (cf. [1,2]), I propose to pay attention to the 'ejection' of water molecules which occurs concurrently with the force-producing isomerization reaction rather than to gross conformational changes of the proteins. Speculatively speaking, one mechanism might be that, *during* the phase transition, the 'contact' region is 'swollen'. If swelling is not isotropic then a net pressure may push the actin filament in a certain, well-defined, direction. If the muscle is held isometrically, tension will develop.

One can decrease the distance between linear macromolecules, such as DNA or collagen triple helices, enclosed in a semipermeable bag, by increasing the osmotic pressure in the outside medium. Part of the water which originally separated the macromolecules will then leave the bag. Since the hydration layers repel each other, the repulsive hydration force increasing exponentially with decreasing distance, this means that osmotic work can overcome the repulsion and bring the macromolecules closer to each other [27]. In active muscle, the liberation of water molecules, which should cause an increase in the thermodynamic activity of water inside the lattice, is equivalent to a decrease in water activity in the medium which is usually achieved by the addition of a polymer. The large positive $\Delta H°$, which is enabled by the increase in entropy associated with the release of water can provide energy and do the osmotic work required in order to overcome the repulsion and bring the protein molecules closer, i.e. to a point where all the energy is exhausted and the repulsive force is then maximal (Fig. 1). The proteins do not have to form a rigor complex, i.e. a complex in which the proteins are strongly ('chemically') bound together by hydrogen bonds and salt linkages (as must be the case when muscle is in the rigor state). If the force has a component which is parallel to the actin filament (e.g. if the active domain of the heads interacts with a domain on actin whose active surface is somewhat perpendicular to the filament axis) then F-actin will move *away* from the head, parallel to an adjacent myosin filament in muscle. Indeed, it has recently been unambiguously claimed [1,2] that in isometrically contracting muscle the c.b.s are practically perpendicular to the thin filaments (and that is the only tilt angle). Hence the force acting on the filaments must be generated at a surface at 90° with their axis. At the same time, the repulsive force will cause the retreat of the myosin head to a point at which the repulsive force vanishes. After binding fresh ATP, the process will be repeated.

In conclusion, it is speculated that the water molecules released from the interface between the myosin heads and actin play an *active* role in tension generation and movement, without necessitating major conformational changes of any of the proteins and, particularly, any rotation of the myosin heads. Formulated differently, the real actors are water molecules – the proteins serve just as the stage [28].

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