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Validity of Hill's Equation in an Artificial Actomyosin Streaming System

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ABSTRACT

The rotation of an actomyosin motor (9,11), assembled from blades, one side onto which F-actin of uniform polarity was attached, suspended in a solution of heavy meromyosin (HMM), was modelled as due to sliding of HMM over the margins of the blades, whereby the work resulting from ATPase activity is used for pushing bulk fluid containing HMM from the leading surface of the blade over the force-generating filaments to the back surface, which leads to increased sliding velocity. The amount of HMM contributing to force-generation is divided into one component perpendicular to the filaments, which is diffusion-limited and regulated by a component parallel to the filaments, represented by the movement of the bulk fluid, the supply of new HMM and the observable velocity of rotation of the blade. Using Hill's equation (6) which essentially states that the product of a virtual force and a virtual velocity is constant within the range of observable forces and velocities, the force can be expressed as velocity, giving a simple first order differential equation. A solution to this equation can be fitted to the observed data with physically meaningful constants in terms of mass, viscosity, the force-velocity constant and a constant expressing the quality of the preparation. Control experiments were performed, using fixed blades. In these experiments, bulk flow around the margins did not increase as a result of ATPase activity and no movement or streaming was observed. The results show that Hill's equation may be applicable to streaming caused by actomyosin irrespective of its confinement to skeletal muscle and independently of preferred molecular model, and leads to several verifyable predictions.

INTRODUCTION

Hill's equation is an empirical equation which describes the force-velocity relationship of contracting skeletal muscle (6). It can be derived from considerations of the molecular mechanism of actomyosin interaction, providing a mathematical foundation for the sliding-filament model of muscular contraction (7). This shows that the macroscopic behaviour of whole muscle may follow from postulated molecular events (7,8) and raises the question how close to the experimental molecular level Hill's equation may actually be valid. In the present report, it is shown that the movement of an artificial actomyosin streaming system, the "actomyosin motor" (9,11), is compatible with Hill's equation. This takes Hill's equation from whole muscle, bypassing the cellular and sarcomeric levels, down to the supramolecular level as represented by the joint actin strands on the blades of the actomyosin motor. Therefore, the present analysis justifies models where the actomyosin interaction in supramolecular structures itself, regardless of elasticity or other macroscopic parameters of whole skeletal muscle or sarcomeres, provides the basis of Hill's equation.

METHODS

The methods have been described before (9,11). Briefly, actin was prepared from contracted acetone dry powder (3,10) and heavy meromyosin by tryptic digestion of myosin for 5 min at 20 ^{o}C at a weight ratio (trypsin/myosin) of 1/200. The myosin had been extracted from minced muscle in 0.2 M phosphate buffer, pH 6.4-6.5, containing 0.5 M potassium chloride and in addition phosphocreatine to avoid accumulation of ADP as previously cautioned (5). The actin was polymerized onto polylysine-coated teflon attached to mica on an "actomyosin motor" as described previously (9,11). The actomyosin motor has six blades with alltogether 24 margins. In a control experiment, 24 blades were attached to the inner surface of a cylindrical container, with free margins facing a 2 mm slit and the bottom of the container. The actomyosin motor and the control devise are schematically illustrated in Fig. 1 A and B, respectively. After polymerization, native tropomyosin (4), containing both troponin and tropomyosin was equilibrated with the actin, the blades washed briefly in buffered KCl and stored in the cold room overnight. Before the experiment, the devise was trans-

ferred to a solution containing 1 g/l of HMM in 90 mM KCl, 4 mM MgCl, 10 mM Mops, pH 7.0 followed by the addition of ATP to 2 mM and Ca ions to 30 uM. The movement was observed using a low-magnifying microscope and video equipment and subsequently quantified by manually measuring the angle of movement on the TV screen.

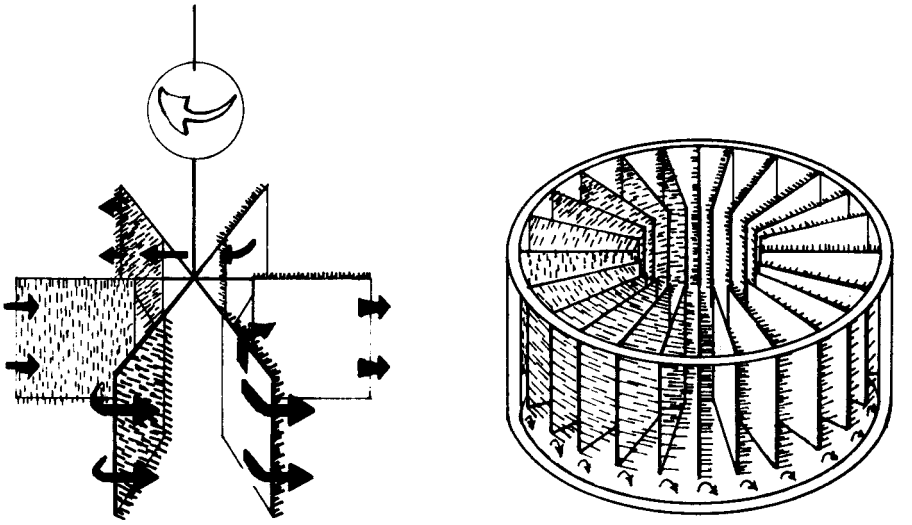

Fig. 1. A. Illustration of the flow of HMM-containing buffer around the margins of the "actomyosin motor" as it rotates in the solution where it is suspended by a floating cork The actomyosin motor consists of six blades, one side onto which actin of uniform polarity has been attached, the arrowhead direction pointing towards the blade. As a result of force-generation due to the interaction of HMM with actin, the blades are pushed forward and solution starts to flow around the margins (indicated by black arrows). B. Illustration of the flow of solution at the margins of the control devise in which the blades are fixed. In this case, the force can not be utilized for pushing more HMM from the anterior side of the blades onto the marginal filaments.

RESULTS AND DISCUSSION

The movement of fluid around the margins of the six suspended blades of the actomyosin motor and the control devise are illustrated in Fig. 1 A and B, respectively. In the actomyosin motor, the force generated is presumed to be proportional to the amount of HMM which can diffuse onto the actin filaments at the initial velocity times the amount of HMM newly supplied by the bulk flow around the margins, which is proportional to the observed velocity

of rotation, v. In the control devise, the blades are fixed to the wall of the cylinder and, as a consequence, no bulk fluid can be pushed around the margins and the streaming will remain at the initial diffusion-limited velocity.

The average intermolecular distance in a solution containing 1 g/l of HMM (which is sufficient to observe movement) is approximately 1000 A, and the diameter of the active globular portion of one sub-unit of HMM is in the range of 100 A, approximately 10 times less. If it is assumed that the HMM which interacts with actin is trans-located under no load along the filament with a velocity of at least 12 um/s (Fig. 2) it would have to be replaced within less than 0.8 ms which is the time it takes for it to leave an elementary space of 100 A on the filament. Since the inter-molecular distance in solution is, on average, 10 times longer than that, and the orientation of HMM is random, the probability of such instantaneous replacement from the solution of HMM molecules moving on the filaments is evidently low. The contribution to the probability given by each molecule is provided by equation (1), which describes the three-dimensional diffusion case:

$$p_t(x,y,z)\,dx^3 = \left(\frac{1}{4\pi Dt}\right)^{3/2} \left(e^{-\frac{x^2}{4Dt}}\right)^3 dx^3 \qquad (1)$$

In this equation, dx is of the order of magnitude of the molecular dimensions of the HMM molecule which is to be replaced, D the diffusion coefficient of HMM and x a multiple of the average inter-molecular distance between HMM molecules in the solution. The relevant time is shorter than 1 ms which corresponds to the distance of "elementary" displacement of an HMM molecule with two heads assuming that its maximal velocity is higher than 12 um/s, which is the observed velocity of rotation in the experiment analysed (Fig. 2). Equation (1) does not allow for steric factors in that only certain orientations of on-diffusing HMM can contribute to force generation.

From the considerations above, it seems reasonable to assume that the bulk flow around the margins supplies HMM to sustain movement, and that as more HMM is added when the velocity of streaming increases, the force development would also increase as a function of the amount of HMM supplied. This situation resembles that when the degree of overlap in the sarcomere of skeletal muscle deter-

mines how much myosin is supplied to the force-generating system and is independent on whether the HMM is supplied individually or in the form of small aggregates. The presence of aggregates of HMM in the present system would strongly favour the arguments presented because of the longer inter-molecular distance between the oligomers and their slower diffusion. Finally, the idea that the flow around the margins supplies HMM to the present force-generating system is compatible with the recent observation of a comparatively slow velocity of streaming (1 um/s) in a system with either one or several hundred closely apposed but immobile margins of polarized actin (12) relative to the maximal velocity of movement observed in the present system, which is 40 um/s (11).

Assuming laminar flow, the supply of HMM to the force-generating site is a linear function of velocity, which is described by:

$$F_{tot} = K_v \, v \, F_u \qquad (2)$$

This equation expresses that the total force development, F_{tot}, is regarded as proportional to a product of two components, one of which is the extra supply of HMM in the laminar flow, the "overlap", represented by v, which is the observed velocity of streaming, and the other of which is the specific force development of HMM, related to a unitary amount of that protein and denoted by F_u. The index "u" stands for "unitary". K_v is a constant which relates the velocity of streaming to the additional force provided by some of the HMM molecules supplied in the flow when they interact with the actin strands. The unitary force, F_u, which can not be related to any molecular mechanism in the present treatment would be relevant to it and to the observed movement and acceleration of the actomyosin motor. The force produced by the elementary cycles of HMM leads to acceleration of the devise until its rotational velocity is balanced by the flow friction. This can be described by:

$$m \, a = K_e \, F_{tot} - c \, v \qquad (3)$$

where m is the mass of the actomyosin motor, K_e an experimental constant relating to the length of the margins, the quality of the proteins and of the preparation and presumably the length of the actin filaments and their coherence (cf. 2). c is the fric-

tional constant. If (2) is inserted into (3), we get:

$$m\,a = K_v F_u - c\,v \qquad (4)$$

where F_u is the unitary force. K_v and K_e are incorporated into this new constant K. This expression provides the observed acceleration dv/dt, which is given by:

$$\frac{dv}{dt} = \frac{K\,F_u\,v}{m} - \frac{c\,v}{m} \qquad (5)$$

Hill's equation, usually given in the form (P+a) * (v+b) = c, where P is the tension of contracting muscle, v the velocity of contraction, and a and b constants of dimension force and velocity, respectively (c is the force-velocity constant), essentially states that the product of a virtual force and a virtual velocity of contraction is constant within the range of observable forces and velocities and thus, that:

$$F^* v^* = k \qquad (6)$$

where k is the force-velocity constant and F^* and v^* the virtual force and velocity, respectively. In the case of the actomyosin motor, the total force increases linearly with velocity due to the increased overlap resulting from the supply of HMM over the edges while the elementary force F_u contributes to the total force according to eq (2). As the velocity increases, the flow friction, and consequently the tension increases, and therefore, the situation is analogous to that of contracting muscle, the only difference being that the overlap also increases when the velocity increases. Since Hill's equation, relating tension to velocity, holds irrespective of the degree of overlap in skeletal muscle, it is appropriate to use it also for the "unitary" force in the present treatment. Using (6), the force can be eliminated from eq (5) so that it takes the form:

$$\frac{dv^*}{dt} = \frac{Kk}{m} - \frac{cv^*}{m} \qquad (7)$$

or,

$$\frac{dv^*}{dt} + A\, v^* = B \qquad (8)$$

where A and B are constants given by c/m and Kk/m, respectively. To make the substitution (7) is essentially to state that Hill's equation is valid at the unitary level defined in (2). In the following, some consequences of this postulate are examined.

A solution to equation (8) is:

$$v = (b - \frac{B}{A})\, e^{-At} - (b - \frac{B}{A}) \qquad (9)$$

where b is Hill's constant, b, or,

$$x = (\frac{B}{A^2} - \frac{b}{A})\, e^{-At} + (\frac{B}{A} - b)\, t - (\frac{B}{A^2} - \frac{b}{A}) \qquad (10)$$

which is equivalent to:

$$x = (\frac{Kkm}{c^2} - \frac{bm}{c})e^{-At} + (\frac{Kk}{c} - b)t - (\frac{Kkm}{c^2} - \frac{bm}{c}) \qquad (11)$$

where x is the observed angle of rotation, measuring distance, and b is Hill's constant, b. With experimentally determined constants this equation fits qualitatively the observed angle of rotation in preparations of high enough quality to allow sustained rotation and determination of the ratio B/A (Fig. 2). The decreased velocity of rotation observed after 30-50 min in the figure is most likely due to ATP depletion, and corresponds to the time required to observe a shift of the absorption at 284 nm of HMM, which is due to the accumulation of ADP (Cerven, 1985, unpublished observations; cf. ref. 5). In experiments where the rotation slows down earlier, perturbation of the actin can not be excluded to be an additional factor. In the present study, such rapid cessation of rotation after an initial burst was observed when the actomyosin motor was used immediately after preparation. The maximal velocity is given by the slope of the curve, or:

$$v_{max} = (\frac{Kk}{c} - b) = (Kk - bc)\frac{1}{c} \qquad (12)$$

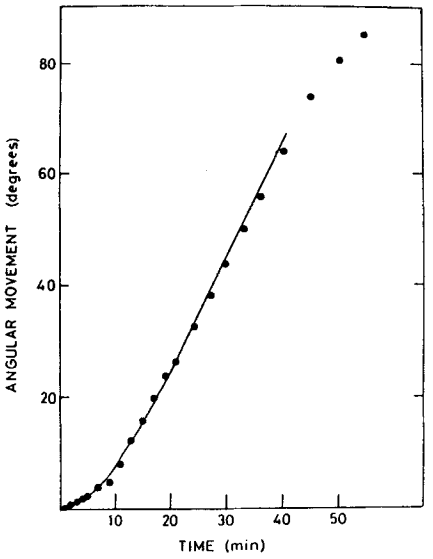

Fig. 2. Experimental results using an "actomyosin motor" (9, 11, Fig. 1 A). The angular movement is plotted against time. The experimental constants were determined by curve-fitting and the curve is described by the equation:

$x = 20.75e^{-0.1t} + 2.1t - 20.75$

and, as expected, is proportional to the force constant representing the quality of the preparation and the flow - supply coupling of HMM, K, the force-velocity constant, k, and inversely proportional to the frictional constant, c, or the viscosity. The observed velocity at time t is given by:

$$v = (bc - Kk)\frac{1}{c}e^{-\frac{c}{m}t} - (bc - Kk)\frac{1}{c} \qquad (13)$$

and the acceleration by:

$$\frac{dv}{dt} = \frac{(Kk - cb)}{m}e^{-\frac{c}{m}t} \qquad (14)$$

As expected, the mass of the rotor does not affect the maximal velocity but, providing inertia, appears in the exponential term delaying the acceleration of the rotor. Also the friction constant and consequently the viscosity decreases the acceleration term, but this dependence may not be straightforward since a medium of higher viscosity could provide a higher counterforce relative to which the observable acceleration takes place. The present treatment does not elaborate on Hill's constant b, the nature of which is poorly known, but may be related to the chemical reaction rate or the "increase of energy rate per g weight

decrease of load" (6). The chemical reaction rates in unidirectional streaming systems may involve considerable utilization of ATP and may be qualitatively different from those during contraction of muscle where the symmetry of the sarcomere provides an ideal framework for futile cycles or ATP-regenerating circuits.

The present analysis indicates that Hill's equation expresses an inherent property in vectorially contracting actomyosin assemblies, irrespective of muscle elasticity or force transmission in whole muscle and even irrespective of the presence or absence of a myosin filament, and leads to several verifyable predictions. These predictions, for example those relating to the influence of mass and viscosity on the coordinate and velocity of movement, are intuitively acceptable, and could be checked for example by changing the mass of the rotor or the viscosity of the medium by altering for example the concentration of HMM or the temperature. Because of the difficulty in obtaining exactly equivalent preparations of actomyosin motors, which would require accuracy at the nanometer level, and since one preparation can only be used once, it is at present impossible to perform such comparative experiments. The main conclusion of the present theoretical and semi-empirical results relate to the validity of Hill's equation and particularly its inverse force-velocity relationship. As such, the present results based on the assumption of a unitary force exerted by HMM are the first indication that the force-velocity relationship may hold outside the sarcomere. This information would be important to understand the molecular mechanism of actomyosin contraction, which is not yet known, but for which several more or less exact theories have been proposed.

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