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Cooperative behaviour of the elementary sarcomere units and the cross-bridge step size

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Abstract

We present a model of muscle contraction based on purely physical grounds and modulated by a parameter, k, related to the visco-elastic hindrances of the contractile apparatus. The model predicts a strong cooperation among sarcomere units and proposes that viscous hindrance is a fundamental component of the economy of the contraction. The concept of cross-bridge step size is also discussed and it is concluded that the step size is of various and probably undeterminable length.

Keywords: Sarcomere; Units; Cooperativity

1. Introduction

The relationship between load and rate of muscle contraction is usually explained by the Eyring's theory [1] of the visco-elastic behaviour of high molecular weight polymers and by the Kramers' theory of chemical reactions [2]. Essentially the rate constant of the contraction, k_+, is assumed to depend exponentially on the free energy required to attain the transition state and that this free energy barrier changes with the elastic energy associated with the load. Thus the rate constant, k_+, depends on the load. On the contrary the rate constant for the reverse reaction, k_-, is independent on the load. On these grounds fairly accurate models were presented suitable to describe even subtleties of muscle contraction [3–5].

Our present aim is to describe muscle contraction on purely physical grounds. The energy made available by the hydrolysis of ATP promotes the sliding of the thin filament and of the attached load. The rate of the contraction is modulated by a parameter, k, related to the visco-elastic hindrances of the contractile apparatus. The model, perhaps naïve, reproduces the force–velocity curve and demonstrates that contractile mechanisms are inescapably influenced by viscous hindrance. The model also predicts a strong cooperation among sarcomere units. The concept of cross-bridge step size is also discussed and it is concluded that the step size is of various and probably indeterminable length.

2. The model

2.1. The elementary unit of the sarcomere

We define as the elementary unit of the sarcomere each thick filament with the surrounding crone of the six thin filaments. During contraction thin filaments move towards the center of the sarcomere. The thin filaments are anchored to the *Z*-disc so that, when sarcomere shortens, *Z*-disc and the thin filament move, all together, toward the center of the sarcomere [25]. The linear rate of the *Z*-disc and of the thin filaments is therefore the same as the rate of sarcomere shortening. Thus, although the elementary units can be considered separately, these in fact belong to a single, coherent system, the whole sarcomere.

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The sarcomere is assumed to be composed by $n=2000$ elementary units [6], its cross-section is,

$$s_S = n\ 3\ \sqrt{3}/2\ r^2, \tag{1}$$

and the fraction of the half sarcomere mass, m_1, which might move with the Z-lines, thin filaments and sarcoplasm separating thin from thick filaments, is,

$$m_1 = n\left(\rho\ 3\ \sqrt{3}/2\ r^2\ l_S/2 - 300\ \mathrm{MW_M}/(2N)\right), \tag{2}$$

where $\rho=1.035$ g cm^{-3} is the density of frog sartorius muscle [7]; $r=25$ nm is the distance between the centers of two adjacent actin filaments; $l_s=2.7$ μm is the sarcomere length; 300 is the number of the myosin molecules in the tick filament; $\mathrm{MW_M}$ is the molecular mass of myosin, 407 kDa [10]; and N is the Avogadro's number.

2.2. The power stroke

The power stroke is powered by the hydrolysis of ATP, which, in muscle conditions, releases approximately 7.44 10^{-8} pJ per molecule (E_{ATP}) [8]. Power strokes occur randomly and the sequence of these events produces muscle contraction.

Energy and force delivered by the power stroke are linked by,

$$E_{\mathrm{ATP}} = F_1 l, \tag{3}$$

where F_1 is the average force over the distance l at the beginning of the contraction and l is the sliding of thick and thin filaments past each other, provided that E_{ATP} is used up completely and that the movement occurs without hindrance.

In the presence of a load per unit area, P, the opposing force, F_2, is

$$F_2 = 2 s_S P, \tag{4}$$

(the factor of 2 accounts for the fact that only about half of the total cross-section of the fiber is occupied by the contractile machinery [9]) and the driving acceleration is,

$$a_{\mathrm{d}} = (F_1 - F_2)/(m_1 + m_2), \tag{5}$$

m_2 is the mass of the load pertaining to the sarcomere section,

$$m_2 = s_S P/a_L, \tag{6}$$

where a_{L} is the acceleration associated with the load, not necessarily the acceleration of gravity.

2.3. The effect of the power strokes in the absence of any hindrance

The force generated by a single power stroke is about 8 pN [9,11] and, in general, it is much lower than F_2 so that it is necessary to sum up the energy delivered by the power strokes. This is possible if the frequency of the power strokes exceeds a given level, so that not all the energy provided by a power stroke is used up before the following power stroke, performed by another attached cross-bridge, occurs. This condition is fulfilled if the space, l_{A}, traveled in the time between two power strokes, is lower than $l=E_{\mathrm{ATP}}/F_1$, so that the fraction left of the original energy, $|l_A|/l$, adds to the energy provided by the subsequent power stroke. To summarize, l is constant, its value is defined by Eq. (3), where the value of F_1 is that at the starting of the contraction. In the transient phase of the contraction both F_1 and l_{A} change. At the steady state both F_1 and l_{A} are constant the driving acceleration, a_{d}, approaches zero. Thus bound cross-bridges make a translation of $|l_{\mathrm{A}}|<l$ whose value changes with the progress of the contraction and becomes fairly constant at the steady state

The iteration procedure is as follows,

The energy left after the $(i-1)$th cycle is,

$$E_{R(i-1)} = E_{T(i-1)}|l_{A(i-1)}|/l, \tag{7}$$

the total energy available in the ith cycle is,

$$E_{T(i)} = E_{\mathrm{ATP}} + E_{R(i-1)}, \tag{8}$$

$$F_{1(i)} = E_{T(i)}/l, \tag{9}$$

$$a_{\mathrm{d}(i)} = \left(F_{1(i)} - F_2\right)/(m_1 + m_2), \tag{10}$$

l_{A} is given by,

$$l_{\mathrm{A}(i)} = v_{(i-1)} t_{\mathrm{AT}} + a_{\mathrm{d}(i)} t_{\mathrm{AT}}^2/2, \tag{11}$$

and the velocity $v_{(i)}$,

$$v_{(i)} = v_{(i-1)} + a_{\mathrm{d}(i)} t_{\mathrm{AT}}, \tag{12}$$

where t_{AT} is the time between the power strokes.

Thus at any cycle the energy available, the contractile force and the driving acceleration change. In the first cycle, $E_{\mathrm{T}}=E_{\mathrm{ATP}}$ and $v=0$.

2.4. The effect of the power stroke in the presence of viscous hindrance

According to Szymanski [12] and Muller [13], in the presence of a viscous hindrance, an hyperbolic form is assigned to the velocity, v_{V}, of the masses, m_1 and m_2, which move under the effect of the force F_1

$$v_{\mathrm{V}} = k\ a_{\mathrm{d}}\ t/(k+t), \tag{13}$$

where the reciprocal of the constant, k, defines the hindrance. In our system, since driving acceleration is changing at every cycle,

$$v_{\mathrm{Vi}} = v_{\mathrm{v}(i-1)} + i_{\mathrm{vv}}, \tag{14}$$

where i_{vv} is the increment of velocity in the time t_{AT} is,

$$i_{\mathrm{vv}} = k\ a_{\mathrm{d}}\ i\ t_{\mathrm{AT}}/(k + i\ t_{\mathrm{AT}}) - k\ a_{\mathrm{d}}\ (i-1)\ t_{\mathrm{AT}} /(k + (i-1)\ t_{\mathrm{AT}}), \tag{15}$$

and the space, l_V, traveled in the ith cycle of time length, t_{AT}, is,

$$l_V = v_V \cdot t_{AT}. \quad (16)$$

Actually to avoid calculation overflow in the iterative procedure the time increment, t_I, must be much lower than t_{AT}. The value selected was $t_I = 10^{-10}$ s. t_I thus replaces t_{AT} in Eqs. (11), (12), (15) and (16) while Eq. (8) is modified accordingly,

$$E_T = E_{ATP}\ t_I/t_{AT} + E_R. \quad (8bis)$$

2.5. Operative features

Data on the force–velocity curve were taken from Fig. 6 of He et al. [14], with $P_0 = 190$ kN m^{-2}, $\alpha/P_0 = 0.42$, and $b = 0.51$.

The ATPase rate constant as a function of the applied shortening velocity were taken from Fig. 8 of He et al. [14]. ATP consumption was assumed to be due only to the actomyosin ATPase since the experiments were performed in permeabilized muscle fibers. From these data the time between the power strokes, t_{AT}, was calculated as follows,

$$t_{AT} = 1/(n\ 300\ 0.96\ k_{AT}), \quad (17)$$

where n is the number of thick filaments in the half sarcomere, 300 is the number of the myosin heads in the half sarcomere, 0.96 is the fraction of myosin heads involved in the process, and k_{AT} is the mean ATPase rate constant which changes with the load [14].

The program was operated into two steps.

a. In the first step and in the presence of an external load, the minimum level of F_1 capable to promote shortening in the absence of viscous hindrance was first determined. This because with the starting force of 8 pN, the force usually assigned to the power stroke, positive values of the driving acceleration were not obtained and sarcomere failed to shorten.
b. In the second step the values of k capable to equate the calculated velocity, v_V, to the observed velocity, v_O, were determined. The program was stopped at $v_V/v_O < 1.001$.

3. Results and discussion

3.1. Mimicking the contraction of the half sarcomere

He at al. [14] studied the isotonic contraction of rabbit psoas muscle at 12 °C. They provided data on the rate of contraction as well as on ATP consumption as a function of the load. We employed the force and ATP consumption data to run our model and to calculate the rate of the contraction. This was done by testing, for each single load, the minimum value of F_1 capable to start the contraction and the value of k capable to approach the calculated to the experimental rate. As it is shown in Table 1 the trial was successful, in fact the calculated velocity rates approach the experimental ones (fifth column of Table 1).

The ratio initial F_1/F_2 (third column of Table 1) increases with the ratio P/P_0, being 0.055 at $P/P0 = 0.053$ and 0.726 at $P/P_0 = 0.8947$. This deserves comments. Although our model displays a device to accumulate the unemployed energy of the power strokes (Eqs. (8)–(11)), consequently to increase the driving force, the mechanism does not work below a given threshold. Apparently active muscle must prepare itself to contraction by developing forces adequate to the load that it will raise. This behaviour reminds that, in active muscle, shortening is preceded by a considerable ATPase activity not attributable to sarcomere shortening but accompanying a period of rapid force development [14,15]. This feature, apparently, represents the preparation of muscle to raise the load. In vivo ATPase activity preceding shortening does not change with the load [14,15] while in our model initial F_1 increase with the load. There is however reason to believe that the force delivered by a single cross-bridge is graduated by the load. In fact when load increases the fraction of attached cross-bridges also increases. We know that cross-bridge attachment per se increases the chemical potential and therefore the elastic energy of the contractile structures [16]. Thus when the fraction of attached cross-bridges increases it is likely that the force per cross-bridge also increases. In conclusion the same ATPase activity can be associated with a different number of cross-bridges and with different contractile forces at different loads.

According to our model, $1/k$ (fourth column of Table 1) is the expression of the viscous hindrance. This hindrances

Table 1
The values of F_1/F_2 and of $1/k$ capable to reproduce the rate of the isotonic contraction

P/P_0	t_{AT} (ns)	F_1/F_2	$1/k$ (s^{-1})	Calculated/ experimental rate
0.0526	99.0	0.055	17065	0.99964
0.105	103.0	0.108	25608	1.0006
0.158	107.5	0.160	55157	1.0005
0.21	112.4	0.209	62422	1.00035
0.263	117.7	0.257	76336	1.0001
0.3158	123.5	0.303	75643	1.0004
0.3684	129.9	0.347	95785	1.00097
0.421	137.0	0.391	108696	0.9997
0.4736	144.9	0.433	140351	1.0007
0.526	153.7	0.473	116059	1.0006
0.579	163.7	0.513	166667	0.9996
0.631	175.1	0.550	117647	0.9991
0.684	188.2	0.588	235294	1.0009
0.7368	203.4	0.624	294551	1.0004
0.789	221.2	0.659	394477	1.0003
0.842	242.5	0.692	286123	1.0009
0.8947	268.2	0.726	566893	1.0002

Temperature: 12 °C; sarcomere length: 2.7 μm. $P_0 = 190$ kN m^{-2}. The values of t_{AT} (time between two subsequent hydrolytic events) are calculated by Eq. (17), by making use of the ATPase rate constants provided by He et al. [14].

increases with the load being 17065 s^{-1} at $P/P_0=0.0526$ and 566893 s^{-1} at $P/P_0=0.8947$.

3.2. The cooperative behaviour of the elementary sarcomere units

To assess whether the elementary sarcomere units display a cooperative behaviour the model was tested on virtual sarcomeres composed by different number of elementary units (Table 2). It was assumed that the virtual sarcomeres displayed the same isometric tension, $P_0=190$ kN m^{-2}; the same $P/P_0=0.3684$; the same rate of contraction; and the same $k_{AT}=13.368$ s^{-1}. Of course since the number of the elementary unit of the virtual sarcomeres was different also the corresponding t_{AT} were different and inversely related to the number of the elementary units.

The ratio F_1/F_2 (third column of Table 2) increases with the decrease of the number of the elementary units of the sarcomeres, being 0.347 for $n=2000$ and 0.978 for $n=1$. This means that the elementary units are cooperating to overcome the load and that, in the case of the single unit where no cooperation is possible, F_1 must almost reach F_2 in order to promote contraction.

$1/k$, on the contrary, decreases from 95785 s^{-1} for $n=2000$ to 7102 s^{-1} for $n=1$. This shows that the increase of the number of the elementary units increases the viscous hindrance of the sarcomere.

The behaviour of the driving acceleration as a function of time was also analyzed. At 2000 elementary units driving acceleration undergoes large, damped oscillations so that after 10 μs their amplitude is ±0.0000723 m s^{-2} (Fig. 1, upper part). At 20 elementary units damping is much lower so that after 150 μs the amplitude of the oscillation is ±0.00326 m s^{-2}. With a single elementary unit, oscillations are small, damping is very low and after 800 μs the amplitude of the oscillations is still 0.072 m s^{-2}. This behaviour confirms that assembly of the elementary units is accompanied by the onset of cooperativity.

3.3. The step size

Originally the cross-bridge step size was fixed at 15 nm [17]. Worthington and Elliott with their impulsive force theory [18–23] criticized this choice. They proposed that

Table 2
The behaviour of virtual sarcomeres composed by different numbers of elementary units

Number of units	t_{AT} (μs)	F_1/F_2	$1/k$ (s^{-1})	Calculated/ experimental rate
2000	0.1299	0.347	95785	1.0003
200	1.299	0.72	46083	1.0008
20	12.986	0.904	20534	1.00003
5	52.0	0.951	18348	1.00045
1	259.7	0.978	7102	0.99964

Temperature: 12 °C; sarcomere length: 2.7 μm. $P_0=190$ kN m^{-2}. $P/P_0=0.3684$.

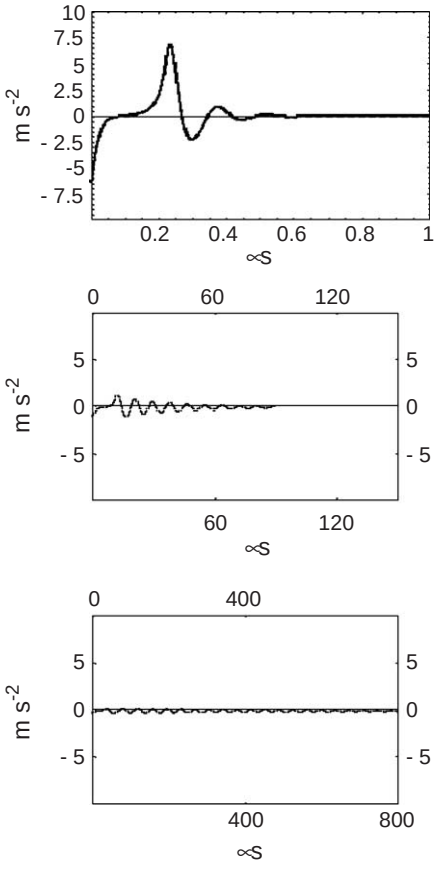

Fig. 1. Driving acceleration as a function of time. Upper part: 2000 elementary units; middle part: 20 elementary units; lower part: one elementary unit. Temperature: 12 °C; sarcomere length: 2.7 μm. $P/P_0=0.347$.

cross-bridge step size is smaller, 2 nm, and of variable length. This view found experimental support from the work of Reconditi et al. [24] who showed that working stroke is smaller and slower at higher load. An important feature of the work of Worthington and Elliott is the introduction of the concept of step size, z, i.e. the net filament sliding per ATP split. It is important to notice that the ATP split is referred to a single actin filament.

$$z = v_{max}\left(\text{nm s}^{-1}\text{hs}^{-1}\right)/(\text{molecules of ATP hydrolysed /actin filament/second}) \text{ [19]},$$

where hs is the half sarcomere length.

We observe that, in the same fiber, net filament sliding does not depend on the cross-section while the number of molecules of ATP hydrolyzed/second does increases proportionally with the cross-section (volume) considered. As an example the z referring to the cross-section of 1 mm^2 would be 100 times larger than the z referring to the cross-section of 1 cm^2. In the case of a sarcomere composed by 2000 elementary units, thus of 2000 thick filaments, thus of 4000 thin filaments, the z would be 4000 times smaller than the z referring to a single thin filament.

The calculation of z is based on the assumption that the number of molecules of ATP hydrolyzed/second decreases proportionally with the mass of muscle considered. Below a given level this is not true. Chemical events occur randomly but, if the number of molecules involved is large, they show up smoothly. In the whole sarcomere randomness is somewhat masked because of the large number of cross-bridges involved, on the contrary, in the single elementary unit, the hydrolysis of ATP shows up inescapably as a random event. This means a discontinuous power input in front of a constant load, thus the reversal of sliding when power input is lacking. As a consequence the rate of the contraction of an insulated elementary unit must be lower than that of the whole sarcomere where cooperativity is present.

In Section 3.3 we too assumed k_{AT} and contraction velocity to be the same for the whole half sarcomere and for the insulated, single elementary unit, but this was made possible only by increasing the F_1/F_2 initial ratio from 0.347 for the whole sarcomere to 0.978 for the single elementary unit where no cooperativity is possible.

We thus consider the step size (net filament sliding per ATP split per actin filament) as a parameter of reference useful to compare the behaviour of different muscle fibres but without significance with regard to the actual cross-bridge step size in muscle.

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