Distribution of Cross-Bridges in Isometrically Contracting Muscle: Its Deduction from the Huxley-Simmons Theory and the Effect of Filament Elasticity

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Summary The author attempted to deduce a possible cross-bridge (XB) distribution as a function of x , the amount of stretch of the XB, by applying the Huxley-Simmons theory with their recent experimental data to a steady isometric contraction. I found no XB distribution which satisfied at once the Huxley-Simmons theory, their data and observations in muscle energetics and biochemistry. Further, a computation with a simple model suggested that the XB distribution could be broadened by the elasticity of the thick and/or the thin filament if it existed.

Key words: muscle, contraction, elasticity.

In the cross-bridge (XB) models which assume the elasticity of the XB, the XB distribution can be expressed as a function of x , the amount of stretch of the elastic part of the XB. A constant (HUXLEY, 1957) and a Gaussian (HILL, 1974) distribution of the XBs, for example, have been used to construct kinetic XB models. In this paper, the author attempted to deduce a possible XB distribution as the function of x from the Huxley-Simmons theory (HUXLEY and SIMMONS, 1971), which has been very influential in subsequent studies. The effect of the elasticity of the thick and the thin filaments on the XB distribution is also considered using a simple model.

XB distribution deduced from the Huxley-Simmons (HS) theory

The HS theory suggests that the activation energy for the transition of the XB from one attached state to the next is given by the sum of the basic activation energy (E_0) and the energy stored in the elastic element of the XB (W). As the tilting proceeds in an isometric state, i.e., with increasing x , the activation energy increases. Therefore, provided that the frequency factor in Arrhenius' equation, A , takes the same value in all the reactions concerned, the rate constant r given by $r =$

$A \exp\{-(E_o + W)/kT\}$ for the reaction decreases with increasing x .

Suppose that the XBs turn over steadily at a plateau of isometric tetanus. That is,

where N_0 represents the detached state and the others the attached state (backward reactions are not considered for simplicity). From the condition of the steady state, $r_i[N]_i = \text{constant}$, where $[N]_i$ represents the number of the XBs at i -th attached state. It follows that the number of the XBs increases as the tilting proceeds, i.e., with increasing x . Since the T_1 and T_2 curves obtained by FORD *et al.* (1977) appear to intercept the length axis at about -4 and -12 nm, respectively, the distribution function of the XBs, $N(x)$, will take some values in the range of $0 \leq x \leq 12$ nm at the plateau of isometric tetanus (the XB distribution in the ranges of $x < 0$ and $x > 12$ nm due to thermal fluctuation of the XBs is neglected for simplicity). A shift of $N(x)$ by -4 nm reduces the tension to zero by balancing the positive tension with the negative one.

Consider what kinds of $N(x)$ satisfy the HS theory and the experimental results. Four typical types of $N(x)$ were examined first in the case in which the attached XB undergoes many tilting steps (Fig. 1). Type (a): a monotonically increasing function (Fig. 1a). Though this is the function predicted from the HS theory because of decrease of r with increasing x and $r_i[N]_i = \text{constant}$, this form does not satisfy the T_1 curve. Type (b): a monotonically decreasing function (Fig. 1b). Since $r_i \propto 1/[N]_i$, the detachment of the XB after completion of the tilting steps is the fastest process. This is unlikely. Type (c): an initially increasing and subsequently decreasing function (Fig. 1c). To explain the decrease in the number of the XBs at a later phase of the tilting steps, it is necessary to assume such routes that the XBs are allowed to detach before completion of the whole tilting steps, in

Fig. 1. Four typical types of the XB distribution in the case of many tilting steps. A monotonically increasing (a), a monotonically decreasing (b), an initially increasing and subsequently decreasing type (c), and a type which takes positive values in a limited range of x (d).

Fig. 2. Simple XB distributions in cases of one (a) and two (b) tilting steps. Corresponding XB states are shown below. N_0 represents the detached state.

addition to the forward-going process. Such routes for the detachment have not been suggested with reasonable rate constants by the biochemical studies. Type (d): a function which takes some values only in the range of $0 \leq x \leq 8$ nm (Fig. 1d). This contradicts the initial assumption that the XBs turn over steadily at the plateau of isometric tetanus. Further, to satisfy the T_2 curve, the XB must undergo an additional step when the contractile unit is made to shorten.

To examine the cases of a few tilting steps, simple forms of the XB distribution were assumed, which satisfy both the T_1 and T_2 curves (Fig. 2). The case of a single tilting step, i.e., two attached states (Fig. 2a), is the simplest form of type (b) distribution discussed above. From the steady state condition and since the ratio $[N]_1/[N]_2$ should be two to satisfy the T_1 curve, $[N]_1/[N]_2 = (r_{21} + r_2)/r_{12} = 2$, in which r_{12} is for the forward and r_{21} for the backward reaction in the attached state and r_2 is for the detachment. Since $r_{12} > r_{21}$, $r_2 > r_{12}$. Thus, the detachment is the fastest process, as in the case of type (b) in the case of many tilting steps.

In the case of two tilting steps such as shown in Fig. 2b, where the backward reaction in the second tilting step is neglected because of the reason described later, the ratio of the rate constants for the two steps (r_2/r_{12}) can be obtained if the frequency factor in the Arrhenius' equation is the same in the two reactions. Then, $r_2/r_{12} = \exp\{(W_1 - W_2)/kT\} = 0.056$, in which the stiffness of the elastic part of the XB is assumed to be 3×10^{-4} N/m at 273 K. Since $r_3 = r_2[N]_2/[N]_3$, $r_3 = 0.056 \times r_{12}[N]_2/[N]_3$. The ratio $[N]_2/[N]_3$ cannot be so small that the rate constant for the detachment (r_3) becomes comparable to r_{12} . Comparing the time course of the rapid tension recovery after a quick release with that of the XB detachment studied by photolysis of caged ATP (GOLDMAN *et al.*, 1984), the rate constant for the XB detachment is not so large as that for the initial tilting step. So, such a case is

unlikely. The presence of the rate constant with an appreciable value in the backward reaction of the second tilting step requires a greater rate constant for the XB detachment to explain a significant amount of ATP hydrolysis in an isometric tetanus. Increasing the number of the tilting steps resulted in the same conclusion.

Consequently, the XB distribution deduced from the HS theory with their experimental data appeared to conflict with the observations in muscle energetics and biochemistry. If the type (c) is the case, the nature of the reaction at a later phase in a series of the tilting steps may be quite different from that at an early phase.

Effect of filament elasticity on the XB distribution

So far, the elasticity of the thin and the thick filaments has been ignored to construct a kinetic XB model because of their rigidity except for Thorson and White's computation (THORSON and WHITE, 1969) about the mechanical properties of the insect muscle. Here, the effect of the elasticity of the filaments on the XB distribution was examined using a very simple model, which consists of a single thin filament, one-half of the thick filament, and the XBs between them (Fig. 3). It is assumed that the XB attaches to the thin filament at $x=0$ and instantaneously stretches its elastic part by a fixed amount, generating force. This force, in turn, stretches both the thin and the thick filaments, resulting in reduction of x not only in newly attached XBs but also in already present, force-generating XBs. No detachment of the XBs was considered because the present purpose is not to give a kinetic XB model but to examine the effect of the filament elasticity on the XB distribution.

When the XBs are numbered in the order of attachment, the force exerted by i -th XB in the presence of n XBs ($i \leq n$), $f_{i,n}$, is expressed as follows:

$$f_{i,n} = f_{i,n-1} - K(\Delta l_{i,n} + \Delta L_{i,n}) \quad (1)$$

where K is the stiffness of the elastic part of the XB, $\Delta l_{i,n}$ and $\Delta L_{i,n}$ are new changes in the thin and the thick filament lengths caused by the n -th XB at the points of the i -th XB along the thin and the thick filaments, respectively, from the reference point (the reference point is the z-band end in the thin filament and the center in the thick filament). The following equation concerning the elongation of the filament by the XB forces is applied to each of the two filaments:

$$F_y = E dl_y / l_y \quad (2)$$

where E is the filament stiffness times unit length, F_y the force by the XBs on a point y distant from the reference point along the filament, and dl_y / l_y the fractional increment of the filament length at y . Starting from $n=1$, the XB forces and the resulting disturbances in the filament lengths were computed one by another with increasing x using Eqs. (1) and (2). Examples of the changes in the XB forces and the filament lengths are shown in Fig. 3. For the computation of this example, the following assumptions were made: E is the same in the thin and the thick filament lengths and amounts to 2×10^4 K; the XB stretches its elastic part initially by 5 units

Fig. 3. The model (upper) used for the computation of the changes of x (middle) and the filament lengths (lower) with increasing number of the XBs (n , abscissa). A, thin filament; M, one-half of the thick filament; XB, cross-bridge; Z, z-band. The XBs are assumed to form in the order of the number shown above each XB so that they distribute evenly along the filament. Ordinates in the middle and the lower graphs are in arbitrary but the same units. The lower graph shows the sum of the changes in the thin and the thick filament lengths; a straight line is drawn for convenience.

before stretching the filaments; the thin and the thick filament lengths in the model are the same and 1,000 units. Since in vertebrate skeletal muscle the ratio of the number of the thin to the thick filament is 2, i.e., the number of the force-generating XBs on the thick filament is twice that on the thin filament, the above assumptions correspond to the situation wherein the thick filament is twice as stiff as the thin filament. The example shown in Fig. 3 indicates that the XB distribution becomes broad with increasing number of the attached XBs in the presence of some elasticity in the filaments.

Suppose that one-half of the 3-standed thick filament (3 XBs per 14.3 nm) is $0.8\ \mu\text{m}$ long, and that 20–35% of the total XBs are attached to the thin filament during maximal contraction (ARATA and SHIMIZU, 1981; COOKE *et al.*, 1982), the present computation suggests that the thin and the thick filaments are elongated by 0.5% each at their free ends at most. If, as suggested by X-ray studies (HUXLEY and BROWN, 1967; HASELGROVE, 1975), there is no elongation of the thin filament during contraction, the present computation is equivalent to 1% elongation at the tip of the thick filament. This value coincides with the X-ray data.

In reality, the attachment of the XB may occur not only at $x=0$ but with some distribution along x such as a Gaussian as described by HILL (1974). Therefore, the real distribution of the force-generating XBs as the function of x may be given by the convolution of the two functions: one represents the distribution of the XBs on attachment, and the other concerns the reduction of x in the attached XBs due to the elasticity of the filaments. If the latter is dominant, the XB distribution at the plateau of isometric tetanus is rather constant along x . Though the example shown in Fig. 3 is the case in which there is no XB turnover, the present result can be applied to be case where the XBs turn over steadily.

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