A Program for Developing a Comprehensive Mathematical Description of the Crossbridge Cycle of Muscle

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ABSTRACT We describe a computer modeling system for determining the changes of force, fraction of attached crossbridges, and crossbridge flux rate through a specifiable transition in response to length changes imposed on a crossbridge model of muscle. The crossbridge cycle is divided into multiple attached and detached states. The rates of transition from one state to another are defined by rate coefficients that can either be constant or vary with the position of the crossbridge relative to the thin-filament attachment site. This scheme leads to a system of differential equations defining the rates of change for the fractions of bridges in each state. Solutions for this system of equations are obtained at specified times during and after a length change using a method for systems with widely varying time constants (C. W. Gear, 1971, Numerical Initial Value Problems in Ordinary Differential Equations, Prentice-Hall, Englewood Cliffs, NJ). Crossbridges are divided into discrete populations that differ both in their axial displacement with respect to thin filament attachment sites and with respect to the twist of the actin helix. Separate solutions are made for the individual populations and are then averaged to obtain the ensemble response. Force is determined as the sum of the product of the force associated with each state multiplied by the fraction of bridges in that state. A measure of metabolic rate is determined as the net flux through one of the crossbridge transitions. When the force-extension characteristics of the individual crossbridges are linear and the filaments are noncompliant the fraction of attached bridges is equivalent to sarcomere stiffness. To illustrate the operation of the program, we also describe here some results obtained with a simplified scheme.

INTRODUCTION

It is generally believed that muscle contraction is generated by cyclical interactions between myosin crossbridges and actin thin filaments, with ATP hydrolysis providing the energy (Huxley, 1957). It is also well accepted that the crossbridge cycle is composed of at least several physical and chemical steps (Lymn and Taylor, 1971; Eisenberg and Greene, 1980; Huxley, 1980, p. 94; Geeves, 1991; Geeves et al., 1984). Whereas many experiments are designed to study just one or two of these transitions, the cyclical nature of the actomyosin interaction often precludes such experimental isolation. The exact interpretation of the results therefore demands some prediction of the effect of each intervention on the entire cycle. To make these predictions we have developed a computer program that is capable of determining the response to any length change of a system that can have as many transitions as are known to occur in muscle. We intend to use the program to develop a model of the crossbridge cycle that will explain both our experimental results and those of others.

A distinction must be made between a program, which we describe here, and a model. In the context of this paper, a model is defined to be a specific crossbridge scheme whose transitions are all defined. In contrast, the program is a tool for deriving the response of a particular model. The program makes no judgments about the model, and as such it is quite possible to have the program obtain a solution for a model that is physically impossible. In this sense, the program differs from earlier published models, which have attempted to predict muscle behavior on the basis of equations developed from specific theoretical considerations, but it does allow the features of earlier models to be incorporated. These features include: physical transitions such as the power stroke (Huxley and Simmons, 1971) and crossbridge attachment and detachment (Huxley, 1957; Podolsky et al., 1969); chemical reactions (Lymn and Taylor, 1971); the specification of the sequence of transitions (Julian et al., 1974; Hill, 1974, 1975; Huxley, 1980; Eisenberg and Hill, 1978, 1985); and multiple thin-filament attachment sites (Schoenberg, 1985). The program also allows the rate functions for attachment and detachment to vary with the twist of the actin helix, on the expectation that these functions could vary with the changing orientation of the surface of the thin filament facing the crossbridges.

As explained in the Methods section, the program operates by calculating the fraction of the total crossbridge population that exists in each state in the cycle, termed the "state fraction." The total force in the muscle is calculated as the sum of the force generated by the bridges in each state multiplied by the fraction of bridges in that state. The program also calculates the total crossbridge fraction attached to the thin

Received for publication 31 March 1994 and in final form 15 July 1994.

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0006-3495/0/01.02.03.04.05.06.07.08.09.10.11.12/1669/09 $2.00

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filament at each solution time. When crossbridge force-extension properties are linear and the filaments are non-compliant, this fraction is equivalent to total muscle stiffness. Finally, the program calculates the net flux of bridges through a specifiable transition so as to yield a measure of metabolic rate at each instant in the solution.

The program techniques used here are similar to those developed by Lombardi and Piazzesi (1990) to develop their model of the contractile response to steady lengthening of stimulated muscle, except that: 1) the present program provides that crossbridges attach at discrete thin filament attachment sites; 2) it uses a new version of the transition rates for the force-producing power stroke; 3) it allows the functions for crossbridge attachment and detachment to be modulated by the twist of the actin helix; and 4) most importantly, it allows a wide variety of schemes to be tested. The main purpose of the present description of our program is to facilitate its use by other investigators. It is available over the Internet.

METHODS

Principle of the method

The crossbridge is presumed to cycle through several states. A linear first-order differential equation is written for the rate of change of the fraction of bridges in each state. This rate of change is simply the difference between the rates at which bridges move into and out of the state. These rates are determined as the product of the fraction of bridges in each state multiplied by the rate coefficient for each transition out of that state. In some cases the rate coefficients are constant values. In others, they vary with filament sliding, as the position of a reference point on the myosin crossbridge moves with respect to the actin attachment sites. These varying rates are defined by "rate functions." The resulting equations are solved using Gear's (1971) method for systems having widely varying rate constants. The force in each attached crossbridge is determined from 1) its elastic modulus, which is usually but not necessarily assumed to be linear, and 2) the axial distance between the bridge and its attachment.

To account for neighboring crossbridges having different relationships to their actin attachment sites, solutions are made for individual populations that vary from one another in terms of both their location relative to the actin attachment site and their position relative to the actin helix. The results are then averaged to obtain the ensemble response.

Equation development

The only assumption made in developing the model is that each step or transition in the cycle occurs as a separate stochastic process, so that the rate of each transition is simply the product of the fraction of crossbridges in the starting state multiplied by the rate coefficient for that transition. Although some of the rate coefficients vary with filament sliding, for any given axial displacement of the attachment site relative to the crossbridge the rate coefficient is invariant.

A simple four-state model (Fig. 1) is used here to illustrate how the equations are developed. In this model, there is one detached and three attached states. After attaching, the crossbridge undergoes a power stroke followed by a chemical transition. This leads to the following set of equations, describing the rates of change in each state:

$$d\alpha_1/dt = G \cdot \alpha_2 + I \cdot \alpha_4 - (F + H) \cdot \alpha_1 \quad (1a)$$

$$d\alpha_2/dt = F \cdot \alpha_1 + S \cdot \alpha_3 - (R + G) \cdot \alpha_2 \quad (1b)$$

$$d\alpha_3/dt = R \cdot \alpha_2 + b \cdot \alpha_4 - (S + a) \cdot \alpha_3 \quad (1c)$$

$$d\alpha_4/dt = H \cdot \alpha_1 + a \cdot \alpha_3 - (I + b) \cdot \alpha_4 \quad (1d)$$

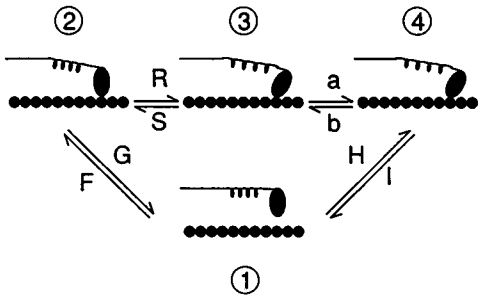

FIGURE 1 A four-state model of the crossbridge cycle with one detached state (state 1), a power stroke (between states 2 and 3), and one chemical transition (between states 3 and 4).

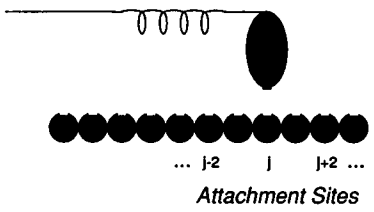

FIGURE 2 Multiple thin-filament attachment sites are within range of the cross-bridge.

where the terms α_i represent the state fractions, the terms F, G, H, I, R, and S represent rate functions, and a and b represent rate constants. By definition, in the steady state, all of the transition rates are 0. However, simply setting all of the $d\alpha_i/dt$ terms to 0 results in an ill-posed problem with no unique solution. This can be resolved by replacing one of the equations with the following, which is obtained from the conservation law (i.e., the sum of all the state fractions is 1):

$$\alpha_1 + \alpha_2 + \alpha_3 + \alpha_4 = 1 \quad (2)$$

Solving this new system of equations yields the steady-state fractions. These fractions serve as the starting condition and represent the isometric steady-state condition immediately before an intervention is made. The rate function values are then updated as length changes progress, and the system of equations given by Eqs. 1a–1d is solved to yield the response of the system.

Multiple attachment sites

Because the crossbridge range of motion is assumed to be greater than the distance between attachment sites, the equations must allow the crossbridges to attach to more than one site (Fig. 2). The addition of another site, as illustrated in Fig. 3, expands the four-state model described in Eq. 1 to the following seven-state model:

$$d\alpha_1/dt = G \cdot \alpha_2 + I \cdot \alpha_4 - (F + H) \cdot \alpha_1 \quad (3a)$$

$$d\alpha_2/dt = F \cdot \alpha_1 + S \cdot \alpha_3 - (R + G) \cdot \alpha_2 \quad (3b)$$

$$d\alpha_3/dt = R \cdot \alpha_2 + b \cdot \alpha_4 - (S + a) \cdot \alpha_3 \quad (3c)$$

$$d\alpha_4/dt = H \cdot \alpha_1 + a \cdot \alpha_3 - (I + b) \cdot \alpha_4 \quad (3d)$$

$$d\alpha_5/dt = F \cdot \alpha_1 + S \cdot \alpha_6 - (R + G) \cdot \alpha_5 \quad (3e)$$

$$d\alpha_6/dt = R \cdot \alpha_5 + b \cdot \alpha_7 - (S + a) \cdot \alpha_6 \quad (3f)$$

$$d\alpha_7/dt = H \cdot \alpha_1 + a \cdot \alpha_6 - (I + b) \cdot \alpha_7 \quad (3g)$$

Note that the same rate constants and functions are used for both attachment sites. For example, the function F specifies the transitions from the single detached state α_1 to attached states α_2 and α_5. In the case of the rate functions,

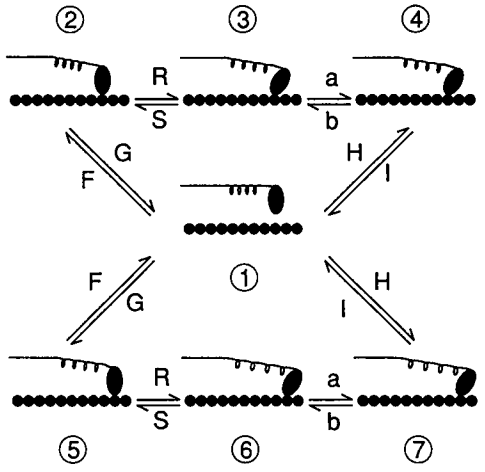

FIGURE 3 Four-state model with two attachment sites.

the program accounts for differences in the distance from the crossbridge to the attachment site by calculating the transition rates separately for each attachment site.

To limit the number of different state fractions associated with different attached states, the program considers a fixed number of sites in both the shortening and lengthening direction at each instant. When filament sliding brings a new site within range in one direction, an old site is removed from consideration in the other. Immediately before a site is recycled, i.e., removed from consideration at one end of the crossbridge range and added at the other, all attached bridges at that site are detached. This provision must be kept in mind when specifying model parameters, as explained below.

Multiple populations

Because the axial spacing of the crossbridges is not an integer multiple of attachment site spacing, neighboring crossbridges will have different orientations relative to the thin-filament attachment sites. The program accounts for these differences by solving the system of equations for several separate crossbridge positions (Fig. 4). The total pool of crossbridges is divided equally into a number of populations that differ only with respect to the axial distance from the crossbridge reference point to the actin attachment site. Because all of the crossbridges move together as the filaments slide, they do not change populations. This allows the responses of the individual populations to be calculated separately and then averaged to obtain the ensemble response for the entire pool.

The minimum number of required crossbridge populations will depend on the characteristics of the rate functions. Rate functions that are discontinuous or that vary sharply within a range where a crossbridge is likely to be attached cause the isometric forces in each population of bridges to be very different before and after a length perturbation. In such cases the transients for the individual populations look very different from the ensemble transients obtained by averaging several populations, so that it may be necessary to average 10 to 15 populations to obtain results that do not vary with the number averaged. In the absence of any sharply varying functions, as few as three populations can produce a response that is not substantially different from that obtained with more populations.

Each crossbridge population can be further subdivided into additional populations that vary with respect to the orientation of the actin helical "twist" relative to the myosin bridge. The effect of these orientation differences is to weight the attachment and detachment functions by multiplying the values in each specified function by a factor that varies with the cosine of the distance along the half-helix. Although the orientations change as the filaments slide, the change is the same for all the bridges within a given population, so that again the bridges do not move from one population to another.

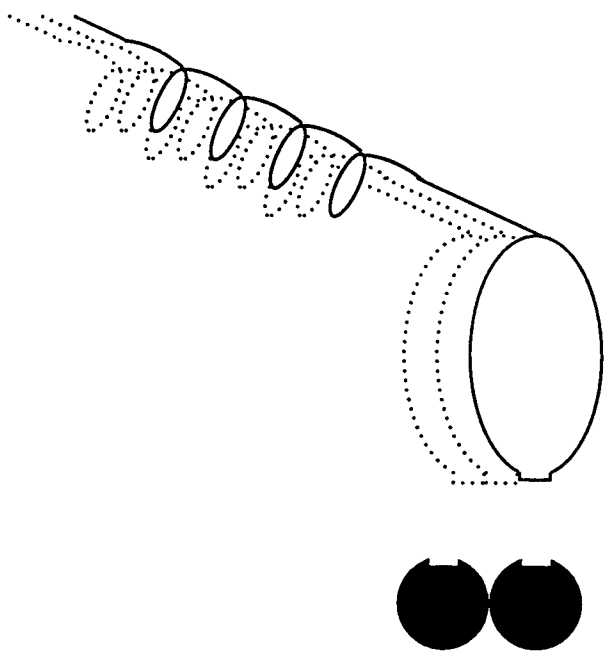

FIGURE 4 To account for the differences in the axial spacings of the crossbridges and the attachment sites, the distance between attachment sites is divided into several intervals, with a separate population (Pi) for each interval. Separate solutions are made for each population and these individual solutions averaged to obtain the ensemble response.

Rate constant and function specification

Rate constants are specified simply in terms of their numeric value (in units of ms^{-1}). To simplify the specification of the rate functions, the user can choose from a number of default functions that are specified by a minimal number of defining parameters. Typically, a gaussian function (Fig. 5*a*) is used for attachment. This function is specified in terms of its peak amplitude, the position of its peak relative to the crossbridge displacement that produces

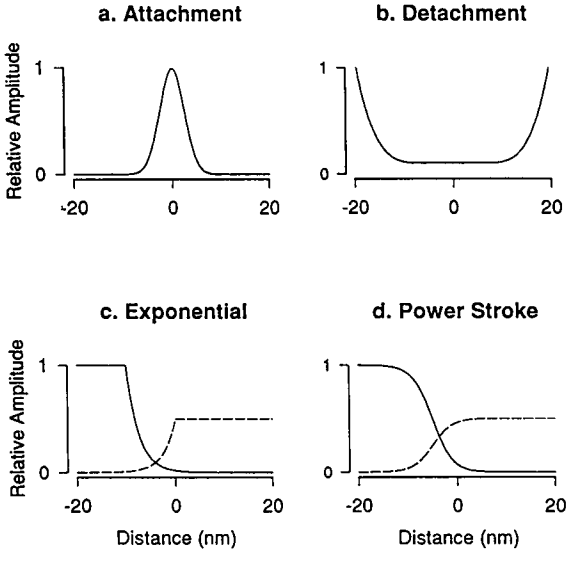

FIGURE 5 Typical rate functions for (*a*) crossbridge attachment, (*b*) crossbridge detachment, and (*c*) forward and reverse exponentials, (*d*) forward and reverse crossbridge power-strokes. Interrupted curves in (*c*) and (*d*) represent reverse rate functions.

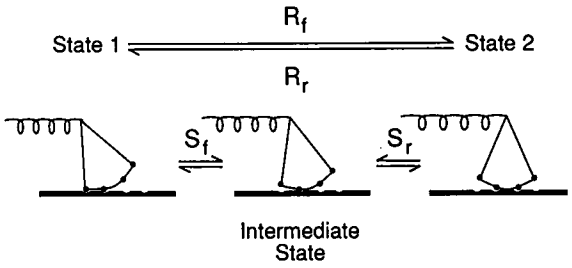

FIGURE 6 Crossbridge scheme illustrating that two separate processes contribute to the power-stroke functions. In going between state 1 and state 2 the bridge must pass through an intermediate state. The rates (R_f and R_r) of going from one state to the other are defined as the product of the rate (S_f and S_r) for leaving one state multiplied by the probability that it will proceed to the next state from the intermediate state.

zero force, and its standard deviation, which defines its width. Detachment functions (Fig. 5 *b*) are typically specified as having a central constant region and steeply rising sides that are specified by separate, single, high-order polynomials.

Some earlier models (e.g., Julian et al., 1974; Lombardi and Piazzesi, 1990) specified the rates for the forward and reverse power strokes as functions that increased exponentially with crossbridge displacement over the range where the power stroke increased the energy stored in the bridge spring. These functions had constant values over the region where the energy in the bridge spring was decreased by the power stroke. Typical forward and reverse functions of this sort are shown in Fig. 5 *c*. (Note that there is a discontinuity in the first derivative of the functions at the transition point from constant value to exponential function.) The rationale for these functions is that the rate constant for going from one state to the next is determined by the sum of the activation energy needed to leave the first state plus the positive work needed to go to the next state. On the other hand, when the work done in going to the next state is negative it does not reduce the activation energy required to leave the first state, so that the transition rate does not continue to increase when force in the spring becomes negative. Functions of this sort, called "forward" and "reverse" exponentials can still be used in the present program to facilitate comparisons with earlier models, but at present we use functions of the type shown in Fig. 5 *d*, termed "forward" and "reverse" power strokes. Note that these functions do not have any discontinuities and that they are symmetrical about their midpoint.

The rationale for these latter functions, illustrated in Fig. 6, is that the power stroke occurs in two steps. In the first it overcomes the activation energy barrier required to leave the first state for an intermediate state, where it is free to oscillate. From this intermediate state it can either settle back into its first state or pass on to the next state. The rate R of transition from one state to another is therefore the product of two components, the rate S at which the bridge leaves the first state for the intermediate state multiplied by the probability P that it will continue to the next state from the intermediate state., i.e.,

$$R = S \cdot P \tag{4}$$

The rate S of leaving the first state is simply specified as a constant in the program, but its value should conform to the Arrhenius equation

$$S = A \cdot \exp(-a/kT) \tag{5}$$

where A is a constant, a is the activation energy barrier, k is Boltzmann's constant, and T absolute temperature. The probabilities of going forward, P_f, or backward, P_r, from the intermediate state are derived from two considerations, 1) that the bridge must go to either one state or the other so that the sum of the probabilities is 1, i.e.,

$$P_f + P_r = 1 \tag{6}$$

and 2) that the ratio of the probabilities is the same as the ratio of time spent very near the two states, which is in turn defined by the difference in the work (w) of going to one or the other, according to

$$P_f/P_r = \exp(-w/kT) \tag{7}$$

The work is in turn equal to the product

$$w = s \cdot h \cdot (x - x_m) \tag{8}$$

where s is the bridge stiffness, h is the distance the bridge spring will be stretched in going between the two states, and $x - x_m$ is the displacement from a position x_m midway between the two states. Combining Eqs. 6, 7, and 8 leads to the following expressions for probabilities of going forward and backward from the intermediate state

$$P_f = \frac{\exp(-s \cdot h \cdot (x - x_m)/kT)}{1 + \exp(-s \cdot h \cdot (x - x_m)/kT))} \tag{9a}$$

$$P_r = 1/(1 + \exp(-s \cdot h \cdot (x - x_m)/kT)) \tag{9b}$$

Combining Eqs. 4 and 5 with Eq. 9 leads to the following expressions for the functions for the forward, R_f, and reverse, R_r, rates of the power stroke (see also Fig. 5 *d*):

$$R_f = \frac{S_f \cdot \exp(-s \cdot h \cdot (x - x_m)/kT)}{(1 + \exp(-s \cdot h \cdot (x - x_m)/kT))} \tag{10a}$$

$$R_r = S_r \cdot 1/(1 + \exp(-s \cdot h \cdot (x - x_m)/kT)) \tag{10b}$$

In specifying power-stroke functions, it is only necessary to specify the values of S_f or S_r, which correspond to the maximum values of the functions; the program calculates the remainder of the function from the absolute temperature, the distance between the states h, the spring constant s, and the displacement from the midpoint of the step $(x - x_m)$. Thus, when using these functions, the dependence of the speed of Phase 2 force recovery on displacement is determined to a large extent by the spring constant and distance moved during the power stroke.

In addition to the standard functions defined above, more elaborate functions can be created using piecewise combinations of polynomial functions. Finally, to allow complete flexibility, the program can import one or more external files containing the function values over a fixed range. The user must create these files in advance, and specify their filenames and directory paths in the "definition files" described below.

To minimize the time taken in calculations, the values of the rate functions are calculated and tabulated only once, when the program is first run. When values are required at other times, they are determined from interpolation in the table. We mention this operation here because the functions are calculated over a range of displacement between −50 nm and +50 nm from the initial position. When external functions are used, the user must provide the values of the functions over the same range. The specifications for these files will be given in the documentation provided with the programs.

A final important point to be made about the operation of the program is that when length changes bring new attachment sites within range of a bridge, old sites are removed from consideration. Just before an attachment site is recycled in this way all bridges on the site are detached. To avoid inaccuracies and discontinuities due to this sudden detachment, it is essential to specify detachment functions that have very high values over the region of filament displacement that causes a bridge to approach the recycling point. Such precautions are less essential when investigating small length steps of the type used in tension-transient experiments. They are very important, however, when long shortening ramps are used to assess isotonic force. These high values of detachment with large displacements mimic the forcible detachment of bridges that must incur large amounts of filament sliding.

Changes in rate coefficients

Some experimental interventions, such as the release of caged compounds, have the effect of altering the rate coefficients for one or more transition in

the cycle. To mimic these alterations, the program can solve the system up to a specifiable time, vary one or more of the rate coefficients, and continue the solution.

General parameter specification

The following parameters must be specified for each model: the number of attached and detached states, the distance between successive actin attachment sites, the length of the working stroke(s), the force-extension characteristics of the crossbridge spring, temperature in degrees Celsius (needed for power stroke calculations), the rate constants and functions for each transition, the order of the transitions, the number of axial and helical crossbridge populations, and the magnitude and phase of the helical weighting factor acting on the attachment and detachment functions. This specification is implemented through the use of a "definition" file, which is read by the main model-solving routine. A sample definition file is listed in the Appendix.

Implementation

At present, the program is implemented in terms of three separate modules: 1) DEF.EXE, a "definition file" editor, which simplifies the specification of all of the model parameters; 2) MDL.EXE, the actual model solver; and 3) DISPLAY.EXE, a graphical display program which simultaneously displays two records and thus facilitates the comparison of various solutions; this program may also be used to compare a theoretical solution with an experimental record.

In addition to the definition file, the model solver requires a time-length file specifying the length deviation from the initial isometric state at each time a solution is desired. It also creates a solution file that contains the values of time, length deviation, force, fraction of attached crossbridges and net flux rate through a specified transition. Finally, switches in the definition file can be set to cause the program to save the solutions for the individual populations and/or save the values of all the state fractions for all populations at all solution times.

The module-based approach facilitates "batch-mode" operation that allows the three programs to be run in different orders. In some cases, for example, it is convenient to first specify a large number of definition files using a batch command, run them in succession with a second batch command, and later display the solutions sequentially using a third batch command. In other cases it is more convenient to run the three programs sequentially for each separate definition file.

At present, all modules are designed to be run on Intel 80386/80486/Pentium-based personal computers having a minimum of 4 MB of memory and a math co-processor.

The main advantage of the DEF.EXE programs is that it eliminates much of the typing required to create a definition file and minimizes the opportunity for mistakes in defining the matrix of rate coefficients. In its present form, it cannot be used to modify the definition files, and an editor program must be used for this purpose. A separate program, FDISP.EXE, can be used to display the rate functions created by the DEF.EXE program.

RESULTS

Results of the simplified scheme

The following general observations were made in the course of implementing the simple four-state scheme described earlier (Fig. 1) with the modification that many more attachment sites are considered.

The two attachment transitions (F and H) were represented by gaussian functions (Fig. 5 *a*) on the assumption that the attachment probability would be highest directly above a thin-filament attachment site and decline steeply as the crossbridges move away from this site. The two detachment transitions (G and I) were represented by a function with a central constant region bordered by steeply rising second-order polynomial functions (Fig. 5 *b*).

The forward and reverse power-stroke transitions (R and S, respectively) were represented by power-stroke functions described in Methods (Figure 5 *d*). Finally, the a and b transitions were simply represented by rate constants. Table 1 lists the rate constant and function values employed.

Fig. 7 shows the simulated responses to three different length steps. As shown, these responses are qualitatively similar to published experimental records (Huxley and Simmons, 1971; Ford et al., 1977). The force transients have the four characteristic phases described by Huxley and Simmons (1971, 1972), and the speed and extent of the early force recovery vary with step size in the manner observed.

Solution times

Although we have not made a systematic study of the various factors that affect solution times, the following observations were made. Because separate solutions are made for each population and then averaged, the solution times vary in direct proportion to the number of populations. Solution times

TABLE 1 Rate constant and function values for the four-state model

Parameter	Type	Value(s)
a	Constant	0.250
b	Constant	0.020
F	Attachment Function	(1.00, 0.00, 2.50)*
G	Detachment Function	(1.0000, −5.0, 2, 0.00010, 5.0, 2, 0.00010)‡
H	Attachment Function	(0.0005, 0.00, 2.00)
I	Detachment Function	(0.0050, −5.0, 2, 0.00010, 5.0, 2, 0.00010)
R	Forward Power-Stroke	(2.50)§
S	Reverse Power-Stroke	(1.50)¶

* A gaussian function with a peak of 1/msec at 0 nm displacement and a standard deviation of 2.5 nm.

‡ A detachment function with a constant central value of 1.000/ms between −5.0 and 5.0 nm. The function has the value $1.000 + 0.0001\ (x - 5.0)^2$ for values of displacement, x (in nm), greater than 5 nm and as $1.000 + 0.0001\ (5.0 + x)^2$ for values of displacement less than −5.0 nm.

§ A forward power stroke function with the value of 2.50/ms for very large values of negative displacement, and declining to 0 for positive displacements (Fig. 5 *d* and Eq. 10a). In the present case, the absolute temperature is specified as 2°C, the crossbridge throw as 10 nm, and the crossbridge stiffness as 0.400 pN/nm (see Appendix).

¶ A reverse power stroke with a constant value of 1.50/ms for large positive displacements and declining to 0 with negative displacements (Eq. 10b and Fig. 5 *d*).

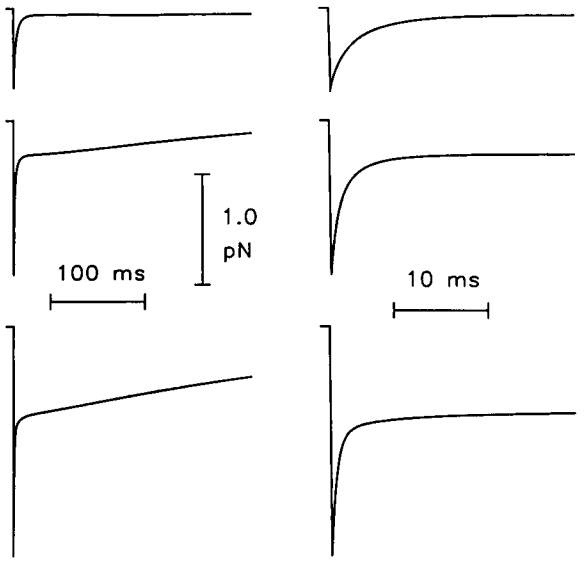

FIGURE 7 Responses obtained by solving the model defined by Table 1 for 2, 4, and 6 nm releases. The early parts of the complete records on the left are repeated on a 10 times expanded time scale on the right to resolve the early events associated with the steps.

also increase with the number of states, particularly attached states, given that they are associated with multiple attachment sites.

Another factor that increases these times is the "stiffness" of the system (i.e., the degree to which the different time constants differ). These effects appear to be greatest when the sarcomere length changes continuously, as during ramp shortening, presumably because the processes with fast time constants continue to contribute to the results throughout the solution period.

Using a 33-MHz 80486-based IBM-compatible computer with 8 MB of memory, we obtained a solution to a 5-nm/half-sarcomere instantaneous release in less than 10 s for the model specified by Table 1 in which we considered five axial populations and one helical population. Increasing the number of attached states to five and detached states to two increased the solution time to 20 s.

DISCUSSION

We describe here a computer program for solving models of the muscle crossbridge cycle. Our understanding of the cycle has progressed to the point where one of the major frontiers in muscle physiology is the determination of the exact sequence and rate coefficients of the transitions in the cycle. An understanding of this sequence is very likely to elucidate the mechanism by which the chemical energy of ATP hydrolysis is converted to mechanical work. For example, some investigators have made the proposal that the energy is released in several power strokes (Huxley and Simmons, 1971) and that phosphate release and rebinding gates the mechanical energy release (Seow and Ford, 1993). A proof of this or any other scheme will ultimately require a match between the experimental data and the proposed scheme. The final scheme for the cycle will require that the rate and order of each transition in the cycle be specified exactly. The program described here can facilitate the exact specification of this scheme. For example, experiments by some of us on the effects of varied ADP concentrations in skinned fibers suggest that elevated ADP concentrations detain crossbridges in high-force states (Seow and Ford, 1992). We found this result difficult to match with a model in which ADP release immediately followed the power stroke (Slawnych et al., 1993). In such a model, the increased rebinding of ADP caused more bridges to accumulate in low-force states that preceded the power stroke. A qualitative match to the experimental results was more readily obtained when we introduced a relatively rapid transition between the power stroke and the ADP release. Such an extra transition might correspond to a second isomerization that has been described in biochemical experiments (Sleep and Hutton, 1980). This experience does not prove by any means that such an extra transition exists, but it does illustrate how extra transitions can isolate various features in the experimental records and affect results that are determined largely by other transitions in the cycle.

The results presented here were obtained from a simple four-state model. We avoided the examination of more comprehensive and realistic schemes to focus on the modeling method. The most important difference between this simplified model and any realistic scheme is the reduced number of states. Such reduced-state models can satisfactorily demonstrate that some aspects of a crossbridge scheme will account for particular aspects of muscle behavior. Nevertheless, as the behavior becomes defined in greater detail, progressively more complete schemes are required. The increasing need for more detailed schemes is therefore a necessary part of our greater understanding of muscle function.

Parameter determination

Although the inherent flexibility in the model may make it difficult to determine unique values for the rate constants and functions values associated with a particular crossbridge scheme, a number of guidelines can be followed.

1. The cycling rate should be comparable to known, experimental values. For example, the osmotically compressed skinned rabbit psoas fibers used in our experiments at 1–2°C produce a maximum power equivalent to about 10 W/l (Seow and Ford, 1991, 1993). If the total free energy available from ATP hydrolysis is 55 kJ/mol and the mechanical efficiency is 50%, the maximum power would suggest a maximum ATPase rate of 0.36 mM/s. If the concentration of crossbridges is 0.28 mM, this ATPase rate would indicate a maximum crossbridge cycling rate of 1.3/s. The assumption that the isometric ATPase rate is ⅓ of the maximum rate (Kushmerick and Davies, 1969) further suggests that the isometric crossbridge cycling rate is about 0.4/s. Allowing that our calculations are imprecise, we generally try to arrange the magnitude of the rate functions so that the isometric cycling rate is between 0.2 and 0.6/s.

2. The fraction of attached bridges should stay within a reasonable range. Although there is very little experimental evidence to indicate a firm value for this fraction, we generally attempt to keep it in the range of 0.4 to 0.8 (cf. Huxley, 1957).

3. The force-velocity properties of the theoretical system should match experimental values. These properties can be readily determined by subjecting the system to shortening ramps sufficiently large to bring force to a steady level.

4. Lastly, by examining the individual state fractions before, during, and after the length perturbation, one should be able to confirm whether or not the particular scheme is operating as expected.

Use of the program in designing experiments

Whereas a model is usually used to interpret experimental findings, it can also be very useful in designing future experiments. Given that experimental measurements must be limited by the time taken to collect the data and by the limited number of conditions that can be tested, being able to anticipate optimal conditions for obtaining the anticipated result can greatly shorten the number of experimental observations that must be made. Another hope for the program therefore is that it will be used to determine the best types of experiments to advance our knowledge of crossbridge function.

APPENDIX I: SAMPLE "DEFINITION" FILE LISTING

```
ASTATES          Number of attached states
  3
DSTATES          Number of detached states
  1
P                Number of axial cross-bridge populations
  3
S                Number of helical populations
  1
V                Half period of actin helix
  36.000
MinF             Helical weighing factor for attachment functions
  1.00000
MinG             Helical weighing factor for detachment functions
  1.00000
PhaseF           Helical phase shift for attachment functions
  0.00000
PhaseG           Helical phase shift for detachment functions
  0.00000
K                Spring constant (pN/nm)
  LINEAR
  0.40000
DA               Distance between consecutive attachment sites
  5.46000
H(i)             Crossbridge movement vector
  0.00000
  10.00000
  0.00000
T                Temperature (Celsius)
  2.0
RATE MATRIX
  - G * I
  F - S *
  * R - b
  H * a -
RATE CONSTANTS
  2
a                0.003000
b                0.000020
RATE FUNCTIONS
  6
F
GAUSSIAN
     5.000000 0.000000 2.500000
G
DETACHMENT
     0.500000
     -5.00000 2 0.000100
     5.000000 2 0.000100
H
GAUSSIAN
     0.000500 0.000000 2.00000
I
DETACHMENT
     0.100000
     -5.0000 2 0.00100
     5.0000 2 0.00100
R
FORWARD POWER STROKE
     2.500000
S
REVERSE POWER STROKE
     0.10000
Flux mode 0→ no flux monitoring 1→ flux monitoring enabled
  1
D1A1 Monitors flux from detached state 1 to attached state 1
Graph mode 0→ no plot 1→plot
  0
Population Save mode (six options, as explained below)
  0
State Fraction Save mode (three options, as explained below)
  0
Caged release mode
  1
  200 2
  a .005000
F
GAUSSIAN
     0.500000 0.000000 2.500000
```

In all cases, rates are in units of ms^{-1}. Lengths are in units of nm with the 0 reference being the value of crossbridge position, relative to the thin-filament site, where force in the spring is 0 and the crossbridge has not undergone an internal, power-stroke type of displacement. The values of the variables are generally preceded by a text line explaining the meaning of the variable. Some lines containing text strings can be variables. Examples of these are strings such as "LINEAR" and "GAUSSIAN." The transitions between states are specified in terms of a rate matrix, as illustrated above. The position of a letter in this matrix designates the name of the rate constant or function for the transition proceeding from the state specified by the column to the state specified by the row. For example, the function "F" specifies the transition from α_1 to α_2. To facilitate the interpretation of these matrices, we generally follow a convention in which rate functions are specified by upper-case letters and rate constants are specified by lower-case letters, but this is not required. The values of the rate functions and constants are specified following the rate matrix. The names of the coefficients must be specified by a one- or two-character string variable with the first character being a letter and the second character, if present, either a number or a letter. Other definitions that require explanation follow.

1) Attachment and detachment functions can be altered according to the helical twist of the thin filaments. This provision is activated when the number of helical populations is >1. Every value in the attachment or detachment function is then multiplied by a cosine function of the actin helical twist. The value of the function varies from 1, at phase angle 0, to the value specified by MinF and MinG, at phase angle π. The values of MinF and MinG must be positive and can be >1, so that the values of the functions can be increased as well as decreased. In addition, the phase angle for each of the two weighing factors can be set independently.

2) The spring constant can be designated as either LINEAR or NONLINEAR. When it is linear, as in the present example, the next line gives the value (0.400) of the spring constant. If it is NONLINEAR the extension-force characteristics will be quasi-sigmoidal and defined by a function that is linear from the origin to designated positive and negative values of displacement, beyond which the slopes flatten as the functions exponentially approach limiting values of force. When a function is designated as NONLINEAR, the next line must have eight values, the first four specifying the function for positive displacements and the last four specifying the function

for negative displacements. These four values specify, in order, the slope of the linear part of the function, the value of displacement at which the function becomes exponential, the final value of force approached by the exponential, and the length constant of the exponential. At present, we have not yet developed a provision for calculating the power stroke functions using nonlinear spring functions, so when the spring is designated as nonlinear the power stroke functions must be specified in another way.

3) The crossbridge movement vector defines the distance that a bridge moves when it undergoes the transition from attached state $i - 1$ to attached state i. It is likely that the H(1) transition, representing initial attachment, will always have zero movement, as will chemical transitions, but the program does not preclude length steps accompanying these transitions.

4) The specification of ATTACHMENT, GAUSSIAN, and POWER STROKE functions are explained in Table 1. FORWARD and REVERSE EXPONENTIAL functions (Fig. 5 *c*) are specified by three numbers, the maximum amplitude that defines the plateau level, the transition point where the function becomes exponential, and the length constant for the exponential. To minimize mistakes, only the values of displacement can be negative. The length constant for the REVERSE EXPONENTIALs, e.g., are specified as positive. In addition to these encoded functions, the user can specify the function as "external" with three lines of code. The first line contains the one- or two-character string designating the name of the function; the second line contains the string "EXTERNAL;" and the third contains the filename, including any necessary path specifications, of the file containing the values of the function. The user must create and save this file in advance. Because the program may be changed in the future, precise instructions for these files are not given here but will be given in the documentation supplied with the software.

5) When flux monitoring is disabled by setting flux mode to 0 there will be no data line following the 0. When flux monitoring is enabled by setting flux mode to 1, the next line will contain a four-character code specifying the transition that is to be monitored. In the example shown, the transition is from Detached state 1 to Attached state 1.

6) When graph mode is set to 1, the program displays a graph of the solution. This provision has not often been used since the DISPLAY.EXE program was developed.

7) The Population Save Mode switch determines which populations will be saved and whether the columns will have headers. In all cases, four or five columns of data are saved. These are, in order: Time, Length, Force, Fraction of bridges attached, and (when flux monitoring is enabled) Flux rate through a specified transition. The first two columns are derived directly from the time-length input files. When the switch is set to 0, 2, or 4 there are no headers on the five columns (a necessary provision for programs such as the DISPLAY.EXE, which accept only numeric data). When the switch is set to 1, 3, or 5 the columns have headings (a useful provision for examining the data in tabular form). When the switch is set to 0 or 1 only the data for the ensemble population is saved. (The ensemble population is the average of the state fraction values at each time point for all the populations.) When the switch is set to 2 or higher, there is saved, in addition to the ensemble data, the data for the individual populations. The operation of the different switches is explained in the README.DOC file supplied with the programs.

8) The State Fraction Save Mode switch enables the individual state fractions to be saved. When the switch is set to 0, no state fraction data is saved. The use of a mode other than 0 will greatly slow program operation because the higher modes result in a great deal of tabular data being appended to files stored on disk. When the switch is set to 1, the program saves a file containing the state fractions for each solution time when a solution is specified. The state fraction values for attached states are averaged for the different thin filament sites. When the switch is set to 2, the program creates and saves several files containing the state fractions at every time point in each population, with a separate file for all the detached states and one file each for the attached states on each thin filament site. There are separate groups of files for each separate step (called a run). The need for multiple files arises because of the large possible number of state fractions, each associated with a different attachment site. The need for separate groups of files for each step arises because different step sizes cause the program to consider different numbers of thin filament sites within reach of the crossbridge, so that there may be more files saved for larger steps. The nature of these output files is described in greater detail in the README.DOC file supplied with the programs.

9) There is an additional program, FDISP.EXE, that will display these state fractions.

10) When the caged release mode is set to 1, a solution is obtained up to a specified time and then continued with one or more altered rate coefficients. The next line specifies the time up to which solutions are to be made with the old rate coefficients and the number of coefficients that are to be changed. The following lines specify the new rate coefficients in the same manner as in their original specification. In the example shown, a solution would be obtained up to 200 ms with the original rate coefficients. At 200 ms the value of the rate constant a would change from 0.004 to 0.005, the peak height of the F attachment function would change from 1.0 to 0.5, and the solution would continue until the last time specified by the time length file. This provision was not implemented in creating the records shown in Fig. 7.

Supported in part by a Fogarty International Fellowship to Dr. Ford, a Senior Research Fellowship of the American Heart Association of Metropolitan Chicago to Dr. Seow, U. S. Public Health Service grant HL 44398, a grant from the European Commission to Prof. Huxley, and a grant from the American Heart Association of Metropolitan Chicago.

We thank Dr. B.H. Bressler for advice and encouragement.

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