Simulation of molecular steps in muscle force generation

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A previously described matrix method was adapted for solving the kinetics of reaction schemes. The method was used for simulation of the kinetics of a simplified acto-myosin ATPase cycle in muscle fibres: the simulated tension (=force) transients for pressure and temperature perturbations possess the basic features observed in the actual experimental records.

Keywords: muscle force generation; matrix method; tension transients; **Q**-matrix; temperature jump

1. INTRODUCTION

Solving the kinetics of reaction schemes is often necessary in the elucidation of the underlying mechanisms of physiological processes. The method commonly used is the numerical integration of the relevant differential equations (see Gutfreund 1995). In this paper, we describe another method—an adaptation of the matrix method described by Colquhoun & Hawkes (1995). The matrix method gives results directly in the form of the reciprocal relaxation times (apparent rate constants) and amplitudes for the sum of the exponentials representing the time-course of the formation and decay of each state of the system. Therefore, it can be considered equivalent to the insight obtained from analytical solutions of the rate equations. To obtain such information from numerical integration of the set of differential equations the data generated have to be fitted (by nonlinear least-square) to an equation for the sum of the exponentials. The matrix method also permits rapid comparison with the reaction scheme and the simulation of relaxations of perturbations of equilibria and steady states. However, it can only be applied to systems that are linear or can be approximated (buffered) to be so.

The particular reaction scheme used for illustration is that proposed in a preceding paper (Ranatunga 1999). In that paper, the rate of force generation induced by a standard temperature jump (endothermic force generation) was examined in single-skinned (rabbit psoas) muscle fibres when they were exposed to different levels of inorganic phosphate (Pi, a product released during ATP hydrolysis in active muscle). The steady force was reduced by increased Pi, but the apparent rate constant (reciprocal relaxation time) for force generation increased and exhibited saturation at higher Pi levels. Similar saturation kinetics for Pi dependence were obtained in previous studies (Fortune *et al.* 1991), where force generation was examined using the hydrostatic pressure perturbation technique. In an attempt to accommodate the findings from the two perturbation techniques, we propose a

scheme for the molecular steps involved in Pi release and force generation in active muscle fibres. According to this scheme, Pi release from attached cross-bridges involves three reversible and sequentially faster steps. Step I is a moderately slow, pressure-sensitive, pre-force generation step, step II is moderately fast and represents temperature-sensitive (endothermic) cross-bridge force generation and step III is a very rapid Pi release.

The aim of this paper is to present the matrix method and to apply it for simulation of the kinetics of a simplified acto-myosin ATPase cycle during pressure and temperature perturbation of muscle fibres.

2. METHODS

The $n \times n$ transition matrix (**Q**-matrix) for a system that exists in n states is constructed as follows. For instance, for the four-state system A₁ to A₄ each element q_{ij} (except for $i=j$) of the **Q**-matrix is the rate constant k_{ij} for the transition from state A_i to state A_j. For the diagonal elements we enter the sum of the negative values of the rate constants for the decay of the state described by that row. The result is that the sum of the elements in each row is zero.

$$Q = \begin{matrix} & \begin{matrix} A_1 & A_2 & A_3 & A_4 \end{matrix} \\ \begin{matrix} A_1 \\ A_2 \\ A_3 \\ A_4 \end{matrix} & \begin{bmatrix} -(k_{12}+k_{13}+k_{14}) & k_{12} & k_{13} & k_{14} \\ k_{21} & -(k_{21}+k_{23}+k_{24}) & k_{23} & k_{24} \\ k_{31} & k_{32} & -(k_{31}+k_{32}+k_{34}) & k_{34} \\ k_{41} & k_{42} & k_{43} & -(k_{41}+k_{42}+k_{43}) \end{bmatrix} \end{matrix}$$

The n eigenvalues (λ_i) of an $n \times n$ **Q**-matrix give the reciprocal relaxation times for the system. The principle of the transition matrix comes from the treatment of Markov chains in stochastic theory (see for instance Feller 1968; Crow & Kimura 1970). Multiplication of the current state of a system, the vector $\mathbf{y}(t)$, by the **Q**-matrix gives the change $\mathbf{y}(t + \Delta t) - \mathbf{y}(t)$, where $\mathbf{y}(t)$ is a vector of the occupations of all the states at time t and Δt is the time increment. This can be written as

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$$y(t + \Delta t) = y(t)(1 + Q\Delta t).$$

The Q -matrix for a complex system can be considered to be analogous to the single rate constant (k) of the simple process $A \rightarrow B$. In that case

$$c_A(t + \Delta t) = c_A(t)(1 + k\Delta t).$$

As stated above, the method was originally developed for stochastic processes involving transition probabilities in the elements of the Q -matrix. Two factors have to be considered. The values for the probabilities have to be $p \leq 1$ and the units of rate constants are s^{-1} and, therefore, Δt has to be small enough for $Q\Delta t$ and $k\Delta t$ to be < 1 . If these conditions are not fulfilled changes per unit time will not produce linear increments.

As described so far the use of the Q -matrix provides a simple form of stepwise numerical simulation—a ‘look-alike’ of the Euler stepping method for numerical integration. Generally available software such as Excel can be used for multiplication of matrices. However, analogous to the simple rate equation, the matrix equation can be integrated as follows:

$$\frac{dy}{dt} = Qy \text{ and } \int \frac{dy}{y} = Q \int dt$$

and

$$y(t) = y(0) \exp(Qt)$$

A useful observation in matrix algebra is that a function of a matrix (M) can be expressed in terms of the function of its eigenvalues (λ_i) and its spectral matrices (S_i) by the following sum:

$$f(M) = \sum_{i=1}^n f(\lambda_i) S_i.$$

The spectral expansion into n (for an $n \times n$ matrix) spectral matrices S_i first involves the evaluation of the n eigenvalues (λ_i) and the n_i eigenvectors (e_i) of the Q -matrix (see Gutfreund (1995) for elementary definitions and applications). The n eigenvectors form an $n \times n$ matrix (E) which, together with its inverse (E^{-1}), are used to construct the spectral matrices. Rows e_i of E and columns e_j of E^{-1} , for $i=j$, are used to evaluate S_i as the products $e_i e_j^T$.

(a) A numerical example

A simple numerical example will illustrate the sequence of operations as well as the information obtained from eigenvalues and the spectral matrices. There are a number of checks to ascertain that the matrices have been set up correctly. The determinant of Q must be zero, the sum of the spectral matrices must be an identity matrix and the product of the spectral matrices must be a zero matrix. The software used here is Mathcad 7, but these operations can also be performed in Maple, Mathematica, Matlab, Derive and, no doubt, in other mathematical programs.

The model used for the numerical illustration is a four-state reversible sequence:

The elements of the Q -matrix are entered here as values for the rate constants as described above and can be easily changed for another simulation:

$$k_{11} = -k_{12}, k_{12} = 20, k_{13} = 0, k_{14} = 0, k_{21} = 35, \\ k_{22} = -(k_{21} + k_{23}), k_{23} = 50, k_{24} = 0$$

Figure 1. The time-course of the approach to equilibrium of the occupation of the four states. The plots were generated and displayed by Mathcad, using the initial amplitudes and rate constants as described in the text (t is time in seconds).

$$k_{31} = 0, k_{32} = 135, k_{33} = -(k_{32} + k_{34}), k_{34} = 1000, \\ k_{41} = 3, k_{42} = 0, k_{43} = 5000, k_{44} = -(k_{43} + k_{41}).$$

The number of states is $n=4$ and the eigenvalues and the matrix of eigenvectors are obtained by single commands in Mathcad:

$$\lambda = \text{eigenvals}(Q), \quad E = \text{eigenvecs}(Q), \quad i = 1 \text{ to } n,$$

$$\lambda = \begin{bmatrix} 0 \\ -40.862 \\ -176.532 \\ -6025.61 \end{bmatrix} \quad E = \begin{bmatrix} 0.5 & -0.368 & 0.047 & 0 \\ 0.5 & 0.384 & -0.37 & 0.002 \\ 0.5 & 0.597 & 0.644 & -0.2 \\ 0.5 & 0.601 & 0.668 & 0.98 \end{bmatrix},$$

$$E^{-1} = \begin{bmatrix} 1.099 & 0.624 & 0.23 & 0.046 \\ -1.191 & 0.703 & 0.406 & 0.082 \\ 0.25 & -1.129 & 0.729 & 0.151 \\ -0.001 & 0.02 & -0.863 & 0.844 \end{bmatrix}.$$

The n spectral expansion matrices S_1 to S_n are derived as described above with the Mathcad commands

$$S_i = \text{submatrix}(E, 1, n, i, i) \times \text{submatrix}(E^{-1}, i, i, 1, n).$$

The initial occupation of the states is defined by the vector $y(0)$ with n elements. The time-course for the occupancy of the n states is given by the sums of the exponentials

$$y_1(t) = \sum_{i=1}^n y(0)_i \cdot \text{submatrix}(S_i, 1, n, 1, 1) \cdot \exp(\lambda_i t) \\ y_2(t) = \sum_{i=1}^n y(0)_i \cdot \text{submatrix}(S_i, 1, n, 2, 2) \cdot \exp(\lambda_i t) \\ y_3(t) = \sum_{i=1}^n y(0)_i \cdot \text{submatrix}(S_i, 1, n, 3, 3) \cdot \exp(\lambda_i t) \\ y_4(t) = \sum_{i=1}^n y(0)_i \cdot \text{submatrix}(S_i, 1, n, 4, 4) \cdot \exp(\lambda_i t).$$

Figure 1 is a plot of the above functions against time and illustrates the time-course of approach to equilibrium for the occupancy of the four states as generated by Mathcad, following definition of the initial amplitudes.

The final equilibrium (or steady-state) concentrations (end-points) are given by the vector

Figure 2. Simulation of tension (=force) transients for the three-step phosphate release mechanism (see the text). The sum of occupancy of state 3 ($AM^*.ADP.Pi$) and state 4 ($AM^*.ADP$) is assumed to represent force (ordinate: the scale is relative to force at the 1 mM phosphate level, i.e. with no added phosphate). (a) Tension transients upon rapid release of Pi (from 1 mM up to 32 mM). Note increased but faster force depression with increased Pi concentration. The dotted curve shows one tension transient (8 mM Pi release) on a faster time-scale (upper labelling on the abscissa): a short initial delay is seen, as in experiments (see Dantzig *et al.* 1992). (b) Depression of steady force with increased Pi concentration; open squares are steady tensions measured from simulated transients as in (a) and the curve fitted to them is a logarithmic one (Cooke & Pate 1985). Mean (s.e.m.) experimental data for 12 °C are also shown (filled circles). Note the depression is 40–50% between 1 mM (no added Pi) and *ca.* 25 mM of Pi. Simulated tension transients following (c) temperature jumps and (d) hydrostatic pressure release at 1 mM (upper) and 8 mM (lower) added Pi. Note that temperature jump transients are faster than pressure jump transients, but in both cases the observed rate is increased at high Pi concentration, as obtained in the experiments (see Ranatunga 1999). In addition, a short initial delay is seen in transients in (d), as observed in slow fibre pressure jump experiments (N. S. Fortune, M. A. Geeves and K. W. Ranatunga, unpublished data).

$$\mathbf{y}(\infty) = \begin{bmatrix} 0.55 \\ 0.312 \\ 0.115 \\ 0.023 \end{bmatrix}$$

For the simulation of a relaxation phenomenon, after a steady state is reached, the rate constants of the temperature- (or pressure-) sensitive step are changed to correspond to the required change in the equilibrium constant. The occupations of the previous final state ($\mathbf{y}(\infty)$) are entered into $\mathbf{y}(0)$ and the program will calculate reciprocal relaxation times (λ_i) and amplitudes for the relaxation to the new conditions.

3. RESULTS AND DISCUSSION

(a) Simulation of force generation

As has been done in previous studies (Fortune *et al.* 1991; Dantzig *et al.* 1992), a simplified acto-myosin ATPase cycle was used in the simulation of phosphate dependence of tension transients at 12 °C. It consisted of four states in a linear scheme as

Step IV, i.e. $AM^*.ADP \rightarrow AM.ADP.Pi$, was taken as irreversible and rate limiting ($5\text{--}10\text{ s}^{-1}$; see Dantzig *et al.*

Figure 3. A comparison of simulated and experimentally recorded temperature jump tension transients. (a) The experimental tension transients from one fibre, which were illustrated in fig. 1 of Ranatunga (1999), are superimposed on those produced by simulation (smooth curves). The upper record (left ordinate) was made with no added Pi (i.e. at 1 mM Pi) and the lower record (right ordinate) with 12.5 mM added Pi (i.e. at 13.5 mM). Note that the model simulates only the fast, Pi-sensitive component (phase 1 or endothermic force generation) of the tension transient. (b) A comparison of the Pi dependence of phase 1 amplitude. The experimental data are shown by filled circles and the solid curve (from fig. 3 of Ranatunga 1999) and the simulated data by the open squares and the dotted curve. The amplitudes are given as percentages of the post-temperature jump tension and those from simulation were scaled (halved) to make the comparison. Note that the normalized phase 1 amplitude tends to be larger at higher Pi levels.

1992): it represents all the steps necessary to reprime the cross-bridges for the next cycle. Thus, the four states A_1 , A_2 , A_3 and A_4 in the scheme used in the previous section now correspond to $\text{AM} \cdot \text{ADP} \cdot \text{Pi}$, $\text{AM}' \cdot \text{ADP} \cdot \text{Pi}$, $\text{AM}^* \cdot \text{ADP} \cdot \text{Pi}$ and $\text{AM}^* \cdot \text{ADP}$ cross-bridge states, respectively. As discussed in a preceding paper, step I is the pressure-sensitive pre-force generating step and its forward and reverse rate constants (i.e. k_{12} and k_{21}) were taken from the pressure jump study (Fortune *et al.* 1991). Step II is the temperature-sensitive force generation step and the rate constants k_{23} and k_{32} were taken from a preceding (temperature-jump) paper (Ranatunga 1999). $\text{AM}^* \cdot \text{ADP} \cdot \text{Pi}$ and $\text{AM}^* \cdot \text{ADP}$ are high (and equal) force states and the sum of their occupancy was considered to

represent muscle force (= tension). Step III is the rapid phosphate release step, where k_{34} was set as 10^3 s^{-1} and k_{43} was adjusted to obtain an appropriate level of phosphate sensitivity of tension depression; the range of k_{43} values used corresponds to a second-order rate constant of $> 1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for phosphate binding.

Figure 2a shows a family of tension transients generated from simulated rapid phosphate release experiments; note the increased rate of tension decrease with phosphate level. The dotted trace is one of the simulated tension traces displayed on a faster time-scale (upper labelling of time axis); it shows a brief (*ca.* 1 ms) initial delay before the tension decrease, as obtained in the actual experiments (see Dantzig *et al.* 1992). The open symbols in figure 2b represent the steady tension values from simulated tension transients at a number of phosphate levels and a logarithmic curve (dotted curve) is fitted to them, as done in previous studies (see Dantzig *et al.* 1992). The tension depression obtained at *ca.* 25 mM added phosphate is *ca.* 40–50%—as in the actual experiments. For comparison, the experimental data (mean $\pm$ s.e.m.) for 12 °C in figure 2a of Ranatunga (1999) are also shown (filled symbols). Figure 2c,d shows two simulated tension transients generated by a standard temperature jump from *ca.* 9 to 12 °C (figure 2c) and by a standard pressure release of *ca.* 15 MPa (figure 2d) at 1 mM (i.e. no added phosphate, upper trace) and at 8 mM (lower trace) of phosphate. Note that tension transients are slower from pressure-release simulation than from temperature-jump simulation—as found experimentally—and the rate of force generation in both is faster with higher phosphate level (lower traces). An initial delay of *ca.* 2–3 ms is seen in pressure-release tension transients (figure 2d). This was not observed in our experiments on fast (psoas) fibres, but tension oscillations resulting from pressure release may have masked this. Indeed, an initial delay was seen in experiments on slow fibres, probably due to their slower kinetics (N. S. Fortune, M. A. Geeves and K. W. Ranatunga, unpublished data).

A direct comparison of two experimental tension transients and their simulations (smooth curves) is illustrated in figure 3a. The experimental traces are from one fibre (given in fig. 1a and 1b in Ranatunga (1999)). The upper trace was recorded when the fibre was exposed to no added phosphate (assumed to be at 1 mM) and the lower trace was recorded when exposed to 12.5 mM added phosphate (i.e. at 13.5 mM). The amplitudes of the simulated transients are scaled (halved) and superimposed. It is seen that the proposed model only accounts for the initial fast component (phase 1, endothermic force generation) of the tension transient. The slow phase 2 of the tension transient, induced by temperature jump or pressure jump, is largely phosphate insensitive and it may reflect the presence of a rate-limiting step after the release of Pi in the cross-bridge cycle (see Ranatunga 1999). Figure 3b shows the phosphate dependence of phase 1 amplitude from the experiments (filled symbols and solid curve) and from simulations (open symbols and dotted curve); in both cases, the amplitude represented as a percentage of the post-temperature-jump tension tends to be larger at higher phosphate levels. The half-time measurements made on the simulated tension transients with the three different types of perturbations showed that the apparent

rate constant increased two- to fourfold and saturated as the phosphate level was increased to > 25 mM. The dissociation constant for phosphate release (K_d), determined from analyses of the reciprocal half-times of simulated tension transients at different phosphate levels, increased in sequence: pressure jump (3 mM) $<$ temperature jump (7 mM) $<$ phosphate release (10 mM). Although the exact values were different, the same ascending sequence was obtained (3 mM pressure jump $<$ 8 mM temperature jump $<$ 12 mM phosphate release) in actual experiments.

The primary aim of this paper was to demonstrate the potentialities of the matrix method for simulating kinetic reaction schemes. The simulations presented here used an acto-myosin ATPase cycle with a simplifying assumption that a given perturbation altered a single step: incorporation of rapid temperature-sensitive steps before step I (e.g. ATP hydrolysis), and/or after step IV (e.g. ADP release), did not substantially alter the basic findings. However, the model used is restricted to phase 1 (endothermic force generation): it does not account for the slower, phosphate-insensitive phase 2 that was obtained both in temperature jump and in pressure jump experiments. The purpose of comparing simulations with the experimental results was to provoke thoughts towards future improvement of the model. It would be of interest to extend the three-step phosphate release and force generation mechanism to a more comprehensive acto-

myosin ATPase cycle including, in addition, any strain-dependent features.

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