# Supporting Text

## Effect of MgATP and Pi on Tension

Similar to the $fw_{max}$ dependencies, maximal $Ca^{2+}$-activated isometric tension ($T_{max}$) of IFI and EMB fibers exhibited qualitative differences in response to MgATP and almost opposite dependencies on Pi. For both fiber types $T_{max}$ decreased exponentially with increasing [MgATP] (Fig. 4A). However, an approximate 4-fold higher concentration of MgATP was required to reach a level of saturation in IFI fibers (5-10 mM) than in EMB fibers. $T_{max}$ of IFI fibers was relatively insensitive to [Pi] at saturating levels of MgATP (15-20 mM) (Fig. 4B), whereas at 5 mM MgATP, $T_{max}$ increased 20% as Pi was raised from 0 to 16 mM. The increase in tension is most likely due to competition between Pi and MgATP for A.M. (see Fig. 3C) during the MgATP induced detachment step. The unusually low affinity of IFI myosin for MgATP causes this effect to be prominent in IFI muscles. In contrast, EMB $T_{max}$ declined by 30% over the same range of [Pi], showing the same inhibitory effects of Pi usually observed in vertebrate skeletal and cardiac muscle (1-5).

## Sinusoidal Length Perturbation Analysis

Fig. 5 illustrates the method of sinusoidal analysis (details are reported elsewhere (6, 7)). At top, small amplitude sinusoidal length perturbations are applied and the phase and amplitude relation between the applied length change ($\Delta L$) and resulting force change ($\Delta F$) were measured over a range of frequencies ($f$ = 0.5-1000 Hz). Small amplitude length changes (0.25% muscle length (ML) peak to peak) were used as above 0.50% ML the force response becomes markedly non-linear with muscle length sinusoidal perturbation (unpublished observations, D.W.M.), the application of which would have made our analysis inaccurate. The complex modulus y(f) is calculated as the ratio of tension change ($\Delta F$ divided by fiber cross-sectional area) and the fractional change in muscle length ($\Delta L/L_o$, equal to 0.00125 under our standard conditions). In the exemplar Nyquist plot shown at bottom right, the viscous modulus (the $90^o$ out-of-phase component of y(f)) is plotted versus elastic modulus (the in-phase component of y(f)). We deconvolve the Nyquist plot to yield components *A, B* and *C*, where $y(f) = A\,(2\pi\, if/\alpha)^k - B\, if/(b+if) + C\, if/(c+if)$, where i = , $\alpha$ = 1 Hz, and k is a unitless exponent. Coefficients A, B and C are the magnitudes of *A, B,* and *C*, expressed as mN per $mm^2$ fiber cross-sectional area. The characteristic frequencies of *B* and *C* are b and c, expressed in Hz. Note that the viscous modulus of *B* is negative, which denotes work-producing cross-bridge processes, whereas the viscous modulus of *C* is positive, which denotes work-absorbing cross-bridge processes. Work (J $m^{-3}$) is equal to $\pi E_v (\Delta L/L)^2$, where $E_v$ is the viscous modulus, and $\Delta L/L$ is the amplitude of the sinusoidal length change divided by the length of the fiber between the two T-clips. Power (W $m^{-3}$) is equal to frequency (f) times $\pi E_v (\Delta L/L)^2$.

**Adequacy of the MgATP Regeneration System**

We investigated whether differences in ability to adequately buffer MgATP might contribute to the 8-fold higher [MgATP] required to saturate IFI fiber kinetics compared to that of EMB. The different response to [MgATP] between IFI and EMB fibers would be expected to occur if the creatine phosphate/creatine kinase (CP/CK) MgATP regeneration system is able to maintain adequate levels of MgATP (and/or is able to keep MgADP levels near zero) inside EMB fibers, but is not able to do so in IFI which may have a much higher ATPase rate. To test the adequacy of the buffer system, we increased [CP] step-wise from 1 to 50 mM, with [CK] at 900 U/ml (Fig. 6). We used the frequency of maximum power output, $f_{Wmax}$, as a kinetic parameter particularly sensitive to MgATP and MgADP levels. At 5, 7.5 and 10 mM MgATP, CP concentrations equal to or less than 15 mM limited $fw_{max}$, but above 20 mM CP, the regeneration system was adequate since no further effect of increasing [CP] on $fw_{max}$ was observed. Varying [CK] (300-900 U/ml) had a minor effect on kinetics at 5 mM MgATP, with no effect at higher MgATP concentrations. At CP > 20 mM and MgATP > 10 mM, $f_{Wmax}$ did not depend on fiber diameter (correlation coefficient, $r^2 < 0.5$). Together, these results indicate that the IFI and EMB kinetic differences can not be explained by diffusion limitations or inadequate buffering of MgATP and MgADP levels.

**Determining the Position of the Rate-Limiting Step from the Calculated Responses of Eight Cross-Bridge Models to Changes in [MgATP] , [MgADP], and [Pi].**

Following the method of Zhao & Kawai (8), we obtained steady-state solutions of 8 alternate versions of the cross-bridge scheme (main text Table 1) that differed only in the position of the rate-limiting step. The steady state solution was obtained by assuming that, for each version, the following steps are approximated by the mass action law:

| Scheme | Steps obeying mass action | Rate limiting step |
|---|---|---|
| 1 | 4,1 | 1 |
| 2 | 8,4,1 | 2 |
| 3 | 8,1 | 3 |
| 4 | 8,4,1 | 4 |
| 5 | 8,4,1 | 5 |
| 6 | 8,4,1 | 6 |
| 7 | 8,4 | 7 |
| 8 | 8,4,1 | 8 |

Let each state in the cross-bridge scheme be represented as a probability of occupancy, i.e.:

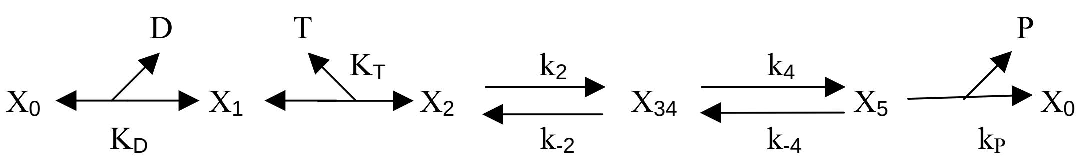
| Scheme | State | Probability of occupancy ($X_n$) |
|---|---|---|
| 1 | A.M | $X_1$ |
| | A.M.T | $X_2$ |
| | A.M.T* + M.T + M.D.P + A.M.D.P | $X_{34}$ |
| | A.M.D.P* | $X_5$ |
| | A.M.D | $X_0$ |
| 2 | A.M | $X_1$ |
| | A.M.T | $X_2$ |
| | A.M.T* + M.T + M.D.P + A.M.D.P | $X_{34}$ |
| | A.M.D.P* | $X_5$ |
| | A.M.D | $X_6$ |
| | A.M.D* | $X_0$ |
| 3 | A.M* | $X_1$ |
| | A.M.T | $X_2$ |
| | A.M.T* + M.T + M.D.P + A.M.D.P | $X_{34}$ |
| | A.M.D.P* | $X_5$ |
| | A.M.D | $X_6$ |
| | A.M | $X_0$ |
| 4 | A.M | $X_1$ |
| | A.M.T | $X_2$ |
| | A.M.T* + M.T + M.D.P + A.M.D.P | $X_{34}$ |
| | A.M.D.P* | $X_5$ |
| | A.M.D | $X_0$ |
| 5 | A.M | $X_1$ |
| | A.M.T* | $X_2$ |
| | A.M.T** + M.T + M.D.P + A.M.D.P | $X_{34}$ |
| | A.M.D.P* | $X_5$ |
| | A.M.T | $X_6$ |
| 6 | A.M | $X_1$ |
| | A.M.T | $X_2$ |
| | A.M.T* + M.T | $X_3$ |
| | M.D.P + A.M.D.P | $X_4$ |
| | A.M.D.P* | $X_5$ |
| | A.M.D | $X_0$ |
| 7 | A.M | $X_1$ |
| | A.M.T | $X_2$ |
| | A.M.T* + M.T + M.D.P + A.M.D.P | $X_{34}$ |
| | A.M.D.P* | $X_5$ |
| | A.M.D.P** | $X_6$ |
| | A.M.D | $X_0$ |
| 8 | A.M | $X_1$ |
| | A.M.T | $X_2$ |

| | | |
|---|---|---|
| | A.M.T* + M.T + M.D.P + A.M.D.P | $X_{34}$ |
| | A.M.D.P* | $X_5$ |
| | A.M.D | $X_6$ |

where A = actin, M = myosin, T = MgATP, D = MgADP, and P = inorganic phosphate. The sum of $X_n = 1$ for each scheme.

Composite state $X_{34}$ (including $X_3$ and $X_4$) does not support force, whereas the other states do, so transitions into or out of $X_{34}$ manifest as changes in dynamic stiffness (6, 8). Sinusoidal length change analysis perturbs strain-sensitive elementary rate constants (6). The instability produces a shift to a new steady-state distribution of cross-bridge states that is observed as tension transients in length-jump analysis and as exponential processes in sinusoidal analysis (2, 8, 9). Given independent variables T, D and P, their corresponding association constants $K_T$, $K_D$, and $K_P$, and the elementary rate constants $k_2$, $k_{-2}$, $k_4$ and $k_{-4}$, it follows from Zhao & Kawai (8):

**Scheme 1**: **IFI Myosin, Rate-Limiting Step Is Phosphate Release**




Assuming that $k_P$ << other rate constants,

$$\dot{X}_0 + \dot{X}_1 + \dot{X}_2 = -k_2X_2 + k_{-2}X_{34} \quad (1)$$

$$\dot{X}_{34} = k_2X_2 - (k_{-2} + k_4)X_{34} + k_{-4}X_5 \quad (2)$$

$$\dot{X}_5 = k_4X_{34} - k_{-4}X_5 \quad (3)$$

and

$$K_T = X_2/TX_1 \quad (4)$$

$$K_D = X_0/DX_1 \quad (5)$$

Where $X_n$ is the probability of each state, T=[$MgATP^{2-}$], D=[$MgADP^{1.5-}$], and P=[Pi].

Express $X_0$ and $X_1$ in terms of $X_2$, $X_{34}$, and $X_5$:

(4) → $X_1 = X_2/K_T T$ (6)

(5+4) → $X_0 = X_1 K_D D = X_2 K_D D/K_T T$ (7)

let $1/\eta \equiv 1 + 1/K_T T + K_D D/ K_T T$ (8)

$$\frac{d}{dt} \quad = - \quad (9)$$

Eigen equation:

$$r^3 - (\eta k_2 + k_{-2} + k_4 + k_{-4})\, r^2 + (\eta k_2 k_{-4} + k_{-2} k_{-4} + \eta k_2 k_4) r = 0 \quad (10)$$

Therefore $r_1 = 0$ (11)

$$r_2 + r_3 = \eta k_2 + k_{-2} + k_4 + k_{-4} \quad (12)$$

$$r_2 r_3 = \eta k_2 k_4 + \eta k_2 k_{-4} + k_{-2} k_{-4} \quad (13)$$

If $\eta k_2 + k_{-2} >> k_4 + k_{-4}$

Then, $r_2 \approx \eta k_2 + k_{-2}$ (14)

And $r_3 \approx (\eta k_2 k_4 + \eta k_2 k_{-4} + k_{-2} k_{-4})/(\eta k_2 + k_{-2})$

$$= [\eta k_2/(\eta k_2 + k_{-2})]\, k_4 + k_{-4} \quad (15)$$

where, again $1/\eta \equiv 1 + 1/K_T T + K_D D/ K_T T$ (8)

<u>Predictions:</u>

When T=0, $r_2 = k_{-2}$
When T=∞, $r_2 = k_2 + k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) increases with increasing T

$r_2$ (i.e., $2\pi c$) is independent of P since no P term in equations 14 or 8

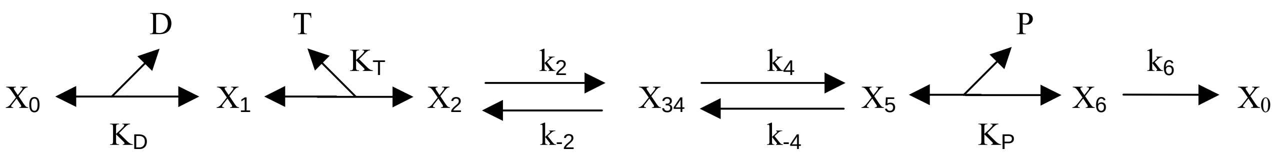
When $D=\infty$, $r_2 = k_{-2}$
When $D=0$, $r_2 = k_2\,[(1/(1+ 1/K_T\,T)] + k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) decreases with increasing D

When $T=0$, $r_3 = k_{-4}$
When $T=\infty$, $r_3= k_4\,/\,[1/(1+k_{-2}/k_{-2}\,)] + k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) increases with increasing T

$r_3$ (i.e., $2\pi b$) is independent of P since no P term in equations 15 or 8

When $D=0$, $r_3= [k_2/(\,k_2+ k_{-2}(1+1/K_T\,T)]\,k_4 + k_{-4}$
When $D=\infty$, $r_3= k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) decreases with increasing D

**Scheme 2: EMB Myosin, Rate-Limiting Step Is an Isomerization Before MgADP Release**




Assuming that $k_6 <<$ other rate constants,

$$\dot{X}_0 + \dot{X}_1 + \dot{X}_2 = -k_2X_2 + k_{-2}X_{34} \qquad (1)$$

$$\dot{X}_{34} = k_2X_2 - (k_{-2} + k_4)X_{34} + k_{-4}X_5 \qquad (2)$$

$$\dot{X}_5 + \dot{X}_6 = k_4X_{34} - k_{-4}X_5 \qquad (3)$$

and

$$K_D=X_0/DX_1 \qquad (4)$$

$$K_T=X_2/TX_1 \qquad (5)$$

$$K_P=X_5/PX_6 \qquad (6)$$

Where $X_n$ is the probability of each state, $T=[MgATP^{2-}]$, $D=[MgADP^{1.5-}]$, and $P=[Pi]$.

(5) → $$X_1 = X_2/K_TT \qquad (7)$$

(4+5) → $$X_0 = (K_DD/K_TT)X_2 \qquad (8)$$

(6) → $X_6 = X_5/K_P P$ (9)

let $\eta \equiv K_T T / (1 + K_D D + K_T T)$ (10)

and $\xi \equiv K_P P / (1 + K_P P)$ (11)

$$\frac{d}{dt}\begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} = - \qquad\qquad \begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} \tag{12}$$

Eigen equation:

$$r^3 - (\eta k_2 + k_{-2} + k_4 + \xi k_{-4})\, r^2 + (\eta k_2 k_4 + \eta k_2 \xi k_{-4} + k_{-2}\xi k_{-4})r = 0 \tag{13}$$

Therefore $r_1 = 0$ (14)

$$r_2 + r_3 = \eta k_2 + k_{-2} + k_4 + \xi k_{-4} \tag{15}$$

$$r_2 r_3 = \eta k_2 k_4 + \eta k_2 \xi k_{-4} + k_{-2}\xi k_{-4} \tag{16}$$

If $\eta k_2 + k_{-2} >> k_4 + \xi k_{-4}$

Then, $r_2 \approx \eta k_2 + k_{-2}$ (17)

And $r_3 \approx (\eta k_2 k_4 + \eta k_2 \xi k_{-4} + k_{-2}\xi k_{-4})/(\eta k_2 + k_{-2})$

$$= [\eta k_2/(\eta k_2 + k_{-2})]\, k_4 + \xi\, k_{-4} \tag{18}$$

where, again $\eta \equiv K_T T / (1 + K_D D + K_T T)$ (10)

and $\xi \equiv K_P P / (1 + K_P P)$ (11)

<u>Predictions:</u>

When $T=\infty$, $r_2 = k_2 + k_{-2}$
When $T=0$, $r_2 = k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) increases with T

When P=0 and $\infty$, $r_2 = k_2\, [(K_D D / K_T T) + 1/K_T T + 1)] + k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) is independent of P

When $D=\infty$, $r_2 = k_{-2}$
When $D=0$, $r_2 = k_2\, [K_T T / (1 + K_T T)] + k_{-2}$;

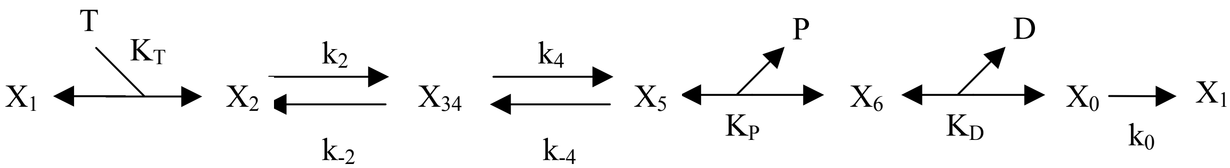
Thus $r_2$ (i.e., $2\pi c$) decreases with D

When T=0, $r_3 = k_{-4} K_P P /(K_P P +1)$
When T=∞, $r_3 = k_4 / [(k_{-2}/k_{-2})( K_D D /K_T T) + 1/K_T T +1)]$
Thus $r_3$ (i.e., $2\pi b$) increases with T

When P=0, $r_3 = k_4 / [1+(k_{-2}/k_{-2})(K_D D /K_T T) + 1/K_T T +1)]$
When P=∞, $r_3 = k_4 / [1+(k_{-2}/k_{-2})(K_D D /K_T T) + 1/K_T T +1)] + k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) increases with P

When P=0, $r_3 = (k_4 + k_{-4})( K_T T/ (1 +K_T T)$
When P=∞, $r_3 = k_{-4} K_T T/ (1 +K_T T)$
Thus $r_3$ (i.e., $2\pi b$) decreases with D

**Scheme 3: Rate-Limiting Step Before MgATP Release**




Assuming that $k_0$ << other rate constants,

$$\dot{X}_1 + \dot{X}_2 = -k_2X_2 + k_{-2}X_{34} \quad (1)$$

$$\dot{X}_{34} = k_2X_2 - (k_{-2} + k_4)X_{34} + k_{-4}X_5 \quad (2)$$

$$\dot{X}_5 + \dot{X}_6 + \dot{X}_0 = k_4X_{34} - k_{-4}X_5 \quad (3)$$

and

$$K_T = X_2/TX_1 \quad (4)$$

$$K_P = X_5/PX_6 \quad (5)$$

$$K_D = X_6/DX_0 \quad (6)$$

Where $X_n$ is the probability of each state, T=[$MgATP^{2-}$], D=[$MgADP^{1.5-}$], and P=[Pi].

Express $X_0$, $X_1$, and $X_6$ in terms of $X_2$, $X_{34}$, and $X_5$:

(4) → $$X_1 = X_2/K_TT \quad (7)$$

(5) → $X_6 = X_5/K_P P$ (8)

(6+8) → $X_0 = (1/K_D D)X_6 = (1/(K_P P K_D D))X_5$ (9)

let $\xi \equiv K_T T/(K_T T + 1)$ (10)

and $\eta \equiv K_D D\, K_P P/(K_D D\, K_P P + K_D D + 1)$ (11)

then

$$\frac{d}{dt}\begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} = - \qquad\qquad \begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} \tag{12}$$

Eigen equation:

$$r^3 - (k_4 + \eta k_{-4} + \xi k_2 + k_{-2})r^2 + (\xi k_2 \eta k_{-4} + k_{-2}\eta k_{-4} + \xi k_2 k_4)r = 0 \tag{13}$$

Therefore $r_1 = 0$ (14)

$r_2 + r_3 = \xi k_2 + k_{-2} + k_4 + \eta k_{-4}$ (15)

$r_2 r_3 = \xi k_2 \eta k_{-4} + k_{-2}\eta k_{-4} + \xi k_2 k_4$ (16)

If $\xi k_2 + k_{-2} >> k_4 + \eta k_{-4}$

Then, $r_2 \approx \xi k_2 + k_{-2}$ (17)

And $r_3 \approx (\xi k_2 \eta k_{-4} + k_{-2}\eta k_{-4} + \xi k_2 k_4)/(\xi k_2 + k_{-2})$

$= \sigma k_4 + \eta k_{-4}$ (18)

where $\sigma \equiv \xi k_2/(\xi k_2 + k_{-2})$ (19)

and, again, $\xi \equiv K_T T/(K_T T + 1)$ (10)

and $\eta \equiv K_D D\, K_P P/(K_D D\, K_P P + K_D D + 1)$ (11)

<u>Predictions:</u>

When T=0, $r_2 = k_{-2}$
When T=∞, $r_2 = k_2 + k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) increases with T

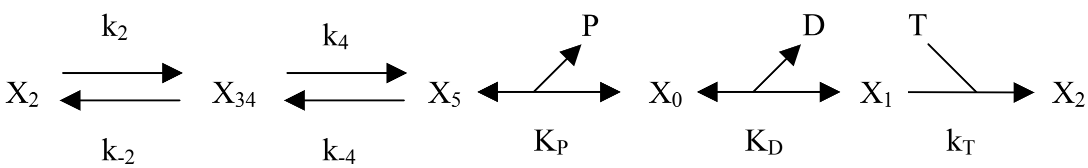
$r_2$ (i.e., $2\pi c$) independent of P and D

When T=0, $r_3 = \sigma k_{-4}$
When T=∞, $r_3 = [k_2/(1+k_{-2})]\, k_4 + \sigma k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) increases with T

When P=0, $r_3 = [\xi k_2/(\xi + k_{-2})]\, k_{-4}$
When P=∞, $r_3 = [\xi k_2/(\xi + k_{-2})]\, k_{-4} + k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) increases with P

When D=0, $r_3 = [\xi k_2/(\xi + k_{-2})]\, k_{-4}$
When D=∞, $r_3 = [\xi k_2/(\xi + k_{-2})]\, k_{-4} + [K_P P/(K_D D\, K_P P + 1)]\, k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) increases with D

**Scheme 4: Rate-Limiting Step MgATP Binding**




Assuming that $k_T$ << other rate constants,

$$\dot{X}_2 = -k_2X_2 + k_{-2}X_{34} \quad (1)$$

$$\dot{X}_{34} = k_2X_2 - (k_{-2} + k_4)X_{34} + k_{-4}X_5 \quad (2)$$

$$\dot{X}_5 + \dot{X}_0 + \dot{X}_1 = k_4X_{34} - k_{-4}X_5 \quad (3)$$

and

$$K_P = X_5/PX_0 \quad (4)$$

$$K_D = X_0/DX_1 \quad (5)$$

Where $X_n$ is the probability of each state, T=[$MgATP^{2-}$], D=[$MgADP^{1.5-}$], and P=[Pi].

Express $X_0$ and $X_1$ in terms of $X_2$, $X_{34}$, and $X_5$:

(4) → $$X_0 = X_5/K_PP \quad (6)$$

(5+6) → $$X_1 = (1/K_DD)X_0 = X_5/(K_PP\, K_DD) \quad (7)$$

let $\eta \equiv 1/(1+ 1/(K_D D\, K_P P) + 1/(K_P P))$ (8)

then

$$\frac{d}{dt}\begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} = - \qquad\qquad \begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} \quad (9)$$

Eigen equation:

$$r^3 + (k_4 + \eta k_{-4} + k_2 + k_{-2})r^2 + (\eta k_2 k_{-4} + \eta k_{-2} k_4 + k_2 k_4)r = 0 \quad (10)$$

Therefore $r_1 = 0$ (11)

$r_2 + r_3 = k_4 + \eta k_{-4} + k_2 + k_{-2}$ (12)

$r_2 r_3 = \eta k_2 k_{-4} + \eta k_{-2} k_4 + k_2 k_4$ (13)

If $k_2 + k_{-2} >> k_4 + \eta k_{-4}$

Then, $r_2 \approx k_2 + k_{-2}$ (14)

And $r_3 \approx (\eta k_2 k_{-4} + \eta k_{-2} k_4 + k_2 k_4)/(k_2 + k_{-2})$ (15)

where, again $\eta \equiv 1/(1+ 1/(K_D D\, K_P P) + 1/(K_P P))$ (8)

Predictions:

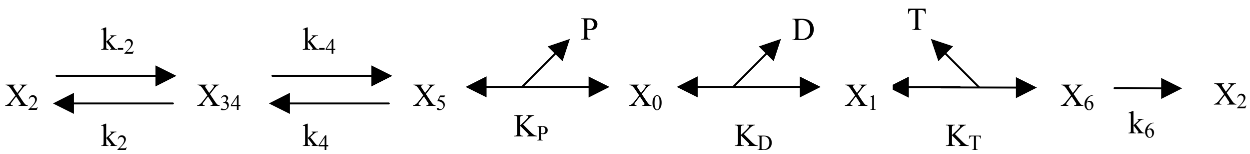
When P=0 & ∞ $r_2 = k_2 + k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) independent of T, P, and D

When T=0 & ∞ $r_3 = (\eta k_2 k_{-4} + \eta k_{-2} k_4 + k_2 k_4)/(k_2 + k_{-2})$
Thus $r_3$ (i.e., $2\pi b$) independent of T

When P=0, $r_3 = k_2 k_4/(k_2 + k_{-2})$
When P=∞, $r_3 = (k_2 k_{-4} + k_{-2} k_4 + k_2 k_4) / (k_2 + k_{-2})$
Thus $r_3$ (i.e., $2\pi b$) increases with P

When D=0, $r_3 = k_2 k_4/(k_2 + k_{-2})$
When D=∞, $r_3 = [[(k_2 k_{-4} + k_{-2} k_4) / (1 + 1/K_P P)] + k_2 k_4] /(k_2 + k_{-2})$
Thus $r_3$ (i.e., $2\pi b$) increases with D

**Scheme 5: Rate-Limiting Step After MgATP Binding**




Assuming that $k_6 <<$ other rate constants,

$$\dot{X}_2 = -k_2X_2 + k_{-2}X_{34} \qquad (1)$$

$$\dot{X}_{34} = k_2X_2 - (k_{-2} + k_4)X_{34} + k_{-4}X_5 \qquad (2)$$

$$\dot{X}_5 + \dot{X}_0 + \dot{X}_1 + \dot{X}_6 = k_4X_{34} - k_{-4}X_5 \qquad (3)$$

and

$$K_P = X_5/PX_0 \qquad (4)$$

$$K_D = X_0/DX_1 \qquad (5)$$

$$K_T = X_6/TX_1 \qquad (6)$$

Where $X_n$ is the probability of each state, T=[$MgATP^{2-}$], D=[$MgADP^{1.5-}$], and P=[Pi].

Express $X_0$, $X_1$, and $X_6$ in terms of $X_2$, $X_{34}$, and $X_5$:

(4) → $$X_0 = X_5/K_PP \qquad (7)$$

(5+7) → $$X_1 = X_0/K_DD = X_5/(K_PP\ K_DD) \qquad (8)$$

(6+8) → $$X_6 = X_1\ K_TT = X_5\ K_TT\ /(P_PP\ K_DD) \qquad (9)$$

let $$1/\eta \equiv 1 + 1/K_PP + 1/(K_PP\ K_DD) + K_TT\ /(K_PP\ K_DD) \qquad (10)$$

then

$$\frac{d}{dt}\begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} = - \qquad\qquad \begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} \qquad (11)$$

Eigen equation:

$$r^3 + (k_4 + \eta k_{-4} + k_2 + k_{-2})r^2 + (\eta k_2k_{-4} + \eta k_{-2}k_{-4} + k_2k_4)r = 0 \qquad (12)$$

Therefore $r_1 = 0$ (13)

$$r_2 + r_3 = k_4 + \eta k_{-4} + k_2 + k_{-2} \quad (14)$$

$$r_2 r_3 = \eta k_2 k_{-4} + \eta k_{-2} k_{-4} + k_2 k_4 \quad (15)$$

If $k_2 + k_{-2} >> k_4 + \eta k_{-4}$

Then, $r_2 \approx k_2 + k_{-2}$ (16)

And $r_3 \approx (\eta k_2 k_{-4} + \eta k_{-2} k_{-4} + k_2 k_4)/(k_2 + k_{-2})$

$$= \sigma k_4 + \eta k_{-4} \quad (17)$$

where $\sigma \equiv k_2/(k_2 + k_{-2})$ (18)

and, again $1/\eta \equiv 1 + 1/K_P P + 1/(K_P P\ K_D D) + K_T T/(K_P P\ K_D D)$ (10)

<u>Predictions:</u>

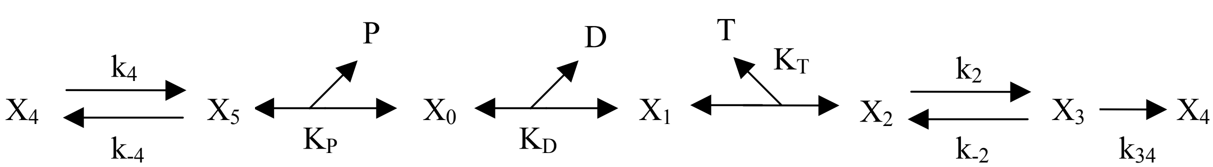
$r_2$ (i.e., $2\pi c$) independent of T, P and D

When T=0, $r_3 = \sigma k_4 + [1/(1 + (1/K_P P) + (1/K_P P K_D D))]\ k_{-4}$
When T=∞, $r_3 = \sigma k_4$
Thus $r_3$ (i.e., $2\pi b$) decreases with T

When P=0, $r_3 = \sigma k_4$;
When P=∞, $r_3 = \sigma k_4 + k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) increases with P

When D=0, $r_3 = \sigma k_4$;
When D=∞, $r_3 = \sigma k_4 + [1/(1 + (1/K_P P))\ k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) increases with D

**Scheme 6: Rate-Limiting Step in a Detached State**




Assuming that $k_{34}$ << other rate constants,

$$\dot{X}_4 = -k_4 X_4 + k_{-4} X_5 \quad (1)$$

$$X_3 \;=\; k_2X_2 - k_{-2}X_3 \tag{2}$$

$$\dot{X}_5 + \dot{X}_0 + \dot{X}_1 + \dot{X}_2 = k_4X_4 - k_{-4}X_5 - k_2X_2 + k_{-2}X_3 \tag{3}$$

and

$$K_D = X_0/DX_1 \tag{4}$$

$$K_T = X_2/TX_1 \tag{5}$$

$$K_P = X_5/PX_0 \tag{6}$$

Where $X_n$ is the probability of each state, $T=[MgATP^{2-}]$, $D=[MgADP^{1.5-}]$, and $P=[Pi]$.

Express $X_0$, $X_1$, and $X_5$ in terms of $X_2$, $X_3$, or $X_4$:

(5) → $$X_1 = X_2/K_TT \tag{7}$$

(4+7) → $$X_0 = X_1\, K_DD = X_2\, K_DD/K_TT \tag{8}$$

(6+8) → $$X_5 = X_0\, K_PP = X_2\, K_DD\, K_PP/K_TT \tag{9}$$

let $$1/\eta \equiv 1 + K_DD\, K_PP/K_TT + K_DD/K_TT + 1/K_TT \tag{10}$$

$$\xi \equiv K_DD\, K_PP/K_TT \tag{11}$$

then

$$\frac{d}{dt}\begin{pmatrix} X_2 \\ X_3 \\ X_4 \end{pmatrix} = - \qquad\qquad\qquad \begin{pmatrix} X_2 \\ X_3 \\ X_4 \end{pmatrix} \tag{12}$$

Eigen equation:

$$r^3 + (\eta k_2 + k_{-2} + k_4 + \eta\xi k_{-4})r^2 + (\eta k_2k_4 + k_{-2}k_4 + \eta\xi\, k_{-2}k_{-4})r = 0 \tag{13}$$

Therefore $$r_1 = 0 \tag{14}$$

$$r_2 + r_3 = \eta k_2 + k_{-2} + k_4 + \eta\xi\, k_{-4} \tag{15}$$

$$r_2r_3 = \eta k_2k_4 + k_{-2}k_4 + \eta\xi\, k_{-2}k_{-4} \tag{16}$$

If $\eta k_2 + k_{-2} \gg k_4 + \eta\xi\, k_{-4}$

Then, $$r_2 \approx \eta k_2 + k_{-2} \tag{17}$$

And $r_3 \approx (\eta k_2 k_4 + k_2 k_{-4} + \eta\xi\, k_{-2} k_{-4})/(\eta k_2 + k_{-2})$

$$= [\eta k_2/(\eta k_2 + k_{-2})]k_4 + [(k_2 + \eta\xi\, k_{-2})/(\eta k_2 + k_{-2})]k_{-4} \quad (18)$$

Again, $1/\eta \equiv 1 + K_D D\, K_P P / K_T T + K_D D / K_T T + 1/K_T T$ (10)

and $\xi \equiv K_D D\, K_P P / K_T T$ (11)

<u>Predictions:</u>

When T=0, $r_2 = k_{-2}$;
When T=∞, $r_2 = k_2 + k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) increases with T

When P=0, $r_2 = k_2 [1/(1 + K_D D/K_T T + 1/K_T T)] + k_{-2}$
When P=∞, $r_2 = k_{-2}$;
Thus $r_2$ (i.e., $2\pi c$) decreases with P

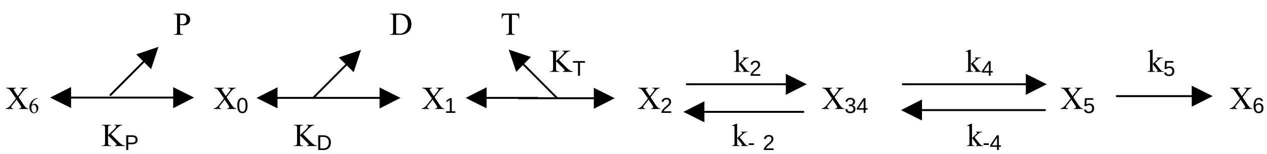
When D=0, $r_2 = k_2 [1/(1 + 1/K_T T)] + k_{-2}$
When D=∞, $r_2 = k_{-2}$
Thus $r_2$ (i.e., $2\pi c$) decreases with D

When T=0, $r_3 = (k_2/k_{-2})k_4 + [k_2 + k_{-2}/(1+1/K_P P + 1/K_D D\, K_P P)]/(k_2 + k_{-2})]\, k_{-4}$
When T=∞, $r_3 = (k_2/k_{-2})k_4 + k_2/(k_2 + k_{-2})k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) decreases with T

When P=0, $r_3 = k_{-2}/(k_2 + k_{-2}[1 + K_D D/K_T T + 1/K_T T]]\, k_4 + (k_2 + k_{-2})/k_{-2})]\, k_{-4}$
When P=∞, $r_3 = (k_{-2}/k_2)\, k_4 + [(k_2 + k_{-2})/k_{-2}]\, k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) decreases with P

When D=0, $r_3 = [k_{-2}/(k_2 + k_{-2}[1 + 1/K_T T]]\, k_4 + [k_2/(k_2/(1+1/K_T T) + k_{-2})]\, k_{-4}$
When D=∞, $r_3 = [k_2/k_{-2} + 1/(1 + 1/K_P P)]\, k_{-4}$
Thus $r_3$ (i.e., $2\pi b$) decreases with D

**Scheme 7: Rate-Limiting Step Before Phosphate Release**




Assuming that $k_5 \ll$ other rate constants,

$$\dot{X}_6 + \dot{X}_0 + \dot{X}_1 + \dot{X}_2 = -k_2X_2 + k_{-2}X_{34} \quad (1)$$

$$\dot{X}_{34} = k_2X_2 - (k_{-2} + k_4)X_{34} + k_{-4}X_5 \quad (2)$$

$$\dot{X}_5 = k_4X_{34} - k_{-4}X_5 \quad (3)$$

and

$$K_P = X_6/PX_0 \quad (4)$$

$$K_D = X_0/DX_1 \quad (5)$$

$$K_T = X_2/TX_1 \quad (6)$$

Where $X_n$ is the probability of each state, T=[$MgATP^{2-}$], D=[$MgADP^{1.5-}$], and P=[Pi].

Express $X_0$, $X_1$, and $X_6$ in terms of $X_2$, $X_{34}$, and $X_5$:

(6) → $X_1 = X_2/K_TT$ (7)

(5+7) → $X_0 = (K_DD/K_TT)\, X_2$ (8)

(4+8) → $X_6 = (K_DD/K_TT)\, K_PP\, X_2$ (9)

let $\eta \equiv 1 / (K_PP\, K_DD/ K_TT + K_DD/ K_TT + 1/K_TT + 1)$ (10)

$$\frac{d}{dt}\begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} = - \qquad \begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} \quad (11)$$

Eigen equation:

$$r^3 - (\eta k_2 + k_{-2} + k_4 + k_{-4})\, r^2 + (\eta k_2k_4 + \eta k_2k_{-4} + k_{-2}k_{-4})r = 0 \quad (12)$$

Therefore $r_1 = 0$ (13)

$$r_2 + r_3 = \eta k_2 + k_{-2} + k_4 + k_{-4} \quad (14)$$

$$r_2r_3 = \eta k_2k_4 + \eta k_2k_{-4} + k_{-2}k_{-4} \quad (15)$$

If $\eta k_2 + k_{-2} \gg k_4 + k_{-4}$

Then, $r_2 \approx \eta k_2 + k_{-2}$ (16)

And $r_3 \approx (\eta k_2 k_4 + \eta k_2 k_{-4} + k_{-2} k_{-4})/(\eta k_2 + k_{-2})$

$$= \sigma k_4 + k_{-4} \quad (17)$$

where $\sigma \equiv k_2 / (k_2 + k_{-2}/\eta)$ (18)

and, again, $\eta \equiv 1 / (K_P P\, K_D D/ K_T T + K_D D/ K_T T + 1/K_T T + 1)$ (10)

Predictions:

When T=0, $r_2 = k_{-2}$;
When T=∞, $r_2 = k_2 + k_{-2}$
Thus $r_2$ (i.e., 2πc) increases with T

When P =0, $r_2 = k_2\, [1/(1+ (1/K_T T) + K_D D/ K_T T)] + k_{-2}$;
When P =∞, $r_2 = k_{-2}$
Thus $r_2$ (i.e., 2πc) decreases with P

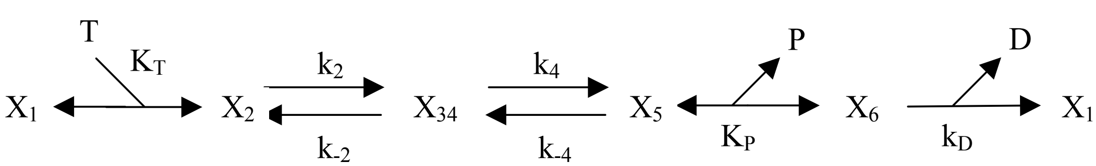
When D =0, $r_2 = k_2\, [1/(1+ (1/K_T T)] + k_{-2}$;
When D =∞, $r_2 = k_{-2}$
Thus $r_2$ (i.e., 2πc) decreases with D

When T=0, $r_3 = k_{-4}$
When T=∞, $r_3 = k_4/(1+k_{-2}/k_2) + k_{-4}$
Thus $r_3$ (i.e., 2πb) increases with T

When P =0, $r_3 = [k_2 /[(k_2 + k_{-2}\, (1+ (1/K_T T) + (K_D D/ K_T T)]]\, k_4 + k_{-4}$
When P =∞, $r_3 = k_{-4}$
Thus $r_3$ (i.e., 2πb) decreases with P

When D =0, $r_3 = [k_2 /[(k_2 + k_{-2}\, (1+ 1/K_T T)]]\, k_4 + k_{-4}$;
When D =∞, $r_3 = k_{-4}$
Thus $r_3$ (i.e., 2πb) decreases with D

**Scheme 8: Rate-Limiting Step MgADP Release**




Assuming that $k_D$ << other rate constants,

$$\dot X_1 + \dot X_2 = -k_2X_2 + k_{-2}X_{34} \qquad (1)$$

$$\dot X_{34} = k_2X_2 - (k_{-2} + k_4)X_{34} + k_{-4}X_5 \qquad (2)$$

$$\dot X_5 + \dot X_6 = k_4X_{34} - k_{-4}X_5 \qquad (3)$$

and

$$K_T = X_2/TX_1 \qquad (4)$$

$$K_P = X_5/\,PX_6 \qquad (5)$$

Where $X_n$ is the probability of each state, T=[$MgATP^{2-}$], D=[$MgADP^{1.5-}$], and P=[Pi].

Express $X_1$ and $X_6$ in terms of $X_2$, $X_{34}$, and $X_5$:

(4) → $X_1 = X_2/K_TT$ (6)

(5) → $X_6 = X_5/K_PP$ (7)

let $\xi \equiv K_TT/(K_TT + 1)$ (8)

and $\eta \equiv K_PP/(K_PP + 1)$ (9)

then

$$\frac{d}{dt}\begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} = - \qquad\qquad \begin{pmatrix} X_2 \\ X_{34} \\ X_5 \end{pmatrix} \qquad (10)$$

Eigen equation:

$$r^3 - (k_4 + \eta k_{-4} + \xi k_2 + k_{-2})r^2 + (\xi k_2\eta k_{-4} + k_{-2}\eta k_{-4} + \xi k_2k_4)r = 0 \qquad (11)$$

Therefore $r_1 = 0$ (12)

$$r_2 + r_3 = \xi k_2 + k_{-2} + k_4 + \eta k_{-4} \qquad (13)$$

$$r_2r_3 = \xi k_2\eta k_{-4} + k_{-2}\eta k_{-4} + \xi k_2k_4 \qquad (14)$$

If $\xi k_2 + k_{-2} >> k_4 + \eta k_{-4}$, as appears to be the case (text Fig. 3),

then, $r_2 \approx \xi k_2 + k_{-2}$ (15)

and $r_3 \approx (\xi k_2\eta k_{-4} + k_{-2}\eta k_{-4} + \xi k_2k_4)/(\xi k_2 + k_{-2})$

$$= \sigma k_4 + \eta k_{-4} \quad (16)$$

where $\sigma \equiv \xi k_2 /(\xi k_2 + k_{-2})$ (17)

and, again, $\xi \equiv K_T T/(K_T T +1)$ (8)

and $\eta \equiv K_P P/(K_P P +1)$ (9)

Predictions:

When T=0, $r_2 = k_{-2}$
When T=∞, $r_2 = k_2 + k_{-2}$
Thus $r_2$ (i.e., 2πc) increases with T

$r_2$ (i.e., 2πc) independent of P and D

When T=0, $r_3 = \eta k_{-4}$;
When T=∞, $r_3 = [1/(1+ k_2 /k_{-2})]\, k_4 + \eta k_{-4}$
Thus $r_3$ (i.e., 2πb) increases with T

When P=0, $r_3 = \sigma k_4$
When P=∞, $r_3 = \sigma k_4 + k_{-4}$
Thus $r_3$ (i.e., 2πb) increases with P

$r_3$ (i.e., 2πb) independent of D

**Derivation of Cross-Bridge Rate Constants**

The kinetic constants of each cross-bridge scheme were derived by fitting algebraic expressions 12 and 13 of Scheme 1 to the [MgATP] sinusoidal rate constant plots (Fig. 7) following the method of Kawai et al.(8). Because the apparent rate constants are more accurately expressed as a sum, $r_2 + r_3$ ($2\pi b + 2\pi c$), and product, $r_2 r_3$ ($2\pi b \times 2\pi c$), in the steady-state solution of each cross-bridge scheme, we plotted both sum and product (rather than separately plotting 2πb and 2πc) as functions of [MgATP]. Plots of sum and product as functions of [MgADP] and [Pi] (not shown) were not used to calculate the kinetic constants of Scheme 1 because of the irreversible effects of [MgADP] and the insensitivity of the apparent rate constants to [Pi].

## Estimate of MgADP Release Rate in Fibers

We estimated the IFI MgADP release rate in IFM by comparing rates measured in fibers with those previously measured using isolated IFI S-1 in solution. In solution, the 2$^{nd}$ order MgATP-induced detachment rate constant for IFI myosin is 750 $mM^{-1}s^{-1}$ (22$^{o}$C) (10). In fibers (15 $^{o}$C), the corresponding rate constant ($K_{ATP}k_2$) is 703 $mM^{-1}s^{-1}$ (Table 1, main text). Given a $Q_{10}$ estimate of 2, at 22$^{o}$C $K_{ATP}k_2$ should be 1195 $mM^{-1}s^{-1}$, which is ~1.6-fold greater than the rate measured using S-1 at 22$^{o}$C. This higher 2$^{nd}$ order detachment rate in fibers is likely due to stress or strain myosin experiences in fibers, but does not experience in solution measurements. If MgADP release is also elevated by a similar factor due to stress or strain, as many studies suggest MgADP release is strain dependent (11), and given the solution determined S-1 ADP release (10) rate of 4,090 $s^{-1}$, we estimate that the MgADP release rate in fibers may be as high as 6,540 $s^{-1}$ (= 1.6 × 4,090 $s^{-1}$) at 22$^{o}$C. This estimated value for fiber MgADP release rate is highly unlikely to be limiting during flight. This view accords with that of Silva et al (12) who conclude that MgADP release must be > 4,000 $s^{-1}$ at 22$^{o}$C to not limit filament sliding speed during flight.


1. Wang, G. & Kawai, M. (1996) *Biophys. J.* **71,** 1450-1461.
2. Fujita, H., Sasaki, D., Ishiwata, S. & Kawai, M. (2002) *Biophys J* **82,** 915-28.
3. Galler, S., Wang, B. G. & Kawai, M. (2005) *Biophys J* **89,** 3248-3260.
4. Pate, E. & Cooke, R. (1989) *Pflugers Arch* **414,** 73-81.
5. Hibberd, M. G., Dantzig, J. A., Trentham, D. R. & Goldman, Y. E. (1985) *Science* **228,** 1317-1319.
6. Kawai, M. & Brandt, P. W. (1980) *J. Muscle Res. Cell Motil.* **1,** 279-303.
7. Mulieri, L. A., Barnes, W., Leavitt, B. J., Ittleman, F. P., LeWinter, M. M., Alpert, N. R. & Maughan, D. W. (2002) *Circ. Res.* **90,** 66-72.
8. Zhao, Y. & Kawai, M. (1993) *Biophys. J.* **64,** 197-210.
9. Kawai, M. (1982) in *Basic Biology of Muscles: A Comparative Approach*, eds. Twarog, B. M., Levine, R. J. C. & Dewey, M. M. (Raven Press, N.Y.), pp. 109-130.

10. Miller, B. M., Nyitrai, M., Bernstein, S. I. & Geeves, M. A. (2003) *J. Biol. Chem.* **278,** 50293-50300.
11. Nyitrai, M. & Geeves, M. A. (2004) *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **359,** 1867-1877.
12. Silva, R., Sparrow, J. C. & Geeves, M. A. (2003) *J. Muscle Res. Cell Moti.l* **24,** 489-498.
13. Maughan, D. & Swank, D. M. (2005) in *Nature's Versatile Engine: Insect Flight Muscle Inside and Out*, ed. Vigoreaux, J. (Springer-Verlag, Boston), pp. 251-269.