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Supporting Material

A metabolite-sensitive, thermodynamically-constrained model of cardiac cross-bridge cycling: Implications for force development during ischemia

Kenneth Tran, Nicolas P. Smith, Denis S. Loiselle, and Edmund J. Crampin

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Kenneth Tran
Auckland Bioengineering Institute
University of Auckland, New Zealand

Nicolas P. Smith
University Computing Laboratory
University of Oxford, UK

Denis S. Loiselle
Auckland Bioengineering Institute
and Department of Physiology
University of Auckland, New Zealand

Edmund J. Crampin[1]
Auckland Bioengineering Institute
and Department of Engineering Science
University of Auckland, New Zealand

[1]Corresponding author: email: e.crampin@auckland.ac.nz

Description of Model Parameters

The following outlines the number of parameters that describe the metabolic model of contraction along with the experimental data that were used to characterise and determine their values. The model is based on the Rice et al. (1) model of Ca^{2+} activation and cross-bridge kinetics, adjusted for guinea pig myocytes. It captures a wide range of experimentally observed myofilament behaviour of the Rice et al. (1) as well as metabolic regulation from MgATP, MgADP, Pi and pH. The model is sensitive to changes in temperature (15-37 °C) and includes extracellular elements for simulating whole-muscle response. Note that the Rice et al. (1) has been successfully integrated into models of electro-mechanics, providing further tissue validation of the model (2, 3).

The whole model is characterised by a total of 46 parameters which are broken down as follows:

- Sarcomere geometry (5)
- Ca^{2+} activation and thin filament regulation (7)
- Cross-bridge cycling (12)
- Temperature dependence (9)
- Metabolite regulation (3)
- Passive force and complete muscle response (10)

These 46 parameters are determined from 24 independent studies comprising of 51 curves and 125 data points. This shows that even though the model is complex, it is by no means over-parameterised as the parameters that characterise the model are determined by a large range and number of experimental data.

Sarcomere geometry parameters

Sarcomere mechanics in the model is described using the classic sliding filament theory (4). The fraction of cross-bridges that can strongly bind and generate force is proportional to the overlap between the thick and thin filaments. The relationship between developed force and sarcomere length is piecewise linear. In the model, five parameters are used to describe the sarcomere geometry (Table 1). The values of these parameters are specific to cardiac muscle and differ from those of skeletal muscle (5).

(6 independent studies)

Parameter	Value	Units	Reference
$SL_{\max}$	2.4	μm	(6–8)
$SL_{\min}$	1.4	μm	1.4-1.5 (9, 10)
$length_{\text{thick}}$	1.65	μm	1.67 (4)
$length_{\text{hbare}}$	0.1	μm	0.15 (4)
$length_{\text{thin}}$	1.2	μm	*a*

Table 1: Sarcomere geometry parameters. [a] Derived.

Ca^{2+} activation and thin filament regulation parameters

The parameters for modelling the binding of Ca^{2+} to troponin C (two-state model) are similar to previous studies (11, 12) (Table 2). The separation of troponin regulatory sites into two populations provides a phenomenological way of capturing the cooperative effect of strongly-bound cross-bridges increasing the binding affinity of nearby regulatory units (RUs). The binding rate for Ca^{2+} is assumed to be the same for both populations but the unbinding rate of the higher affinity population is assumed to be 10 times smaller following estimates from experimental studies (13, 14).

The steep Ca^{2+} sensitivity of cardiac muscle during activation (F-Ca relationship) is a result of nearest-neighbour interactions. This behaviour can be described using spatially explicit models. In order to build this behaviour into a simple ODE model, nonlinear functions of intracellular [Ca^{2+}] are used as a phenomenological representation of the nearest-neighbour effect. This produces two parameters in the form of $perm_{50}$ and n_{perm}, which, along with forward and reverse binding rates between the permissive and non-permissive states ($k_{\text{n\_p}}$ and $k_{\text{p\_n}}$) are fitted to the data of Dobesh et al. (15) (Fig. 2A) which has approximately 50 data points (5 curves).

(3 independent studies comprising a total of 50 data points)

Cross-bridge kinetics and temperature-dependence parameters

The basic framework for the model is derived from Razumova et al. (16), where the calculated active force is proportional to the fractional occupancy of the strongly-bound cross-bridges multiplied by the respective mean distortion of these states. Three states make up the cross-bridge cycle, two of which are strongly-bound

Parameter	Value	Units	Reference
k_{on}	50	$\mu\text{M}^{-1}\text{s}^{-1}$	40 (11)
k_{offL}	250	s^{-1}	(13, 14) [a]
k_{offH}	25	s^{-1}	20 (12)
$perm_{50}$	0.5	Unitless	Model fit (15)
n_{perm}	15	Unitless	Model fit (15)
$k_{\text{n\_p}}$	50	s^{-1}	Model fit (15)
$k_{\text{p\_n}}$	500	s^{-1}	Model fit (15)

Table 2: Ca^{2+} activation and thin filament regulation parameters. [a] k_{offL} is set to be 10 times larger than k_{offH} following experimental estimates.

states. The base rates in the model (Table 3) are different and, in general, larger than those from Razumova as they correspond to the default temperature of 37 °C.

gslmod is a parameter that builds strain dependence into the detachment step, g_{appT}, in order to simulate the increase in rate of relaxation as sarcomere length decreases (17) (Fig. 2, 7 curves). Strain-dependence is also added to the isomerization step, h_{bT}, to reproduce shortening velocities that are comparable to experimental measures (18). The strain-dependence of the detachment step, g_{xbT}, is retained but σ_{p} is set larger than σ_{n} to increase the ATPase rate more for shortening than for lengthening protocols.

In the model, force is proportional to the mean strains in each of the two strongly-bound states. These mean strains are described by two nonlinear ODEs and are affected by two separate mechanisms: the net motion between thick and thin filaments (velocity of sarcomere length) and the gain or loss of distortion as cross-bridges change states. The parameter, ϕ, is an empirically derived scaling term which apportions the extent to which the mean strains are affected by these two mechanisms. The value of ϕ is set so that the shortening velocities that are comparable to experimental measures as function of temperature (18). This provides a balance between strain added by rotating cross-bridge head and strain lost due to sarcomere shortening. At higher rates of crossbridge cycling, this can produce larger mean strain values in the post-rotated state which allow for faster shortening velocities at higher temperatures. The parameter, x_0, represents the average distortion of a cross-bridge after undergoing a power-stroke and is set similar to the study of Pate and Cooke (19). The $xbmod_{species}$ parameter represents the adjustment from rat to guinea pig cross-bridge kinetics. All the rate constants in the cross-bridge cycle are effectly slowed down by a factor of 5 (20).

Parameter	Value	Units	Reference	Value[b]
f_{app}	500	s^{-1}	50 (16)[a]	32
g_{app}	70	s^{-1}	400 (16)[a]	4.5
$gslmod$	6	Unitless	Model fit (17, 21)	-
h_{f}	2000	s^{-1}	8 (16)[a]	128
$hfmdc$	5	Unitless	Model fit	-
h_{b}	400	s^{-1}	6 (16)[a]	25.6
g_{xb}	70	s^{-1}	4 (16)[a]	4.5
σ_{p}	8	Unitless	1 (16)[a]	-
σ_{n}	1	Unitless	8 (16)[a]	-
x_0	0.007	μm	0.0075 (19)	-
ϕ	2	Unitless	Model fit	-
$xbmod_{species}$	0.2	Unitless	(20)	-

Table 3: Cross-bridge cycling parameters. [a] Parameter value from Razumova et al. (16). [b] Model value after adjusting to 22 °C

The model captures the temperature dependence using a Q_{10} multiplicative factor in each of the rate constants. The effect of the Q_{10} term is to decrease the rate constants from a default temperature of 37 °C. In the model, $Qk_{\mathrm{on}} > Qk_{\mathrm{off}}$ because Ca^{2+} sensitivity has been shown to decrease with lower temperature (22–24) (Table 4).

The default Q10 values for the for most of the rate constants in the cross-bridge cycle are set to 6.25, similar to reported values of 6.7 (25). The exception is Qg_{app} which is set to 2.5 following Wang and Kawai (25). This produces a maximal Ca^{2+}-activated force that increases with temperature which is consistent with experimental observations of Harrison and Bers (22) (Fig. 5, 6 curves) and de Tombe and Stienen (24) (Fig 2A, 3 curves, 16 data points total). The model is also consistent with temperature-induced changes in maximal shortening velocity (26) (Fig. 7, 3 curves), twitch duration (27) (Fig. 2A and 2C, data atr 2 temperatures, 10 curves for each temperature) and tension redevelopment (K_{tr}) (24) (Fig. 4A, 3 curves, 16 data points total).

(8 independent studies comprising of 36 curves and 32 data points)

Metabolite regulation parameters

The implementation of metabolite regulation in the cross-bridge model was achieved through the addition of three additional parameters (Table 5). Two of these parameters relate to the modelling of pH dependence (k_{dHCa} and m). Experimental

Parameter	Value	Units	Reference
Qk_{on}	1.5	Unitless	(22–24)
Qk_{off}	1.3	Unitless	(22–24)
$Qk_{\mathrm{n\_p}}$	1.6	Unitless	Model fit
$Qk_{\mathrm{p\_n}}$	1.6	Unitless	Model fit
Qf_{app}	6.25	Unitless	(24, 26, 27)
Qg_{app}	2.5	Unitless	(24, 25, 27)
Qh_{f}	6.25	Unitless	(24, 26, 27)
Qh_{b}	6.25	Unitless	(24, 26, 27)
Qg_{xb}	6.25	Unitless	(24, 26, 27)

Table 4: Temperature dependence parameters

data from Orchard and Kentish (28) (Fig. 4, 4 curves, 27 data points total) was used to characterise these parameters as well as the placement of the H^+ binding step within the cross-bridge cycle. The value of the third parameter (k_{dADP}) was determined using steady-state data from Godt and Nosek (29) (Fig. 6, 4 data points). The position of Pi binding within the cross-bridge cycle was also determined by data from Godt and Nosek (29) (Fig. 3, 6 data points). Moreover, the model was able to qualitatively and semi-quantitatively simulate the MgATP-dependence and force-redevelopment data of Ebus et al. (30) (Fig. 2A, 6 data points and Fig. 5A, 3 curves) as well as the sinusoidal perturbation experiments of Kawai et al. (31) (Fig 1A and 1B, 6 curves total and Fig. 4A and 4B, 6 curves total).

(4 independent studies comprising of 15 curves and 43 data points)

Parameter	Value	Units	Reference
k_{dHCa}	2×10^{-5}	mM	Model fit (28)
m	1	Unitless	Model fit (28)
k_{dADP}	4	μM	Model fit (29)

Table 5: Metabolite regulation parameters

Passive force and complete muscle response parameters

The modelling of complete muscle force response involves the contribution of passive forces and visco-elastic elements (Table 6). At the cellular level, there is a passive force from titin while at the trabecular (tissue) level, there is an additional passive force arising from collagen. The resting sarcomere length (SL_{rest}) corresponds to a point where there is no passive force (10, 18). The

passive force is assumed to be reflected around the resting length, where increases in sarcomere length will lead to a positive passive force that increases total force whereas decreases in sarcomere length will lead to a negative passive force which will decrease the total force (32). For trabeculae, additional component to passive force increases steeply at $SL > 2.2\mu$m which limits the sarcomere length to a maximum of 2.3 μm. The modelling of both these passive force components matches the curvature and steepness from experimental observations (10, 18).

Visco-elastic effects are modelled using a visco-elastic element that is assumed to be parallel to the active and passive forcs. This is set to be 0.3% of $F_{\max}$ (18). A small mass term is also added to prevent instantaneous changes in muscle shortening velocity and therefore prevent instabilities occuring during the solving process. This parameter is tuned to improve response time and stability during solving. A linear elastic element is also implemented in the model to simulate the effect of end compliance that often exist in real muscle preparations.

(3 independent studies)

Parameter	Value	Units	Reference
SL_{rest}	1.9	μm	1.9-2.1 (10, 18)
$PCon_{\text{titin}}$	0.002	Normalized force	(10, 18, 32)
$PExp_{\text{titin}}$	10	Unitless	(10, 18, 32)
SL_{collagen}	2.25	μm	(10, 18)
$PCon_{\text{collagen}}$	0.02	Normalized force	(10, 18)
$PExp_{\text{collagen}}$	70	Unitless	(10, 18)
Mass	0.00005 (rat)	(Normalized force) $\text{s}^2\mu\text{m}^{-1}$	Model fit[a]
	0.00025 (rabbit/guinea pig)	(Normalized force) $\text{s}^2\mu\text{m}^{-1}$	Model fit[a]
Viscosity	0.003	(Normalized force) $\text{s}\mu\text{m}^{-1}$	(18)
$F^{\text{constant}}_{\text{afterload}}$	0.0 - 1.0	Normalized force	Adjustable[b]
KSE	1-200	(Normalized force) μm^{-1}	Adjustable[b]

Table 6: Passive force and complete muscle response parameters. [a] The Mass parameter is tuned to improve the stability of the integration to improve response times. [b]These are adjusted depending on the simulation protocol.

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