Online Supplementary Data

Roles of Phosphorylation of Myosin Binding Protein-C and Troponin I in Mouse Cardiac Muscle Twitch Dynamics

Carl W. Tong[1, 2], Robert D. Gaffin[1], David C. Zawieja[1], Mariappan Muthuchamy[1]*

Running Title: Roles of MyBP-C and TnI Phosphorylation

Key words: Force-Frequency, Myosin binding protein-C, Troponin I

1. 336 Reynolds Medical Building, Cardiovascular Research Institute and Department of Medical Physiology, College of Medicine, Texas A&M University System Health Science Center, College Station, Texas 77843-1114.
2. Present address: Department of Internal Medicine, Duke University Medical Center, Durham, North Carolina 27710

***Corresponding Author:**
Telephone #: 979-847-9251
Fax#: 979-862-4638
E-mail: marim@tamu.edu

Data Analysis

The data analyses of calcium-force loop are detailed here. **First,** we analyzed the measured calcium and force values at specific points, A, B and C, during a contraction cycle that consists of: (1) point “A” represents relaxation stage, (2) point ‘B” represents maximum calcium concentration “B”, and (3) maximum force occurs at point “C”. These three points intended to provide a snapshot of pivotal transitional phases of the force-calcium contraction cycle. Analyses were performed on changes in force and changes in calcium concentrations between these points.

Gain (G). Gain is defined as the total force/area versus calcium concentration ratio. This was mainly used in the point “A”.

$$G = \frac{Force / Area}{[Ca^{2+}]}$$

Delta Gain (DG: Delta Gain (DG) is defined as the active force/area versus the change in calcium concentration from the point “A” ratio. This was used in the “B” and “C” points.

$$DG = \frac{\Delta Force / Area}{\Delta [Ca^{2+}]} = \frac{(Total\_Force - Force\_at\_\mathrm{Re}st) / Area}{([Ca^{2+}]_{N\_State} - [Ca^{2+}]_{\mathrm{Re}sting\_State})}$$

Then the ratio of DG of a specific frequency to the maximum DG obtained by the muscle bundle of the experiment was calculated to provide the normalized delta gain (NDG). This provided value between 0 and 1. The maximum DG almost always occurred at 3 Hz.

$$NDG = \frac{DG_{(N\_Hz)}}{DG_{(Maximum)}} = \frac{\left(\dfrac{(\Delta Force / Area)}{\Delta [Ca^{2+}]}\right)_{(N\_Hz)}}{\left(\dfrac{(\Delta Force / Area)}{\Delta [Ca^{2+}]}\right)_{Maximum}}$$

Second, three different equations were derived to describe the three segments, A to B, B to C and C to A of the contraction cycle. Two new equations provided models for A to B and B to C segments. The Hill equation described C to A segment. These equations then provided descriptors that characterized the path of the segments. These descriptors obtained for the control and experimental fibers were compared.

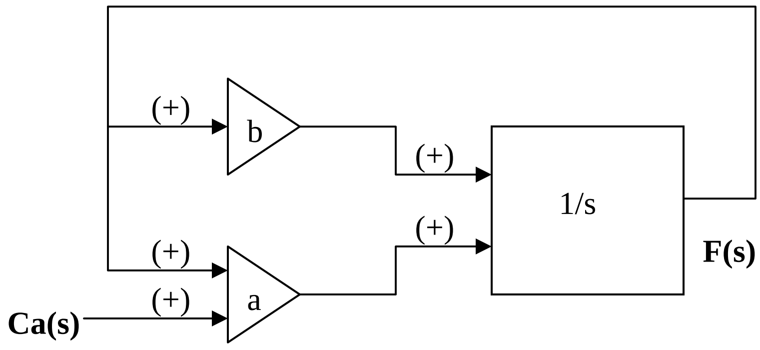
Theoretical Basis For A to B Segment

The data points of the calcium activation segment (A to B) of the calcium force loop fit the following equation.

$$Force = a \cdot [Ca2+]_{in} \cdot e^{(a+b)\cdot[Ca2]_{in}} \qquad \textbf{(Eq 1)}$$

The initial derivation is done in the Laplace domain for the ease of being able to use multiplications and divisions in place of integration and differentiation. After the relationship has been derived; the inverse Laplace will place the relationship back into the time domain. The derivation is based on the following scheme:

Scheme for Calcium Activation



Calcium causes crossbridge attachment through thin filament activation and other calcium-dependent process such as regulatory myosin light chain (RLC) activation with an over factor “a”. Calcium in the Laplace domain Ca(s) is ($Ca(s) = \int_0^\infty Ca(t) \cdot e^{-st} dt$). The integration (1/s) of accumulating attached crossbridges provides the output force (*F(s)*).

$$F(s) = \frac{a}{s} \cdot Ca(s) \qquad \text{(Eq a)}$$

The strongly attached crossbridge keeps TM in the “open” state to allow either the second crossbridge of the pair or others to attach in a positive feedback manner with factor “b” that includes conversion from force to crossbridge and attachment rate. Furthermore, the strongly attached crossbridges also help with calcium-dependent mechanisms such as increasing calcium sensitivity of TnC and promoting the TM from “closed” to “open” state. The positive feedback effects then cause the equation change to the following.

$$F(s) = \frac{a}{s} \cdot Ca(s) + \frac{b}{s} \cdot F(s) + \frac{a}{s} \cdot F(s) \qquad \text{(Eq b)}$$

Collecting and isolating *F(s)* to one side produces the following.

$$F(s) \cdot \left(\frac{s-(a+b)}{s} \right) = \frac{a}{s} \cdot Ca(s) \qquad \text{(Eq c)}$$

Writing *F(s)* in terms of *Ca(s)* gives the following.

$$F(s) = Ca(s) \cdot \left(\frac{a}{s-(a+b)} \right) \qquad \text{(Eq d)}$$

Considering that calcium activation of the crossbridge cycling is similar to amplitude modulation (AM) of a message signal multiplying/enabling a carrier of the AM radio, then the multiplication relationship in the Laplace domain is changed to a convolution relationship.

$$F(s) = Ca(s) \otimes \left(\frac{a}{s-(a+b)} \right) \qquad \text{(Eq e)}$$

Now, taking the inverse Laplace transform back into the time domain provides the following.

$$F(t) = a \cdot Ca(t) \cdot e^{(a+b)\cdot Ca(t)} \qquad \text{(Eq f)}$$

In this equation, the factors “a” and “b” could be indexes of the calcium-dependent and non-calcium-dependent processes, respectively. In addition, the $a \cdot Ca(t)$ part may represent the direct effects and the $e^{(a+b)\cdot Ca(t)}$ part may represent the feedback effects.

Equation for B to C Segment

The B to C segment of the calcium force loop fits the following equation with G as the gain factor, k may be the [Ca^{2+}] where 50% of active force occurs, n may be a measure of cooperativity associated with calcium, and Offset is the force at maximum calcium “B”. Unlike the previous equation, this segment is not easily derived based on first principles. It actually relies on the fraction of crossbridge at the strongly bound state. The data fits the following equation with k as rough substitute for strongly bound crossbridges.

$$Force = G \cdot \frac{k^{n}}{k^{n} + [Ca^{2+}]^{n}} + Offset \qquad \textbf{(Eq 2)}$$

Equation for C to A Segment

The relaxation phase of the calcium force loop from maximum force back to resting force (C to A segment) fits the well-known Hill’s equation with offset. F0 is the maximum active force, k may be the $[Ca^{2+}]_{in}$ where 50% of maximum active force is reached, and n may be a measure of calcium-dependent cooperativity.

$$Force = F0 \cdot \frac{[Ca2+]_{in}^{\ n}}{k^{n} + [Ca2+]_{in}^{n}} + Offset \qquad \textbf{(Eq 3)}$$