

Identification of the intracellular control of the cardiac force-length relationship: Analysis of the hystereses in the force-length plane.

Yael Yaniv^{1,2}, Amir Landesberg³ and Raphael Sivan¹

¹Faculty of Electrical Engineering, ²Faculty of Mechanical Engineering and the ³Department of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa, Israel
amir@biomed.technion.ac.il³

ABSTRACT

Revealing the mechanisms underlying the regulation of the cardiac muscle force-length relation is of immense importance for the understanding the normal and failing heart functions. Our model of the intracellular control of contraction includes two feedback loops: 1. The cooperativity (positive feedback) mechanisms, whereby the number of force generating cross-bridges (XBs) affects the rate of new XB recruitment. 2. The mechanical feedback, whereby the sarcomere velocity determines the time over which the XBs are at the strong state. The study aims to verify the ability of the model to explain the experimentally observed hystereses in the force-length relations (at constant activation) and to determine the role of each feedback loop. The force response to large (>4%) sarcomere length (SL) oscillation lags the length changes at low frequencies (<4Hz), leading to a counter-clock wise hysteresis. At high frequencies (>4Hz) the force precedes the SL oscillation and clockwise hysteresis appears. The model of the sarcomere, that couples calcium kinetics with XB dynamics, was built on Simulink. The force responses to SL oscillations were simulated at constant calcium concentration (activation). When the mechanical feedback is opened and only the cooperativity feedback exists, the force lags the SL. Opening the cooperativity mechanism and leaving only the mechanical feedback yields the opposite phenomenon and the force precedes the SL. The cooperativity dominates at low frequencies, when both feedbacks exist, while the mechanical feedback dominates at higher frequencies, in accordance with the experimental observations. The study emphasizes the role of each feedback. The cooperativity mechanism allows the muscle to adapt its energy consumption to the prevailing loading conditions, within a single contraction. The change over frequency, from CCW to CW hysteresis defines the muscle maximal working frequency at which the muscle can adapt to the loading conditions.

INTRODUCTION

The heart produces larger external work and greater pressure as the ventricle volume just before the contraction (end diastolic volume or the preload) increases. This phenomenon, denoted as the Frank-Starling Law, allows the heart to adapt to the prevailing loading conditions and to increase its output when the venous return increases. This basic law of the heart is derived from the steep increasing force-length relation at the contractile filament (sarcomere) sub-cellular level [1].

The muscle force is proportional to the number of strong, force generating cross-bridges (XBs). Each myosin head that creates a strong cross-bridge, is a nano-motor that generate about 2pN. The density of the available myosin heads is in the order of $40 \cdot 10^{12}$ per cubic mm. The number of activated XBs is determined by the regulatory proteins (the troponin-tropomyosin complexes) that are spread along the contractile filaments (the sarcomeres) and allow to switch on and off the activity of the adjacent XBs. Electrical activation of the myocytes (Fig-1) produces a transient increase in the free calcium concentration within the cell, due to the opening of calcium channels in the cell membrane. The inflow of calcium into the cell matrix is further amplified by additional calcium release from intracellular calcium stores (The Sarcoplasmic Reticulum) by mechanism denotes as calcium induce calcium release. Calcium binding to the troponin regulatory complexes turns

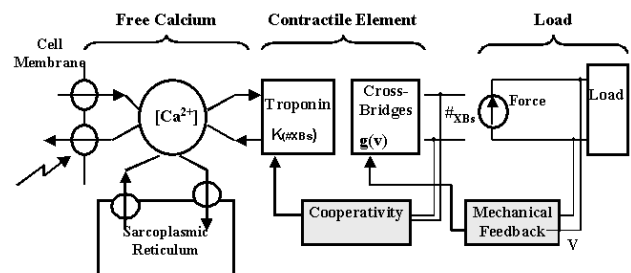


Figure 1. Block diagram of the intra-cellular control of sarcomere contraction.

the regulatory proteins to the activated state and allows XB recruitment and force generation. Muscle relaxation is produced by reuptake of calcium into the intracellular stores and calcium sequestration outside of the cell by membranous calcium pumps.

Modeling the intracellular control of contraction requires to couple the kinetics calcium binding to troponin with the regulation of XB cycling (Fig.1). Our theoretical studies [3-6] suggest that the three basic characteristics of the cardiac muscle: the steep force-length relation, the hyperbolic force-velocity relation and the linear relationship between the energy consumption and the generated mechanical energy, can be explained by the existence of two feedback mechanisms: a positive and a negative one (Fig 1). The positive feedback, denoted as the cooperativity mechanism, whereby the force or the number of strong XBs determines the affinity of the regulatory proteins for calcium [3], regulates the force length relation and provides the adaptive mechanism whereby the loading conditions affect XB recruitment. The negative feedback, denoted as the mechanical feedback, suggests that the sarcomere shortening velocity determines the time over which the XBs can be in the strong conformation [4]. The mechanical feedback provides an analytical solution for the Force Velocity relationship and the linear relation between energy consumption and the generated mechanical work.

These feedback loops predict that there is no constitutive law that describes a unique force length relation, but that the instantaneous force depends on the instantaneous length and on the history of contraction.

The force-length relationship is measured usually at steady state conditions (skinned fiber, tetanus) or at the peak isometric force of the twitch contraction. These measurements do not describe any dynamic relationship between the force (output) and the length (input). Recent studies have established the existence of hystereses in the force-calcium relations at constant Sarcomere length [2,9] and in the force-length relations at constant free calcium [7]. The analysis of these hystereses allows to gain insight into the mechanisms underlying the regulation of XB recruitment.

Counterclockwise hystereses in the force calcium relation were observed in skinned skeletal and cardiac fibers [2,9]. In these studies the authors kept the length constant and have changed the intracellular calcium concentration. Calcium concentration was slowly changed from the relaxing

to the fully activating concentration, back and forth. The force was higher during stepping down in calcium concentration than during stepping up in the free calcium. Hystereses in the force-length relations were recently described in the intact, tetanized rat cardiac trabeculae [7]. The shapes and directions of the hystereses depend on the frequency of oscillations and on the initial SLs. The force response lagged the sarcomere length oscillations at frequencies below 4 Hz and hystereses in the counterclockwise (ccw) directions were obtained in the force-length plane; at a given SL the force was higher during shortening than during lengthening. At frequencies above 4Hz the force responses preceded the length oscillations and a clockwise (cw) loops were observed at the force length plane. These observed hystereses in the force-length and force calcium relations confirm the hypothesis that the instantaneous force depend on the history of contraction. The study aims to verify that the suggested feedback loops can explain the observed phenomena and to establish the role of each feedback loop.

METHODS

The simulation describes the force response to length perturbations at constant calcium concentration. The assumptions relevant for the analysis of the tetanus contraction (constant calcium concentration) were derived from our previous studies [3-6]. The XBs cycle between two conformations, the strong force generating conformation and the weak non-force generating conformation. The troponin regulatory proteins also exist in two conformations: with and without bound calcium. The regulatory units consist of a single regulatory protein complex (The troponin-complex) with the adjacent actin binding sites for the myosin and the nearby myosin heads. There are about 26 regulatory units along the thin filament within the sarcomere (The width of a single sarcomere is about 45nm and the physiological length of the cardiac sarcomere is between 1.6 and 2.1 μ m).

To couple calcium binding to troponin with XB dynamics, where each kinetic includes two states, the regulatory units must have four possible states, as it described in figure 2. *State R* represents the rest state, where the XBs are in the weak conformation and no calcium is bound to the troponin. Calcium binding to troponin leads to *state A*. *State A* describes an “activated” regulatory unit with

bound calcium but where the adjacent XBs are still in the weak conformation. XBs turnover from the weak to the strong conformation leads to *state T*, in which calcium is bound to the troponin and the XBs are in the strong conformation. Calcium dissociation at *state T* leads to *state U*, in which the XBs are still in the strong conformation but without bound calcium.

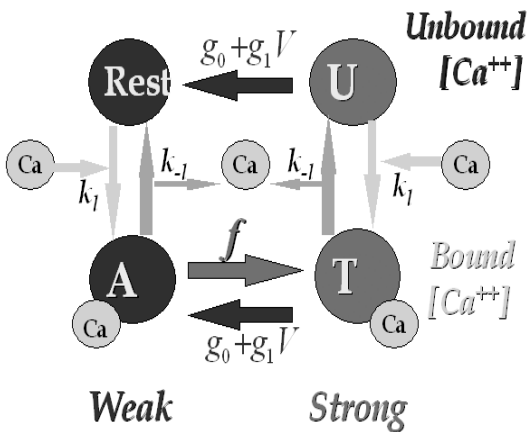


Figure 2: The transitions between the four states of the regulatory units are determined by calcium kinetics and XBs cycling.

The state variables R, A, T and U describe the probability of having the regulatory unit at each of the above states. The transitions between the states are determined by calcium kinetics and XB cycling. The rate constants k_{ℓ} and $k_{-\ell}$ relate to the kinetic of calcium binding and dissociation form troponin, respectively. The rate constants f , and g_0 relate to the kinetic of XB turnover between the weak and strong states.

The system is non-linear since the rate kinetics are not constant and depend on the state variables through the feedback loops. While the rate of calcium binding to troponin is constant (k_{ℓ}), the rate of calcium dissociation ($k_{-\ell}$) is determined by calcium affinity. To simplify the simulation the affinity is assumed to be a linear function of number of strong XBs [3], i.e function of the state variables T,U, and SL:

$$(1) \quad k_{-\ell} = \frac{k_{\ell}}{K_0 + K_1 \cdot SL \cdot (T + U)}$$

The rate (g) of XB turnover from the strong to the weak conformation (weakening) is also not constant

and is determined by the sarcomere shortening velocity (V), based on the mechanical feedback [4]:

$$(2) \quad g = g_0 + g_1 \cdot V$$

Where g_1 is the mechanical feedback coefficient .

The system includes four state variables and the state equations are:

$$(3) \quad \begin{cases} \frac{dA}{dt} = k_{\ell}[Ca](1-A-T-U) - (f+k_{-\ell})A + (g_0 + g_1V)T \\ \frac{dT}{dt} = fA - (g_0 + g_1V)T + k_{\ell}[Ca]U \\ \frac{dU}{dt} = k_{-\ell}T - (g_0 + g_1V + k_{\ell}[Ca])U \\ \frac{dSL}{dt} = -V \end{cases}$$

where $k_{-\ell}$ is given in Eq. 1. The force, the output equation, is given by:

$$(4) \quad F = F_0 \cdot SL \cdot (T + U)$$

Since the force is proportional to the number of strong regulatory units within the single overlap length between the contractile filaments [3,4].

The model was built on Simulink. The SL oscillations were imposed after the tetanus contraction had reached steady force level. Hence, the force responses to SL oscillations were examined at constant calcium concentration. The parameters were identified based on a literature survey regarding the following measurements: Peak of Ca transient, time to peak Ca and Hill's parameters for the FVR.

The effect of each feedback was studied by "opening the loop" and observing the effect of the remaining feedback on the force responses to the SL oscillations.

To study the stability, controllability and observability of the system, linearization of the model including the suggest feedback was done and the approximate solutions for small oscillations were derived. The trajectories were chosen after the state variables have reached a steady state points (due to the constant activation).

RESULTS

For the simple forward system that does not include any feedback loop ($g_1 = K_1 = 0$) the force follows the SL oscillations without any phase shift, for all frequencies of oscillations, as shown in figure 3. Without the feedback loops the rate of XB weakening and the rate of calcium dissociations are constant ($g = g_0$; $k_{-\ell} = k_{\ell} / K_0$) and the state

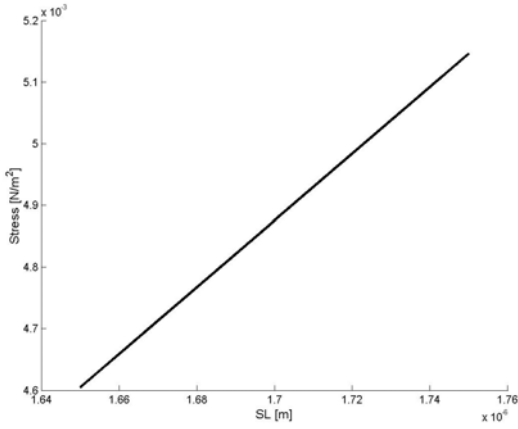


Figure 3: Phase plot of the force response to the SL oscillation for the system without any feedback loop. (freq = 3Hz $K_1 = 0$ $g_1 = 0$).

variables A, T and U (Eq. 3) become constant at constant activation level. Consequently, a linear relation is obtained between the force and the SL (Eq. 4).

The force response precedes the SL oscillations when the cooperativity feedback is opened and hysteresis in the *clockwise* direction is obtained in the Force-length plane. When the mechanical feedback prevails the number of strong XBs depends on the sarcomere shortening velocity (Eq. 2) and the muscle behaves like a visco-elastic element, where work is done on the muscle during the oscillations.

The force lags the SL oscillations and *counterclockwise hystereses* are obtained (Fig. 4) at all oscillation frequencies when the mechanical feedback loop is opened. The counterclockwise hystereses are obtained due the recruitment of new Xbs during the oscillation, through the cooperativity mechanism.

The force response of the whole system, with both feedback loops, presents clockwise and counterclockwise hystereses, at the different frequency of oscillations. At slow frequencies ($f < 4$ Hz) the positive feedback dominates and a counterclockwise hysteresis is obtained. At higher frequencies the mechanical feedback prevails and clockwise hysteresis appears, in accordance with the experimental studies. The muscle generates external work at low oscillation frequencies while at high frequencies it absorbs energy.

The nominal state trajectory and the nominal output were calculated under the assumption that the system

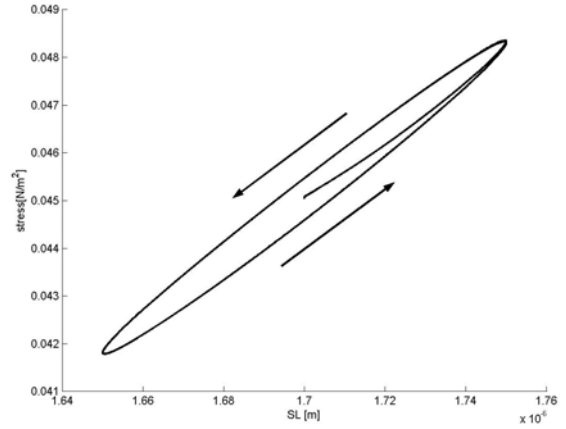


Figure 4: Phase plot of the force response to the SL oscillation, when only the cooperativity mechanism exists. (freq = 1Hz , $K_1 = 40e6$, $g_1 = 0$).

was long time at the nominal condition. The linearized system is described by:

$$(5) \quad \begin{aligned} \delta \dot{x}(t) &= E(t) \cdot \delta x(t) + B(t) \cdot \delta u(t) \quad \delta x(t_0) = \delta x_0 \\ \delta y(t) &= C(t) \cdot \delta x(t) + D \cdot \delta u(t) \end{aligned}$$

when:

$$(6) \quad E(t) = \begin{bmatrix} -86.0987 & -1.1416 & -11.1416 & 214.5315 \\ 40 & -24.4046 & 31.694 & 428.3494 \\ 0 & 14.4046 & -41.694 & -428.349 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

$$(7) \quad B(t) = \begin{bmatrix} -5.3e-10 \\ -1.07e-9 \\ 1.07e-9 \\ -1 \end{bmatrix}$$

$$(8) \quad C(t) = [0 \quad 1.667e3 \quad 1.667e3 \quad 5.213e4]$$

$$(9) \quad D(t) = [-1.3e-13]$$

The pools of the system are: 0, -12.41, -51.94 and -87.84. The transfer function, calculated from Eq.6, is:

$$(10) \quad \frac{-52130 \cdot (s+19.3891) \cdot (s+46.0953) \cdot (s+86.712)}{s \cdot (s+12.4094) \cdot (s+51.9442) \cdot (s+87.8437)}$$

The system has four pools, three at the left side and one at the origin. Therefore the system has BIBO stability. The system is minimal and is consequently observable and controllable.

DISCUSSION

In our previous studies we have shown that the model provides analytical explanation to the basic mechanical characteristics of the muscles, as the force-velocity relation [4] and the regulation of energy conversion [6]. The model describes wide spectrum of phenomena, as the cardiac force-length relation and the regulation of relaxation [3-5]. The present study establishes that this model of the intracellular control of contraction is stable BIBO. Verification of its controllability and observability further strengthen its utilization as a unit in a closed loop system.

The suggested model of the intracellular control of contraction provides the explanation to the experimentally observed hystereses in the force-length relationships, at constant calcium concentration. The simulated results are in accordance with the experimental observations [7]. At low frequencies (<2.5Hz) counterclockwise hystereses are obtained, where at a given SL the force is larger during shortening than during lengthening (Fig. 4), while clockwise hysteresis is obtained at higher oscillation frequencies (>2.5Hz). The analysis reveals the role of each feedback loop. Without the suggested feedback loops the force is independent of the history of contraction and a unique force-length relation is obtained (Fig. 3). The cooperativity mechanism, that regulates calcium affinity and XB recruitment, produces only counterclockwise hystereses (at all frequencies). In contrast, the mechanical feedback, that regulates the rate of XB weakening, generates clockwise hysteresis (at all frequencies). The phase of the hysteresis depends on the frequency of oscillation due to the interplay between the opposite effects of these two feedback loops. At low frequencies the cooperativity mechanism dominates. As the frequency increases, the shortening velocity increases and the role of the mechanical feedback becomes prominent.

Clockwise hystereses are observed in various physical *passive* elements, as visco-elastic and magnetic elements. Clockwise hysteresis infers that work is done upon the muscle and energy dissipates during the cycle and more energy is consumed in stretching the muscle than is gained during muscle shortening. Hence, at high frequencies the muscle behaves as a shock absorber.

The counterclockwise hysteresis is of immense importance, and infers that work is none by the

muscle. Eighty years ago Fenn have shown one of the most importance characteristic of muscle physiology, denoted as the Fenn Effect, where an increase in the loading conditions feeds back and increases the rate of XB recruitment. The underlying mechanism was obscure (8) but is explained by the suggested intracellular feedback loops [5,6]. The ccw hysteresis is attributed to the cooperativity mechanism that allows the muscle to recruit new Xbs during each cycle. At low frequencies, an increase in the loading condition is associated with an increase in XB recruitment through the cooperativity mechanism. The changeover frequency from ccw to cw hystereses described here defines the maximal rate that the muscle can adapt to the prevailing loading conditions. Describing the intracellular control of contraction is an important step toward understanding the cardiac function.

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