Published in final edited form as:

Cell Mol Bioeng. 2013 June 1; 6(2): 183–198. doi:10.1007/s12195-013-0269-8.

Slowed Dynamics of Thin Filament Regulatory Units Reduces Ca^{2+} -Sensitivity of Cardiac Biomechanical Function

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Abstract

Actomyosin kinetics in both skinned skeletal muscle fibers at maximum Ca^{2+} -activation and unregulated *in vitro* motility assays are modulated by solvent microviscosity in a manner consistent with a diffusion limited process. Viscosity might also influence cardiac thin filament Ca^{2+} -regulatory protein dynamics. *In vitro* motility assays were conducted using thin filaments reconstituted with recombinant human cardiac troponin and tropomyosin; solvent microviscosity was varied by addition of sucrose or glucose. At saturating Ca^{2+} , filament sliding speed (s) was inversely proportional to viscosity. Ca^{2+} -sensitivity ($p\text{Ca}_{50}$) of s decreased markedly with elevated viscosity ($\eta/\eta_0 \sim 1.3$). For comparison with unloaded motility assays, steady-state isometric force (F) and kinetics of isometric tension redevelopment (k_{TR}) were measured in single, permeabilized porcine cardiomyocytes when viscosity surrounding the myofilaments was altered. Maximum Ca^{2+} -activated F changed little for sucrose 0.3 M ($\eta/\eta_0 \sim 1.4$) or glucose 0.875 M ($\eta/\eta_0 \sim 1.66$), but decreased at higher concentrations. Sucrose (0.3 M) or glucose (0.875 M) decreased $p\text{Ca}_{50}$ for F . k_{TR} at saturating Ca^{2+} decreased steeply and monotonically with increased viscosity but there was little effect on k_{TR} at sub-maximum Ca^{2+} . Modeling indicates that increased solutes affect dynamics of cardiac muscle Ca^{2+} -regulatory proteins to a much greater extent than actomyosin cross-bridge cycling.

Keywords

skinned myocyte; myosin; actin; troponin; tropomyosin; *in vitro* motility assay; kinetics of isometric tension redevelopment; microviscosity; monosaccharide glucose; disaccharide sucrose

INTRODUCTION

Muscle contraction is a highly intricate process at both the macroscopic and microscopic levels. Microscopically, myosin hydrolyzes ATP leading to conformational changes

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

associated with force generation and/or displacement of actin filaments (sarcomere shortening) which at the macroscopic level corresponds to muscle contraction. There is much interest in dynamic motions of both the myosin motors¹⁴ and the thin filament regulatory proteins during Ca^{2+} -activation of striated muscles,^{29,56} particularly in cardiac muscle.^{19,44} Various aspects of protein dynamics can be elucidated by modulating solvent viscosity.^{8,26} Elevated solvent viscosity by the addition of low molecular weight sugars affects actomyosin cycling at saturating Ca^{2+} ,¹⁰ or in the absence of regulatory proteins¹¹ in a manner consistent with diffusion control of some kinetic process. In permeabilized fibers from rabbit psoas muscle, the velocity of unloaded shortening (V_{us}) and the kinetics of isometric tension redevelopment (k_{TR}) both decreased in inverse proportion to solvent viscosity within the myofilament lattice. In motility assays with unregulated filamentous-actin (F-actin), filament sliding speed also decreased inversely with solvent viscosity, consistent with V_{us} measurements in muscle fibers at saturating Ca^{2+} . These results suggested that some aspect of cross-bridge dynamics is diffusion-controlled at saturating, physiological ATP levels. Here we hypothesized that the dynamics of the thin filament Ca^{2+} regulation process might also be diffusion-limiting to function, particularly at submaximum Ca^{2+} levels. Because sub-saturating Ca^{2+} levels are more physiologically relevant in the heart than in skeletal muscle, we focused on cardiac preparations for these experiments.

Ca^{2+} -regulated motility assays and assays of permeabilized myocyte mechanics can provide information at the level of single thin filaments or individual regulatory units, respectively, where effects of solvent viscosity might be easiest to detect. Contraction kinetics can be considered as a combination of two processes in the presence of thin filament Ca^{2+} -regulatory proteins troponin (Tn) and tropomyosin (Tm): the kinetics of the actomyosin interaction or cross-bridge cycle, and the kinetics of Ca^{2+} -activation of the thin filament. Landesberg and Sideman²⁸ and Hancock et al.²⁰ modeled the processes of Ca^{2+} binding and force generation as two-state, first-order reactions with forward and reverse rate constants, leading to four possible thin-filament states (see Fig. 2 in Hancock et al.²⁰). This relatively simple model is remarkably able to capture key aspects of striated muscle function^{20,28,47} and has formed the basis for more complex models.^{6,49,60} Since Ca^{2+} binding experiments suggest that Ca^{2+} affinity at the regulatory site (site II) of cardiac troponin C (cTnC) increases with force, they assumed that in cardiac muscle, Ca^{2+} remains bound to a regulatory unit as long as the unit is occupied by a force generating cross-bridge (i.e., Ca^{2+} dissociation rate constant $k'_{OFF} \sim 0$) resulting in a reduced, three-state model (Scheme 1; also see Fig. 3 in Hancock et al.²⁰). While k_{TR} can be expressed as the sum of cross-bridge association (f) and dissociation (g) rate constants ($k_{TR} = f + g$) at saturating Ca^{2+} ,³ additional parameters are required at sub-saturating Ca^{2+} ,^{4,55} and Scheme 1 provides a straightforward and mechanistically plausible resolution with a minimum number of extra parameters. Analysis using Scheme 1 shows that k_{TR} reflects force-generating processes of actomyosin interactions at maximal Ca^{2+} activation^{4,9,40,55} while, under typical circumstances, k_{TR} is dominated by the thin filament regulatory unit dynamics at submaximum Ca^{2+} activation.^{9,45}

Here, we examine whether diffusion-limited processes could be rate-limiting for cardiac contractile kinetics at sub-saturating Ca^{2+} levels. To investigate the effects of viscosity on cardiac myofilament kinetics at both saturating and intermediate Ca^{2+} levels, we used single, skinned porcine cardiomyocytes for isometric mechanics assays, or thin filaments reconstituted with recombinant human cardiac troponin (rHcTn) and α -tropomyosin (rH α -Tm) for unloaded motility assays; bathing solution microviscosity was varied by adding low molecular weight sugars. Elevation of solvent viscosity by adding mono- or di-saccharides inhibited maximum Ca^{2+} -activated force and k_{TR} in myocytes, and maximum Ca^{2+} -activated sliding speed in motility assays. Increasing viscosity resulted in reduced Ca^{2+} -sensitivity of both force in myocytes and speed of regulated thin filaments in motility assays.

In contrast to inhibition observed at saturating Ca^{2+} , there was little or no effect of elevated solute concentration on k_{TR} at submaximum Ca^{2+} . The observed changes in steady-state isometric force and k_{TR} can only be described with biomechanically relevant parameters in the 3-state model of Scheme 1^{20,28,47} if elevated solute concentrations and solvent viscosity affects thin filament dynamics to a much greater extent than cross-bridge cycling in cardiac muscle; the predicted effects on thin filament dynamics were greater than expected for a simple, diffusion-limited process.

MATERIALS AND METHODS

Solution Viscosity

Viscosity of solutions was varied by adding low molecular weight sugars, disaccharide sucrose or monosaccharide glucose, as described previously.^{10,11} An Ostwald-type viscometer (CANNON Instrument Company, State College, PA) was used to measure the relative viscosity (η/η_0) of solutions, relative to water at 30°C for *in vitro* motility and 15°C for myocyte mechanics. The viscometer was kept in a water bath at the experimental temperatures. Note that high molecular weight dextran or methyl cellulose (MC) was added after the viscosity measurements on solutions containing sucrose or glucose. Relative viscosity was calculated according to:

$$\frac{\eta}{\eta_0} = \frac{\rho \Delta t}{\rho_0 \Delta t_0} \quad (1)$$

where ρ is density, Δt is the flow time measured in the viscometer, η_0 , ρ_0 and Δt_0 indicate measurements on ddH₂O.³⁹

To estimate η/η_0 in experimental solutions as a function of added low molecular weight (MW) sugars, nonlinear least-squares regression was used to fit the data for mono- and disaccharides separately to the following expression:

$$\frac{\eta[\text{solute}]}{\eta_0} = A[\text{solute}]^B + \frac{\eta(0)}{\eta_0} \quad (2)$$

where A and B were the fitted parameters. $\eta(0)/\eta_0$ was constrained to be equal to the value of η/η_0 measured in the absence of added sugar at the relevant temperature;¹⁰ the viscosity of control relaxing and activating solutions was slightly higher than that of water (i.e., $\eta(0)/\eta_0 > 1$) at the same temperature due to the presence of solutes other than sugars such as salts and buffers. Addition of low MW sugars increased η/η_0 in a nonlinear manner, as expected.⁶¹ Increase in η/η_0 by addition of disaccharide sucrose was approximately twice that measured when the same concentration of monosaccharide glucose was added. Relative viscosity values obtained at 15°C for fiber mechanics solutions (data not shown) were consistent with previously published data measured at 12°C;¹⁰ similarly, η/η_0 values obtained at 30°C for motility solutions (data not shown) were consistent with previously published measurements.¹¹

In Vitro Motility Assays

Animal use—The Florida State University's Institutional Animal Care and Use Committee approved all procedures and protocols involving vertebrate animals. Adult New Zealand White rabbits were handled in accordance with the current National Institutes of Health/National Research Council Guide for the Care and Use of Laboratory Animals.

Protein preparations—Myofilament proteins necessary for *in vitro* motility assays, F-actin, heavy meromyosin (HMM), rHcTn and rH α -Tm were prepared as described previously.^{5,11,17,52,54,58} Actin was extracted from acetone powder prepared from rabbit back and leg muscles.⁴³ Rabbit skeletal myosin was prepared as described from rabbit back muscle³³ and was subjected to mild chymotryptic digestion to obtain HMM.²⁵ ATP-insensitive ‘dead heads’ were removed from HMM preparations by ultracentrifugation in the presence of MgATP and F-actin on the day of use.²⁵ F-actin was fluorescently labeled with rhodamine phalloidin (RhPh). rHcTn and rH α -Tm were obtained by bacterial expression as described.^{52,54,58}

Solutions and flow cell preparation—*In vitro* motility assays were performed to measure the sliding speed of fluorescently labeled F-actin or regulated thin filaments over HMM coated surfaces. Experimental protocols, solution preparation, and flow cell preparation were essentially as previously described^{11,52,58} with BSA⁴¹ as the surface passivation reagent. Assays were carried out at 30°C, with temperature in the flow cell maintained by a heating coil (OH30 heater and B600 controller, 20-20 Technology, Inc., Wilmington, NC) on the objective of a Nikon Eclipse TE2000-U inverted fluorescence microscope.⁵⁴

For unregulated filaments (i.e., F-actin without Tm and Tn), the motility buffer was actin buffer (AB), composed of 25 mM KCl, 25 mM imidazole, 4 mM MgCl₂, 1 mM EGTA, 1 mM DTT, at pH 7.4²⁵ with modifications as described below. For regulated thin filament motility assays to measure the Ca²⁺ dependence of regulated filament speed, thin filaments were reconstituted by incubating fluorescently labeled F-actin with rH α -Tm and rHcTn complex in the flow cell as described below; motility buffers (MB) consisted of rHcTn and rH α -Tm added to solutions of defined pCa (pCa = $-\log[\text{Ca}^{2+}]$, where $[\text{Ca}^{2+}]$ is in molar). For each pCa solution, concentrations of Ca²⁺, Mg²⁺, Na⁺, K⁺, Tris, and acetate were determined,^{5,24,30,52,58} taking into account known metal ion binding constants and their enthalpies to obtain an ionic strength of 0.085 M, pH 7, pMg 3, $[\text{K}^+] = 50 \text{ mM}$, $[\text{Na}^+] = 15 \text{ mM}$, and appropriate amount of calcium to achieve the desired pCa. pCa stock solutions for motility assays were prepared at evenly spaced pCa values ranging from pCa 9 (relaxing) to pCa 4 (activating); intermediate pCa's were obtained by mixing calculated volumes of the next higher and lower pCa stock solutions. Immediately before each motility assay, 2 mM ATP, 16.7 mM glucose, 100 $\mu\text{g/ml}$ glucose oxidase, 18 $\mu\text{g/ml}$ catalase (Worthington Biochemical Corp., Lakewood, NJ), 0.3% MC, and an additional 40 mM DTT were added to MB. This minimal amount of glucose plus glucose oxidase, catalase, and extra DTT were added to all motility solutions to minimize photo-bleaching of the RhPh label and photo-oxidative damage to the proteins.^{17,25} Solvent viscosity was increased by adding 0.3 – 0.75 M sucrose to MB, which corresponds to up to ~2.5 fold increase in η/η_0 .

For assays with regulated thin filaments, rHcTn and rH α -Tm were added to AB buffer (regulated AB) that was applied to the flow cell immediately before MB, and regulated AB was incubated for 3 min (to reconstitute thin filaments on the flow cell surface) before MB containing the same concentrations of Tn and Tm was applied.

Fluorescence microscopy and data acquisition—Myosin-propelled motility of RhPh labeled F-actin and reconstituted thin filaments was observed by fluorescence microscopy on a Nikon TE2000-U inverted fluorescence microscope at 100 – 150 $\times$ magnification,⁵⁴ imaging 6 – 8 fields from varying areas on each flow cell. Field images were recorded as 30 – 60 s clips using a VE1000 SIT camera (Dage-MTI, Inc, Michigan City, IN) and a Sony RDR-GX7 DVD recorder. The DVD clips were converted to AVI movies using AoA DVD Ripper (ver. 5.1.8; AoAMedia.com, 2008) and processed into movie stacks consisting of ~1000 frames using Virtual Dub (ver. 1.6.19; Avery Lee, 2007).

Stacks were imported into ImageJ (ver. 1.37v; National Institutes of Health, USA), making a “flip book” of every 5 – 10 frames for total length of 500 – 600 frames, or ~16 – 20 s of data. The filament sliding movement was manually tracked using a Plug-In called MTrackJ, by following the leading edge of a filament for the period contained in the flip book and measuring the total number of pixels traveled. About 10 filaments were tracked per field, with ~ 6 fields per flow cell; a total of ~ 60 filaments were tracked per condition. The filament sliding speeds from each flow cell were averaged, and then normalized against either the average control value (no added sugar) for reconstituted thin filament speed at pCa 5, or the average speed of unregulated F-actin, measured on the same day.

Data analysis—The relationship between regulated thin filament sliding speed (s) and pCa was fit to the following, modified version of the Hill binding equation by nonlinear least square regression:

$$s = \frac{s_{max}}{\left(1 + 10^{n_H(pCa - pCa_{50})}\right)} + s_{min} \quad (3)$$

where s_{max} and s_{min} are the speeds at high and low $[Ca^{2+}]$, respectively, pCa_{50} is the pCa at which $s = s_{max}/2 + s_{min}$, and n_H is the Hill coefficient. The Hill coefficient in Eq. 3 reflects the apparent cooperativity of Ca^{2+} -regulation of unloaded sliding of regulated thin filaments.

Myocyte Mechanics

Animal use—Myocytes were obtained from porcine hearts from a local abattoir.⁵³ All procedures were approved by the The Florida State University’s Institutional Animal Care and Use Committee.

Preparation of single, glycerinated cardiomyocytes—A small portion of ventricular wall was removed from porcine hearts for myocyte preparation as described³⁸ with minor modifications. Complete Protease Inhibitor Cocktail (Roche Applied Science, Indianapolis, IN) or a combination of 1 μ M leupeptin, 1 μ M pepstatin A, and 200 μ M phenylmethylsulfonyl fluoride (PMSF) was added to all pCa 9 relaxing solutions. Heart tissue was minced and homogenized using a Wheaton tissue grinder. Homogenized tissue was subjected to centrifugation for 2 min at ~ 165 g. Then cells were skinned by re-suspending the pellet for 5 min in 0.3% Ultrapure Triton X-100 in relaxing solution. The pellet was washed three times with ice cold relaxing solution, and then re-suspended and stored in 50% v/v relaxing/glycerol solution at -20 °C for up to one month.

Skinned myocytes were attached to a motor (High-Speed Length Controller model 312C with 300 μ s step time set by data acquisition and control software; Aurora Scientific Inc., Ontario, Canada) to control overall cell length at one end and to a capacitive-type force transducer (model 403A; $f_c = 3.3$ kHz; Aurora Scientific Inc.) at the other end. Foam sealant with rapid (~ 10 – 15 min) curing time (Great Stuff, Dow Chemical Company, Wilmington, IL) was used to glue myocyte ends to hooks on the motor and force transducer. Initial sarcomere length (L_s) was set to 2.2 μ m, and length and width (measured with an optical micrometer) were measured prior to the start of each experiment. Periodic (0.2 Hz) unloading (i.e., fiber shortening followed by restretch to the isometric length) was used to maintain myocyte structure and mechanical properties, as previously used for skinned skeletal fibers and cardiac trabeculae.^{2,51}

Solutions—Relaxing and activating solutions were prepared as described previously⁵¹ and contained 5 mM MgATP, 15 mM phosphocreatine, 1 mM Pi, 10 mM EGTA, 50 mM MOPS, 45 mM free Na^+ , 100 mM free K^+ , 1 mM DTT, 200 – 400 units/ml creatine

phosphokinase, and 4% w/v Dextran T-500 (Fisher Scientific Co., Pittsburgh, PA). To alter solution $[Ca^{2+}]$, appropriate amounts of $Ca(acetate)_2$ were added, taking into account desired free $[Ca^{2+}]$ and the metal-binding constants (and their enthalpies) of all solutions constituents, as described for motility assay solutions. Ca^{2+} solutions were prepared at evenly spaced pCa values ranging from pCa 9 (relaxing) to pCa 4 (maximally activating); intermediate pCa solutions were obtained by mixing calculated volumes of the next higher and lower pCa stock solutions. Ionic strength was maintained constant (180 mM) by varying Tris and acetate. Solution pH was adjusted to 7.0 at 15°C. Experimental solutions were held in 175 μ l or 200 μ l anodized aluminum wells. The bottom of each well consisted of a glass, number 1 thickness cover slip. The temperature was set at 15°C and was controlled to within 1°C during individual experiments using an ATR-4 adaptable thermoregulator (Quest Scientific, North Vancouver, BC, Canada).

Experimental control, data acquisition, and data analysis—Steady-state isometric force (F) and the kinetics of isometric tension redevelopment (k_{TR}) were determined as described previously^{9,42,48,51,53} with minor modifications. Data were discarded if maximum Ca^{2+} -activated $F(F_{max})$ in control conditions decreased more than 20% of the initial value. k_{TR} was characterized by obtaining an apparent rate constant (expressed as reciprocal seconds) from the linear transformation of the half-time estimate ($t_{1/2}$) extrapolating from 50% to 63.2% ($k_{TR} = \tau^{-1} = -\ln 0.5 \cdot (t_{1/2})^{-1}$).⁴⁷ Alternatively, mono-exponential fits to the data, obtained using the Simplex method for nonlinear least-squares regression, gave similar results.

The relation between F and pCa was fitted by a nonlinear least-squares regression to a form of the Hill binding equation:

$$\frac{F}{F_{max}} = \frac{1}{(1 + 10^{n_H(pCa - pCa_{50})})} \quad (4)$$

where F/F_{max} is the relative force at a given pCa. Comparable to parameters in Eq. 3, n_H is the Hill coefficient, and pCa_{50} is the pCa at which half maximum Ca^{2+} -activated force is achieved. The Hill coefficient in Eq. 4 reflects the apparent cooperativity of Ca^{2+} -regulation of isometric force generation.

Statistical Analyses

Statistical analyses were performed using Excel (version 2003; Microsoft, Redmond, WA) or Sigma Plot for Windows (version 10.0; Systat Software Inc., San Jose, CA). Student's t -test was used to compare means, and statistical significance was accepted at the $P < 0.05$ level.

Numerical Modeling of Myocyte Mechanics

A three-state model (Scheme 1) that describes Ca^{2+} -activation and cross-bridge cycling in cardiac muscle^{20,28} was implemented numerically as a system of homogenous 1st order linear ordinary differential equations:

$$\frac{d}{dt} \begin{pmatrix} NB(t) \\ NC_{Ca}(t) \\ FO_{Ca}(t) \end{pmatrix} = \begin{pmatrix} -k_{on} \times [Ca^{2+}] & k_{off} & 0 \\ k_{on} \times [Ca^{2+}] & -(f + k_{off}) & g \\ 0 & f & -g \end{pmatrix} \times \begin{pmatrix} NB(t) \\ NC_{Ca}(t) \\ FO_{Ca}(t) \end{pmatrix} \quad (5)$$

In this model, $NB(t)$ represents the time-dependent occupancy of cross-bridges in Non-force-generating states because they are associated with regulatory units in the Blocked state

(no Ca^{2+}).³⁶ $NC_{Ca}(t)$ represents the time-dependent occupancy of cross-bridges in Non-force-generating states because they are associated with regulatory units in the Closed state (with bound Ca^{2+}). $FO_{Ca}(t)$ represents the time-dependent occupancy of cross-bridges in Force-generating states because they are associated with regulatory units in the Open state (with bound Ca^{2+}). Ca^{2+} association and dissociation rate constants, k_{ON} and k_{OFF} , are linked with regulatory units turning on and off, respectively. Rate constants f and g represent the forward and reverse kinetics of force generation associated with strong cross-bridge formation and cross-bridge dissociation, respectively. At the start of each simulation, the initial condition was set with full occupancy of the NB state and null occupancy of the NC_{Ca} and FO_{Ca} states, i.e., the relaxed state. $[\text{Ca}^{2+}]$ was increased in a stepwise manner, and, for any given set of kinetic parameters (i.e., k_{ON} , k_{OFF} , f , g), the myocyte force was considered to be linearly proportional to $FO_{Ca}(t)$ in the model. The general solution of $FO_{Ca}(t)$ can be obtained from the analytical solution consisting of a linear superposition of three exponentials:

$$FO_{Ca}(t) = c_1 \times e^{\lambda_1 t} + c_2 \times e^{\lambda_2 t} + c_3 \times e^{\lambda_3 t} \quad (6)$$

where c_i are constants determined by initial conditions, and λ_i are constants determined by eigenvalues of the coefficient matrix in Eq. 5. For each set of $[\text{Ca}^{2+}]$ and kinetic parameters, cross-bridge release after initial tension development had achieved steady-state was simulated by momentary increase in cross-bridge dissociation rate (g increased to 100 s^{-1} for 0.5 s), from which the initial condition for tension redevelopment was attained. F and k_{TR} were then obtained from simulated isometric tension redevelopment, using analyses equivalent to those used for analysis of experimental data as described for cardiac myocytes.

RESULTS

Unloaded Sliding of Regulated Thin Filaments

To test the hypothesis that elevated solvent viscosity inhibits the maximum sliding speed (s_{max}) of regulated thin filaments, as previously observed with unregulated F-actin, we performed motility assays at pCa 5 with varying amounts of sucrose added to the motility buffer. Control s_{max} for regulated thin filaments (no added sucrose, pCa 5) was $8.1 \pm 1.8 \mu\text{m s}^{-1}$. s_{max} for regulated thin filaments (pCa 5) decreased as sucrose concentration was increased (Fig. 1A), and s_{max} was linearly and inversely related to the solvent viscosity (Fig. 1B), similar to previous results with unregulated F-actin.¹¹

We also examined speed-pCa relationships in the presence of 0.3 M and 0.6 M sucrose or in its absence (Fig. 2). Control s_{max} for regulated thin filaments (no added sucrose, pCa 5) was $5.2 \pm 0.2 \mu\text{m s}^{-1}$; on the same experimental days, s for unregulated F-actin (no added sucrose) was $4.3 \pm 0.2 \mu\text{m s}^{-1}$. Speed-pCa relationships for thin filaments reconstituted with rHcTn and rHa-Tm were steep as previously reported.^{52,58} In all instances, speed saturated at pCa > 5, thus confirming that the data in Fig. 1 represent s_{max} under conditions of elevated viscosity. Increased solvent viscosity was associated with a marked reduction (rightward shift) of Ca^{2+} -sensitivity for filament sliding. For 0.3 M sucrose, ΔpCa_{50} was 0.32 pCa units (Table 1); due to the substantial reduction in s_{max} , pCa_{50} was not well resolved at 0.6 M sucrose although ΔpCa_{50} appeared to be shifted to a similar extent as at 0.3 M sucrose (Fig. 2).

Steady-State Isometric Force in Permeabilized Cardiomyocytes

Steady-state and transient kinetic measurements of isometric force were determined from different portions of tension time courses obtained with permeabilized cardiomyocytes; example records are shown in Fig. 3. Effects of increased viscosity via addition of low MW

sugars sucrose or glucose on steady-state isometric force generation, determined from the initial portion of such records were explored first.

Maximum Ca^{2+} -activated force ($F_{\max}$)

Fig. 4A shows the effect of increased solute concentration on $F_{\max}$ (pCa 4). Isometric force at pCa 4 decreased dramatically in the presence of 0.4 M sucrose or higher, whereas little or no effect of glucose was observed for [glucose] < 1.0 M. As also found with permeabilized rabbit psoas fibers,¹⁰ $F_{\max}$ was not strictly proportional to $(\eta/\eta_0)^{-1}$ over the range of sucrose and glucose concentrations examined (Fig. 4B). Based on the data in Fig. 4A, 0.3 M sucrose ($\eta/\eta_0 \sim 1.39$) and 0.875 M glucose ($\eta/\eta_0 \sim 1.66$) were chosen for studies at submaximal Ca^{2+} -activation of cardiomyocytes; these solute concentrations yielded a large change in viscosity with minimal effect on $F_{\max}$.

Isometric force at submaximum Ca^{2+} levels

To determine the effects of elevated sucrose or glucose concentration on Ca^{2+} -activation of isometric force, permeabilized cardiomyocytes were subjected to two consecutive cycles of increasing Ca^{2+} concentrations ranging from pCa 9 (relaxed) to pCa 4 (maximally activated). In some experiments, the myocytes were exposed first to control pCa solutions (without added sugar) followed by pCa solutions with elevated sugar concentration, while the reverse order was used in other experiments; no difference was observed in results from these two protocols—indicating that effects of added low MW sugars were fully reversible—and thus the data within each condition were combined. We found that 0.3 M sucrose decreased Ca^{2+} -sensitivity by 0.30 pCa units (rightward shift in the force-pCa relationship, Fig. 5A; Table 1). Similarly, addition of 0.875 M glucose also shifted the force-pCa relationship rightward, but by an even greater extent, 0.44 pCa units (Fig. 5B; Table 1). There was no statistically significant effect of either sucrose (Fig. 5A) or glucose (Fig. 5B) on n_H ($P > 0.05$).

Kinetics of Isometric Tension Redevelopment (k_{TR}) in Permeabilized Cardiomyocytes

To understand the effects of elevated sucrose or glucose concentration on biomechanical kinetics of actomyosin interaction and Ca^{2+} activation of myofilaments, kinetics of tension redevelopment were also studied. At maximum Ca^{2+} -activation (pCa 4), k_{TR} decreased monotonically with increasing glucose (squares) or sucrose (triangles) concentration (Fig. 6A); note in Fig. 6, that k_{TR} was normalized to that obtained in the same preparation in the absence of sucrose or glucose. k_{TR} at maximum Ca^{2+} -activation decreased in inverse proportion to η/η_0 (dashed line in Fig. 6B) when glucose was added, while the inhibition by sucrose was greater than expected from the linear relationship.

Effects of added sugars on the Ca^{2+} -activation dependence of k_{TR} are illustrated in Fig. 7, where k_{TR} is shown as a function of Ca^{2+} -activated force; note that in Fig. 7, F was normalized to that obtained at pCa 4 for the same preparation in the absence of sucrose and glucose. The trend of increasing k_{TR} as F increased was greatly diminished in the presence of either sucrose (0.3 M; Fig. 7A) or glucose (0.875 M; Fig. 7B). In other words, the inhibition of k_{TR} by elevated sugar concentration at low Ca^{2+} -activation levels was less than that at or near saturating Ca^{2+} , effectively rendering k_{TR} independent of the level of Ca^{2+} activation when low MW sugars were added. At the lowest Ca^{2+} -activated force levels at which k_{TR} was measurable for each condition, there was no significant effect of elevated sugar concentrations on k_{TR} (Fig. 7).

The relationship between Ca^{2+} -activation-dependence of k_{TR} and F can be described for a wide variety of conditions by a relatively simple kinetic model.^{20,28,42,47} To distinguish between the influence of solutes on cross-bridge kinetics and regulatory unit dynamics,

computational modeling (Materials and Methods) was used to identify parameter sets (k_{ON} , k_{OFF} , f and g in Scheme 1) that are physically and biochemically sensible, and yielded reasonable descriptions of key elements of the myocyte mechanics data (Figs. 4 - 7; Table 1) obtained in four conditions (0.3 M sucrose and control; 0.875 M glucose and control). Initial attempts to model viscosity-dependent changes involved simply decreasing diffusion limited kinetic parameters (i.e., f and k_{ON}) by the inverse ratio of relative viscosities (Table 1), but none of the resulting predictions adequately met the selection criteria specified below. Thus, more comprehensive searches of parameter space were conducted. Wide ranges (several orders of magnitude) of the kinetic parameters (k_{ON} , k_{OFF} , f and g) were explored independently for each of the control and elevated viscosity data sets. When searching for the best parameter sets to describe the sucrose (or glucose) and respective control data, k_{ON} was logarithmically sampled and other parameters were varied in steps of 0.0001.

The following criteria were used to select best fits of model predictions to the four data sets, considered as pairs of control and high solute concentration (0.3 M sucrose and control; 0.875 M glucose and control). First, model values of pCa_{50} for steady-state isometric force were matched for all four conditions (Fig. 5) with differences from experimental data less than 10^{-5} ; predicted values of pCa_{50} depend primarily on k_{ON} and k_{OFF} , as expected, with lesser but still significant influences of f and g . The lists of possible parameter sets for the two control conditions were refined by identifying those with the lowest sum-of-squared errors with respect to experimental data (Fig. 7). Pairs of parameter sets (elevated viscosity and respective control) were then chosen by identifying simulations that approximately matched the observed, solvent-induced decrease in maximum isometric force (Table 1) and maximal k_{TR} values (Figs. 6, 7) relative to the predicted, respective controls, and with little effect on k_{TR} values computed at very low forces ($\lim_{F \rightarrow 0} k_{TR}$). For each sampled k_{ON} , an initial best set of k_{OFF} , f and g that approximately satisfied the above criteria (usually up to differences of 10^{-3} in corresponding units) were selected as a seed. Further optimization was carried out by varying k_{OFF} , f and g independently by 15 to 25 steps around the respective seed value to iteratively improve upon the match to the above criteria, with final respective deviations being less than 10^{-5} in corresponding units. The parameter sets that best satisfied the above criteria for each experimental dataset are given in Table 2 and the predicted force- k_{TR} relationships are shown in Fig. 7. Sensitivity of the computational solutions to variation in parameter space was demonstrated by evaluating simulations with k_{ON} , k_{OFF} , f , and g altered 5% above or below the final values (Fig. 7 dotted and dashed lines, respectively).

DISCUSSION

For this study, we hypothesized that dynamic aspects of thin filament Ca^{2+} -regulation are diffusionally controlled processes and thus susceptible to modulation by solvent viscosity, as previously observed for actomyosin function at maximum Ca^{2+} -activation or in assays with unregulated F-actin.^{10,11} To assess myofilament function at submaximum Ca^{2+} -activation, motility assays were performed to determine the speed of regulated thin filament sliding, and single skinned porcine cardiomyocytes were used to measure steady-state force and k_{TR} . Microscopic viscosity surrounding the myofilaments was elevated by adding the low MW sugars glucose or sucrose to the bathing solutions. The major findings of this study were: (i) maximum Ca^{2+} -activated isometric force decreases non-linearly with increased solute concentration and thus solvent viscosity (Fig. 4); (ii) kinetic parameters s_{max} (motility) and k_{TR} (myocytes) at high Ca^{2+} varied linearly with $(\eta/\eta_0)^{-1}$ (Figs. 1, 6); (iii) Ca^{2+} -sensitivity of motility and myocyte isometric force decreased with added solutes (Figs. 2, 5); and (iv) increased η/η_0 inhibited k_{TR} at saturating Ca^{2+} (Figs. 6, 7), while in marked contrast there was little or no effect of added solutes on k_{TR} at submaximum Ca^{2+} (Fig. 7). These results are best explained in the model of Scheme 1 when dynamics of thin filament Ca^{2+} -

regulation (k_{ON} , k_{OFF}) are affected to a much greater extent by elevated sucrose or glucose than is cross-bridge cycling (f , g) in cardiac muscle.

Experimental Quality

In *in vitro* motility assays, sliding speed of thin filaments reconstituted with rHcTn and rH α -Tm at pCa 5 with no added sucrose (control in Figs. 1, 2) was higher than unregulated F-actin speed (Results, Fig. 2), consistent with our previously published observations with these^{5,52,54,58} and regulatory proteins from other sources.^{17,18,24,30} Although HMM and RhPh-labeled F-actin were from rabbit skeletal muscle for the motility experiments conducted in this study, our motility results should capture the essential features of low MW solvent effects on cardiac thin filaments because, in our previous comparison of motility of regulated thin filaments (reconstituted with human rH α -Tm and rHcTn complex, as in the motility experiments reported here) when the motors were rabbit skeletal HMM versus porcine cardiac myosin, there was no difference in the speed-pCa relationships other than the five-fold difference in s_{max} due to myosin source.⁵² Our skinned porcine myocyte average size, control (i.e., no added sugar) F_{max} , maximum k_{TR} , and pCa_{50} values were generally similar to values reported previously by others.^{12,22} To ensure the integrity of each myocyte's sarcomeric structure, F_{max} was measured and compared between the beginning and end of each experiment. Mechanics data for myocytes that lost F_{max} of $> 20\%$ were discarded. Also to demonstrate reversibility of added sugar treatment, some myocytes were treated with control condition first and high [sugar] solution second, and others in the opposite order; there was no significant difference found between those two groups (data not shown).

To vary the experimental viscosity, we choose two low MW sugars that we have used previously,^{10,11} disaccharide sucrose and monosaccharide glucose, for the following reasons. First, these molecules are small enough to freely permeate into the myofilament lattice (interfilament spacing $\sim 16 - 20$ nm^{7,34}) of permeabilized, striated muscle cells, and therefore the interfilament viscosity would be the same as bulk solution viscosity. Second, these sugar molecules do not affect ionic strength of the solution because they are uncharged.

It is also important to consider the addition of 4% w/v Dextran T-500 to myocyte bathing solutions and the use of 0.3 % MC in the motility buffers. Dextran T-500 and MC are large polymers compared with the proteins under study. Dextran T-500 acts as an osmotic agent to compress the diameter of muscle preparations, and $\sim 4 - 6\%$ dextran provides an optimal myofilament lattice spacing for maximum contraction.^{37,59} MC restricts lateral, but not axial motions of thin filaments in motility assays, which promotes retention of filaments on the HMM-coated surface.²³ Although Dextran T-500 and MC do not contribute to microscopic viscosity significantly because of their large size relative to the proteins that are the focus of our study, they both markedly increase the viscosity of the bulk solution, i.e., the macroscopic viscosity measured experimentally using a viscometer (Materials and Methods); therefore, these high MW components that were included in myocyte (Dextran T-500) or motility (MC) buffers for experiments (Materials and Methods) were omitted from solutions used to determine relative viscosity (η/η_0).

Effects of Viscosity at Maximum Ca²⁺ Activation

In our previous study with unregulated F-actin which used rabbit skeletal HMM,¹¹ it was shown that s was inversely proportional to the viscosity of the medium when various low MW solutes (< 20 kDa, including glucose and sucrose) were used to alter viscosity, and that slowing of filament sliding was not due to a viscous "load" or drag on F-actin. Also, in the rabbit psoas fiber study at saturating Ca²⁺ levels,¹⁰ two kinetic measures of actomyosin

mechanics within the intact sarcomere—maximal velocity of shortening (unloaded) and k_{TR} (isometric)—were both slowed in inverse proportion to increased solution viscosity. Our new data from both regulated *in vitro* motility and cardiomyocyte mechanics studies at maximum Ca^{2+} levels confirm that those findings also apply to regulated cardiac systems (Figs. 1, 6). In addition, it shows that these viscosity effects on a diffusion-limited reaction are independent of absolute rates of actomyosin kinetics, i.e., regulated vs. unregulated motility speed, and fast skeletal (rabbit psoas) vs. slow (porcine cardiac) β -myosin heavy chain isoforms.⁵⁰ The idea that cross-bridge diffusion to its binding site on actin is the limiting factor in determining those kinetic parameters at high Ca^{2+} remains a possible explanation for these observations.^{10,11}

Decrease in maximum Ca^{2+} -activated isometric force (Fig. 4), however, cannot be explained fully by elevated solution viscosity. We observed that when maximum isometric force was plotted against relative solution viscosity, the curves for two sugars did not converge (Fig. 4B), which is consistent with our previous study.¹⁰ Endo et al.¹³ reported the same phenomenon in toad fibers, concluding that relative viscosity measured using an Ostwald viscometer is not the sole determinant of inhibition of maximum isometric force at the highest solute concentrations examined in their study. Effects of sucrose on force are also somewhat greater than other disaccharides at the same concentration.^{10,13} In contrast, Lamb et al.²⁷ reported little or no effect of sucrose concentrations up to 0.7 M on maximum isometric force of mechanically skinned toad fibers. Endo et al.¹³ suggested the possibility that the effect of low MW sugars occurs via changes in the activity of water (a_w); we have previously considered this possibility but estimated, however, that only a small change in a_w (< 3%) is expected over the range of solute concentrations tested.¹⁰

Effects of Low MW Sugars at Submaximum Ca^{2+} Activation

To investigate the influence of low MW sugars on steady-state isometric force and k_{TR} at submaximum Ca^{2+} -activation levels, we chose concentrations of sucrose (0.3 M) and glucose (0.875 M) that increased η/η_0 substantially ($\eta/\eta_0 \sim 1.39$ and 1.66, respectively, Table 1) with minimal effects on F_{max} (Fig. 4, Table 1). Although k_{TR} at high Ca^{2+} has a linear, inverse dependence on η/η_0 (Fig. 6), there was little or no effect of elevated viscosity on k_{TR} at submaximum Ca^{2+} -activation level, regardless of the type of low MW sugar used (Fig. 7). In the presence of elevated sucrose or glucose, k_{TR} values were essentially unchanged throughout the range of $[\text{Ca}^{2+}]$ tested here (Fig. 7A and B, respectively). There are several possible explanations for this observation: (i) increased solute concentration and/or elevated viscosity do not affect thin filament Ca^{2+} regulatory kinetics, i.e., diffusion-controlled kinetic parameters (if any) associated with regulatory protein dynamics are not limiting to cardiac muscle function at sub-saturating Ca^{2+} ; (ii) increased solute concentration and/or elevated viscosity slow the dynamics of thin filament regulatory units to such an extent that it limits k_{TR} over the entire range of $[\text{Ca}^{2+}]$; or (iii) differences between k_{TR} for cardiomyocytes at low Ca^{2+} in control and viscous condition could not be resolved in our apparatus.

Under near-physiological conditions, k_{TR} at saturating Ca^{2+} generally reflects the kinetics of cross-bridge cycling, while k_{TR} at sub-saturating Ca^{2+} reflects the dynamics of individual regulatory units.^{9,47} A supporting argument for the first case comes from a study to design Ca^{2+} -sensitizing mutants of cTnC.⁵⁷ Ca^{2+} affinity of cTnC was increased by 2.1 – 15.2 fold by substituting individual, hydrophobic residues in the N-domain of cTnC with polar glutamine. Surprisingly, the increased affinity was largely due to faster Ca^{2+} association rates (2.6 – 8.7 fold) rather than to slower Ca^{2+} dissociation rates (1.2 – 2.9 fold). The fact that Ca^{2+} association rate could be increased implies this rate, while fast, is not diffusion-limited,⁵⁷ and therefore may not be influenced by elevated viscosity. A supporting argument for the second case comes from a previous study on a constitutively activated

cTnC, aTnC.^{9,21} In aTnC-reconstituted skeletal muscle fibers, there was a flat relationship between k_{TR} and steady-state isometric force (i.e., number of regulatory units containing aTnC). What this observation suggests is that when regulatory unit dynamics are slowed dramatically—as might also occur in viscous solvents as suggested by the modeling of data in Fig. 7— k_{TR} varies very little with activation level. As noted in the third case, however, in our experience the smaller k_{TR} value gets, the more difficult it becomes to accurately measure it; this might be evident in relatively larger error bars for low Ca^{2+} /force levels (Fig. 7). While we cannot completely eliminate this possibility, we have previously measured k_{TR} under a wide variety of conditions,^{9,42,45-47,51,53} and confidence is bolstered in this study because the average trends (Fig. 7) are readily evident in our raw data (Fig. 3C, D).

Even though we did not observe any effect of elevated viscosity on k_{TR} at submaximum Ca^{2+} , Ca^{2+} -sensitivity of both *in vitro* motility assays and myocyte mechanics was greatly decreased by added low MW sugars (Figs. 2, 5, and Table 1). Such large changes are supportive of the second case above. Lower Ca^{2+} -sensitivity is in agreement with two reports using skinned toad skeletal fibers. Endo et al.¹³ reported that 1 M glucose caused ~ 0.5 pCa unit decrease of Ca^{2+} -sensitivity of isometric force, and Lamb et al.²⁷ reported that 0.4 M sucrose decreased Ca^{2+} -sensitivity of isometric force by 0.3 pCa units and 0.7 M sucrose caused a 0.5 pCa unit decrease. Endo et al.¹³ dismissed the idea of sugars binding to Ca^{2+} and lowering the actual $[\text{Ca}^{2+}]$ by noting that further increase in $[\text{Ca}^{2+}]$ above the maximum activated isometric force level does not affect the force developed.

To distinguish between the first two cases outlined above, we evaluated a computational model based on Scheme 1 (Materials and Methods). An extensive search was conducted across a broad parameter space, as described in Results, to simulate sucrose- and glucose-dependent changes in isometric force and k_{TR} (Fig. 7) with additional constraints from key elements of the mechanics data, including $p\text{Ca}_{50}$ and F_{max} , in Figs. 4 - 6 and Table 1. Initially, we attempted to describe the effects of sucrose and glucose in the model simply by decreasing f and k_{ON} , i.e., steps that may be diffusion limited, by a factor inversely proportional to the increase in solvent viscosity (Table 1); it was not possible, however, to achieve a good fit to the data using these assumptions. To match the changes in k_{TR} and F_{max} , cross-bridge attachment and detachment rates f and g were decreased by 34% and 21%, respectively, in the presence of 0.3 M sucrose, and by 40% and 25%, respectively, in the presence of 0.875 M glucose (Table 2). The slightly larger effects of 0.875 M glucose compared with 0.3 M sucrose on model kinetic parameters are consistent with the higher relative viscosity with glucose (Table 1). The inhibition of both f and g suggests that overall cross-bridge cycling, and not just the attachment phase, is slowed by approximately the extent expected according to $(\eta/\eta_0)^{-1}$ (Table 1). The relatively greater inhibition of f compared with g is consistent with expectation for processes that might involve a diffusional component. To match the changes in Ca^{2+} -sensitivity of isometric force and the force- k_{TR} relation, thin filament regulatory unit rates k_{ON} and k_{OFF} were markedly decreased by 99.9% and 99.7%, respectively, in the presence of 0.3 M sucrose, and by 99.98% and 99.95%, respectively, in the presence of 0.875 M glucose. Fig. 7 shows that relatively small variations ($\pm 5\%$) in the final parameters lead to significant changes in the predicted curves, indicating that the final parameters represent uniquely good fits to the data. The modeling is consistent with case two above, leading to the conclusion that the major effect of low MW sugars and elevated solvent viscosity at sub-saturating Ca^{2+} levels is on dynamics of thin filament Ca^{2+} -regulation.

The model-deduced effects of low MW sugars on k_{ON} and k_{OFF} (Table 2) are dramatically greater than would be expected from elevated solvent viscosity alone (Table 1), which may reflect the complex nature of activation. The most straightforward explanation is that this is

due to combined effects of solutes on the multiple, dynamic molecular motions that occur between Ca^{2+} binding to TnC and displacement of Tm on the thin filament (inclusive); this activation process and its reversal are included in the model of Scheme 1 as lumped parameters (k_{ON} , k_{OFF}), and both were markedly altered by low MW solutes in the model (Table 2). An additional mechanism that might explain solute-dependent reduction in Ca^{2+} -sensitivity of isometric force generation (Fig. 5) involves the strong dependence of cardiac activation on cross-bridges.^{16,19} Although not explicitly included in the model of Scheme 1, regulatory units can be cooperatively activated by cross-bridges, and low MW solutes could interfere with this process. Slowed cross-bridge cycling, however, would be expected to cooperatively increase Ca^{2+} -sensitivity of isometric force-generation, in contrast with the observed decrease caused by low MW solutes (Fig. 5, Table 1). Changes in cooperative activation could, in principle, also be due to changes in cross-bridge number although that would not likely be the case in this instance (Figs. 4, 5). Furthermore, Ca^{2+} -sensitivity of unloaded filament sliding—a condition involving substantially fewer cross-bridges than isometric force generation—was also markedly reduced by low MW solutes (Fig. 2, Table 1), although this is distinct from the lack of change in the speed-pCa relation (other than s_{max}) when cross-bridge cycling was slowed five-fold by utilizing porcine cardiac myosin in place of rabbit skeletal HMM.⁵² Since the primary effects of low MW solutes on myocyte mechanics could be explained by the simple kinetic model in Scheme 1 (Results, Table 2), and previous experiments indicate that k_{TR} primarily reflects events within single regulatory units,^{9,42} we have not explored more complex models that incorporate additional parameters such as models that include cross-bridge-dependent cooperativity,⁶ although such models could be examined in the future to evaluate possible contributions of cross-bridges to activation of the cardiac thin filament.

Conclusion and Future Directions

Combining experiments and modeling, we have demonstrated the effects of low MW sugars and viscosity on cross-bridge dynamics and even greater effects on thin filament dynamics in cardiac muscle. Although cytoplasmic viscosity of myocytes is not anticipated to be altered in cardiovascular diseases, these experiments demonstrate the utility of low molecular weight solutes and solvent viscosity as probes of cardiac Ca^{2+} -regulatory unit dynamics, and may be of utility to understand altered activation of the heart impaired by mutations in the thin filament such as in cardiomyopathies, especially if such mutations alter the dynamics of Ca^{2+} -activation as has been suggested from molecular investigations of thin filament mutations.^{1,5,15,31,32,35,58}

Acknowledgments

This work was supported by National Institute of Health grants HL63974 (PBC), a Florida State University Center for Materials Research and Technology (MARTECH) Pre-doctoral Fellowship (MAB), and American Heart Association Pre-doctoral Fellowships 0615164B (AKT) and 0815127E (CKPL). We thank Bradley's Country Store, Tallahassee, FL, for generously supplying porcine hearts.

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FIGURE 1.

Sucrose (A) and elevated solvent viscosity (B) inhibit regulated thin filament sliding at saturating Ca^{2+} (pCa 5). *In vitro* motility assays were conducted at 30°C using rabbit skeletal muscle HMM and thin filaments reconstituted with RhPh-labeled F-actin (also from rabbit skeletal muscle) plus rHcTn and rH α -Tm (Materials and Methods). Filament sliding speeds were determined as described in Materials and Methods, averaged for each flow cell, and normalized to control (0 M sucrose) from the same experimental series. Points are the mean of means ($\pm$ SD) for 1 to 6 flow cells; number of flow cells (N) is shown next to each point in panel A. Control speed (regulated pCa 5) was $8.1 \pm 1.8 \mu\text{m s}^{-1}$ (N = 6). The line in panel A indicates the trend of the data. The straight line in panel B was constrained to pass through both the control (rightmost point) and the origin. Inset: fluorescence microscopy image of regulated thin filaments at pCa 5 (control; no sucrose added).

FIGURE 2.

Speed-pCa relationships for regulated thin filaments at sucrose concentrations 0 M (control; circles), 0.3 M (filled triangles), and 0.6 M (open triangles). Filament sliding speeds were normalized to unregulated F-actin (0 M sucrose) from the same experimental series. Each point is the mean ($\pm$ SEM) for approximately 60 filaments from one flow cell. Unregulated F-actin speed was $4.3 \pm 0.2 \mu\text{m s}^{-1}$ ($N = 1$ flow cell). Lines are the nonlinear least squares regressions of Eq. 3 on each dataset. Inset: fluorescence microscopy image of regulated thin filaments at pCa 6 and 0.6 M sucrose.

FIGURE 3.

Example records from a single, permeabilized myocyte from porcine heart for determining isometric force and k_{TR} . (A-D) Fast time-base digital recordings of force from a single cardiac myocyte were obtained at pCa 5.7 (light gray) or 4 (black), in the absence (A, B) or presence (C, D) of 0.3 M sucrose. Two force records are superimposed within each panel; to simplify comparisons, the records in A and C were normalized to the initial, isometric force of each record and replotted in B and D, respectively. (E) Length record applies to all.

FIGURE 4.

Influence of (A) elevated sucrose (solid triangles) or glucose (solid squares), and (B) elevated solvent viscosity on maximum isometric force (pCa 4) of permeabilized porcine myocytes. Isometric force was determined from the beginning of force records as illustrated in Fig. 3, and was normalized to the control value obtained in the absence of sugars in the same myocyte as described in Materials and Methods. Points are the mean $\pm$ SEM from 5 – 7 myocytes. Control force (open circles) was 17.8 ± 2.38 mN mm⁻² for glucose and 24.6 ± 5.94 mN mm⁻² for sucrose. Lines in panel A indicate the trends of the data. The straight line in panel B was constrained to pass through both the control and the origin.

FIGURE 5.

Elevated solvent viscosity reduces Ca^{2+} -sensitivity of isometric force generation by permeabilized cardiac myocytes. Solvent viscosity was increased (A) 39% by adding 0.3 M sucrose (solid triangles) or (B) 66% by adding 0.875 M glucose (solid squares). Force-pCa data for both control (circles; no added sugar) and either sucrose or glucose were obtained from the same myocyte (for details of experimental protocol, see Materials and Methods); data for each condition were normalized to the value obtained at pCa 4 from that condition in the same myocyte. Points are mean $\pm$ SEM from 5 – 9 myocytes. Control force at pCa 4 was $33.9 \pm 5.18 \text{ mN mm}^{-2}$ in panel A, and was $23.3 \pm 3.40 \text{ mN mm}^{-2}$ in panel B. Lines are the nonlinear least squares regressions of Eq. 4 on each dataset.

FIGURE 6.

(A) Sucrose (filled triangles), glucose (filled squares) and (B) elevated solvent viscosity inhibit the kinetics of tension redevelopment (k_{TR}) of permeabilized cardiac myocytes at saturating Ca^{2+} (pCa 5). Isometric k_{TR} was determined from force records (Fig. 3) and was normalized to the control value obtained in the absence of sugars in the same myocyte as described in Materials and Methods. Points are the mean $\pm$ SEM from 5 – 7 myocytes. Control k_{TR} was $0.53 \pm 0.025 \text{ s}^{-1}$ for glucose and $0.45 \pm 0.037 \text{ s}^{-1}$ for sucrose. Lines in panel A indicate the trends of the data. The straight line in panel B was constrained to pass through both the control and the origin.

FIGURE 7.

Elevated sucrose or glucose concentrations affect k_{TR} less at submaximal than maximum Ca^{2+} -activation in permeabilized cardiac myocytes. (A) 0.3 M sucrose (solid triangles) increased solvent viscosity by 39% or (B) 0.875 M glucose (solid squares) increased solvent viscosity by 66% (Table 1). Ca^{2+} -dependence of isometric force and k_{TR} for both control (circles; no added sugar) and either sucrose or glucose were obtained from the same myocyte as illustrated in Fig. 3 (for details of experimental protocol, see Materials and Methods). Force data for each condition were normalized to the control value obtained at pCa 4 in the same myocyte; normalized force data for each condition were binned as described in Materials and Methods. Points are mean $\pm$ SEM from 5 – 9 myocytes. Solid lines are predictions of a 3-state model (Scheme 1) using parameters in Table 2, with gray lines representing control conditions (0 M sucrose or glucose), and black lines representing high (0.3 M) sucrose (A) or high (0.875 M) glucose (B). In each condition, dotted (upper) lines represent model prediction when all four parameters were increased by 5%, and dashed (lower) lines represent model prediction when all four parameters were decreased by 5% relative to the values in Table 2 (solid lines in panels A and B).

SCHEME 1.

3-state model for simulation of cardiac muscle activation. Shown schematically are two structural regulatory units (SRU) of a thin filament and one myosin subfragment 1 (S1) “head” of a thick filament. Each SRU is comprised of 7 actin monomers, one Tm molecule, and one Tn complex. There are 4 rate constants (k_{ON} , k_{OFF} , f and g) that are lumped parameters describing transitions between states. $k_{ON}[\text{Ca}^{2+}]$ is the rate of Ca^{2+} association and is generally thought to be diffusion-limited. k_{OFF} is the rate of SRU deactivation. f is the rate of strong cross-bridge formation. g is the rate of cross-bridge dissociation. The 3-state model was adapted from Hancock et al.²⁰

TABLE 1

Effects of increased solvent viscosity (η/η_0) on F_{max} , s_{max} , and pCa_{50} from *in vitro* motility experiments (Fig. 2) and myocyte mechanics (Fig. 5).

<i>In Vitro</i> Motility	η/η_0	$(\eta/\eta_0)^{-1}$	Δs_{max}	ΔpCa_{50}
0.3M Sucrose	1.29	0.77	-19% *	0.32**
0.6M Sucrose	2.03	0.49	-71% **	$-0.02 < \Delta pCa_{50} < 0.28$
Myocyte Mechanics	η/η_0	$(\eta/\eta_0)^{-1}$	ΔF_{max}	ΔpCa_{50}
0.3M Sucrose	1.39	0.72	-13% *	0.30**
0.875M Glucose	1.66	0.60	-18% **	0.44**

Significance expressed as *, $P < 0.05$; **, $P < 0.01$ when compared with the control (0 M sugar) value.

TABLE 2

Model parameters (Scheme 1) for simulations shown in Fig. 7.

	Cross-bridge kinetics		Regulatory unit dynamics	
	f (s ⁻¹)	g (s ⁻¹)	k_{ON} (s ⁻¹ μ M ⁻¹)	k_{OFF} (s ⁻¹)
[Sucrose] (M)				
0	0.158	0.273	32	96.244
0.3	0.1042	0.2159	0.044	0.2612
[Glucose] (M)				
0	0.1441	0.2995	32	121.5199
0.875	0.0869	0.2236	0.006	0.0644

Parameter estimates for the kinetic biomechanical model in Scheme 1 (Materials and Methods) were identified by optimizing the correspondence of predictions to portions of the myocyte mechanics data in Figs. 5, 6 and 7 and Table 1 as described in Results. Parameters correspond to simulation predictions in Fig. 7: sucrose (0 M) control (Fig. 7A, gray solid line); elevated (0.3 M) sucrose (Fig. 7A, black solid line); glucose (0 M) control (Fig. 7B, gray solid line); elevated (0.875 M) glucose (Fig. 7B, black solid line).