

ATP-induced reconfiguration of the micro-viscoelasticity of cardiac and skeletal myosin solutions

Cite as: Appl. Phys. Lett. **125**, 173702 (2024); doi: 10.1063/5.0224003

Submitted: 18 June 2024 · Accepted: 11 October 2024 ·

Published Online: 21 October 2024



View Online



Export Citation



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ABSTRACT

We study the high-frequency, micro-mechanical response of suspensions composed of cardiac and skeletal muscle myosin by optical trapping interferometry. We observe that in low ionic strength solutions, upon the addition of magnesium adenosine triphosphate (MgATP^{2-}), myosin suspensions radically change their micro-mechanics properties, generating a viscoelastic fluid characterized by a complex modulus similar to a suspension of worm-like micelles. This transduction of energy, from chemical to mechanical, may be related to the relaxed states of myosin, which regulate muscle contractility and can be involved in the etiology of many myopathies. Within an analogous generic mechanical response, cardiac and skeletal myosin suspensions provide different stress relaxation times, elastic modulus values, and characteristic lengths. These discrepancies probably rely on the dissimilar physiological functions of cardiac and skeletal muscle, on the different MgATPase hydrolysis rates of cardiac and skeletal myosins, and on the observed distinct cooperative behavior of their myosin heads in the super-relaxed state. *In vitro* studies like these allow us to understand the foundations of muscle cell mechanics on the micro-scale, and may contribute to the engineering of biological materials whose micro-mechanics can be activated by energy regulators.

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Myosin is a molecular machine whose isoforms are involved in different processes of eukaryotic cells, such as cell division and movement, or intracellular transport.¹ This molecule is well-known because it drives muscle contraction by binding to F-actin with Mg^{2+} -ATPase activity.² Importantly, myosin in its relaxed state, in particular from cardiac muscle, can be related to cardioprotective mechanisms.³ A striated muscle myosin II molecule consists of three main structural components: two globular heads (subfragment 1) that can hydrolyze ATP and bind to actin; a double-stranded coiled-coil α -helical segment (subfragment 2) that lacks ATPase activity; and a rigid, rod-like coiled-coil section known as light meromyosin, which tends to aggregate and form thick filaments of myosin molecules (see schematics in Refs. 22 and 27).

During muscle contraction, the head structure of these molecules is considered the main part of the protein responsible of the transduction of energy, from chemical to mechanical,⁴ and the time that a myosin head needs to hydrolyze is related to muscle thermogenesis and

metabolic activity.⁵ Myosin molecules are contained in the thick filament of the striated sarcomere, while the thin filament contains actin and regulatory proteins (tropomyosin and troponin). The interaction of thick and thin filaments generates a phenomenon named super-precipitation,^{6,7} which has been considered as the *in vitro* contraction.⁸ When it comes to *in vitro* experiments, studies on actomyosin (AM) solutions traditionally provide simple models for exploring the interactions between actin and myosin, and the mechanical properties of AM networks.^{9–11}

Sarcomeric myosin molecules aggregate leading to filament formation at low ionic strength, e.g., for KCl concentrations below 0.6 M.¹² Hence, it is not possible to study monomeric myosin in solution under physiological conditions.¹³ The synthetic myosin filaments have a diameter of 16 nm, and a length of about 1.6 μm , but these quantities vary with solvent conditions,¹² such as the salt content in the solution, and length and width of the filaments decrease when increasing KCl concentration.^{14,15} The myosin minifilaments¹⁶ can be

very uniform in size, with a bipolar structure, a length of $\sim 0.3\text{--}0.4\ \mu\text{m}$,^{17,18} and a diameter of about 8 nm.

The observed rheological properties of dilute myosin solutions are typical of semiflexible structures and allowed to deduce mechanical properties of the myosin molecule.^{19,20} While the rod domain of the protein has been considered semiflexible, a second flexible region appears where the heads attach to the tail, which allows the heads to move freely in solution.²¹ This section links the heads to the thick filament when adding ATP, creating cross-bridges to bound to actin. At this point, some mechanism should temporarily store the energy before the movement of the filaments during muscle contraction is activated, and the elasticity of the cross-bridge is a potential candidate.²²

In this work, we study the micro-mechanical properties of solutions of striated muscle myosins, i.e., cardiac and skeletal, to analyze the changes on their micro-viscoelasticity in the presence or absence of MgATP^{2-} without actin. Here, we search for the mechanical storing of chemical energy in the myosin network under the effect of ATP, something already observed for actin filaments.²³ In fact, the state where the myosin heads action is blocked by molecular switches is called the relaxed state.²⁴ An additional relaxed state, named super-relaxed state, with a very low metabolic rate,³ has been detected for both cardiac and skeletal myosin. It has been proposed that the structural base for this state is the autoinhibitory interactions between the heads.^{25–27}

To retrieve information of the biopolymer fluctuations on the biological scale,²⁸ we study one-particle microrheology at high frequencies by using optical trapping interferometry (OTI), a technique based in the motorization of the Brownian motion of optically trapped single micro-particles inside the fluid with nanometric accuracy at the microsecond timescale.²⁹ The short timescale assures the access to the high-frequency regime of the AM network micro-mechanics, revealing the single filament dynamics.^{30,31} We use skeletal and cardiac myosin [from rabbit skeletal muscle (RS), and porcine cardiac muscle (PC), respectively] purchased from Cytoskeleton, Inc. (with molecular weight $M_w \approx 200\ \text{kDa}$ for the myosin heavy chain), and prepared following established methodologies.^{32,33} Myosin II was dissolved in a high-salt solution [10 mM HEPES pH 7.5, 400 mM KCl, 1 mM DTT] at room temperature. We perform the experiments using cardiac and skeletal myosin with a final concentration of 5 mg/ml, and 10 mM MgCl_2 with and without an equimolar amount of ATP. A single bead is trapped in the center of a sample chamber using optical tweezers³⁴ and its movement is recorded by means of an interferometric position detector (OTI)³⁵ (see the [supplementary material](#) for further details). The probes are melamine resin microbeads (Microparticles, GmbH) with a radius $a = 1.47\ \mu\text{m}$ and density $\rho_p = 1570\ \text{kg/m}^3$ at a work temperature of $T = 21\ ^\circ\text{C}$, providing a relatively high refractive index ($n = 1.68$) and good trapping efficiency in OTI experiments. The resin beads are chemically non-active and, therefore, the protein specific bonding when surrounded by biomaterials is minimized.^{36,37}

In a microrheology tracer experiment, the complex modulus of the surrounding material is $G^*(\omega) = G'(\omega) + iG''(\omega)$, where G' is the storage or elastic modulus and G'' is the loss modulus, and it is usually calculated from the measured mean square displacements $\text{MSD}(t) \equiv \langle [r(t) - r(0)]^2 \rangle$ of the microbeads, where $r(t) = \{x(t), y(t)\}$ is the one-dimensional bead trajectory. In our experiments, we separately analyze the one-dimensional MSDs from the $x(t)$ and $y(t)$ positions of the trapped beads. [Figure 1](#) shows all the

measured MSDs for cardiac and skeletal myosin solutions with and without adding ATP. The measurements contained in [Fig. 1](#) are performed using the weakest optical strength available (see the [supplementary material](#)). The plateau observed at long times in the MSD curves is related to the constant component in the elastic modulus, because of the optical restoring force, or by the fluid itself.^{38,39} The MSDs increase their curvature and decrease their value at long times in [Figs. 1\(b\)](#) and [1\(d\)](#), reflecting that the influence of ATP modifies the viscoelastic behavior and the network structure of the fluid. To extract the complex modulus, $G^*(\omega)$, from the measured MSDs, we apply the standard Mason–Weitz (MW) approach based on the Generalized Stokes–Einstein relation (GSER)⁴⁰ and the Mason’s approximation.⁴¹

For obtaining a single complex modulus for each case, we have blocked all the MSDs plotted in [Fig. 1](#) in 10 points per decade, calculated their complex modulus, and averaged the obtained elastic and loss moduli. The result of this procedure is applied first to myosin solutions with 10 mM of MgCl_2 in the absence of ATP. In [Figure 2](#), we plot the averaged $G'(\omega)$ and $G''(\omega)$ as discrete points and their standard deviation of the mean as shades. [Figures 2\(a\)](#) and [2\(b\)](#) show no appreciable differences for the micro-mechanical response of PC and RS myosin without ATP. The elastic response of these myosin networks is not particularly strong at low frequencies, because their $G'(\omega)$ value corresponds to the value of the optical trap, $G'_k = k/6\pi a = 0.25\ \text{Pa}$. The loss modulus, $G''(\omega)$, grows following a power-law behavior $G''(\omega) \sim \omega^\alpha$ at high frequencies. The elastic modulus should behave similarly, but it shows a characteristic breakup at very high frequencies.^{30,41} Therefore, only $G''(\omega)$ is used to calculate the power-law exponent at high frequencies for both cases. [Figure 2](#) shows power-law exponents $\alpha = 0.89(1)$ and $\alpha = 0.88(2)$, compatible with a 7/8 exponent, which is related to the detection of the longitudinal response of the biopolymers⁴² and has been already observed in fibrin and F-actin experiments using this same experimental technique.^{30,31}

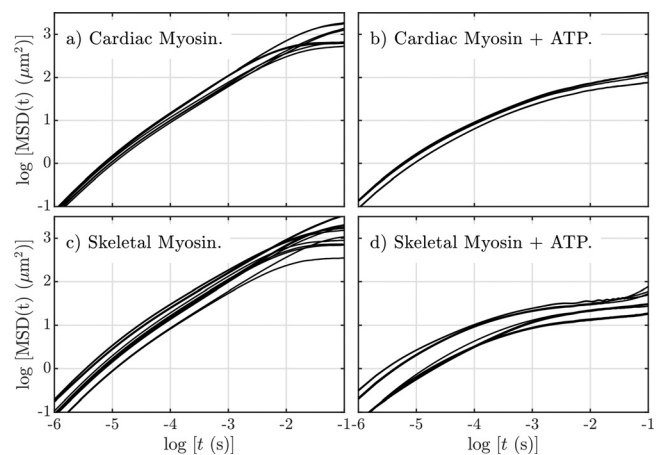


FIG. 1. Log-log plots of the one-dimensional mean square displacements (MSD) for melamine resin trapped beads using the weakest optical strength available, immersed in myosin water solutions with a polymer concentration of 5 mg/ml, and 10 mM MgCl_2 , and (a) porcine cardiac (PC) myosin (8 curves), (b) PC myosin with 10 mM ATP (6 curves), (c) rabbit skeletal (RS) myosin (12 curves), and (d) RS myosin with 10 mM ATP (10 curves). The MSDs appear dispersed because we plot jointly all the curves obtained from different trapped probes.

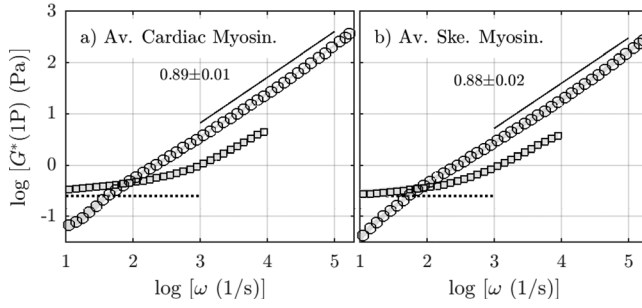


FIG. 2. Log–log plots of the averaged loss (○) and elastic (□) modulus of myosin solutions with 10 mM MgCl_2 in the absence of additional ATP when using (a) porcine cardiac (PC) myosin, or (b) rabbit skeletal (RS) myosin. All data have been blocked in 10 points per decade. Shades represent the standard deviations of the mean. Black lines show the linear regressions to $G''(\omega)$. Dashed lines represent $G'_k = k/6\pi a$, where $k = 7 \mu\text{m}$ is the stiffness of the optical trap.

When we repeat this analysis for the PC and RS myosin solutions with added 10 mM ATP, we obtain the curves plotted in Fig. 3. The micro-mechanical behavior detected by the probe has radically changed. The obtained exponents are $\alpha = 0.74 \pm 0.02$ for PC and $\alpha = 0.74 \pm 0.04$ for RS, and both show a minimum in the loss modulus at lower frequencies, and a substantial increase in the elastic modulus at lower frequencies. Remarkably, the curves for the loss and storage moduli are very similar to those observed for the high-frequency behavior of worm-like micelle solutions,^{43,44} including the characteristic 3/4 exponent for semiflexible polymers.

Theoretically, the complex modulus at higher frequencies for a solution of semiflexible polymers is⁴⁵

$$G_{\text{theo}}^*(\omega) - i\omega\eta_s = \frac{1}{15} \rho_m \kappa l_p \left(\frac{-2i\zeta}{\kappa} \right)^{3/4} \omega^{3/4}, \quad (1)$$

where η_s is the solvent viscosity, ρ_m is the polymer concentration in length per unit volume, κ is the bending modulus, l_p is the persistence length, and ζ is the lateral drag coefficient. We can evaluate most of these quantities using the standard theory of polymers.⁴⁶ The persistence length of the biopolymers is $l_p = (k_B T / 8\eta_s \omega_0)^{1/3}$, where k_B is the Boltzmann constant, ω_0 is the crossover frequency marked in Fig. 3, which allows to calculate the bending modulus by $\kappa = k_B T l_p$. The lateral drag coefficient, ζ , of the filaments is calculated using the mesh size $\xi = (k_B T / G_0)^{1/3}$, where G_0 is the value of the elastic modulus at which the loss modulus has a local minimum, denoted as G''_{\min} (marked in Fig. 3). We need to estimate ρ_m , which we calculate as a free parameter to fit the experimental curves. We obtain $2.0 \times 10^{15} \text{ m}^{-2}$ for cardiac myosin and $4.2 \times 10^{15} \text{ m}^{-2}$ for skeletal myosin. Finally, to characterize the structure of the networks, we calculate the entanglement length of the ATP-added myosin networks, $l_e = \xi^{5/3} / l_p^{2/3}$, and the contour length of their filaments, $L = l_e G_0 / G''_{\min}$. The results of the calculations for all these quantities are summarized in Table I, and the evaluation of the theoretical complex modulus is plotted in Fig. 3 with blue symbols. The agreement with the experimental loss modulus in both cases is notable and indicates how the elastic modulus should grow to follow the 3/4 power-law behavior and remain significantly smaller than $G''(\omega)$. If the complex viscosity is $\eta \equiv |\eta^*(\omega)| = \omega^{-1} [G'(\omega)^2 + G''(\omega)^2]^{1/2}$, this observation implies $\eta \sim G''(\omega) / \omega$ at high frequencies.

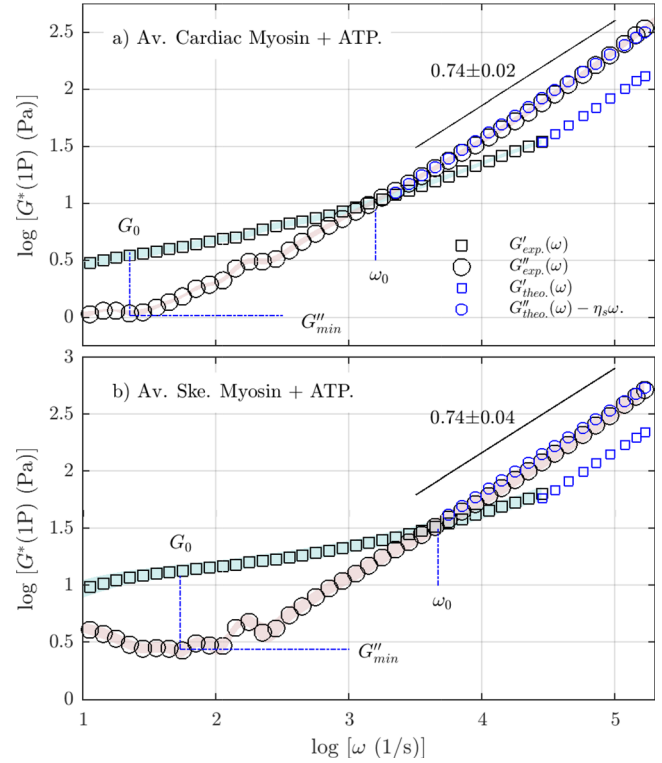


FIG. 3. Log–log plots of the averaged loss (○) and elastic (□) modulus of myosin solutions with 10 mM MgCl_2 , 10 mM ATP, and (a) porcine cardiac (PC) myosin, or (b) rabbit skeletal (RS) myosin. All data have been blocked in ten points per decade. Shades represent the standard deviations of the mean. Black lines show the linear regression to the experimental $G''(\omega)$ data. Blue squares and circles represent the theoretical complex modulus by Eq. (1).

These values allow to evaluate the micro-mechanical behavior of the myosin structures under ATP. For example, the persistence length is considered a measure of the degree of flexibility of the structures and the bending force constant evaluates the resistance to bending. The values obtained for l_p for these myosin filaments are similar to those measured for DNA ($\sim 50 \text{ nm}$)⁴⁷ or worm-like micelles ($\sim 30 \text{ nm}$),^{44,48} but they are half the value for myosin rods without added ATP ($l_p \sim 140 \text{ nm}$).¹³ In relation with the bending force, the fundamental flexural mode of a semiflexible rod is characterized by a time⁴⁹ $\tau_F = 5.53 \times 10^{-3} (\pi\eta_s L^4) / \kappa \ln(L/d)$, where η_s is the solvent viscosity, L is the contour length, and d is the diameter of the rod. Using these calculated values and their uncertainties, we obtain an associated frequency interval of $\omega_F \sim 10^3 \text{ s}$. This value can be identified with the values of Table I for the crossover frequency, ω_0 , which is the inverse of a relaxation timescale of elastic structures of the material. The obtained value for the contour length of these filaments is similar for cardiac and skeletal, $L \sim 0.4 \mu\text{m}$, matching the expected averaged length of myosin minifilaments.^{17,18}

Therefore, the effect of ATP is to render polymeric structures less rigid and more flexible. The results of Table I allow a comparison of the morphology of these filaments with worm-like micelles, already studied using this same experimental procedure.⁴⁴ The characteristic lengths are 2–3 times bigger for myosin, but the proportion $L/l_p \sim 6$

TABLE I. Results obtained by the application of Eq. (1) and the standard theory of polymers to the data of Fig. 3.

Myosin+ATP	ω_0 (s ⁻¹) $\times 10^3$	G_0 (Pa)	G'_{\min} (Pa)	l_p (nm)	ζ (Ns/m ²) $\times 10^{-3}$	ζ (nm)	l_e (nm)	L (μ m)
Cardiac (PC)	1.6 \pm 0.2	2.8 \pm 0.3	1.07 \pm 0.09	68 \pm 3	5.89 \pm 0.11	113 \pm 4	158 \pm 11	0.42 \pm 0.07
Skeletal (RS)	4.4 \pm 0.6	13.6 \pm 1.7	2.7 \pm 0.4	49 \pm 2	7.8 \pm 0.2	67 \pm 3	83 \pm 6	0.41 \pm 0.08

in the case of cardiac myosin is very similar to micelles ($L = 180$ nm, $l_p = 30$ nm) and a little higher for skeletal myosin $L/l_p \sim 8$. This factor indicates a slightly coil-like structure in the filaments,⁵⁰ similar to worm-like micelles, but composed of longer filaments. These coil-like structures have a tendency of bending themselves because of the thermal fluctuations at their independent ends. The similarity of the viscoelastic properties of these myosin solutions with worm-like micelles solutions may have its origin in the behavior of the detached myosin heads in the filaments. The ATP can dissociate the myosin filaments,¹⁷ creating a bipolar structure with heads at both extremes of the filaments. Because of the energy provided by ATP, the myosin heads look for an actin binding site by Brownian search, generating a collective movement with high degree of freedom.⁵¹

Further in-depth, several values in Table I contain appreciable discrepancies for skeletal and cardiac myosin. For example, the stress relaxation time (inverse of ω_0) for cardiac myosin is three times larger than for the skeletal one. The persistence length, which is proportional to the bending modulus, is also significantly larger in the activated cardiac myosin, but the elastic modulus at low frequencies, G'_0 , is five times lower. A considerable difference appears in the fitted values for the area density, ρ_m , where a simple calculation returns the value obtained for cardiac myosin (see the supplementary material), but it is doubled for skeletal. This outcome is rather complex to interpret, because, in the context for the super-relaxed state, different muscle types show substantial dissimilarities in behavior and distribution of the myosin heads.³

These differences between cardiac and skeletal myosin solutions are compatible with a generic mechanical picture of the cardiac and skeletal muscle. Skeletal muscle generates more intense forces in comparison with cardiac muscle, which should regularly contract and relax, retaining mechanical capacities.⁵² In other words, skeletal muscle may remain in a relaxed state more often and has to be able to rapidly generate contraction forces, but cardiac muscle needs to contract and relax more constantly, normally at a steady pacing. Cardiac muscle's longer relaxation time supports sustained contractions, conserves energy, ensures rhythmic beating, and allows adaptation to changing demands, protecting the heart and aligning with the super-relaxed state properties of cardiac myosin.

In conclusion, both cardiac and skeletal myosin solutions increase their high-frequency micro-viscoelastic response under the effect of ATP, transforming the chemical energy into mechanical energy. This process may be related to the relaxed states, in which a mechanism for storing energy prior to muscle contraction is needed. Within a similar generic micro-mechanical response, cardiac and skeletal myosin suspensions provide different values regarding their characteristic viscoelastic parameters, something that may be related to several factors such as different MgATPase hydrolysis rates of cardiac and skeletal myosins, distinct cooperative behavior of the myosin heads, or dissimilar physiological functions of cardiac and skeletal muscle. *In vitro* studies like this one may be of interest to develop materials whose

micro-viscoelasticity can be modified by internal processes, such as energy release or enzymatic activity, with potential application in engineering biological materials,⁵³ and in medicine and biotechnology.⁵⁴

See the [supplementary material](#) for additional information about experimental data and methodology. P.D.G acknowledges support aid by Grant No. PID2020-117080RB-C54 funded by MCIN/AEI/10.13039/501100011033, and J. C. Gómez-Sáez for her proofreading of the texts. J.R.P. acknowledges support from the National Institutes of Health Grant No. R01 HL128683. A.A. acknowledges funding from the Swiss National Science Foundation through Project No. PP00P2_202661.

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Pablo Domínguez-García: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Software (lead); Writing – original draft (lead); Writing – review & editing (lead). **Jose R. Pinto:** Conceptualization (equal); Investigation (equal); Methodology (equal); Resources (equal); Supervision (equal); Validation (equal); Writing – review & editing (equal). **Ana Akrap:** Funding acquisition (equal); Project administration (equal); Resources (equal); Supervision (equal); Validation (equal); Writing – review & editing (equal). **Sylvia Jeney:** Conceptualization (lead); Funding acquisition (lead); Investigation (equal); Project administration (lead); Resources (lead).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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