Viscoelastic retraction of stress fibers in living myoblasts under oxidative stress

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Introduction

Stress fibers (SFs) are intracellular bundles composed of actin filaments. They are crucial in various biological activities. It has been reported that the actin network in cells can be considerably affected by reactive oxidative species (ROS). ROS like hydrogen peroxide (H_2O_2) can build up oxidative stress, leading to pathogenesis of muscle injury. To understand such pathological challenges from the basics, we set out to study the effect of oxidative stress on cell mechanics, particularly the mechanics of SFs. H_2O_2 was used to pose oxidative challenges to the cells. We utilized a femtosecond laser (fs) to sever single SFs in living C2C12 myoblasts and investigated the retraction of the severed SFs. A modified standard linear solid (SLS) model was employed to describe the retraction process. By performing curve fitting using data points obtained from the recorded retraction, we were able to see how H_2O_2 affected the mechanical properties of the SFs.

Methods

1. Experimental setup

Mouse myoblasts (C2C12) were transfected with actin-GFP for the visualization of SFs. Different groups of H_2O_2 treatment, namely, **control (no treatment), 0.5mM for 1h, 2mM for 1h, and 0.5mM for 12h,** were then applied. Femtosecond (fs) laser was used to sever single SFs (**Fig. 1**) immediately after the removal of H_2O_2. The retraction of single SFs was observed and recorded by confocal microscopy.

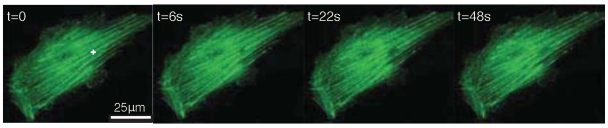

Fig. 1. The retraction of a single SF upon fs laser ablation. The cross denotes the severing point.

2. Theory

A modified standard linear solid (SLS) model, with mathematical expression **$L(t)=L_o[1-\exp(-t/\tau)]+L_p+L_a$**, is adopted to describe the retraction kinetics of a single SF (see **Fig. 2** for illustration of model parameters) (Kumar et al. 2006). Data points (t, L) were obtained from the recorded retraction of SFs. Curve fitting was performed to obtain the values of model parameters L_o, τ, and D_a (i.e., L_p+L_a).

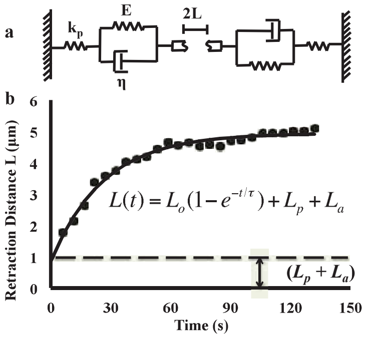

Fig. 2. (a) The mechanical model of the retraction of a single SF.
(b) Representative kinetics of single SF retraction and the model equation, where L is the retraction distance (half the distance between the severed ends), L_o is the maximum retraction, τ is the characteristic time constant equal to the ratio of the material's viscosity η to its elastic modulus E, L_p is the initial passive elastic retraction, L_a is the initial material loss due to laser ablation, and $(L_p + L_a)$ is denoted as the initial gap D_a.

Results and Discussion

1. The effect of H_2O_2 treatment on model parameters

Sample number n = 10, 9, 10, 5 for control, 0.5mM-1h, 2mM-1h, and 0.5mM-12h H_2O_2–treated groups respectively.

1.1. Characteristic time constant τ and maximum retraction distance L_o

As shown in **Fig. 3a**, the time constant τ (i.e., η/E) increased after H_2O_2 treatment. This indicates that H_2O_2 treatment either increased the viscosity or decreased the elasticity of the SFs. The change of L_o in shown in **Fig. 3b**.

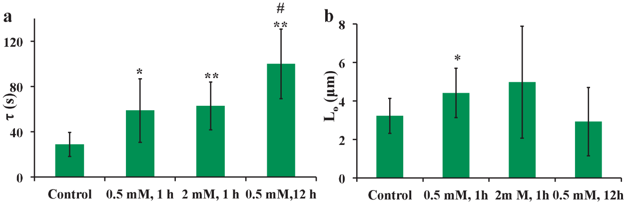

Fig. 3. (a) Characteristic time constant τ and **(b)** maximum retraction distance in all the experimental groups. *: $p < 0.05$ compared to control; **: $p < 0.01$ compared to control (same in other figures). #: $p < 0.05$ compared to 0.5mM-1h group.

1.2. Initial gap D_a (i.e., $L_p + L_a$)

The initial gap D_a (the distance between the two severed ends immediately after laser ablation) is contributed by both the initial passive elastic retraction L_p and the laser-induced material loss L_a, where L_a can be quantified by severing SFs in fixed cells.

D_a increased after H_2O_2 treatment (**Fig. 4a**), while we found L_a insensitive to laser ablation energy and H_2O_2 treatment (data not shown). Hence, we conclude that the increase in D_a is mainly contributed by the increase in L_p. L_p, according to the above SLS model, is proportional to σ/k_p (prestress/spring constant), where prestress is the preexisting tensile stress borne by the SF. Additionally, it has been reported that cell stiffness is proportional to the pretress in cell (Wang et al. 2001). And cell stiffness in myotubes increased after 0.5mM/2mM-1h H_2O_2 treatment (**Fig. 4a**) according to AFM test conducted by Singwan Wong. Combining these two pieces of evidence, we think that H_2O_2 treatment increased the prestress in the cell.

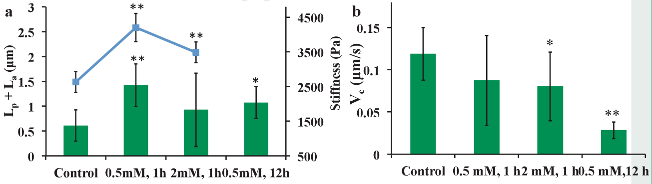

Fig. 4. (a) the initial gap width D_a ($L_a + L_p$) and **(b)** the characteristic retraction velocity V_c in all the experimental groups.

1.3. Characteristic retraction velocity V_c

The characteristic retraction velocity V_c is defined as L_o/τ, and is reported to be closely related to (motor force – friction) (Colombelli et al. 2009). We believe the decrease in V_c after H_2O_2 treatment (**Fig. 4b**) reflects an increase in the friction experienced by SFs, since the motor force, which is generated via the same mechanism as prestress, did not decrease.

2. The effect of H_2O_2 treatment on the ablation energy

The laser energy required to sever SFs increased after H_2O_2 treatment, indicating the SFs were strengthened.

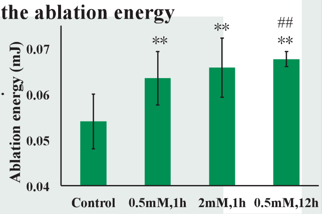

Fig. 5. The ablation energy in all the experimental groups. ##: $p < 0.01$ compared to 0.5mM-1h. Sample number n = 15, 19, 8, 11 for control, 0.5mM-1h, 2mM-1h, and 0.5mM-12h, respectively.

Conclusion

In conclusion, we found that H_2O_2-treated SFs tended to be more viscous and less elastic. H_2O_2 treatment, especially moderate concentration and short duration (0.5mM-1h), was likely to up-regulate the prestress borne by the SFs, though direct evidence was lacking (no direct measurement of prestress in SFs). The friction experienced by the SFs probably also increased after H_2O_2 treatment according to Section 1.3 above. Moreover, H_2O_2 treatment in our experiments was believed to have strengthened the SFs, with the evidence being the increase in the ablation energy.

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