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$_2$O$_2$) 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$. The retraction of single SFs was observed and recorded by confocal microscopy.

2. Theory
A modified standard linear solid (SLS) model, with mathematical expression $L(t)=L_0[1-exp(-t/\tau)]=L^*_p + L^*_t$, 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 (1, t) were obtained from the recorded retraction of SFs. Curve fitting was performed to obtain the values of model parameters $L^*_p$, $L^*_t$, and $D_p$ (i.e., $L^*_p + L^*_t$).

Results and Discussion

1. The effect of H$_2$O$_2$ treatment on model parameters
Sample number $n=10$, 9, 10, 5 for control, 0.5mM-1h, 2mM-1h, and 0.5mM-12h H$_2$O$_2$-treated groups, respectively.

1.1. Characteristic time constant $\tau$ and maximum retraction distance $L^*_p$
As shown in Fig. 3a, the time constant $\tau$ (i.e., $\eta/E$) increased after H$_2$O$_2$ treatment. This indicates that H$_2$O$_2$ treatment either increased the viscosity or decreased the elasticity of the SFs. The change of $L^*_p$ in shown in Fig. 3b.

1.2. Initial gap $D_0$ (i.e., $L^*_p + L^*_t$)
The initial gap $D_0$ (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 $L^*_t$, where $L^*_t$ can be quantified by severing SFs in fixed cells. $D_0$ increased after H$_2$O$_2$ treatment (Fig. 4a), while we found $L^*_p$ insensitive to laser ablation energy and H$_2$O$_2$ treatment (data not shown). Hence, we conclude that the increase in $D_0$ is mainly contributed by the increase in $L^*_t$, according to the above SLS model, is proportional to $\eta/\tau$ (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$_2$O$_2$ treatment (Fig. 4a) according to AFM test conducted by Singwan Wong. Combining these two pieces of evidence, we think that H$_2$O$_2$ treatment increased the prestress in the cell.

1.3. Characteristic retraction velocity $V^*_c$
The characteristic retraction velocity $V^*_c$ is defined as $L^*_p/\tau$, and is reported to be closely related to (motor force – friction) (Colombelli et al. 2009). We believe the decrease in $V^*_c$ after H$_2$O$_2$ 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$_2$O$_2$ treatment on the ablation energy
The laser energy required to sever SFs increased after H$_2$O$_2$ treatment, indicating the SFs were strengthened.

Conclusion

In conclusion, we found that H$_2$O$_2$-treated SFs tended to be more viscous and less elastic. H$_2$O$_2$ 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 is probably also increased after H$_2$O$_2$ treatment according to Section 1.3 above. Moreover, H$_2$O$_2$ 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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References

