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SUPPRESSION OF CROSSBRIDGE MOTIONS OF ISOLATED THICK MYOFILAMENTS IN ATP-FREE MEDIUM BY THIOUREA

Shih-fang Fan[1], M.M. Dewey[2*] and B. Chu[3]

[1]Department of Physiology and Biophysics and [2]Department of Anatomical Sciences, Health Sciences Center, and [3]Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794

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Thiourea is known to suppress the contractile response of *Mytilus* anterior byssus retractor muscle and toad sartorious muscle following electrical or chemical stimulation without abolishing of the electrical responses. In addition, it blocks the shortening of glycerinated sartorious muscle induced by Ca^{2+}. With dynamic laser light scattering method we found that thiourea suppresses the increase of the average linewidth of the photoelectron count autocorrelation function, $\bar{\Gamma}$, of isolated thick filaments of *Limulus* striated muscle induced by depletion of ATP. The results obtained suggest that thiourea prevents the crossbridges from moving away from the thick myofilament backbones which will, in turn, prohibit the crossbridges from interacting with the thin myofilaments.

Thiourea affects the electrical responses and the contraction of muscle differently. For instance, Rüegg *et al* [1] reported that thiourea abolished all contractile responses of the anterior bysuss retractor muscle of *Mytilus* to acetylcholine, cathodal current, isotonic KCl, caffeine and chloroform. However, thiourea did not suppress the depolarization of the muscle by acetylcholine or KCl. Fan *et al* [2] reported that toad sartorious muscle lost its responsiveness to stimulation after treating with 0.7 M thiourea in Ringer's solution for about 15 minutes. After washout of thiourea the ability of muscle to give electrical responses to both direct and indirect stimulation was largely

*Deceased.

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restored. However, there was no corresponding recovery of contractility. Thiourea also suppressed the Ca^{2+}-induced shortening of glycerinated muscle fiber. These results indicate that thiourea can affect the contractile apparatus directly.

The contractile apparatus of muscle is composed of two sets of myofilaments, the thick and the thin ones. Contraction is caused by the pulling of thin myofilaments in between thick myofilaments by the crossbridges which project outward from the thick myofilaments. To achieve this, crossbridges should first moves radially away from the thick myofilament backbones towards the thin myofilaments then attached to the thin myofilaments and cyclically pull them.

Dynamic laser light scattering (DLS), by using of Doppler effect, can detect translational motions of the center of mass and the internal motions of the scatterer. Many reports have shown that DLS could be used to study the dynamics of muscle fibers and their substructures (for examples, see refs [3-7]). We had used this method to study the crossbridge motions and observed two modes of motion in isolated *Limulus* thick myofilaments : (1) the radial motion of the crossbridge which was detected when myofilaments were suspended in an ATP-depleted solution [8] and (2) an ATP dependent, Ca^{2+}-activated cyclic one [9]. Does thiourea affects the crossbridge motions and whether it primarily affects the radial or the cyclic motions is tested in this work with DLS.

METHODS: The telson levator muscles of *Limulus* (*Tachypleus polyphenus*) were used. Effect of thiourea on the shortening of muscle was tested with glycerinated muscle bundles. They were isolated, tied at fixed length and soaked in a 50-50% relaxing solution-deionized glycerol (v/v) mixture at $4^{o}C$. The relaxing solution contained (in mM) 100 KCl, 2 $MgCl_2$, 5 Tris, 2 EGTA and 2 ATP at pH 7.0. After 48 hours, the relaxing solution-glycerol mixture was changed and the temperature lowered to $-20^{o}C$. The fiber bundle was usually used about 1-2 weeks later. The activating solution contained (in mM) 100 KCl, 2 $MgCl_2$, 5 Tris, 5 $CaCl_2$ and 2 ATP at pH 7.0

For DLS studies, isolated and purified thick myofilaments from the telson levator muscles of *Limulus* were used. The methods of preparation of the samples and the light scattering apparatus used were the same as described previously [9,10]. We compared the $\bar{\Gamma}$ values obtained in different experimental conditions. $\bar{\Gamma}$ is the average linewidth of the photoelectron count autocorrelation function. For long flexible filaments

in the semidilute regime ($c << 1/dL^2$, with c being the concentration of the rod in number of rods per milliliter^3) and $KL >> 1$[11]

$$\bar{\Gamma}/K^2 \longrightarrow 2\ D_{Ts} + D_{Ts}\sum_m{}''1$$

where K is the magnitude of the momentum transfer vector and equals $(4\pi/\lambda)\sin(\theta/2)$, with λ the wavelength of the incident light in the medium and θ the scattering angle; D_{Ts} is the sideway tranlational diffusion coefficient, L is the contour length of the filament; d is the diameter of the filament; $\sum_m{}''1$ is the number of bending motions involved in the scattering process, its value lies between $1/(cL^3)$ and $1/(cL^3)^2$. $\bar{\Gamma}$ value is a function of the length, the flexibility and other internal motions of the filament. Decrease in length, increase in flexibility as well as increase in internal motions will increase the $\bar{\Gamma}$ value.

RESULTS AND DISCUSSION: The effect of thiourea on the shortening of *Limulus* striated muscle was first tested with glycerinated muscle fibers. Muscles bundles with only a few muscle fibers were isolated and placed on a Sylgard plate under a dissecting microscope. After repeated rinses with relaxing solution containing only 0.1 mM EGTA, drops of activating solution were added. The fibers shortened almost immediately. If the fibers were first soaked in relaxing solution containing 0.7 M thiourea, no shortening was observed with the addition of activating solution. It usually required more than two hours of presoaking in thiourea solution to completely block shortening. Treatment with 0.5 M thiourea never totally abolished shortening even after soaking for five hours.

The effect of thiourea on isolated thick myofilaments was then studied. When a suspension of thick myofilaments isolated in relaxing solution was dialyzed against an ATP-free solution (relaxing solution with ATP removed and treated with 50 μg/ml hexokinase and 2 mM glucose to remove the residual ATP) the $\bar{\Gamma}$ values at a high scattering angle (e.g. 120^o, which corresponds to $K^2 = 8.8 \times 10^{10}$ cm^{-1}) increased on average about two times as compared with that obtained with myofilaments suspended in relaxing solution with ATP. If 0.7 M thiourea were added, the $\bar{\Gamma}$ value decreased and after about three hours, it dropped to the value obtained with myofilaments suspended in relaxing solution with ATP (Figure 1A, curve marked with ATP-free). In Figure 1A, the curves

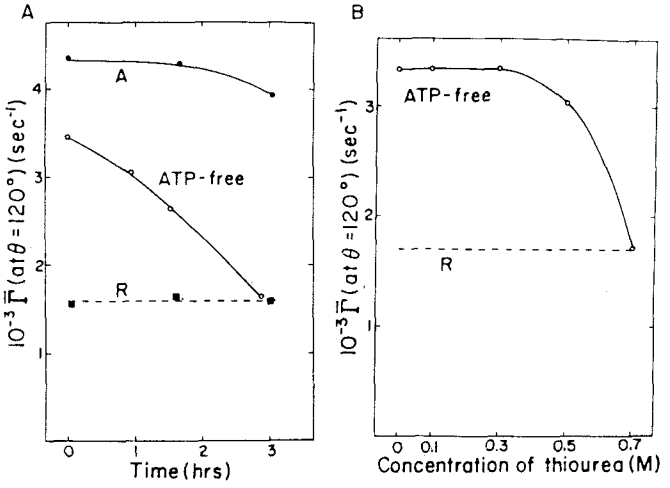

Fig. 1. Effect of thiourea on $\bar{\Gamma}$ values of isolated thick myofilaments suspended in ATP-free solution.

A. 0.7 M thiourea suppresses $\bar{\Gamma}$ values of isolated thick myofilaments suspended in ATP-free solution. Thiourea has no effect on $\bar{\Gamma}$ values of filaments suspended in relaxing solution with ATP and affects $\bar{\Gamma}$ values of filaments suspended in activating solution with a much slower time course.

B. Effect of different concentrations of thiourea on $\bar{\Gamma}$ values of isolated thick myofilaments suspended in ATP-free media. Data were obtained after three hours of treatment.

Curve labeled with ATP - filaments suspended in ATP-free solution; dotted line labeled with R - filaments suspended in relaxing solution; curve labeled with A - filaments suspended in activating solution.

marked with R and A are the experimental results from thick myofilaments suspended in relaxing and activating solution, respectively, with 0.7 M thiourea added at time 0. The $\bar{\Gamma}$ value obtained from myofilaments suspended in activating solution was also decreased by 0.7 M thiourea but with a much slower time course. It took about ten hours to reduce the value to that obtained from myofilaments suspended in relaxing solution. Fig. 1B shows the results obtained with thick myofilaments suspended in mediums with different concentrations of thiourea. Experiment was carried with thick myofilaments isolated from the same muscle. After isolation, the myofilament preparation was split into five equal portions, one dialyzed against regular relaxing solution (i.e., containing ATP) and the other five dialyzed against ATP-free solution. At time 0, different amounts of thiourea were added and the $\bar{\Gamma}$ values determined and

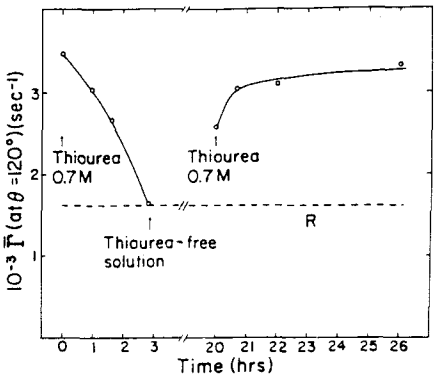

Fig. 2. Reversibility of thiourea effect.
After the increase of the $\bar{\Gamma}$ value was completely suppressed by 0.7 M thiourea, the sample was dialyzed against a thiourea-free solution. $\bar{\Gamma}$ slowly increased, reached about half of its initial value after about 20 hours. However, second treatment with thiourea not only was ineffective in suppresion of $\bar{\Gamma}$ values but, in fact, accelerated recovery process.

followed. The value shown in Figure 1B were taken after three hours of treatment when the $\bar{\Gamma}$ values already showed no further changes. It was shown that 0.5 M thiourea suppressed the increase of $\bar{\Gamma}$ value to less than 30%.

After washout the thiourea, $\bar{\Gamma}$ value slowly increased. After 15 hours or more half of the value suppressed by 0.7 M thiourea was recovered (7 experiments). Figure 2 shows the results of one experiment. One puzzling but interesting phenomenon was that the second treatment of thiourea was not only ineffective in the suppression of Γ values but instead, accelerated the recovery course. Similar results have been obtained in all 7 experiments.

The preliminary effect of thiourea on isolated thick myofilaments was to suppress the increase of $\bar{\Gamma}$ value under ATP-free conditions. After treating with ATP-free solution, the length of the myofilament decreased slightly and the flexibility was increased. However, we have showed that the shortening of filament length and the increase in flexibility of the filaments can only account for a small portion of the increase in $\bar{\Gamma}$ value, the increase is mainly due to the thermal motion of

crossbridges detached from the filament backbone around the S_2 - filament backbone joint [8]. Therefore the results obtained in this study indicate that the suppression of contraction of muscle by thiourea is due to the crossbridges being prevented from moving away from the thick myofilament backbones, in turn, prohibited them from interacting with thin myofilaments.

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