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Dynamic laser light scattering studies of the effects of pyrophosphate on cyclic motions of cross-bridges in isolated thick myofilaments from *Limulus* striated muscle

S.-F. Fan, M. M. Dewey[a]† and B. Chu[b]

Department of Physiology and Biophysics, [a]Department of Anatomical Sciences, Health Sciences Centre and [b]Department of Chemistry, State University of New York at Stony Brook, Stony Brook (New York 11794, USA)
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Abstract. Pyrophosphate (PP_i) is a non-hydrolyzable ATP analogue known to affect the binding between myosin heads and actin. By using a dynamic laser light scattering method, we have shown that 1–2 mM PP_i enhances the increase in $\bar{\Gamma}$ values induced by Ca^{2+} in isolated thick myofilaments from *Limulus* striated muscle. However, similar treatment has no effect on the $\bar{\Gamma}$ values of filaments suspended in either relaxing solution or ATP-free solution. $\bar{\Gamma}$ is the average linewidth of the photoelectron count autocorrelation function of the light scattered. PP_i had no effect on the increase of $\bar{\Gamma}$ values by Sr^{2+} but it substantially increased the $\bar{\Gamma}$ values of the thick myofilaments suspended in Ba^{2+}-substituted Ca^{2+} activating solution. The results show that PP_i also affects the energy-requiring cyclic cross-bridge motions.
Key words. Cross-bridge; dynamic laser light scattering; *Limulus* muscle; pyrophosphate; thick myofilament.

Striated muscles consist of cells that contain structures known as myofibrils. Each myofibril consists of a series of contractile structures called sarcomeres. Each sarcomere consists of a central darker band sandwiched between two lighter regions. The dark bands are known as A bands; the lighter regions are called I bands. In striated muscles, the I band consists only of thin filaments while the A band has both thick and thin filaments. The thick filaments consist mainly of the contractile protein myosin, which has lateral projections called cross-bridges. The thin filaments consist mainly of another contractile protein, actin. When striated muscles contract, the cross-bridges are believed to bind cyclically to the actin molecules and pull the thin filaments toward the center of the sarcomeres. The energy is derived from the breakdown of MgATP. Figure 1 is a conceptual diagram of the scheme[1]. One of the fundamental questions to be solved in studies of muscle is the mechanism of conversion of the free energy of hydrolysis of MgATP into mechanical work. One approach to this problem is to perturb the cross-bridge cycling, and trap the cross-bridges at various intermediate stages, using MgATP analogues.

PP_i is a non-hydrolyzable ATP analogue. It alters the kinetics of both attachment and detachment of the S-1 moiety of cross-bridges to or from actin molecules (e.g. see refs 2–4). It also weakens the binding while the cross-bridges and the thin filaments are attached. However, Makinose and Nagi[5] reported that PP_i enhances contraction elicited by electrical stimulation in living muscles. Subsequent evidence presented by Fan and Li[6] showed that the apparent increase was caused by repetitive firing of muscle fibres following a single direct stimulus. The maximum tension developed during a single twitch is actually reduced. Whether this is due to changes in the interaction between cross-bridges and thin filaments and/or the effect on cyclic cross-bridge motions is unknown. *Limulus* striated muscle has been shown to possess both a direct myosin regulatory system and a troponin-tropomyosin system[7]. Therefore the isolated thick myofilaments from *Limulus* striated muscle can be used to test the effect of PP_i on cross-bridge cyclic motion without the intervention of actin molecules.

Dynamic laser light scattering (DLS) uses the Doppler effect to detect translational motions of the center of mass and the internal motions of the scatterer. This method has been used to study the dynamics of muscle fibers and their substructures (e.g. see refs 8–12). We used it to study cross-bridge movement and observed two types of motion in isolated *Limulus* thick myofilaments: 1) the ATP-dependent, Ca^{2+}-activated cyclic motion[13] and 2) the thermal motion of the cross-bridge in ATP-free conditions[14]. We show in the work described here that PP_i only enhances the first type of motion.

Materials and methods

Isolated and purified thick myofilaments from the telson levator muscles of *Limulus* (*Tachypleus polyphemus*) were used. The sample preparation method and the light scattering apparatus used were the same as those described previously[13,15]. The average linewidths of the photoelectron count autocorrelation function, $\bar{\Gamma}$, were obtained. For long flexible filaments in the semidilute regime ($c \ll 1/dL^2$, with c being the concentration of filaments in number of filament per ml^3, d being the

diameter of the filament, L being the contour length of the filament and $KL \gg 1$ [ref. 16])

$$\bar{\Gamma}/K^2 \rightarrow 2D_{TS} + D_{Ts} \sum_{m}'' l$$

where K is the magnitude of the momentum transfer vector and equals $(4\pi/\lambda) \sin(\theta/2)$, λ is the wavelength of the incident light in the medium and θ is the scattering angle; D_{TS} is the sideway translational diffusion coefficent and $\sum_{m}'' l$ is the number of bending motions involved in the scattering process; its value lies between $1/(cL^3)$ and $1/(cL^3)^2$. As the internal motions of the filament, such as cross-bridge movements, increase, the $\bar{\Gamma}$ value will increase.

Results and discussion

When thick myofilaments were suspended in a relaxing solution (100 mM KCl, 5 mM $MgCl_2$, 2 mM EGTA, 5 mM Tris, 2 mM ATP, pH 7.2), PP_i (1–10 mM) has no effect on the $\bar{\Gamma}$ value at high scattering angles (e.g. 120 °C, which corresponds to $K^2 = 8.8 \times 10^{10}$ cm^{-2}). As thick myofilaments were suspended in an activating solution (100 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM Tris, 2 mM ATP, pH 7.2), low concentrations of PP_i increased the $\bar{\Gamma}$ value. At a scattering angle of 120° it increased 10–25% [(18 ± 5)%, mean ± SD, n = 6] with 2 mM PP_i. Figure 2 shows the results obtained with filaments suspended in media with different concentrations of PP_i. In each experiment, filament suspensions from the same batch of preparation were used. After isolation, the sample was split into seven equal portions. One was dialyzed against PP_i-free relaxing solution, one against PP_i-free activating solution and the other five against activating solutions with different concentrations of PP_i. At PP_i concentrations up to 2 mM, the $\bar{\Gamma}$ values increased. As the PP_i concentration exceeded 5 mM, the increase of the $\bar{\Gamma}$ values by Ca^{2+} was suppressed. 1–10 mM PP_i had no effect on the $\bar{\Gamma}$ values of thick myofilaments suspended in low ATP solution. The table shows the results.

The results obtained indicate:

1) Binding of the S1 moiety of myosin (S1) to ADP is important for muscle contraction. Cross-bridge cycling involves substantial change in the shape of the myosin head as the S1 moiety of myosin molecule interacts with ATP. Such change is believed to occur during the transition of S1 into the S1** · ADP · P_i state[17]. The ^{31}P NMR study of the complexing of S1 with ADP and with PP_i indicates that PP_i binds to the same site of S1 as the phosphate of ADP[18]. Komatou et al.[19] showed that the conformations of S1 in S1 · PP_i and S1 · ADP are different. Therefore PP_i affects the cross-bridge motion, which indicates that S1-ADP binding is important for muscle contraction.

2) PP_i inducing decay of tension and stiffness of rigor muscle is not due to a change in the mechanical properties of cross-bridges. Rigor develops in muscle depleted of ATP. It is believed that the cross-bridges bound to thin filaments can no longer dissociate from actin. PP_i added to muscle in rigor causes an irreversible decay of tension. The possible mechanisms underlying this are: a) cross-bridges become detached from actin molecules but reattach in positions of lower strain[20, 21]; b) the mechanical property of the cross-bridges changes. The increase of $\bar{\Gamma}$ values of isolated thick filament in an ATP-free solution is believed to be due to the thermal motions of the cross-bridge around the S-2-myosin back bone. If the mechanical properties of the cross-bridge are changed, then the $\bar{\Gamma}$ values will also be changed. The fact that PP_i does not affect the $\bar{\Gamma}$ values of thick filaments suspended in an ATP-free solution indicates that the mechanical properties of the cross-bridge are not changed.

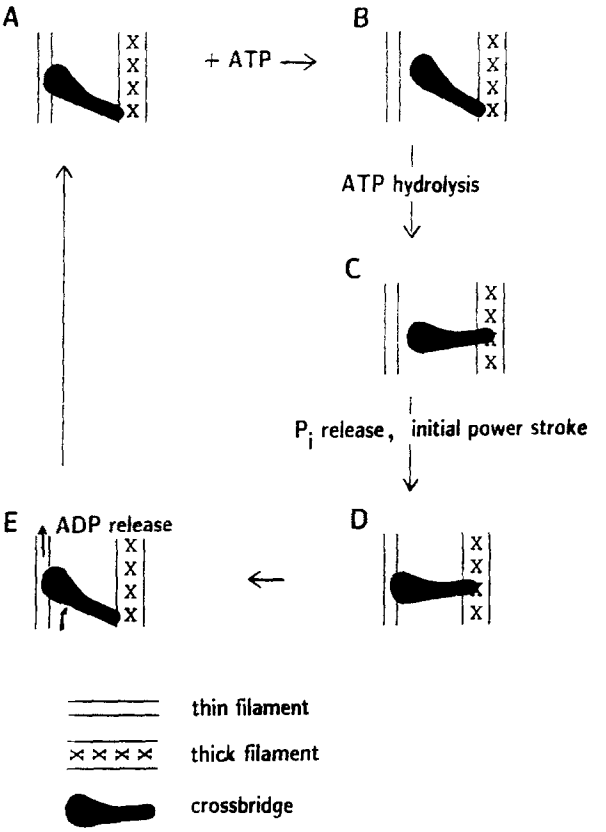

Figure 1. Conceptual diagram of cross bridge cycling and ATP hydrolysis[1]. *A* with ATP, the cross-bridge is detached from the thin filament. *B-D* as ATP is hyrolyzed, the cross-bridge attaches to the thin filament and *E* pulls the thin filament.

Activation of the contractile apparatus of striated muscle by Ba^{2+} and Sr^{2+} has been studied to obtain more information about the mechanism of force generation[22, 23]. In isolated thick myofilaments from *Limulus* striated muscle, the effect of Sr^{2+} had been tested by substituting the Ca^{2+} in the activating solution by an equimolar concentration of Sr^{2+} or Ba^{2+} (fig. 3). While Sr^{2+} was somewhat less effective, Ba^{2+} was almost entirely ineffective in increasing the $\bar{\Gamma}$ values. These

Table. Effect of pyrophosphate (PP_i) on the values of *Limulus* thick filaments suspended in low ATP relaxing solution[1] at a scattering angle of 120°.*

ATP-concentration (μM)	n	Value (mean ± SD)	
		PP_i-free low ATP-relaxing solution	Low ATP-relaxing solution with 2 mM PP_i
0	7	1.75 ± 0.23	1.74 ± 0.25
1	6	1.55 ± 0.21	1.56 ± 0.20

[1]100 mM KCl, 5 mM $MgCl_2$, 5 mM Tris, 2 mM EGTA, pH 7.2.
*The value of thick myofilaments suspended in relaxing solution containing 2 mM ATP was taken as 1.

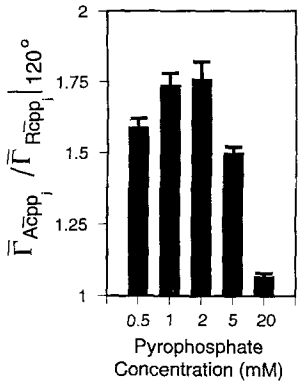

Figure 2. Concentration-response curve of pyrophosphate. Ordinate is the ratio of $\bar{\Gamma}$ values of thick myofilaments suspended in activating solution with 2 mM pyrophosphate to that in relaxing solution with 2 mM pyrophosphate (mean ± SD, n = 8). The ratio obtained with pyrophosphate-free solutions was 1.62 ± 0.04. The scattering angle was 120°. Experimental temperature was 25 °C.

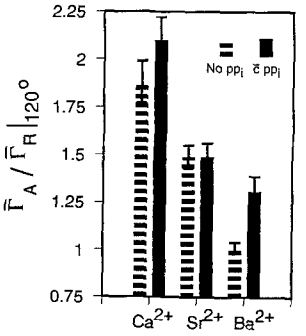

Figure 3. Effect of 2 mM pyrophosphate on the $\bar{\Gamma}$ values of isolated thick myofilaments suspended in activating solution and in activating solutions with Ca^{2+} substituted by equimolar Sr^{2+} or Ba^{2+} (mean ± SD, n = 6). Scattering angle was 120°. Experimental temperature was 25 °C.

results are consistent with those reported by Saito et al.[23]. They found that in skinned cultured chick myotube Sr^{2+} is somewhat less effective and Ba^{2+} is much less effective than Ca^{2+} in inducing tension development. The effect of PP_i is different in the cases of the two ions. It had no effect on the increase of $\bar{\Gamma}$ values by Sr^{2+}, yet substantially increased the $\bar{\Gamma}$ values of filaments suspended in Ba^{2+}-substituted activating solution. That PP_i does not enhance the increase of $\bar{\Gamma}$ values by Sr^{2+} indicates that Sr^{2+} may affect the cross-bridge motions by a different mechanism from Ca^{2+}.

Ba^{2+} is supposed to have dual effects on muscle contraction. It can bind to TnC, which, in turn initiates contraction. On the other hand, it also exerts an 'inhibitory' effect on contraction[19]. The fact that Ba^{2+} is far less effective than Ca^{2+} in initiating the cyclic motions of cross-bridges might be the basis of its 'inhibitory' effect.

In conclusion, the results obtained show that PP_i affects the active cross-bridge cycling but not the mechanical properties of the cross-bridges in isolated thick filaments from the *Limulus* muscle.

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1 Raymetn, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes, K. C., and Milligan, R. A., Science *261* (1993) 58.
2 Greene, L. E., and Eisenberg, E., Proc. natl Acad. Sci. USA *77* (1980) 2616.
3 Brenner, B., Yu, L. C., Greene, L. E., Eisenberg, E., and Schoenberg, M., Biophys. J. *50* (1986) 1101.
4 Schoenberg, M., Biophys. J. *60* (1991) 690.
5 Makinose, M., and Nagai, Y., Jap. J. Physiol. *5* (1955) 49.
6 Fan, S. F., and Li, A. Y., Acta physiol. sin. *26* (1963) 28.
7 Lehman, W., and Szent-Gyorgyi, A. G., J. gen. Physiol. *66* (1975) 1.
8 Newman, J., and Carlson, F. D., Biophys. J. *29* (1980) 37.
9 Haskell, R. C., and Carlson, F. D., Biophys. J. *33* (1981) 39.
10 Suzuki, N., and Wada, A., Biochim. biophys. Acta *670* (1981) 408.
11 Maeda, T., and Fujime, S., Macromolecules *14* (1981) 809.
12 Mochizuki-Oda, N., and Fujime, S., Biopolymers *27* (1988) 1389.
13 Fan, S.-F., Dewey, M. M., Colflesh, D., and Chu, D., Biophys. J. *52* (1987) 859.
14 Fan, S.-F., Dewey, M. M., Colflesh, D., Gaylinn, B., Greguski, R. A., and Chu, B., Biophys. J. *47* (1985) 809.
15 Kubota, K., Chu, B., Fan, S.-F., Dewey, M. M., Brink, P., and Colflesh, D. E., J. molec. Biol. *166* (1983) 329.
16 Maeda, T., and Fujime, S., Macromolecules *17* (1984) 2381.
17 Wakabayashi, K., Tokanaga, M., Kohno, I., Sugimoto, Y., Hawanaka, T., Takezawa, Y., Wakabayashi, T., and Amemiga, Y., Science *238* (1992) 443.
18 Tanokura, M., and Ebashi, S., J. Biochem., Tokyo *113* (1993) 19.
19 Komatsu, H., Emoto, Y., and Tawada, K., J. biol. Chem. *268* (1993) 7799.
20 Schoenberg, M., and Eisenberg, E., Biophys. J. *48* (1985) 863.
21 Arhenden, H., and Arner, A., Biophys. J. *61* (1992) 1480.
22 Grundeman, R. L., de Beer, E. L., van den Berg, C., van Buren, K. J. and Schiereck, P., Am. J. Physiol. *260* (1991) C609.
23 Saito, K., Yoshida, M., and Tanaka, H., J. cell. comp. Physiol. *150* (1991) 45.