Activation dependence and kinetics of force and stiffness inhibition by aluminiofluoride, a slowly dissociating analogue of inorganic phosphate, in chemically skinned fibres from rabbit psoas muscle

P. BRYANT CHASE*, DONALD A. MARTYN† and JAMES D. HANNON‡

1Department of Radiology, SB-05, University of Washington, Seattle, WA 98195, USA
2Center for Biomedical Engineering, University of Washington, Seattle, WA 98195, USA
3Department of Anesthesiology, Mayo Clinic, Rochester, MN 55905, USA

Received 16 July 1993; revised 19 October 1993; accepted 22 October 1993

Summary

To examine the mechanism by which aluminiofluoride, a tightly binding analogue of inorganic phosphate, inhibits force in single, chemically skinned fibres from rabbit psoas muscle, we measured the Ca\(^{2+}\)-dependence of the kinetics of inhibitor dissociation and the kinetics of actomyosin interactions when aluminiofluoride was bound to the crossbridges. The relation between stiffness and the speed of stretch during small amplitude ramp stretches (< 5 nm per h.s.) was used to characterize the kinetic properties of crossbridges attached to actin; sarcomere length was assessed with HeNe laser diffraction. During maximum Ca\(^{2+}\)-activation at physiological ionic strength (pCa 4.0, 0.2 M \(\text{r/2}\)), stiffness exhibited a steep dependence on the rate of stretch; aluminiofluoride inhibition at pCa 4.0 (0.2 M \(\text{r/2}\)) resulted in an overall decrease in stiffness, with stiffness at high rates of stretch (10\(^{3}\)-10\(^{4}\) nm per h.s. per s) being disproportionately reduced. Thus the slope of the stiffness-speed relation was reduced during aluminiofluoride inhibition of activated fibres. Relaxation of inhibited fibres (pCa 9.2, 0.2 M \(\text{r/2}\)) resulted in aluminiofluoride being 'trapped and was accompanied by a further decrease in stiffness at all rates of stretch which was comparable to that found in control relaxed fibres. In relaxed, low ionic strength conditions (pCa 9.2, 0.02 M \(\text{r/2}\)) which promote weak crossbridge binding, stiffness at all rates of stretch was significantly inhibited by aluminiofluoride 'trapped in the fibre. To determine the Ca\(^{2+}\)-dependence of inhibitor dissociation, force was regulated independent of Ca\(^{2+}\) using an activating troponin C (aTnC). Results obtained with aTnC-activated fibres confirmed that there is no absolute requirement for Ca\(^{2+}\) for recovery from force inhibition by inorganic phosphate analogues in skinned fibres; the only requirement is thin filament activation which enables active crossbridge cycling. These results indicate that aluminiofluoride preferentially inhibits rapid equilibrium or weak crossbridge attachment to actin prior to inorganic phosphate analogue dissociation is the primary event regulated by Ca\(^{2+}\).

Introduction

Inorganic phosphate (Pi) release during the actomyosin chemomechanical cycle is thought to be closely associated with force generation in muscle (Hibberd et al., 1985; Hibberd & Trentham, 1986). Consistent with this argument is the observation that elevated [Pi] in skinned skeletal muscle fibres depresses force (Cooke & Pate, 1985; Hibberd et al., 1985; Bowater & Sleep, 1988; Pate & Cooke, 1989; Millar & Homsher, 1990; Martyn & Gordon, 1992; Walker et al., 1992). In addition, elevated Pi suppresses stiffness but to a lesser extent than force, suggesting that the Pi-bound crossbridge generates little or no force when attached to actin (Hibberd et al., 1985; Martyn & Gordon, 1992). Tightly binding analogues of Pi also inhibit force to a greater extent than stiffness, and thus when bound to myosin, may provide a crossbridge intermediate which is analogous to the Pi-bound state occurring early in the crossbridge cycle (Chase et al., 1993). Such analogues may be particularly useful for investigations of the initial steps in the actomyosin interaction leading to force generation since dissociation of the analogue from myosin apparently occurs on a time scale which is orders of magnitude slower than Pi
As has been found for Pi, the dissociation rate of tightly-binding Pi analogues from crossbridges is greatly accelerated by actin (Werber et al., 1992; Phan et al., 1993), and thus shows an apparent dependence on Ca\(^{2+}\) in muscle fibres (Chase & Kushmerick, 1990; Chase et al., 1993). In the absence of Ca\(^{2+}\) (or crossbridge cycling) and at physiological [MgATP], the dissociation of aluminium-fluoride (AlF) or orthovanadate (Vi) from myosin in fibres is sufficiently slow (t\(_{1/2}\) > 15 min) as to appear to be 'trapped' (Dantzig & Goldman, 1985; Chase & Kushmerick, 1990; Chase et al., 1993), although during maximum Ca\(^{2+}\)-activation analogue dissociation became faster and t\(_{1/2}\) decreased to 2–4 min for AlF or <1 min for Vi (Chase & Kushmerick, 1990; Chase et al., 1993). There does not appear to be an absolute requirement for Ca\(^{2+}\), since activation by rigor crossbridges at low [MgATP] increased the Vi dissociation rate in fibres without addition of Ca\(^{2+}\) (Dantzig & Goldman, 1985). However, a modulatory role for Ca\(^{2+}\) on Pi release kinetics and force generation under more native conditions has been indicated by other studies (Chalovich et al., 1981; Brenner et al., 1982; El-Saleh et al., 1986; Brenner, 1988; Walker et al., 1992). Furthermore, Ca\(^{2+}\) may modulate Pi release kinetics and thus the steady state level of force either by binding to troponin C (1nC) alone or to additional sites such as myosin light chain 2 (MLC,) (Metzger & Moss, 1991, 1992). To address this latter possibility, we examined inhibition and recovery of inhibition of force at pCa 9.2 utilizing an activating TnC (aTnC), a structurally modified form of cardiac TnC (cTnC) which, when substituted for endogenous skeletal TnC (sTnC) in skinned skeletal muscle fibres, enables force generation and shortening independent of [Ca\(^{2+}\)] (Hannon et al., 1993; Putkey et al., 1993).

In our previous work (Chase et al., 1993), we were unable to determine whether the residual crossbridge attachments in activated fibres inhibited with AlF are in fact due to crossbridges with inhibitor bound or to normally cycling, inhibitor-free crossbridges. For example, a shift in the population of crossbridges by AlF to weakly attached and/or detached states would be consistent with the observed inhibition of both force and stiffness. Such weakly binding crossbridges do not produce force and have been characterized by their low affinity for actin and rapid equilibrium between attachment and detachment (Brenner et al., 1982; Brenner et al., 1986; Schoenberg, 1988). On the other hand, it is also possible that crossbridges with inhibitor bound could be in a more strongly attached state which produces little or no force. Therefore we have characterized in greater detail the mechanical kinetics of actomyosin interactions in the presence and absence of AlF to test the hypothesis that AlF specifically inhibits weak crossbridge binding to actin. The nature of the initial binding of myosin to actin is particularly important to determine because it has been shown that an actin-binding fragment of caldesmon inhibits both weak crossbridge binding and force in parallel, suggesting that weak binding is an obligatory step in the pathway to force generation (Brenner et al., 1991).

To test for changes in the kinetics of actomyosin interactions in skinned fibres (containing native sTnC), we measured the dependence of stiffness during small amplitude stretches on the rate of stretch (Brenner et al., 1986) which is thought to provide information about the predominant detachment rate constant(s) (Schoenberg, 1985). Compared to maximally Ca\(^{2+}\)-activated controls, we found that AlF at pCa 4.0 preferentially inhibited stiffness at the highest speeds of stretch examined (10\(^{5}\)–10\(^{6}\) nm per h.s. per s), indicating that the crossbridges bound to actin during AlF inhibition are not in a rapid equilibrium and thus are not in a weakly attached state. Stiffness of AlF-bound crossbridges was found to be Ca\(^{2+}\)-sensitive. Furthermore, weak, rapid equilibrium crossbridge attachment in low ionic strength solutions was significantly inhibited by AlF 'trapped' in fibres. To test whether the Pi analogue release step has an absolute requirement for Ca\(^{2+}\), we examined recovery of force from inhibition in the absence of Ca\(^{2+}\) using aTnC-activated fibres. No absolute Ca\(^{2+}\) requirement was observed for recovery from either AlF or Vi. Thus, Pi analogue release appears to depend primarily on thin filament activation and crossbridge cycling. Taken together these results indicate that Ca\(^{2+}\) regulates attachment of AlF-bound crossbridges by modulation of strong myosin crossbridge interactions with actin.

**Materials and methods**

**Experimental preparation and data acquisition**

Single fibre segments were prepared from glycerinated rabbit psoas muscle as previously described (Chase & Kushmerick, 1986; Chase et al., 1993). The ends of the fibre segments were treated by local application of glutaraldehyde to reduce their compliance (Chase & Kushmerick, 1988), and attached to the mechanical apparatus with local application of aluminium foil 'T'-clips. The length of the unfixed portion of the fibre segments (FL), determined as described elsewhere (Chase & Kushmerick, 1988), was 1.47 ± 0.10 mm at an initial relaxed sarcomere length (SL) of 2.54 ± 0.04 μm (mean ± SD, n = 17), fibre diameter was 72 ± 11 μm (mean ± SD, n = 17). Force, FL and SL were measured as described (Chase et al., 1993). SL was continuously monitored using HeNe laser illumination of the fibre. The position of the resulting first order diffraction maximum was measured as described (Chase & Kushmerick, 1988), and attached to the mechanical apparatus with local application of aluminium foil 'T'-clips. The length of the unfixed portion of the fibre segments (FL), determined as described elsewhere (Chase & Kushmerick, 1988), was 1.47 ± 0.10 mm at an initial relaxed sarcomere length (SL) of 2.54 ± 0.04 μm (mean ± SD, n = 17), fibre diameter was 72 ± 11 μm (mean ± SD, n = 17). Force, FL and SL were measured as described (Chase et al., 1993). SL was continuously monitored using HeNe laser illumination of the fibre. The position of the resulting first order diffraction maximum was obtained from a wide bandwidth Schottky photodetector (model PIN-LSC5D, United Detector Technologies, Hawthorne, CA, USA) as described (Chase et al., 1993). To maintain sarcomere homogeneity throughout long periods of activation necessary for this study, fibres were shortened at near maximal velocity and restretched to their original length every 5 s (Brenner, 1983; Chase & Kushmerick, 1988; Chase et al., 1993).