Letters to the Editor

Reversible Inactivation of Myosin Subfragment-1 Activity by Mechanical Immobilization: A Reappraisal

A few years ago we reported that, at ionic strength and protein osmotic pressure comparable to those in vivo, myosin subfragment-1 (S1) is influenced both by the ionic environment and by the presence of MgADP (Grazi et al., 1995). Thus we found the recent report by Highsmith et al. (1998) most interesting, that poly(ethyleneglycol) 3000 (PEG 3000) inhibits S1 MgATPase, suggesting macromolecular osmotic pressure as a playmaker in muscle contraction. We are skeptical, however, of their conclusion that those data provide evidence for "an ATP hydrolysis mechanism in which S1 segmental motion is coupled to its enzymatic activity," because the authors overlook two crucial features of their experimental system:

1. PEGs coordinate Mg^{2+} and buffer its concentration.

2. The addition of KCl influences per se the MgATPase activity of S1.

We tested the effect of PEGs of three different sizes: 2000, 6000. and 40,000 Da, on the MgATPase activity of S1 from rabbit muscle, prepared according to the merthod of Weeds and Taylor (1975). Inhibition was detected only beyond 10% (w/v) PEG. With 25% PEGs 2000 and 6000, the MgATPase activity decreases to 11% of that found in the absence of PEG. With 15% PEG 40,000 MgATPase activity decreases to 61%. These results confirm the data of Highsmith et al. (1998) on MgATPase inhibition by PEG 3000.

PEGs   (HO-CH_2-CH_2-[O-CH_2-CH_2]$_n$-O-CH_2-CH_2-OH) are composed of repeating units of mass 44 Da that contain an etheric oxygen. Thus, in a 25% (w/v) PEG solution, the concentration of the etheric oxygen is 5.68 M, i.e., within an order of magnitude of the water oxygen concentration. Poly(ethyleneoxide)s, in the crystalline state, form helices. Inside these helices ions such as K^+ and NH_4^+ are coordinated by the etheric oxygens, which are 2.85 Å from each other (Lightfoot et al., 1994). Based on these premises, it is expected that the etheric oxygens of PEGs may compete with the oxygen atoms of water for magnesium ions. The expectation was confirmed by titrating the free magnesium ion at 360 nm with 8-hydroxyquinoline (Burton, 1959). It was found that the addition of PEG (2000, 6000, or 40,000) to a solution containing 1 mM ATP and 2 mM $MgCl_2$ decreased free Mg^{2+} concentration, the PEG-Mg^{2+} association constant being 0.648 M^{-1}. The calculation was performed on the basis of the molecular mass of the polymeric unit, 44 Da, so a 25% (w/v) solution of PEG was assigned a concentration of 5.68 M.

The withdrawal of Mg^{2+} by PEGs decreases the concentration of MgATP, the substrate of MgATPase, and thereby decreases the MgATPase activity. To quantitate this effect,

in separate experiments, MgATPase activity of S1 was determined in the presence of 1 mM ATP and decreasing concentrations of total Mg^{2+}. As shown in Fig. 1, MgATPase activity is reduced to ~70% when total Mg^{2+} is decreased from 2 to 1 mM and declines even more steeply below 1 mM total Mg^{2+}.

As observed by Highsmith et al. (1998), in 0.5 M KCl no inhibition by PEGs is observed (Fig. 1), but we disagree with their interpretation. In the absence of KCl the ATPase activity decreases to zero with decreasing total $MgCl_2$ concentration but, in the presence of 0.5 M KCl, the ATPase activity is maintained in the range of 80–100% of the activity found at 2 mM $MgCl_2$. Thus the effect of KCl is unrelated to PEGs or aggregation of S1.

The MgATPase activities recorded either in the presence of PEGs or with decreasing concentrations of total Mg^{2+} were then compared by plotting the activities as a function of the MgATP concentration. For the experiment of Fig. 1 the concentrations of MgATP were calculated from the known total Mg^{2+} and ATP concentrations and the equation

$$a_t = a_m + \frac{a_m \times (1 + K_h \times H^+)}{K_{Mg} \times (m_t - a_m)},$$

where (a_t), (m_t), and (a_m) are total ATP, total Mg^{2+}, and MgATP concentrations, and with the following association by constants: $K_h = 8.9 \times 10^6 \ M^{-1}$ for the reaction $ATP^{4-} + H^+ = HATP^{3-}$ and $K_{Mg} = 10^4 \ M^{-1}$ for the reaction $Mg^{2+} + ATP^{4-} = MgATP^{2-}$ (Alberty, 1968). For the experiments performed in the presence of PEG the concentration of MgATP was calculated from the known, total ATP concentration, the free Mg^{2+} concentrations of Fig. 1, and the following equation:

$$a_t = a_m + \frac{a_m \times (1 + K_h \times H^+)}{K_{Mg} \times m},$$

where (m) is the free Mg^{2+} concentration. The calculated MgATP concentrations were then plotted with the corresponding MgATPase activities (Fig. 2). Inspection of the figure reveals that 1) with PEG 40000 (lowest figure), withdrawal of Mg^{2+} by the polymer accounts fully for the MgATPase inhibition; 2) with PEG 2000 and 6000, withdrawal of Mg^{2+} by the polymer accounts for >50% (lowest figure) and ~20% (middle figure) of the MgATPase inhibition respectively. Interestingly, increasing total Mg^{2+} from 2 to 6 mM led to substantial recovery of the ATPase activity: full recovery with 15% PEG 2000, from 45% to 60% with 15% PEG 6000, from 61% to 90% with 15% PEG 40,000.

In conclusion, we confirm the report of Highsmith et al. (1998) that PEGs reversibly inhibit MgATPase activity of S1 and that the inhibition is not apparent in the presence of 0.5 M KCl. We disagree, however, on the mechanism of this
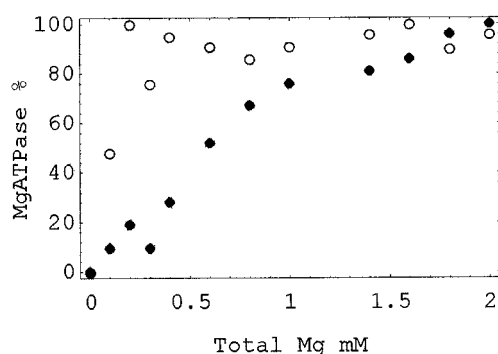
FIGURE 1 Effect of $MgCl_2$ concentration, in the presence and in the absence of 0.5 M KCl, on the ATPase activity of S1. The incubation mixtures (1 ml) contained 1 μM S1, 20 mM imidazole-HCl buffer, 1 mM ATP, and $MgCl_2$, as indicated in the figure. Two series of the incubation mixtures were prepared, the first series without KCl, the second series with 0.5 M KCl. The pH was 7.0. The temperature was 22°C. At time intervals aliquots of the incubations mixtures were taken, quenched by the addition of an equal volume of 3% trichloroacetic acid, and centrifuged, and the supernatant solution was assayed for inorganic orthophosphate according to the method of Tashima and Yoshimura (1975). One hundred percent activity corresponds to an ATPase rate of 0.07 s^{-1}. ●, Without KCl; ○, with KCl.

phenomenon. A substantial fraction of the MgATPase inhibition is due to the decrease in free Mg^{2+} concentration in the presence of PEGs. Our result that inhibition by PEG 6000 is significantly larger than the inhibition by PEG 2000 does not support the "immobilization-inhibition" mechanism proposed by Highsmith et al. (1998), because the osmotic behaviors of PEG 2000 and 6000 are very similar (see http//aqueous.labs.brocku.ca/osfile.html).

We also disagree with the proposal that reversal of the ATPase inhibition by KCl is due to disaggregation of myosin S1. Restoration of the ATPase activity by KCl is independent of the presence of PEGs. Aggregation of S1 is only a side effect of osmotic stress. This is particularly evident when osmotic stress is applied to actin filaments. Association into bundles may take hours because the filaments diffuse slowly, but the physicochemical effects of osmotic stress are apparent very quickly, regardless of the association state (Suzuki et al., 1989).

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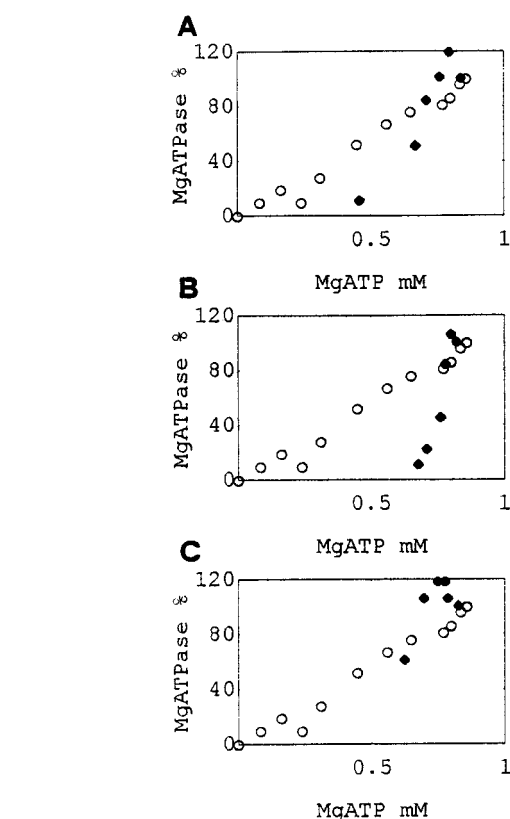
FIGURE 2 MgATPase activity as a function of MgATP concentration. The change of MgATP concentration was obtained either by adding PEGs (●) or by decreasing total Mg^{2+} concentration (data from Fig. 1) (○). One hundred percent activity corresponds to an ATPase rate of 0.07 s^{-1}. (*A*) PEG 2000. (*B*) PEG 6000. (*C*) PEG 40000.

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