Response to Grazi et al.

In the accompanying letter to the editor, Dr. Grazi and his colleagues have suggested an alternative explanation for the interpretation of our recent study of the effects of immobilization on S1. They raise the possibility that the free [Mg^{2+}] is reduced by binding to PEG, which reduces [MgATP], and that this accounts for the inhibition of MgATPase activity when S1 is aggregated by high [PEG]. While it is true that [Mg^{2+}] binds to PEG in solution, a simple calculation demonstrates that the reduction in [MgATP] is not sufficient to account for the observed changes in S1 activity. The association constant for Mg^{2+} binding to PEG reported by Grazi et al. in their letter can be used to calculate an effective association constant, K_{eff}, for Mg^{2+} binding to ATP in the presence of PEG:

$$K_{eff} = K_{MgATP}/(1 + K_{HATP} \times [H+] + K_{MgPEG} \times [PEG]), \quad (1)$$

where K_{MgATP} ($= 1.15 \times 10^4\ M^{-1}$), K_{HATP} ($= 3.2 \times 10^6\ M^{-1}$), and K_{MgPEG} ($= 0.648\ M^{-1}$) are the association constants for Mg^{2+} binding ATP in the absence of PEG, H^+ binding to ATP, and Mg^{2+} binding to PEG, respectively. Using Eq. 1, for 4.5 M PEG (20 wt%) at pH 7, K_{eff} is $2.71 \times 10^3\ M^{-1}$. This calculation is made using the assumption in the letter from Grazi et al. that the stoichiometry of binding is one Mg to each oxygen in PEG, which is undoubtedly an overestimate. Using this estimate of K_{eff} to calculate [MgATP] for 2 mM $MgCl_2$ and 1 mM ATP, one obtains 0.77 mM MgATP, compared to 0.93 mM in the absence of PEG. This shows that although [MgATP] may be reduced because of competition for Mg^{2+} by PEG, the remaining [MgATP] is high enough to saturate the micromolar S1 binding sites. Thus the ATPase inhibition we reported in the presence of PEG is not due to reduced [MgATP].

Direct experimental observations show that inhibition of S1 MgATPase activity is not due to lowered [Mg^{2+}] in the presence of PEG, supporting the calculation given above. We measured the effect of increased $MgCl_2$ on S1 solubility and activity. In the presence of 20 wt% PEG3, 10 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), 1 mM $MgCl_2$, S1 is over 90% aggregated and only 15% active (Table 1). As reported earlier (see Fig. 3 in Highsmith et al., 1998), for these conditions S1 should be sensitive to changes in [Mg^{2+}] if it is relevant. When the [$MgCl_2$] is increased from 1 mM to 2 mM or 10 mM, S1 aggregation and activity are unchanged (Table 1). These data show unequivocally that our observed inhibition of MgATPase activity in the presence of high [PEG] is not due to reduced [Mg^{2+}].

TABLE 1 PEG and Mg^{2+} dependence of S1 solubility and activity

PEG (wt%)	$MgCl_2$ (mM)	Soluble fraction (%)	MgATPase activity (s^{-1})
0	1	100	0.046
20	1	5.4	0.0073
0	2	100	0.044
20	2	4.7	0.0076
0	10	100	0.046
20	10	5.1	0.0068

Crystal structures of S1 · $MgPP_i$ obtained in the presence of 2 mM $MgCl_2$, 2 mM PP_i, and 8.5 wt% PEG8 again show that PEG is not a strong binder of Mg^{2+} (Smith and Rayment, 1995). Magnesium is bound at the active site despite the almost 10-fold lower affinity of Mg^{2+} for PP_i than for ATP.

In addition, PEG was only one of the methods that we used to immobilize S1. The MgATPase activity of S1 was also inhibited by immobilization because of adsorption on the surface of hydroxyapatite beads. We observed an excellent correlation between S1 solubility and activity for all of the conditions that we used.

In conclusion, we find the experimental observations reported by Dr. Grazi and his colleagues interesting. We hope that they would agree that there is a great deal more to be learned about both the effects of PEG and other osmolytes on protein structure and function, and about the effects of mechanical restraint on myosin and other mechanochemical enzymes. However, we do not think that the data in their letter bear directly on our experiments or on our interpretation: immobilization of S1 by a variety of conditions leads to an inhibition of its ATPase activity.

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Received for publication 9 February 1999 and in final form 10 February 1999.

0006-3495/99/06/3351/01 $2.00