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DIFFERENTIAL BEHAVIOR OF HEAVY MEROMYOSIN AND HEAVY MEROMYOSIN SUBFRAGMENT 1 TOWARD A MONOMERIC ACTIN DERIVATIVE

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Actin and myosin in muscle act together to catalyse the hydrolysis of ATP and to couple the chemical energy released in this process to a mechanical event, contraction. In vitro, the Mg^{2+}-ATPase activities of myosin and its proteolytic fragments heavy meromyosin (HMM) and HMM subfragment-1 (S1) are stimulated in the presence of F-actin. Like myosin, both HMM (Trinick and Offer, 1979) and S1 (Ando and Scales, 1985), in the absence of ATP, bind tightly to and induce bundle formation by F-actin filaments.

G-actin can be reacted with fluorescein isothiocyanate (FITC) to give a product, FITC-actin, in which Lys-61 is the prime site of modification (Burtnick, 1984). FITC-actin has the circular dichroism spectrum of native G-actin and inhibits the nuclease activity of DNAse I. However, it does not undergo salt-induced polymerization. This report details initial studies on the ability of this nonpolymerizable species of actin to interact with HMM and S1 by direct observation of the emission properties of FITC-actin and by measurement of FITC-acto-HMM and FITC-acto-S1 Mg^{2+}-ATPase activities.

MATERIALS AND METHODS

FITC-actin was prepared as described earlier (Burtnick, 1984). Chymotryptic HMM and S1, prepared according to Weeds and Taylor (1975), were generous gifts of Dr. C. M. Kay and Dr. R. S. Hodges of the University of Alberta.

Acto-S1 and acto-HMM ATPase assays were performed by following either the inorganic phosphate release (Fiske and SubbaRow, 1925) or the proton release (Talbot and Hodges, 1981) tht accompanies ATP hydrolysis.

Fluorescence measurements were performed on a Perkin-Elmer (Norwalk, CT) MPF-44B spectrofluorimeter equipped with a thermostatted cell holder. Excitation was at 494 nm and emission intensities were measured at 517 nm. Excitation and emission bandwidths were both 4 nm.

Before fluorescence or ATPase studies, FITC-actin was dialyzed extensively against 2 mM Tris-HCl, 1 mM dithiothreitol, 0.2 mM ATP, 0.2 mM $CaCl_2$, pH 7.6 (henceforth called Buffer A) and clarified by centrifugation at 80,000 × *g* for 20 min. Concentrations of FITC-actin solutions were determined by amino acid analysis of duplicate samples that had been hydrolyzed for 20 h at 110°C in tubes that had been sealed under vacuum.

RESULTS

Addition of HMM to solutions of FITC-actin in solutions containing 100 mM KCl, 10 mM Tris-HCl, pH 8.0 resulted in quenching of the emission intensities of the samples to ~80% of their original levels (Fig. 1). The effect began to level off when the mole ratio of HMM:FITC-actin reached ~1.0; that is, when the mole ratio of myosin heads:actin units was ~2.0. When the ionic strength of the sample was reduced, the apparent lag in response of the fluorescence intensity to added HMM became less pronounced. As yet, a satisfactory explanation for this observation is lacking.

In contrast to the quenching of FITC-actin fluorescence that resulted in the presence of HMM, addition of S1 actually enhanced the observed emission intensities (Fig. 2). The enhancement reached 20–25% at S1 to FITC-actin mole ratios near seven. At higher mole ratios, the samples displayed a slight turbidity and the measured fluorescence intensities, as a result, began to decline. The single-headed S1 had an effect on the fluorescence of FITC-actin that required considerably more protein than did that of the double-headed HMM, suggesting a relatively weaker binding affinity of FITC-actin for S1 than for HMM.

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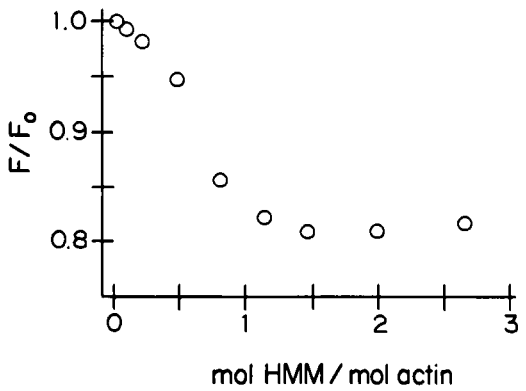

FIGURE 1 Identical samples of 0.648 nmol FITC-actin in 20 μl Buffer A were diluted to 2.00 ml with 100 mM KCl, 10 mM Tris-HCl, pH 8.0. To one sample, aliquots of HMM in 100 mM KCl, 10 mM Tris-HCl, pH 8.0 were added and the fluorescence intensity (F) at 517 nm recorded. To the other sample, equal volumes of solvent alone were added and the fluorescence intensity (F_0) at 517 nm recorded.

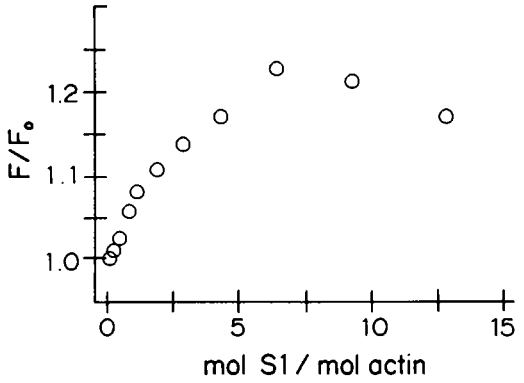

FIGURE 2 Identical samples of 1.22 nmol FITC-actin in 20 μl Buffer A were diluted to 2.00 ml with 100 mM KCl, 10mM Tris-HCl, pH 8.0. To one sample, aliquots of S1 in 100 mM KCl, 10 mM Tris-HCl, pH 8.0 were added and the fluorescence intensity (F) at 517 nm recorded. To the other sample, equal volumes of solvent alone were added and the fluorescence intensity (F_0) at 517 nm recorded.

The distinct difference in the manner of interaction of FITC-actin with HMM and S1 also was apparent in biological activity essays. When compared at identical weight ratios (1:4 and 1:8) of actin to HMM in 33 mM KCl, 25 mM Tris-HCl; 2.5 mM ATP, 2.5 mM $MgCl_2$, and 1 mM EGTA, pH 7.5, FITC-actin was only 20–30% as active as an unlabeled actin preparation in its ability to stimulate HMM Mg^{2+}-ATPase activities. In contrast, at identical weight ratios (1:1 and 1:2) of actin to S1, FITC-actin and an unlabeled actin preparation could not be distinguished in their abilities to stimulate S1 Mg^{2+}-ATPase activities.

DISCUSSION

The fluorescence and biological activity studies demonstrate the ability of FITC-actin to distinguish HMM from S1. The most obvious basis for this ability is the fact that HMM consists of two S1 entities tied together into one double-headed molecule. The presence of a second head on one molecule modulates the ability of HMM to interact with FITC-actin either in a direct way, by providing for additional attachment sites, or by regulating the affinity of the other head in some allosteric manner. More detailed binding studies are required, particularly in the range of HMM-to–actin ratios in which some sigmoidicity is evident in Fig. 1.

The ability of a putative actin monomer to stimulate the Mg^{2+}-ATPase activity of S1 is also worth notice. Several nonpolymeric forms of actin have been identified in nonmuscle cells (Korn, 1982). They may serve roles in such cells more complex than as storage or transport forms of actin.

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