G-ACTIN BINDING QUENCHES INTERNAL MOTIONS IN MYOSIN SUBFRAGMENT-1

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1. Introduction

Chemical energy available from ATP is converted into mechanical energy by an interaction between actin and myosin, the details of which are unknown. Recent high-resolution ^1H NMR measurements indicated that F-actin binding causes a large structural change in myosin subfragment-1 (S1) [1]. In that paper, >20% of the structure of S1 was assigned to NMR peaks too narrow to be modeled by a freely rotately methyl group on a molecule rotating end-over-end at the rate known for S1. To account for the narrow linewidths, additional modes of motion (internal motions involving peptide backbone motion and/or segmental motion) had to be included. The rod portion of myosin and F-actin were immobile in comparison. F-actin binding to S-1 eliminated the narrow peaks in the spectrum and presumably quenched the internal motions. To demonstrate that the observed loss of narrow NMR peaks for S1 was due to an actomyosin interaction rather than a reduction of the rate of rotational Brownian motion, spectra were obtained for S1 with its rate of rotation reduced by means not involving actin [2]. Virtually no loss or broadening of the narrow peaks was observed for actin-free myosin heads slowed to rotational rates approaching that of S1 bound to F-actin, indicating the actin effect was indeed structural.

Another approach, which circumvents the difficulties of slowing free S1 rotation down to rates as slow as that of F-acto–S1, is to obtain spectra for G-acto–S1. The G-actin complex with S1 should rotate less than twice as slowly as free S1, in a range known to have no effect on the NMR spectrum [1,2]. Thus a loss of narrow peak intensity for S1 upon binding G-actin would unambiguously be due to a structural change in S1. There are two potential problems that this strategy provokes. One is losing G-actin to a denatured state because of the low ATP concentrations needed to maximize the binding to S1 [3]. The other is losing G-actin to F-actin, especially by a S1-catalyzed mechanism [4]. Conditions were found where both of these problems were negligible, and G-actin binding to S1 at low ionic strength in the absence of free nucleotide was investigated. These results show that G-actin will bind S1 and alter its NMR spectrum in a way similar but not identical to that due to F-actin. Thus actin binding changes the structure of myosin in a way that quenches the internal motions of the myosin heads, whether actin is in the G-form or the F-form.

Abbreviations: S1, myosin subfragment-1; F-acto–S1, the complex of S1 and F-actin; G-acto–S1, the complex of S1 and G-actin; ATP, adenosine triphosphate; MgPP, magnesium pyrophosphate; NMR, nuclear magnetic resonance

2. Experimental

2.1. *Proteins and chemicals*

Myosin subfragment-1 was prepared from rabbit skeletal muscle as in [5] and used as a mixture of the isozymes containing the alkali-1 and alkali-2 light chains. Actin was prepared by the method in [6]. When G-actin in 2 mM Tris, 0.2 mM ATP, 0.5 mM β-mercaptoethanol (pH 8.0) in ^1H_2O was dialyzed to the same buffer in 99.8% ^2H_2O, the actin polymerized. By reducing the concentration of salts in the ^2H_2O buffer this polymerization could be reduced. Trial and error led to 1 mM phosphate p^{2}H 7.0 at 6°C as the buffer used to dialyze actin to the G-form for the ^2H_2O stock solutions which were diluted for an experiment. S1 and G-actin were dialyzed in the

same dialysate for the final two dialysis changes and the proteins were spun at 100 000 × *g* for 1–3 h to remove any aggregated material before beginning an experiment. Molecular weights of 115 000 and 42 300 and extinction coefficients of 0.77 ml/mg and 1.16 ml/mg at 280 nm were used to calculate the concentrations of S1 and actin, respectively. Stored at 5–6°C, the proteins were stable for 24 h and perhaps longer.

All chemicals were at least reagent grade purity. ^2H_2O was 99.8% ^2H from Biorad.

2.2. *NMR*

The 360 MHz instrument at the Stanford Magnetic Resonance Laboratory and the conditions used to obtain spectra for myosin and actin have been described [1,7]. Measurements were made at 297–298 K. Chemical shifts are in ppm from external tetramethylsilane.

3. Results

G-actin in 1 mM phosphate p^{2}H 7.0 in ^2H_2O was stable at 5–6°C with no added nucleotide for 24 h at least. Solutions at 24–61 μM G-actin had viscosities within a few % of that of the solvent alone. This G-actin polymerized readily. Addition, after as long as 24 h, of KCl and $MgCl_2$ to solutions of G-actin to obtain 50 mM KCl and 5 mM $MgCl_2$ caused polymerization to occur within 1 h such that the solutions were too viscous to flow in the Ostwald viscometer at 24.8°C. When G-actin was polymerized in the NMR tube using solid KCl, with or without $MgCl_2$, the solutions became too viscous to flow in the tube (5 mm diam.) or to allow migration of small air bubbles.

Fig.1 shows spectra for 20 μM G-actin, 20 μM F-actin and the G-actin dialysis buffer. The F-actin spectrum was obtained from the same sample used for the G-actin spectrum, after KCl and $MgCl_2$ were added directly to the NMR tube. The spectrum for G-actin is quite different from that of F-actin, exhibiting increased intensities of broad and intermediate width peaks in the aliphatic and aromatic regions as well as narrow peaks at 0.7, 1.8 and 6.8 ppm and possibly 1.1 ppm. The narrow peak at 1.1 ppm was variable in intensity from one preparation to another and in the buffer; it does not appear to be due to the protein itself. F-actin prepared this way has a ^1H NMR spectrum which is indistinguishable from that prepared in ^1H_2O and dialyzed to ^2H_2O [1]. The virtual absence of peaks at 0.7, 1.8 and 6.8 ppm in the center trace of fig.1 indicates that the G-actin has been fully polymerized by the addition of KCl and $MgCl_2$ under these conditions.

The spectra for 15 μM S1 and for 15 μM S1 plus 20 μM G-actin are shown in the upper two traces of in fig.2. Clearly the center trace in fig.2 is not the sum of the upper traces in fig.1,2. A substantial loss of the intensities of almost all of the narrow peaks has occurred in the spectrum for G-acto-S1. The upper two traces in fig.3 show spectra for concentrations of S1 and S1 plus F-actin identical to those for S1 and S1 plus G-actin shown in fig.2. F-actin has a greater effect on the narrow peaks of S1 and also causes a greater loss of intensity from the envelope of broad and intermediate peaks under the narrow ones. The spectrum shown in the center of fig.3 could be obtained by adding F-actin to S1 or by adding KCl to G-actin and S1 already mixed.

The lower traces in fig.2,3 show spectra for G- and F-acto–S1 complexes plus MgPP. In agreement with earlier results [1], F-acto–S1 was dissociated by MgPP. Somewhat surprisingly, G-acto–S1 was not dissociated by MgPP under conditions where the nucleotide binding site is saturated.

4. Discussion

The crucial question for interpreting the above results is whether or not the solutions of G-actin are contaminated with F-actin. In the absence of S1, it is quite reasonable to conclude that the amount of F-actin is negligible because:

(i) The samples have been spun at 100 000 × *g* for 1 h;
(ii) The solutions have viscosities within a few percent of that of buffer;
(iii) The ^1H NMR spectra is not that of F-actin (or that of denatured actin [1]) but can be converted to that of F-actin by adding KCl, a treatment known to cause polymerization of G-actin.

It is true that the dialysis against ATP-free buffer before the NMR measurements makes the state of the nucleotide unknown–it may be ADP. However, the conclusion that the actin is not in the F-form or denatured is still sound. There may be more than one kind of monomeric actin, depending on the conditions [8,13].

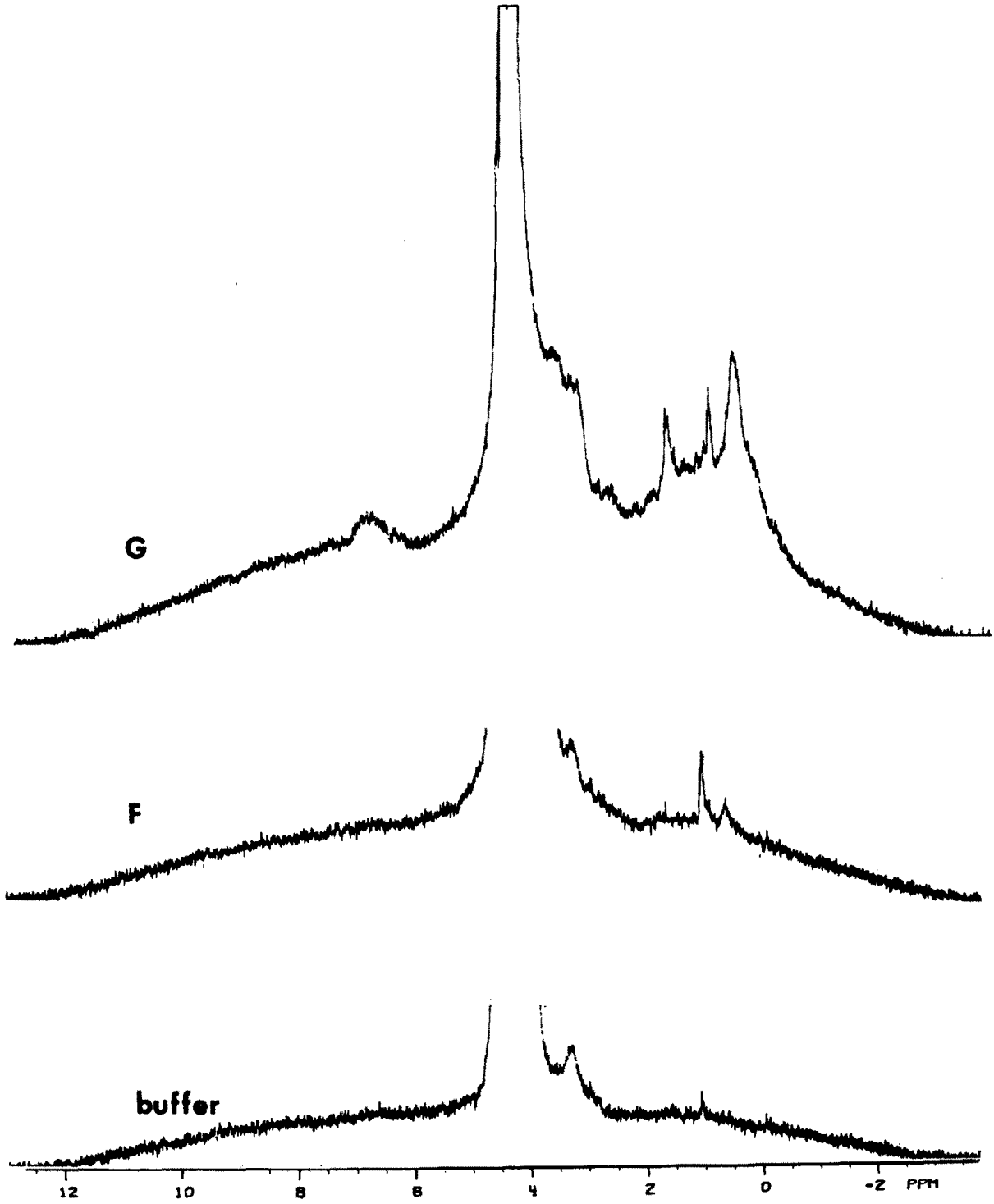

Fig.1. G- and F-actins. The upper trace is 20 μM G-actin in 1 mM phosphate, p²H 7.0 at 23°C in ^2H_2O. The center trace is 20 μM F-actin in 50 mM KCl, 5 mM $MgCl_2$, 1 mM phosphate, p²H 7.0 at 23°C in ^2H_2O. The lower trace is 1 mM phosphate.

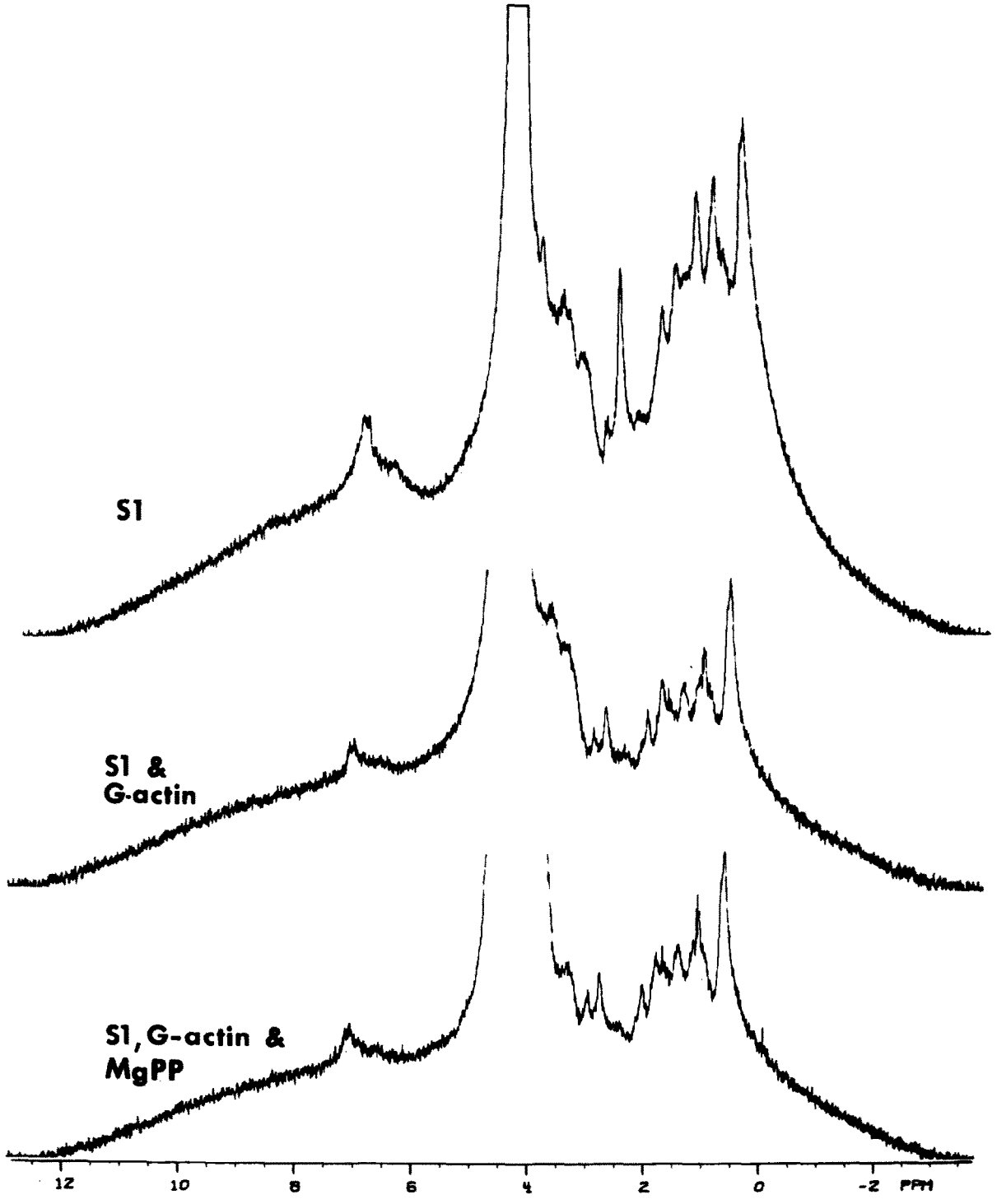

Fig.2. S1, G-actin and MgPP. The upper trace is 20 μM S1. The center trace is 20 μM S1 and 15 μM G-actin. The lower trace is 20 μM S1, 15 μM G-actin, 0.2 mM $MgCl_2$ and 1 mM pyrophosphate. All solutions were in 1 mM phosphate p^{2}H 7.0 at 23°C in ^2H_2O.

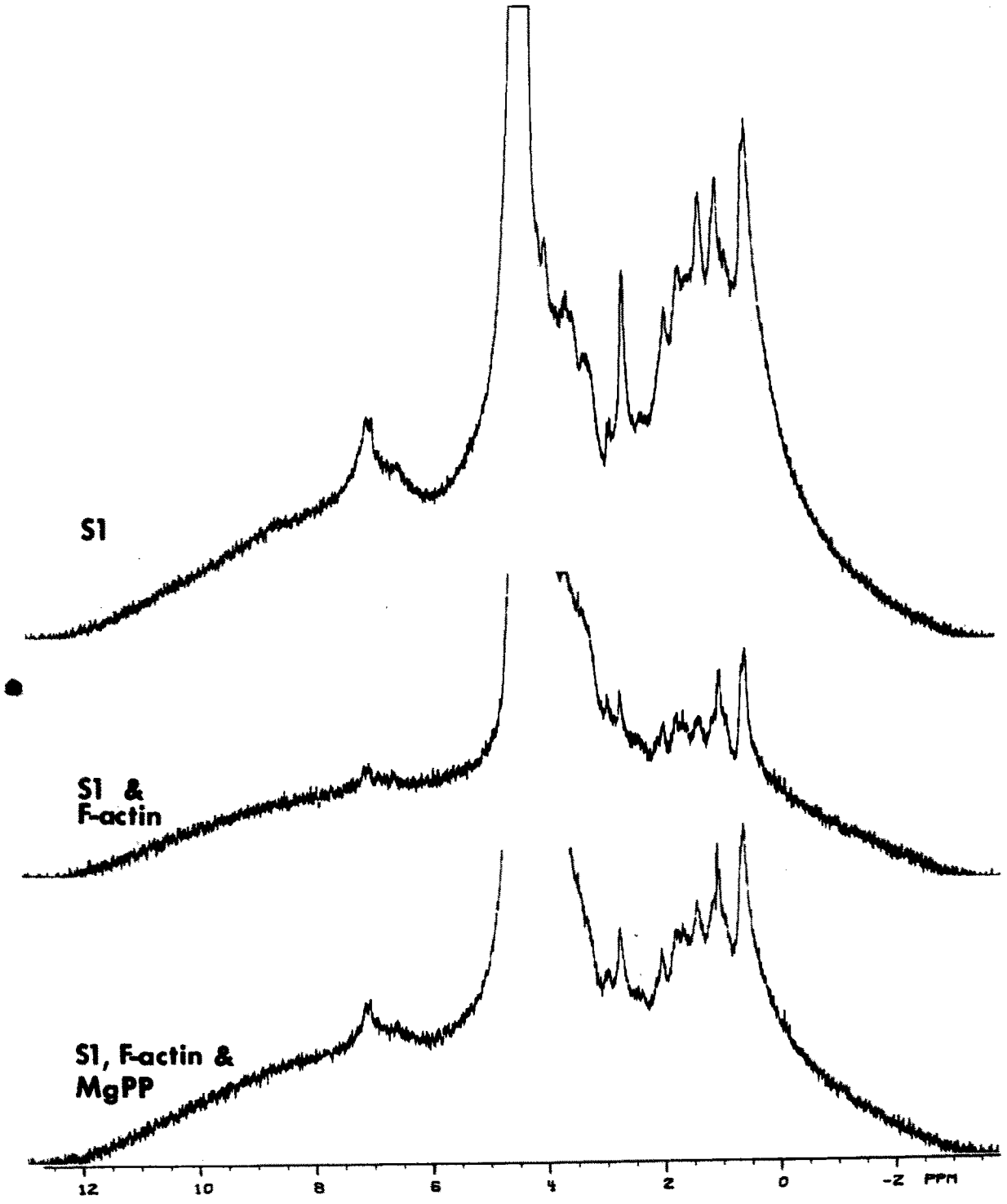

Fig.3. S1, F-actin and $MgPP_i$. The upper trace is 20 μM S1 in 1 mM phosphate. The center trace is 20 μM S1, 15 μM F-actin, 50 mM KCl, 1 mM phosphate. The lower trace is 20 μM S1, 15 μM F-actin, 50 mM KCl, 0.2 mM $MgCl_2$, 1 mM pyrophosphate, 1 mM phosphate. The temperature was 23°C.

Solutions containing S1 require additional scrutiny because there are conflicting reports as to whether or not S1 catalyzes the G- to F-actin transition. Evidence exists indicating that S1 does or does not cause polymerization depending on the conditions and on the protease used to prepare the S1 [4,9,10]. No data exist for the conditions used here; but the following results indicate that S1 does not catalyze polymerization in 1 mM phosphate (p^2H 7.0). The viscosity of the solutions of G-acto-S1 is still low while that of F-acto-S1 was grossly increased. In addition, the G-acto–S1 spectra are different from F-acto–S1 spectra in that the various narrow peaks of S1 are reduced by different degrees with G-acto–S1 rather than uniformly as they are with F-acto–S1. Since G-actin has narrow peaks itself, which may or may not be altered by binding S1, a quantitative comparison of the G-acto–S1 and F-acto–S1 spectra is difficult. However, the effect of G-actin and F-actin on the broad peak in the aliphatic region is clearly different. In addition, since the peak at 2.8 ppm is only due to S1, it is useful in comparing fig.2,3. The 2.8 ppm peak is reduced about equally by binding in both cases, while the broad peak at 0–2.5 is much more affected by F-actin binding because F-acto-S1 is highly immobilized. This differential effect on the peaks along with the large loss of narrow peak intensity rule out the G-acto–S1 spectrum being due to a combination of traces of F-actin which bind S1 and G-actin which does not. Thus the changes in S1 detected by NMR are due to some interaction with G-actin.

The lack of effect due to MgPP shown in the lower trace in fig.2 cannot be explained unambiguously at this time. G-actin could lower the affinity of acto–S1 for MgPP so that higher concentrations are required to form the ternary complex and cause dissociation. Alternatively, the nucleotide site may be saturated but MgPP · S1 may still have a $K_a \geqslant 10^7\ M^{-1}$ for binding G-actin so no S1 is dissociated from actin. The MgPP result may be relevant to contradictory reports on G-actin activation of S1 ATPase activity [8–12].

This report confirms that S1 can bind G-actin [8–11] under some conditions and not cause polymerization [8–10]. This complex may be of interest in itself. More important to the case at hand, it is clear that S1 undergoes a significant structural change when it binds G-actin and this change is similar at least with respect to hydrogen mobility to that caused by binding F-actin. When these results are combined with the earlier results for S1 and F-actin [1,2], they are strong evidence that actin binding causes a significant change in the structure of myosin.

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