Myosin II from rabbit skeletal muscle and *Dictyostelium discoideum* and its interaction with F-actin studied by ^{1}H NMR spectroscopy

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Abstract Mg-F-actin occurs in two conformational states, I and M, where the N-terminal amino acids are either immobile or highly mobile. In the rigor or ADP complex of rabbit myosin S1 with Mg-F-actin the N-terminal acetyl group of actin stays in its highly mobile state. The same is true for the complexes with the myosin motor domain from *Dictyostelium discoideum*. This excludes a direct strong interaction of the N-terminal amino acids with myosin in the rigor state as suggested. An interaction of the N-terminus of F-actin with myosin is also not promoted by occupying its low-affinity binding site(s) with divalent ions. The N-terminal high-mobility region may be part of a structural system which has evolved for releasing inadequate stress applied to the actin filaments © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Nuclear magnetic resonance; Actin; Myosin

1. Introduction

Actin and myosin represent the main components of the contractile system in eukaryotes. Actin filaments (F-actin) can be formed by polymerizing monomeric actin (G-actin) by addition of KCl in moderate concentrations (≥ 30 mM). Actin binds one adenine nucleotide molecule and one divalent metal ion per protomer at its high-affinity binding site. When it contains a Mg^{2+} ion at its high-affinity site (as is expected under in vivo conditions) F-actin occurs in two different conformational states, the M state where the N-terminal amino acids are highly mobile and the I state where they are immobilized [1,2]. The X-ray structure of rabbit G-actin has been determined in complex with DNase I [3], with segment 1 of gelsolin [4], in complex with profilin [5], and uncomplexed in the ADP state [6]. Starting with the X-ray structure of G-actin the atomic structure of F-actin has been solved using X-ray small angle scattering [7,8]. The obtained structure is consistent with most of the extensive biochemical, spectroscopic and electron microscopic data (for a review see [9–11]).

Myosin forms a still expanding family of motor proteins which are able to produce a unidirectional movement of actin filaments under simultaneous hydrolysis of ATP. The crystallization of subfragment S1 from skeletal muscle myosin [12], the myosin motor domain from *Dictyostelium discoideum* [13–15], smooth muscle myosin [16] and scallop S1 [17] with different nucleotides and nucleotide analogs gives detailed structural models which can be related to different functional states. However, some parts of the structure assumed to be mobile or disordered as the N-terminal parts of the light chains are missing in the electron density maps. Myosins of *D. discoideum* have the big advantage that they are easily available to modern techniques of molecular biology.

From the existing structures of F-actin and myosin the structure of the actomyosin complex in different functional states has been modelled on the basis of X-ray small angle scattering or electron microscopic data [18–20].

Nuclear magnetic resonance (NMR) spectroscopy is complementary to X-ray crystallography especially since it shows highly mobile regions of biological macromolecules which are usually not visible in the X-ray crystallography. In the past a large number of NMR studies of the actomyosin system have been published [1,2,21–39]. In rabbit skeletal muscle myosin S1 (A1) the signals of the N-terminal *N*-trimethyl-alanine residue of the essential light chain LC1 can be observed by ^{1}H NMR spectroscopy, which are quenched after binding of myosin S1 to F-actin. This suggests that the N-terminus of LC1 binds to F-actin [32,39–41]. Cross-linking studies support the notion of a direct interaction in the C-terminal region near Glu-361 to Glu-364 [42–44]. Cross-linking indicates that the interaction of LC1 with actin does not involve the same protomer as that of the corresponding heavy chain [45].

2. Materials and methods

2.1. Protein preparation

Actin from rabbit skeletal muscle was prepared from acetone powder as described by Pardee and Spudich [46] modified according to Heintz et al. [2]. Rabbit myosin S1 was prepared according to Weeds and Taylor [47]. Clone M754 encoding amino acids 1–754 of the motor domain of myosin II from *D. discoideum* was used [48]. At its C-terminus it contains the non-myosin sequence LARILGSTRDALHHHHHHHH. It was expressed in *D. discoideum* and purified as described by Manstein and Hunt [48].

2.2. Sample preparation

For ^{1}H NMR experiments the sample was usually dissolved in buffer B (0.2 mM ATP, 0.1 mM $MgCl_2$, 0.2 mM EGTA, 1 mM NaN_3, 2 mM Tris–HCl, pH 7.8, 90% H_2O/D_2O 10%). Polymerization of actin was induced by adding appropriate quantities of a stock solution of 3 M KCl to obtain a final concentration of 30 mM. As internal reference 0.1 mM 3-(trimethylsilyl)-tetradeutero propionic acid (TSP) was added.

2.3. NMR measurements

NMR spectra were measured on Bruker DRX-500, DRX-600 and DMX-800 spectrometers operating at proton frequencies of 500 MHz,

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600 MHz and 800 MHz, respectively. In one-dimensional ^{1}H NMR spectroscopy the water signal was usually suppressed by selective presaturation. Typically, 512 free induction decays were accumulated with a repetition time of 5 s including the water suppression pulse of 1 s.

2.4. NMR data processing and software

Data were processed with the XWINMR package (Bruker) running on a Silicon Graphics Indigo^2 workstation.

3. Results

3.1. Interaction of F-actin with rabbit skeletal muscle myosin

Titration of Mg-F-actin with myosin or myosin S1 should lead to the disappearance of signals of the N-terminal residues of actin if they are involved in a direct interaction with myosin. Fig. 1A shows the results. For establishing a pure rigor state alkaline phosphatase was added which hydrolyzes the nucleotides completely. When the actin concentration is held constant and the myosin concentration is increased, the signal intensity of the *N*-acetyl signal of F-actin remains unchanged. Slightly downfield shifted from the signal of the *N*-acetyl group a new singlet resonance appears at 2.050 ppm when the S1 concentration is increased. This resonance has been assigned earlier to the *N*-acetyl group of myosin light chain LC3 [40] which is also contained in our S1 preparation in low concentration. The myosin LC3 is also not influenced by binding to actin.

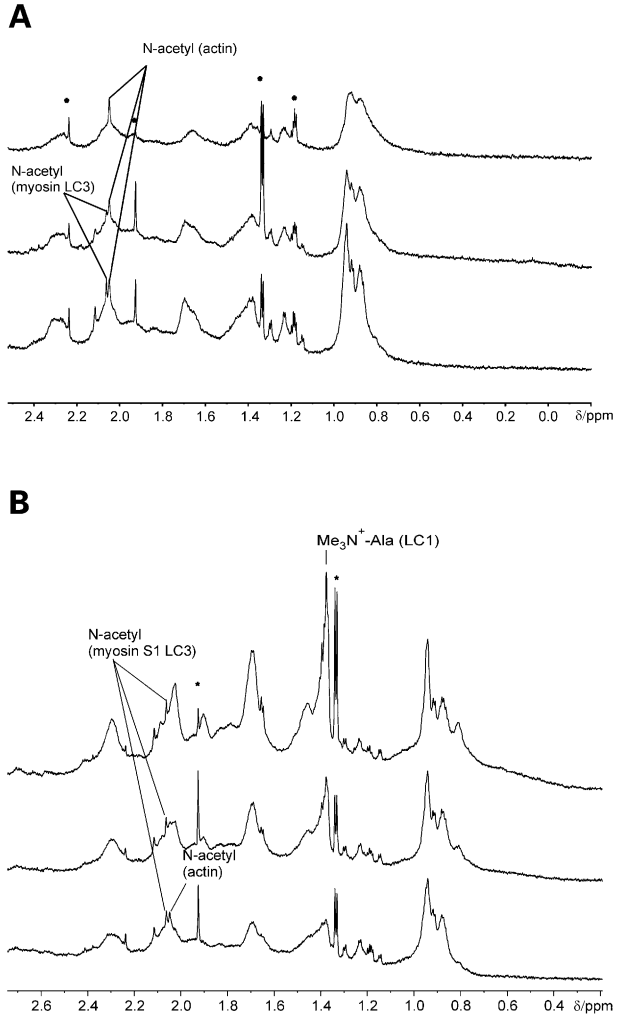

Fig. 1. ^{1}H NMR spectra of rabbit myosin S1 and Mg-F-actin. Spectra were recorded at 800 MHz at 288 K. A: Upper trace: The sample contained 29 μM actin in buffer A consisting of 2 mM Tris–HCl, pH 7.8, 30 mM KCl, 22 μM phalloidin, 1 U/ml alkaline phosphatase, 0.2 mM ATP, 0.1 mM $MgCl_2$, 0.2 mM EGTA, 1 mM NaN_3, in 90% H_2O, 10% D_2O. Alkaline phosphatase was added approximately 10 min after polymerizing actin by addition of KCl. The sample contained in addition 13 μM myosin S1 (middle trace) or 22 μM myosin S1 (lower trace). B: Upper trace: The sample contained 31 μM myosin S1 in buffer A. The sample additionally contained 19 μM actin (middle trace) or 27 μM myosin S1 (lower trace). The star indicates low-molecular-mass impurities, all NMR spectra were recorded under identical experimental conditions.

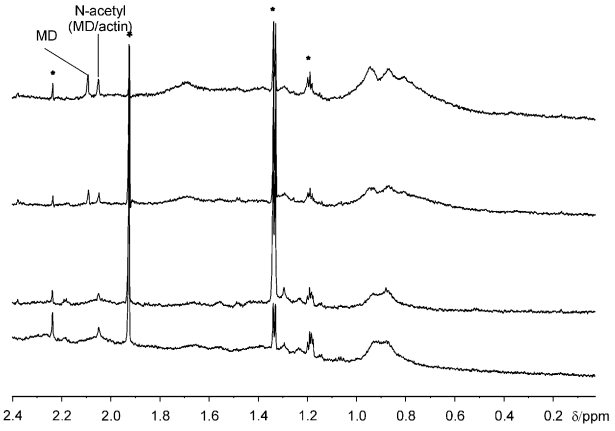

Fig. 2. ^{1}H NMR spectra of the myosin motor domain of *D. discoideum* and Mg-F-actin. Spectra were recorded under identical conditions and were contained in the same buffer as described in Fig. 4 for rabbit myosin. The sample contained 17 μM myosin motor domain of *D. discoideum* and actin in various concentrations; actin concentrations from top to bottom 0 μM actin, 6.3 μM, 25.2 μM, and 21.5 μM. The star indicates low-molecular-mass impurities, all NMR spectra were recorded under identical experimental conditions.

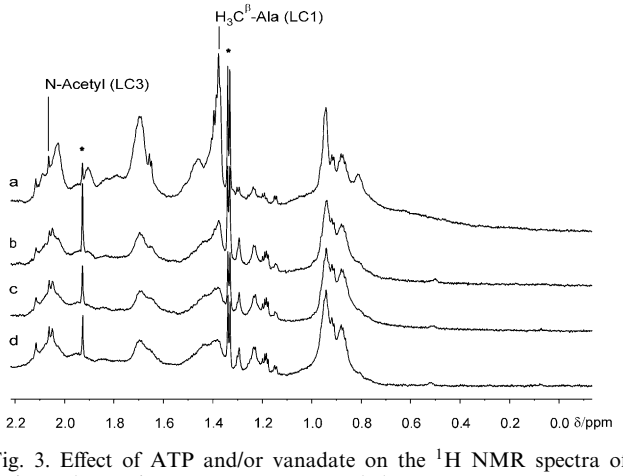

Fig. 3. Effect of ATP and/or vanadate on the ^{1}H NMR spectra of actin and myosin. Spectra were recorded at 800 MHz at 288 K. The samples contained 32 μM rabbit myosin S1 in 2 mM Tris–HCl, pH 7.8, 30 mM KCl, 40 μM phalloidin, 0.1 mM $MgCl_2$, 0.2 mM EGTA, 1 mM NaN_3, in 90% H_2O, 10% D_2O. In addition sample a contained 0.2 mM ATP, sample b 40 μM actin, 4 mM ATP and 1 mM Na_3VO_4, sample c 40 μM actin and 4 mM ATP and sample d 40 μM actin and 2 mM ADP. The star indicates low-molecular-mass impurities, all NMR spectra were recorded under identical experimental conditions.

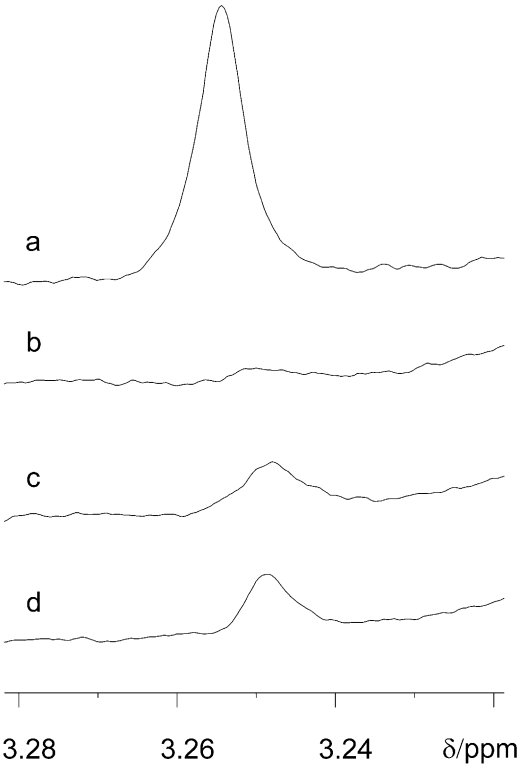

Fig. 4. The signal of the trimethyl amino group in different functional states of myosin S1. Spectra were recorded at 800 MHz at 288 K. a: The sample contained 32 μM rabbit myosin S1 in 2 mM Tris–HCl, pH 7.8, 30 mM KCl, 40 μM phalloidin, 0.1 mM $MgCl_2$, 0.2 mM EGTA, 1 mM NaN_3, in 90% H_2O, 10% D_2O. b: Same sample but with 40 μM actin, 2 mM ADP. c: Same sample but with 4 mM ADP, 1 mM Na_3VO_4 and 15 min after starting the experiment. d: Same as sample c, 2 h after starting the experiment.

In another set of experiments (Fig. 1B) the myosin concentration was held constant but the concentration of actin was varied. In these experiments the changes in the myosin resonances induced by binding of actin can be easily observed. The N-terminal amino acid sequence of myosin LC1 starts with $(CH_3)_3N^+$-Ala-Pro-Lys-Lys-Asp-Val- and contains 12 alanine residues in its first 31 N-terminal residues. The signals of the trimethylamino group give rise to a strong singlet resonance at 3.25 ppm, the signals of the alanine β-methyl groups are expected at 1.37 ppm [39]. The signals of the trimethylamino group (Fig. 4) and of the β-methyl group of mobile alanine residues of LC1 are quenched by binding of myosin indicating that they are immobilized or strongly exchange broadened with binding to actin. A quantitation of the signals around 1.37 ppm disappearing after binding would lead to a signal reduction that corresponds to approximately 20 methyl groups. This rather large number indicates that this signal is composed also of resonances of residues other than alanines but would be in line with the proposition that a large part of the N-terminus of LC1 gets immobilized. In addition, a number of other resonances are quenched with actin binding, e.g. the signal at 0.81 ppm, the broad signals at 1.46 and 1.69 ppm. In contrast, the signal from the N-terminal acetyl group of myosin LC3 (2.05 ppm) is clearly not influenced by actin binding. This is also true for the narrow singlet resonances at 2.06 and 2.11 ppm which originate from the methyl groups of two (not yet assigned) methionine residues of myosin.

3.2. Interaction of actin with the motor domain of myosin from *D. discoideum*

The motor domain of *D. discoideum* exhibits a spectrum typical for a well-folded protein (Fig. 2). Since it contains no light chains, the sharp signals arising from the light chains bound in myosin S1 cannot be present. Nevertheless, the spectrum contains two sharp singlet resonances at 2.05 and 2.09 ppm which present either methyl groups of methionines or an *N*-acetyl group (Fig. 2).

With increasing actin concentrations all signals from the motor domain are quenched. This is also true for the signal at 2.05 ppm which overlaps with the *N*-acetyl signal from F-actin. Only about 50% of the protomers of Mg-F-actin are in the M state, which means that the relative intensity of the *N*-acetyl signal from F-actin should have half the intensity of the methyl signal of the motor domain. Assuming that the myosin signal is quenched after binding but the F-actin signal is not influenced as in rabbit S1, the signal intensity should first decrease, have a minimum when the actin to myosin ratio is 1, and then should increase with increasing actin concentrations. Just such a behavior is observed in Fig. 2. This means that also the motor domain of *D. discoideum* does not interact with the *N*-acetyl of F-actin.

3.3. Actomyosin interaction in the presence of ATP and/or vanadate

Myosin with ATP bound at the active center should interact with F-actin only weakly. The addition of ATP to actomyosin leads to no significant spectral changes when compared with the rigor state (Fig. 3). Especially, the trimethylamino signal of LC1 does not recover. Vanadate binds to myosin.ADP and is assumed to form a transition state similar to the ADP.P_i state of myosin. Fig. 3 shows the result of adding vanadate to actomyosin, the spectral changes observed indicate that some of the myosin bound is released since signals typical for free myosin reappear. This can be best seen when observing the trimethylamino signal from myosin (Fig. 4). This signal is quenched completely in the rigor state but reappears partly after addition of vanadate. However, even after prolonged incubation with vanadate only about 20% of the signal intensity reappears indicating that under our conditions only about 20% of myosin is released. A close inspection of this signal shows that it is shifted upfield by 0.05 ppm relative to the signal of free myosin.ADP.

3.4. Influence of divalent ions on the mobility of the actin N-terminus in actomyosin

When adding Ca^{2+} to a solution of actomyosin in the rigor state no effect on the N-terminal signal of actin is observed (Fig. 5). This is different to the behavior observed in the absence of myosin where the F-actin signal was completely quenched after addition of calcium in millimolar concentrations [2]. The addition of Mg^{2+} ions in millimolar concentrations also has no effect on the signal of the N-terminus of F-actin.

4. Discussion

4.1. Binding of the N-terminus of actin to myosin in the strong binding state

Since up to now no crystal structure of the actomyosin complex has been solved, only reconstructions of the complex

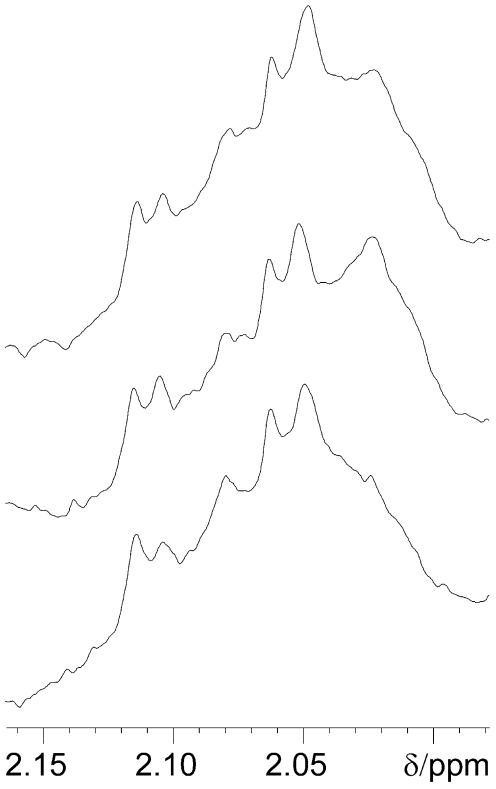

Fig. 5. The effect of divalent ions in the actomyosin complex. The samples contained 26 μM myosin S1 and 38 μM actin in 2 mM Tris–HCl, pH 7.8, 30 mM KCl, 40 μM phalloidin, 0.1 mM $MgCl_2$, 0.2 mM EGTA, 0.2 mM ATP, 0.010 mM TSP, 0.1 mM NaN_3, 90% H_2O, 10% D_2O. The sample contained additionally 5 mM $CaCl_2$ (middle) or 5 mM $MgCl_2$ (bottom).

are available. Due to the inherent low resolution of these data additional structural information is necessary for obtaining reliable structures. Carbodiimide cross-linking of the N-terminus with the cluster of lysines in the loop 626–647 indicates a direct interaction of the negatively charged N-terminus of actin with the positive charged loop of myosin [49]. The deletion of the four N-terminal amino acids in yeast actin or their replacement by neutral amino acids leads to significant changes of the weak and strong binding interaction and to a decrease of the force generation [50]. Our data show unequivocally that there is no direct stable interaction of the N-terminus of Mg-F-actin with myosin in the absence and presence of ADP since the signals of the N-terminal acetyl group of actin is not influenced by binding of myosin S1 or the motor domain of *Dictyostelium discoideum*. This means that in the equilibrium there is no direct interaction between the N-terminus of actin and myosin in the rigor state or in the ADP state.

Our high-resolution data are in line with more indirect functional evidence: blocking of residues 1–7 by antibodies inhibits the weak binding of myosin and the actin-activated myosin ATPase but not much the strong binding of S1 [51,52].

4.2. Binding of the N-terminus of myosin light chains to actin in the strong binding state

In the rigor state the NMR signal of the N-terminus of light chain LC1 of rabbit myosin is quenched. This observation has been reported previously by several groups using LC1 from different sources [32,39–41]. The disappearance of the signal could be caused by an immobilization by (1) a direct interaction or (2) an actin-induced structural change in myosin S1 immobilizing the light chain, e.g. by a direct interaction of the N-terminus of the light chains with the heavy chain of S1. An additional mechanism could be (3) an exchange broadening of the signal when there is more than one conformational state of myosin S1 bound to actin with different environments of the N-terminus of the light chain. Cross-linking studies [43–45,53] indicate mechanism 1. The observation that the much shorter light chain LC3 is not influenced visibly by actin binding would also be a natural consequence from mechanism 1 but would not follow directly from the two alternative mechanisms.

4.3. Binding of myosin to actin in the weak binding state

A transition from the tight binding state to the weak binding state of myosin is expected when ATP or ADP.P_i is bound at the active center of myosin. This should also be observable experimentally in the ^1H NMR spectroscopy when the environment of NMR-observable groups is changed by a change of actomyosin interaction. The addition of 4 mM ATP to the actomyosin solution did not lead to an observable change in the ^1H NMR spectra (Figs. 3 and 4). Especially, the signal of the *N*-acetyl group of actin and the signal of the trimethylamino group of LC1 did not change. This would indicate that in the weak binding state the N-terminus of actin is not involved in an interaction and the N-terminus of LC1 is immobilized (most probably by direct interaction with actin). However, it is more likely that under the experimental conditions used part of ATP is hydrolyzed and only a small proportion of myosin is in the ATP state which cannot be observed in the spectra.

The ADP.VO_4^{3-} complex is thought to represent a rather stable analog to ADP.P_i bound to myosin and thus may produce an NMR-visible weak binding state. Indeed, the signals typically observed in the spectra of free myosin S1 reappear. This can be easily seen at the signal of the trimethylamino group of LC1 which becomes visible again. The time course of this event is rather slow, after 15 min the equilibrium is not reached since the signal intensity of myosin increases with time. After 2 h a stable equilibrium is reached which is still unchanged after incubation of the sample for 24 h. However, only about 20% of the signal reappears indicating either that only this percentage of myosin occurs in an NMR-visible ADP.VO_4^{3-} state or that in this state part of the signal of myosin.ADP.VO_4^{3-} is still quenched by an interaction of LC1 with actin. However, it is known that actin competes with vanadate bound to myosin leading to a mixture of tightly binding myosin.ADP and weakly binding myosin.ADP.VO_4^{3-}. If this is the case then the data indicate that in the weak binding state LC1 is not in contact with actin. The signal of the N-terminus of actin seems to remain unperturbed after addition of vanadate. However, the signal-to-noise ratios obtainable in these experiments were not sufficient to exclude a small decrease of its amplitude.

The NMR data exclude direct interactions of the N-terminus with myosin in the rigor and ADP state with a longer lifetime and significant population but do not exclude an interaction in the weak binding state. This would be in line with the observation that only the number but not the position of

acidic residues of actin suggested to be involved in the weak actomyosin interaction seems to be important for the interaction with myosin [54]. The actomyosin interaction can be increased by addition of positively charged residues in the basic binding loop of the motor domain of *D. discoideum* [55,56]. These experiments suggest that during the initial formation of the collision complex only long-range ionic interactions between myosin and the N-terminal amino acids of F-actin occur [18,19,54,57–60]. In this case they would not be observable by NMR spectroscopy in the present setup.

4.4. Effect of Ca^{2+} or Mg^{2+} ions bound to the weak affinity sites of actin

Actin contains a number of weak binding sites for divalent cations ions with affinities which range from 0.018 mM^{-1} to 6 mM^{-1} [61–65]. These binding sites should be at least partly occupied in the presence of 5 mM $MgCl_2$ or 5 mM $CaCl_2$. In the absence of myosin Mg^{2+} in millimolar concentrations had no effect on the signal of Mg-F-actin indicating that the occupation of the weak binding site(s) by magnesium does not influence the mobility of the N-terminal residues. In contrast, after addition of Ca^{2+} to free Mg-F-actin in millimolar concentrations to the sample, the *N*-acetyl signal of F-actin was not visible [2] in the NMR spectra. The disappearance of the signal was interpreted as being most probably due to the exchange of the Mg^{2+} ion by Ca^{2+} in the active center. Since the typical time needed for starting an NMR measurement is 5 min, the ion exchange should have been completed after 5 min.

A different result is obtained when Mg-F-actin is decorated with myosin. Ca^{2+} in millimolar concentrations does not have any effect on the mobility of the N-terminus of F-actin (Fig. 4). At 288 K even after 90 min no quenching of the actin signal can be seen. This means that the exchange of the divalent ion bound to the active center of actin is strongly delayed by binding of myosin to actin.

The mobility of the N-terminus of actin in the actomyosin complex is also not influenced by the presence of free magnesium in millimolar concentrations. This falsifies one interesting idea that the interaction of the negatively charged N-terminus of actin with myosin requires that a supposed weak binding site is occupied by a divalent ion.

4.5. The function of the high-mobility element in F-actin

The question remains what the reason is for the high mobility of the first half of subdomain 1. At present one can only speculate. There is now increasing evidence that for high-affinity, controlled protein–protein interaction the interaction sites have to have an intrinsic mobility which allows the optimal mutual fitting of the residues located at the interface. A rather well understood example is the Ras binding to effectors whose interfaces both show high mobility [66]. This explanation is excluded by our experiments: at least in the strong binding state there is no direct interaction of the N-terminal acetyl of actin with myosin and most probably of the whole high-mobility region encompassing residues 1–21.

When looking at the geometry of the high-mobility region in F-actin one sees that it is arranged almost parallel to the fiber axis and that it is continued by the region of low interdomain stability caused by the ATP binding cleft of actin. Another, more intriguing function of the high-mobility zone is that the cleft region and the high-mobility region would present elastic elements which can buffer tensile or torsional forces when myosin heads are attached thus preventing the possible destruction of the fiber. This may be especially important in cells other than skeletal muscle where actin is not stabilized by a scaffold of other proteins. The last function could be tested easily by experiment since the more rigid Ca^{2+}-F-actin should be more prone to get destroyed by asymmetrically attacking forces than Mg^{2+}-F-actin.

4.6. Structural consequences

The modeling of the actomyosin complex by using the X-ray structures of free actin and myosin for the reconstruction of the data obtained from low-angle scattering or electron microscopy is the method of choice for obtaining as good as possible models of the complex from low-resolution data. Our NMR results make it possible to check details of these results and may serve as an aid in model building.

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References

[1] Slosarek, G., Heintz, D. and Kalbitzer, H.R. (1994) FEBS Lett. 351, 405–410.
[2] Heintz, D., Kany, H. and Kalbitzer, H.R. (1996) Biochemistry 35, 12686–12693.
[3] Kabsch, W., Mannherz, H.G., Suck, D., Pai, E.F. and Holmes, K.C. (1990) Nature 347, 37–44.
[4] McLaughlin, P.J. and Weeds, A.G. (1995) Annu. Rev. Biophys. Biomol. Struct. 24, 643–675.
[5] Schutt, C.E., Myslik, J.C., Rozycki, M.D., Goonesekere, N.C. and Lindberg, U. (1993) Nature 365, 810–816.
[6] Otterbein, L.R., Graceffa, P. and Dominguez, R. (2001) Science 293, 708–711.
[7] Holmes, K.C., Popp, D., Gebhard, W. and Kabsch, W. (1990) Nature 347, 44–49.
[8] Lorenz, M., Popp, D. and Holmes, K.C. (1993) J. Mol. Biol. 234, 826–836.
[9] Egelman, E.H. (1997) Structure 5, 1135–1137.
[10] Steinmetz, M.O., Stoffler, D., Hoenger, A., Bremer, A. and Aebi, U. (1997) J. Struct. Biol. 119, 295–320.
[11] Schoenenberger, C.A., Steinmetz, M.O., Stoffler, D., Mandinova, A. and Aebi, U. (1999) Microsc. Res. Tech. 47, 38–50.
[12] Rayment, I., Rypniewski, W.R., Schmidt-Base, K., Smith, R., Tomchick, D.R., Benning, M.M., Winkelmann, D.A., Wesenberg, G. and Holden, H.M. (1993) Science 261, 50–58.
[13] Fisher, A.J., Smith, C.A., Thoden, J.B., Smith, R., Sutoh, K., Holden, H.M. and Rayment, I. (1995) Biochemistry 34, 8960–8972.
[14] Smith, C.A. and Rayment, I. (1996) Biochemistry 35, 5404–5417.
[15] Gulick, A.M., Bauer, C.B., Thoden, J.B. and Rayment, I. (1997) Biochemistry 36, 11619–11628.
[16] Dominguez, R., Freyzon, Y., Trybus, K.M. and Cohen, C. (1998) Cell 94, 559–571.
[17] Houdusse, A., Szent-Gyorgyi, A.G. and Cohen, C. (2000) Proc. Natl. Acad. Sci. USA 97, 11238–11243.
[18] Rayment, I., Holden, H.M., Whittaker, M., Yohn, C.B., Lorenz, M., Holmes, K.C. and Milligan, R.A. (1993) Science 261, 58–65.
[19] Schroder, R.R., Manstein, D.J., Jahn, W., Holden, H., Rayment, I., Holmes, K.C. and Spudich, J.A. (1993) Nature 364, 171–174.
[20] Mendelson, R. and Morris, E.P. (1997) Proc. Natl. Acad. Sci. USA 94, 8533–8538.
[21] Cozzone, P.J., Nelson, D.J. and Jardetzky, O. (1974) Biochem. Biophys. Res. Commun. 60, 341–347.
[22] Highsmith, S., Akasaka, K., Konrad, M., Goody, R., Holmes,

K., Wade-Jardetzky, N. and Jardetzky, O. (1979) Biochemistry 18, 4238–4244.
[23] Highsmith, S. and Jardetzky, O. (1980) FEBS Lett. 121, 55–60.
[24] Highsmith, S. and Jardetzky, O. (1981) Biochemistry 20, 780–783.
[25] Barden, J.A., Cooke, R., Wright, P.E. and dos Remedios, C.G. (1980) Biochemistry 19, 5912–5916.
[26] Barden, J.A., Wu, C.S. and dos Remedios, C.G. (1983) Biochim. Biophys. Acta 748, 230–235.
[27] Barden, J.A. and Kemp, B.E. (1989) Eur. J. Biochem. 184, 379–394.
[28] Barden, J.A. and dos Remedios, C.G. (1984) J. Biochem. (Tokyo) 96, 913–921.
[29] Barden, J.A. and dos Remedios, C.G. (1985) Eur. J. Biochem. 146, 5–8.
[30] Barden, J.A. and Kemp, B.E. (1987) Biochemistry 26, 1471–1478.
[31] Barden, J.A. and Phillips, L. (1990) Biochemistry 29, 1348–1354.
[32] Prince, H.P., Trayer, H.R., Henry, G.D., Trayer, I.P., Dalgarno, D.C., Levine, B.A., Cary, P.D. and Turner, C. (1981) Eur. J. Biochem. 121, 213–219.
[33] Shriver, J.W. and Sykes, B.D. (1981) Biochemistry 20, 6357–6362.
[34] Shriver, J.W. and Sykes, B.D. (1981) Biochemistry 20, 2004–2012.
[35] Shriver, J.W. and Sykes, B.D. (1982) Biochemistry 21, 3022–3028.
[36] Brauer, M. and Sykes, B.D. (1981) Biochemistry 20, 2060–2064.
[37] Brauer, M. and Sykes, B.D. (1981) Biochemistry 20, 6767–6775.
[38] Brauer, M. and Sykes, B.D. (1982) Biochemistry 21, 5934–5939.
[39] Trayer, I.P., Trayer, H.R. and Levine, B.A. (1987) Eur. J. Biochem. 164, 259–266.
[40] Henry, G.D., Trayer, I.P., Brewer, S. and Levine, B.A. (1985) Eur. J. Biochem. 148, 75–82.
[41] Henry, G.D., Winstanley, M.A., Dalgarno, D.C., Scott, G.M., Levine, B.A. and Trayer, I.P. (1985) Biochim. Biophys. Acta 830, 233–243.
[42] Sutoh, K. (1981) Biochemistry 20, 3281–3285.
[43] Yamamoto, K. and Sekine, T. (1983) J. Biochem. (Tokyo) 94, 2075–2078.
[44] Andreev, O.A., Saraswat, L.D., Lowey, S., Slaughter, C. and Borejdo, J. (1999) Biochemistry 38, 2480–2485.
[45] Timson, D.J., Trayer, H.R. and Trayer, I.P. (1998) Eur. J. Biochem. 255, 654–662.
[46] Pardee, J.D. and Spudich, J.A. (1982) Methods Cell Biol. 24, 271–289.
[47] Taylor, R.S. and Weeds, A.G. (1977) FEBS Lett. 75, 55–60.
[48] Manstein, D.J. and Hunt, D.M. (1995) J. Muscle Res. Cell Motil. 16, 325–332.
[49] Sutoh, K. (1983) Biochemistry 22, 1579–1585.
[50] Miller, C.J., Wong, W.W., Bobkova, E., Rubenstein, P.A. and Reisler, E. (1996) Biochemistry 35, 16557–16565.
[51] DasGupta, G. and Reisler, E. (1989) J. Mol. Biol. 207, 833–836.
[52] DasGupta, G. and Reisler, E. (1992) Biochemistry 31, 1836–1841.
[53] Sutoh, K. (1982) Biochemistry 21, 3654–3661.
[54] Wong, W.W., Doyle, T.C. and Reisler, E. (1999) Biochemistry 38, 1365–1370.
[55] Furch, M., Remmel, B., Geeves, M.A. and Manstein, D.J. (2000) Biochemistry 39, 11602–11608.
[56] Furch, M., Geeves, M.A. and Manstein, D.J. (1998) Biochemistry 37, 6317–6326.
[57] Bonafe, N., Chaussepied, P., Capony, J.P., Derancourt, J. and Kassab, R. (1993) Eur. J. Biochem. 213, 1243–1254.
[58] Aspenstrom, P. and Karlsson, R. (1991) Eur. J. Biochem. 200, 35–41.
[59] Sutoh, K., Ando, M., Sutoh, K. and Toyoshima, Y.Y. (1991) Proc. Natl. Acad. Sci. USA 88, 7711–7714.
[60] Cook, R.K., Root, D., Miller, C., Reisler, E. and Rubenstein, P.A. (1993) J. Biol. Chem. 268, 2410–2415.
[61] Tellam, R. (1985) Biochemistry 24, 4455–4460.
[62] Carlier, M.F., Pantaloni, D. and Korn, E.D. (1986) J. Biol. Chem. 261, 10785–10792.
[63] Zimmerle, C.T., Patane, K. and Frieden, C. (1987) Biochemistry 26, 6545–6552.
[64] Zimmerle, C.T. and Frieden, C. (1988) Biochemistry 27, 7759–7765.
[65] Pliszka, B., Redowicz, M.J. and Strzelecka-Golaszewska, H. (1989) FEBS Lett. 243, 30–32.
[66] Spoerner, M., Herrmann, C., Vetter, I.R., Kalbitzer, H.R. and Wittinghofer, A. (2001) Proc. Natl. Acad. Sci. USA 98, 4944–4949.