Crystal Structure of Monomeric Actin in the ATP State

STRUCTURAL BASIS OF NUCLEOTIDE-DEPENDENT ACTIN DYNAMICS*

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A nucleotide-dependent conformational change regulates actin filament dynamics. Yet, the structural basis of this mechanism remains controversial. The x-ray crystal structure of tetramethylrhodamine-5-maleimide-actin with bound AMPPNP, a non-hydrlyzable ATP analog, was determined to 1.85-Å resolution. A comparison of this structure to that of tetramethylrhodamine-5-maleimide-actin with bound ADP, determined previously under similar conditions, reveals how the release of the nucleotide γ-phosphate sets in motion a sequence of events leading to a conformational change in subdomain 2. The side chain of Ser-14 in the catalytic site rotates upon Pi release, triggering the rearrangement of the loop containing the methylated His-73, referred to as the sensor loop. This in turn causes a transition in the DNase I-binding loop in subdomain 2 from a disordered state to a helical one. The methylated His-73 is an essential part of many forms of cell motility. A driving force behind actin treadmilling is the hydrolysis of ATP by actin. However, in vivo treadmilling is further regulated by a number of factors including a battery of actin-binding proteins. Actin-depolymerizing factor/cofilin, for instance, binds preferentially to the ADP-actin monomers that accumulate toward the pointed end of the filament, accelerating their dissociation. Other proteins such as profilin and thymosin-β4 bind ATP-actin with higher affinity than ADP-actin, maintaining a pool of ATP-actin monomers ready for incorporation into the barbed end of the filament. The fact that these proteins can “distinquish” between ATP- and ADP-actin suggests that these two states are structurally different. Consistent with this view, biochemical (3–5), spectroscopic (6–8), and electron microscopic (9) evidence has suggested that a conformational change in actin subdomain 2 accompanies the hydrolysis of ATP and the release of inorganic phosphate.

Visualization of the structural details of such a conformational change has come from a comparison of crystal structures of the actin monomer (G-actin) in the ATP and ADP states. ATP-actin structures have been determined from complexes with actin-binding proteins that keep actin in a monomeric state: DNase I (10), profilin (11), gelsolin (12, 13), and vitamin D-binding protein (14, 15). ADP-actin, on the other hand, was crystallized in a monomeric state after binding tetramethylrhodamine-5-maleimide (TMR)1 to Cys-374, which blocks polymerization (16). A comparison of the structures in the two states reveals how the release of the nucleotide γ-phosphate triggers a sequence of events that propagate into a loop to helix transition in the DNase I-binding loop in subdomain 2. However, a proper comparison of the ATP- and ADP-bound states of actin would require for the two structures to be determined under similar conditions.

Meanwhile, an analysis of the existing structural data has led to conflicting interpretations. Recent reports have questioned the validity of the structure of TMR-modified actin with bound ADP as truly representative of the ADP state (17–19). Some (18, 20, 21) think that in the “real” ADP state the cleft that separates the two major domains of actin must be open, as observed in the so-called “open” state structure of the actin-profilin complex determined with bound ATP (22). It has also been suggested that the TMR probe bound to Cys-374 prevents the two domains of actin from opening apart so that actin can assume its “true” ADP conformation (18). The TMR probe, rather than the change in the nucleotide, has also been held responsible for the conformational change observed in actin subdomain 2 (17, 19). According to this hypothesis, long range allosteric effects due to the binding of the TMR probe to the C terminus of actin could have induced the conformational change observed at the opposite end of the molecule in subdomain 2. Another explanation for the loop to helix transition in the DNase I-binding loop, which disregards any role of the nucleotide state, is that it is triggered by crystal packing contacts (18). A structure of monomeric TMR-actin in the ATP state would provide an excellent way to resolve this controversy (23, 24).

In this report, we describe the structure of TMR-modified actin with bound AMPPNP, a non-hydrlyzable ATP analog (25), at 1.85-Å resolution. The symmetry and unit cell param-

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† The abbreviations used are: TMR, tetramethylrhodamine-5-maleimide; ACES, 2-

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Structure of Monomeric Actin in the ATP State

W. F. Stafford, unpublished data.

K. Mabuchi, unpublished data.

RESULTS AND DISCUSSION

General Description of the Structure of TMR-Actin in the ATP State—Crystals of TMR-actin with bound ATP can be obtained under conditions similar to those for ADP-actin (16). However, nucleotide hydrolysis takes place during the time needed to grow these crystals, which is probably the result of the high salt concentration in the crystallization buffer. A way to circumvent this problem was to crystallize TMR-actin with AMP-PNP, a commonly used non-hydrolyzable ATP analog (25). The crystallization conditions for AMP-PNP-actin, although similar to those for ADP-actin (16), had to be modified slightly (see “Experimental Procedures”). An analysis of the diffraction pattern from these crystals revealed unit cell parameters and symmetry identical with those of ADP-actin. However, a characteristic feature of the crystals of AMP-PNP-actin is that they do not diffract the x-rays as strongly as those of ADP-actin. This is probably due to increased disorder in actin subdomain 2 (see below). After screening a large number of crystals at the BioCARS beamline 14-ID-B, a complete x-ray diffraction data set was collected from one of the crystals that diffracted to the resolution of 1.85 Å (Table I). Refinement of the structure converged to a R-factor value of 18.7% and a R-free value of 22.6%.

The position and geometry of AMP-PNP (Fig. 1) is almost indistinguishable from that of ATP in the ATP-bound actin structures (10–15). Indeed, a superimposition of AMP-PNP-actin (henceforth referred to as ATP-actin) with all of the existing actin structures places the nucleotide and associated divalent cation in nearly identical positions (Fig. 2) with the exception of one of the structures, that of the open state of the actin-profilin complex that contains ATP bound in an unusual manner (22) (discussed below). Moreover, a comparison of the ATP and ADP bound structures of monomeric TMR-actin reveals how the ADP moiety of the nucleotide and associated Ca²⁺ ion remain bound after hydrolysis in precisely the same position, linked by a network of conserved interactions that are often mediated by water molecules (Fig. 3).

The structures of TMR-actin in the ADP (16) and ATP states are similar overall (Fig. 4A). However, important differences occur around the nucleotide γ-phosphate site, Ser-14, the loop containing the methylated His-73, and subdomain 2 (Figs. 1, 3, and 4). Most remarkably, the DNase I-binding loop, which formed a well ordered α-helix in the ADP-actin structure (16), is now fully disordered in the ATP structure (Fig. 5). Although the crystals corresponding to the two nucleotide states are
identical, the ATP-actin structure is more flexible overall but significantly more so in subdomain 2. The higher thermal mobility of the ATP structure is reflected by higher temperature factor values, which in this case can be compared directly because of the identity of the crystals (30). The conformational change upon P_i release can be described as a sequence of events that originate at the nucleotide-binding site and the Ser-14 β-hairpin loop, propagating through the His-73 loop into subdomain 2. Similar inserts exist in the actin-related proteins 2 and 3 (Arp2 and Arp9) (31) and in the bacterial actin homologue MreB (32). The conformation of the sensor loop in ATP-actin appears to be less stable than that in the ADP-actin structure, which is reflected by temperature factor values that are well above the average value for the rest of the structure. In contrast, in the ADP-actin structure, the loop displays temperature factors similar to those of the rest of the structure. One element that probably contributes to the stability of the loop in the ADP-actin structure (but which is missing in the ATP structure) is a stacking interaction between the side chains of Glu-72 and the methylated side chain of His-73 (Fig. 3). When the constraints imposed upon the loop in the ATP structure (because of the presence of the γ-phosphate of the nucleotide and resulting orientation of the side chain of Ser-14) are removed upon P_i release, the sensor loop moves toward the γ-phosphate site. Identical changes in Ser-14 and the sensor loop were observed previously between the structures of ATP- and ADP-actin complexed with DNase I (10). However, because the ADP structure in this case was obtained following the hydrolysis of ATP within the crystals in which DNase I is tightly bound to actin subdomains 2 and 4, the changes in the sensor loop could not propagate into a conformational transition in subdomain 2. Such constraints do not exist in the structures of monomeric TMR-actin.

Subdomain 2 and the DNase I-binding Loop—The change in conformation of the sensor loop in the ATP structure is accompanied by a small ~4° rotation of subdomain 2. Note, however, that this rotation does not bring subdomain 2 entirely back to the orientation observed in the other ATP-actin structures (10–15), which would have required a rotation of ~10°. A number of factors could help explain this small difference, including the use of AMPPNP in place of ATP, the binding of TMR to Cys-374, or the fact that this is the first ATP-actin structure to have been determined without any other protein bound to it.

More significant, however, is the increased disorder in subdomain 2 in the ATP state. Indeed, as evidenced by the electron density maps and temperature factor values, subdomain 2 is far more mobile in the ATP-actin structure than in the ADP-actin structure. For instance, witness the striking difference between the 2F_o – F_c electron density maps contoured around subdomain 2 (Fig. 5A) and around a more typical region of the structure such as the catalytic site (Fig. 1A). As previously observed in other ATP-actin structures (12–15), the DNase I-binding loop within subdomain 2 becomes fully disordered with 12 amino acids (His-40 to Asp-51) undetermined in the final structure (Fig. 5A). The average temperature factor for the 25 amino acids (of 37) of subdomain 2 that remain visible in the structure is 42.2 Å² as compared with 32.5 Å² for the rest of the structure. In contrast, in the ADP state, subdomain 2 is well ordered and the DNase I-binding loop forms a stable α-helix (Fig. 5B). Therefore, one reason for the melting of the α-helix in the ATP structure appears to be the increased flex-

### Table I

| Data collection and refinement statistics |   |
|------------------------------------------|---|
| Space group | C2 |
| Unit cell parameters |   |
| a, b, c (Å) | 112.22, 37.37, 85.03 |
| a, b, γ (°) | 70.0, 108.13, 90.0 |
| Resolution range (Å) | 24.4–1.85 (1.97–1.85) |
| Average B factor (Å²) | 12.13 (7.2) |
| Crystal mosaicity (°) | 0.7 |
| R-merge (%) | 9.1 (29.0) |
| Completeness (%) | 98.2 (90.2) |
| Redundancy | 7.5 (4.3) |

*Values in parentheses correspond to last resolution shell.

† R-factor = Σ |I – (I_o / ΣI_o)| / ΣI, where I is the intensity of an individual reflection and I_o is its mean value.

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§ R-free = R-factor calculated for a subset of the reflections (5%), which were omitted during the refinement and used to monitor its convergence.

The Ser-14 β-Hairpin Loop—In the structure of ATP-actin, the presence of the nucleotide γ-phosphate forces the rotation of the side chain of Ser-14 into a common rotamer with that of ADP-actin (Figs. 1 and 3) (16). However, Ser-14 is one of 12 amino acids in the structure that adopted two alternative side chain orientations. The second rotamer of Ser-14, which is identical, the ATP-actin structure is more flexible overall but significantly more so in subdomain 2. The higher thermal mobility of the ATP structure is reflected by higher temperature factor values, which in this case can be compared directly because of the identity of the crystals (30). The conformational change upon P_i release can be described as a sequence of events that originate at the nucleotide-binding site and the Ser-14 β-hairpin loop, propagating through the His-73 loop into subdomain 2.

The Sensor Loop—When the ATP- and ADP-bound structures of TMR-actin are superimposed, it becomes apparent that the two different orientations of Ser-14 result in two different conformations of the loop containing the methylated His-73 (Fig. 4). Because of steric hindrance, the conformation of this loop in the ADP-actin structure, where it had moved toward the β-hairpin loop containing Ser-14, would have been inconsistent with the presence of the intact nucleotide and the resulting orientation of Ser-14 in the ATP structure. Therefore, there exists a direct correlation among the state of the nucleotide (ATP or ADP), the orientation of the side chain of Ser-14, and the conformation of this loop. Thus, we refer to this loop as the sensor loop. The sensor loop, Pro-70 to Asn-78, constitutes an insert between actin subdomains 2 and 1 and appears to function as a switch, linking changes in the nucleotide site to structural transitions in subdomain 2 (Fig. 4). Similar inserts exist in the actin-related proteins 2 and 3 (Arp2 and Arp9) (31) and in the bacterial actin homologue MreB (32). The conformation of the sensor loop in ATP-actin appears to be less stable than that in the ADP-actin structure, which is reflected by temperature factor values that are well above the average value for the rest of the structure. In contrast, in the ADP-actin structure, the loop displays temperature factors similar to those of the rest of the structure. One element that probably contributes to the stability of the loop in the ADP-actin structure (but which is missing in the ATP structure) is a stacking interaction between the side chains of Glu-72 and the methylated side chain of His-73 (Fig. 3). When the constraints imposed upon the loop in the ATP structure (because of the presence of the γ-phosphate of the nucleotide and resulting orientation of the side chain of Ser-14) are removed upon P_i release, the sensor loop moves toward the γ-phosphate site. Identical changes in Ser-14 and the sensor loop were observed previously between the structures of ATP- and ADP-actin complexed with DNase I (10). However, because the ADP structure in this case was obtained following the hydrolysis of ATP within the crystals in which DNase I is tightly bound to actin subdomains 2 and 4, the changes in the sensor loop could not propagate into a conformational transition in subdomain 2. Such constraints do not exist in the structures of monomeric TMR-actin.

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ibility of subdomain 2, which in turn emanates from increased flexibility in the sensor loop. Another reason could be the disruption of a hydrophobic cluster that stabilizes the inner face of the $\alpha$-helix in the structure of ADP-actin. In the ADP-actin structure, amino acids Val-43, Met-44, and Met-47 from the inner face of the $\alpha$-helix form part of a hydrophobic cluster with Pro-38, Tyr-53, Ile-64, and Leu-65 in subdomain 2. In the ATP structure, the main chain carbonyl oxygen of Ile-64, which is connected to the sensor loop by a short $\beta$-strand, is rotated toward the core of this hydrophobic cluster. Such an orientation of the carbonyl of Ile-64 would be inconsistent with the formation of an $\alpha$-helix in the DNase I-binding loop in the ATP structure. However, a comparison of the structures alone does not allow determining whether the rotation of the carbonyl at Ile-64 is a consequence of the melting of the $\alpha$-helix in the DNase I-binding loop or whether, on the contrary, it causes the melting of the $\alpha$-helix in response to the movement of the sensor loop.

The DNase I-binding loop can adopt a broad range of conformational states. In F-actin, this loop is thought to participate in intermonomer interactions (33, 34). In the structure of the actin-DNase I complex, the loop is attached as an additional $\beta$-strand to a $\beta$-sheet in DNase I (10). In a number of ATP-actin structures, including the current structure of TMR-actin, the loop is disordered (12–15). In contrast, in the structure of ADP-actin, the DNase I-binding loop is very stable, forming an $\alpha$-helix (16). Such an array of conformational states is made possible by specific features of the amino acid sequence. For instance, three Gly residues at positions 42, 46, and 48 of the loop may account for its flexibility. The loop contains four hydrophobic amino acids, two valines (Val-43 and Val-45), and two methionines (Met-44 and Met-47). The presence of a sulfur atom within the unbranched side chain of methionine imparts this amino acid with uniquely high flexibility and polarizability (35). Thus, methionines are thought to play an important part in a number of protein-protein contacts by creating highly adaptable surfaces (36, 37). The occurrence of two methionine residues, a relatively rare amino acid (2.3/100), in the loop may reflect the need for conformational adaptability. The DNase I-binding loop is also unique in that it is among the most exposed regions of the actin structure, yet together with the rest of subdomain 2 and the sensor loop, it is one of the most

![Figure 1. Nucleotide binding site.](image-url)

A, all-atom-stereodiagram of the AMPPNP nucleotide analog bound in the catalytic site of actin. The $2F_o - F_c$ electron density map, contoured at 1.2 $\sigma$, is also shown. B, stereodiagram of a superimposition of the catalytic site region in the structures of AMPPNP-actin (cyan) and ADP-actin (red). The AMPPNP analog is colored according to atom type. Note how the change in nucleotide state leads to two different orientations of Ser-14 and the subsequent rearrangement of the loop containing the methylated His-73. Also shown are Ser-33, which marks the beginning of subdomain 2, and Arg-183 from subdomain 4, which changes conformation between the two nucleotide states so that it interacts with the His-73 loop in the ADP state but not in the ATP state.
for ATP, decreased ATPase activity, and altered protease susceptibility in the DNase I-binding loop (39, 40). In full agreement with the structural results described here, the protease digestion pattern of the Ser-14 to Ala mutant with bound ATP becomes similar to that of the ADP-bound state, whereas in wild-type actin, the two nucleotide states are characterized by different protease susceptibilities (39). In other words, the removal of the hydroxyl group of Ser-14 seems to break the coupling mechanism by which cleavage of the DNase I-binding loop becomes susceptible to the state of the nucleotide. Interestingly, the mutation of the residue equivalent to actin Ser-14 in heat shock cognate (Hsc) 70 (41) and BiP (42, 43) also leads to decoupling of ATP binding from a conformational change. Mutation of Ser-14 to Cys in β-actin also results in decreased thermal stability and lower binding affinity for DNase I, which has been interpreted as evidence of a change in the interdomain relationship of actin, i.e. open versus closed (19, 44). However, a different interpretation would be that this mutation affects the conformation of the DNase I-binding loop, thereby changing the binding affinity for DNase I than does ATP-actin.

His-73 occupies a special position within the sensor loop. This amino acid is absolutely conserved in actin, and most commonly it is also methylated, a rare posttranslational modification. However, the function of this modification remains unclear. His-73 mutagenesis studies attest to the importance of the sensor loop in determining the conformation of the DNase I-binding loop. Substitutions of His-73 by basic amino acids (Arg and Lys) have a general stabilizing effect on actin, whereas anionic or neutral substitutions are destabilizing (19, 45). As revealed by limited proteolysis using trypsin, subtilisin, and α-chymotrypsin, mutants of His-73 are characterized by changes in the conformation of subdomain 2 and, in particular, the DNase I-binding loop (45). The effect of a His-73 to Ala mutation has been also investigated using an indirect assay that monitors the DNase I-inhibitory activity of actin (19). Based on the activity of actin, the affinity of this mutant for DNase I was estimated to be less than that of wild-type actin, which was interpreted as evidence that a change had occurred in the degree of opening of the cleft that separates the two major domains of actin. However, as for the Ser-14 to Cys mutant of β-actin described above (44), such an interpretation may be unwarranted since a change in the activity of actin in the presence of DNase I could be interpreted in a number of different ways, including the possibility for a conformational change limited to the DNase I-binding loop. Although these two studies on the mutants of His-73 (19, 45) confirm the existence of a relationship between the sensor loop and the structure of subdomain 2, they do not specifically investigate the role of the nucleotide. However, since interactions of the loop (with Ser-14 and Arg-183 in the ATP and ADP structures, respectively) involve main chain atoms (Fig. 3) and not any specific side chain, mutants of this loop may not always be sensitive to the state of the nucleotide.

Nucleotide-dependent Conformational States of Actin—Similar to G proteins (46) and myosin (47), members of the actin superfamily (48) appear to undergo at least two major nucleotide-dependent conformational changes, one upon release of the nucleotide γ-phosphate and a second one upon ADP release. Members of this superfamily include the Hsp70 molecular chaperones, hexokinase, the sugar kinases, the Arps (31), and the prokaryotic actin-like proteins MreB (32) and ParM (49). Crystal structures corresponding to their different nucleotide states are starting to reveal a general pattern. Structures with bound nucleotide (either ATP or ADP) are characterized by a
closed conformation of the main interdomain cleft, whereas nucleotide-free structures are generally distinguished by an open cleft (Fig. 6). The reason for this is that the nucleotide and associated divalent cation bind at the base of the deep interdomain cleft, forming an elaborate network of hydrogen-bonding interactions (many of which are mediated by solvent molecules). This network of interactions helps to hold the two major domains together. Two homologous β-hairpin loops (actin residues 11DNGSGLVK18 and 154DSGDGVT161), one from each of the two major domains on each side of the nucleotide, account for the majority of the interactions with the nucleotide and divalent cation (Figs. 2 and 3). Although some of these interactions are lost upon release of the nucleotide γ-phosphate, the majority remains because of the presence of the ADP.
FIG. 5. Subdomain 2 and the DNase I-binding loop. A, a superimposition of subdomain 2 in the structures of TMR-modified ADP-actin (red, only the main chain backbone atoms are shown) and ATP-actin (cyan, all of the atoms are shown). The electron density map corresponding to the ATP state structure is also shown contoured at 1.0 σ. Note that in the ATP state structure there is no electron density visible for the region comprising the DNase I-binding loop (top of the figure), reflecting disorder. As a result, 12 amino acids (His-40 to Asp-51) are missing in the structure. B, in ADP-actin structure, in contrast, subdomain 2 is very well ordered and clearly defined in the electron density map with the DNase I-binding loop forming a α-helix.

FIG. 6. Three conformational states of actin. The two major actin domains on each side of the nucleotide, domain I (subdomains 1 + 2) and domain II (subdomains 3 + 4), are colored purple and red, respectively. The domains are connected by a linker α-helix (amino acids Ile-136 to Gly-146, green), which cannot be considered as belonging to either one of the two domains but which during opening of the cleft moves together with domain I. The only other connection between the two domains occurs in a loop centered at residue Lys-336. The major changes upon P_i release occur in the three regions shown in yellow (the Ser-14 β-hairpin loop, the sensor loop containing the methylated His-73, and the DNase I-binding loop). There is also a small rotation of subdomain 2 in a direction perpendicular to that of the plane of the figure (shown by an arrow). Note, however, that these changes do not lead to an open cleft, as interactions mediated by the ADP moiety of the nucleotide and divalent cation hold the two domains together. C, model of nucleotide-free actin built by homology with Arp3 (31). Indeed, although there is no structure of nucleotide-free actin available as yet, this state may be represented by the structures of nucleotide-free Arp2 and Arp3 (31), which share significant sequence similarity with actin. According to these two structures, as well as that of nucleotide free ParM (49), opening of the cleft can be described as a combination of two perpendicular rotations of ~12° each (indicated by the arrows). The α-helix between amino acids Ile-136 and Gly-146 shown in green serves as a hinge for the first of these two rotations (to the right) while the loop at the end of this helix rearranges slightly to accommodate the second rotation.
moiety of the nucleotide and the divalent cation, which remain bound in nearly the same position (Fig. 3). However, once ADP dissociates, very little remains to keep the two major domains together, which then could (but not necessarily have to) open apart (Fig. 6C). As a result, ATP-actin is generally more stable than ADP-actin (50) and nucleotide-free actin denatures rapidly and irreversibly (51). What is the evidence from the crystal structures in support of two conformational transitions?

A well studied member of the actin superfamily is Hsc70 (a representative of the Hsp70 family). The structures of Hsc70 in the ATP and ADP states are both in a closed conformation (52). However, the structure of nucleotide-free DnaK (a bacterial homologue of Hsc70) bound to the nucleotide exchange factor GrpE reveals an open cleft (53). The binding of ATP to the ATPase domain of DnaK dissociates GrpE, presumably because of a conformational change that brings about the closure of the interdomain cleft. Thus, GrpE helps stabilize an open conformation that cannot occur when a nucleotide (either ATP or ADP) is bound to the catalytic site of DnaK. Although the crystal structures of ATP- and ADP-bound Hsc70 do not reveal any major conformational change (52), these two states are characterized, respectively, by low and high substrate affinity, suggesting that they are structurally different. In agreement with this finding, the existence of a conformational change upon release of the nucleotide γ-phosphate has been demonstrated by solution x-ray-scattering experiments (54).

Another example is provided by the recently determined structures of the prokaryotic actin-like protein ParM (49). Two different structures of ParM were determined corresponding to the ADP-bound and nucleotide-free states. Although the nucleotide-free structure is characterized by an open cleft, that of ADP-ParM is in the closed conformation, very similar to ADP-actin (16).

Even more closely related to actin are Arp2 and Arp3. The nucleotide-free structures of Arp2 and Arp3 both reveal an open cleft (31). Binding of nucleotide to Arp2 and Arp3 is predicted to lead to the closure of their respective clefts and the concommitant activation of the Arp2/3 complex (31).

An apparent exception to the rule described here is provided by the structure of nucleotide-free MreB, which is in the closed conformation (32). Therefore, although opening of the cleft is more likely to take place in the absence of a nucleotide, under certain conditions even a nucleotide-free cleft could remain closed. Interestingly, in MreB, a salt bridge between Lys-49 from subdomain 2 and Glu-204 from subdomain 4 helps stabilize the closed conformation. Two additional positively charged amino acids from subdomain 2 of MreB (Arg-63 and Arg-66) face negatively charged amino acids in subdomain 4 (Asp-180 and Glu-200). Such a charge balance between subdomains 2 and 4 does not exist in actin. Another feature that distinguishes MreB from actin is that the structural alignment of their respective polypeptide chains breaks apart toward the C terminus so that a α-helix in MreB (residues Lys-325 to Leu-332) occupies a position right in between subdomains 1 and 3. The presence of this α-helix adds to the number of contacts between the two major domains of MreB as compared with actin. Together, these structural features may help explain why nucleotide-free MreB is quite stable in solution while nucleotide-free actin is not (51).

The Open State Structure of the ATP-Actin-Profilin Complex Cannot Be Equated with That of ADP-Actin—As discussed above, by analogy with other members of the actin superfamily, nucleotide-free actin would be expected to be open. However, because of the general instability of nucleotide-free actin (51), no crystal structure of this state has been determined as yet that would confirm this view. Nevertheless, there exists one structure of actin with an open cleft, namely that of the so-called open state of the actin-profilin complex (22). Although this structure contains ATP bound, it has been interpreted by some as the true ADP-actin structure (18, 21). To understand the meaning of this structure, one must first analyze how it was obtained. Similar to all of the other ATP-actin structures, the original actin-profilin structure in which the crystals had been stabilized in ammonium sulfate-containing solution was characterized by a closed nucleotide cleft (11). But when the crystals that produced that closed cleft structure are stabilized in a different solution, containing 1.8 M potassium phosphate, their unit cell parameters change, giving rise to the open-state structure of the actin-profilin complex (22). Therefore, this is not a nucleotide-dependent but rather a solution-dependent conformational change. Moreover, ATP, which in the original closed-state structure occupied a position similar to that in all of the other ATP-actin structures (11), is shifted upwards from this position in the open-state structure (Fig. 2). Such a displacement of the nucleotide has not been observed for any other member of the actin superfamily. Possibly, the nucleotide in the open actin-profilin structure is being competed out of its site by the high concentration of free phosphate ions in the solution. Moreover, as currently modeled, the stereochemistry of the nucleotide and associated divalent cation in this structure (PDB code 1HLU) does not fall within the range of accepted values, suggesting poor definition in the electron density map. The displacement of the nucleotide and divalent cation from their genuine binding sites has the effect of breaking the linkage that they would normally exert between the two major actin domains, thereby allowing the cleft to open (Fig. 2), within the limits allowed by the crystal contacts.

It has been suggested that the two domains are not allowed to open apart in the structure of TMR-modified ADP-actin because of steric hindrance between the TMR probe and actin subdomains 1 and 3 (18). However, that ADP-actin is in a closed conformation is in full agreement with the fact that the nucleotide cleft is also closed in the ADP-bound structures of other members of the actin superfamily including ParM (49) and Hsc70 (52). Moreover, considering that TMR does not make any direct contact with subdomain 3, it is unlikely that it would interfere with the opening of the cleft. Profilin, on the other hand, makes extensive contacts with both subdomains 1 and 3 yet does not prevent the cleft from opening apart when the crystals are transferred into 1.8 M potassium phosphate (22). As it stands, the open-state structure of the actin-profilin complex has far more in common with a nucleotide-free structure than with either an ATP- or ADP-bound actin structure (Fig. 6).

CONCLUSIONS

This work and previous evidence (16) reveal a conformational change in actin upon P1 release, culminating in a disordered loop to ordered α-helix transition in the DNase I-binding loop in subdomain 2. Such a conformational change would affect the monomer-monomer interface in P-actin (33, 34), which may explain why ADP-actin dissociates more readily from the filament than ATP- or ADP-P1-actin. This change could also provide the basis for why certain actin-binding proteins, such as actin-depolymerizing factor/cofilin, specifically target ADP-actin for accelerated dissociation from the filament, thereby regulating treadmilling in the cell (2). It is also concluded that the conformational change upon P1 release does not lead to an open nucleotide cleft in G-actin. Neither does the cleft appear to be open in the actin filament. Indeed, Holmes et al.4 have recently used the structure of ADP-actin (16) to

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4 K. C. Holmes, I. Angert, J. Kull, W. Jahn, and R. R. Schroeder, submitted for publication.
refine a 6-Å resolution model of the actin filament obtained from fiber diffraction. This is the highest resolution structure of F-actin so far available, and it does not reveal an open actin cleft. Because the structures of nucleotide-free Arp2, Arp3 (31), ParM (49), and DnaK (53), as well as nucleotide-free and glucose-free hexokinase (55), all display an open cleft, it is proposed here that nucleotide-free actin will be open as well. Although in vitro nucleotide-free actin is unstable, in the cell this open-cleft state may be stabilized by actin-binding proteins, such as profilin, that promote nucleotide exchange during treadmilling.

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