Actin Structure and Function: What We Still Do Not Understand*

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Emil Reisler‡ and Edward H. Egelman§

From the ‡Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90095 and the §Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, Virginia 22909

Actin is interesting. We state this not just because actin is so plentiful that it is the single most abundant protein in many eukaryotic cells. Actin is interesting not only because it is so highly conserved that between humans and chickens there have been no amino acid changes in the 375 residues present in the skeletal muscle isoform (1). We think that actin remains interesting because after more than 60 years of study (2) fundamental questions about actin structure and dynamics and how these determine function remain unanswered. We will not attempt to summarize what is known about actin in this very brief review. Indeed, a search of PubMed for “actin” retrieves 53,234 publications! Rather, we will focus on a few related issues involving the structure of the actin monomer and polymer and spend as much time discussing what we still do not know about actin as we spend reviewing what we do know.

An Actin-like Superfamily

Actin is found in two main states: G-actin (G for globular) is the monomeric form (Fig. 1), whereas F-actin (F for filamentous) is the helical polymer (Fig. 2). The determination of the first crystal structure for G-actin was a major breakthrough (3), and the concurrent determination of a crystal structure for the ATPase domain of the heat shock protein HSC-70 (4) led to the immediate realization that both of these proteins share the same fold as the enzyme hexokinase (5). All three proteins contain two major domains with a nucleotide bound in the cleft between these domains. The existence of the three structures, combined with the available information that the domains in hexokinase undergo substantial rotations with respect to each other, led to the development of an algorithm to predict other proteins in this superfamily based upon the comparison of crystal structures (6). Subsequent structural work has revealed that this superfamily is indeed vast and includes bacterial (7, 8) and archaeal (9, 10) counterparts of actin as well as enzymes such as propionate kinase (11) that have no common function with actin, but share a common fold and thus presumably share a common ancestral protein. We think that looking at these actin-like proteins can be informative and may tell us something about actin itself.

Of the ~25 actin crystal structures that have been published, only one shows a significant opening of the nucleotide-binding cleft (12). There has been some controversy about whether an opening of the cleft might be associated with the change in the bound nucleotide from ATP to ADP and whether there are other conformational changes within the subunit associated with the change in the bound nucleotide. The reason for interest in this topic is the vast literature showing how actin filament dynamics are regulated by ATP hydrolysis (13) and the observation that within F-actin there is an apparent opening of the cleft associated with ATP hydrolysis and phosphate release (14). The interactions of actin-binding proteins such as profilin and cofilin are regulated by the nucleotide bound to actin, and because these proteins are not expected to directly contact the nucleotide, they must sense the state of actin itself that is a function of the nucleotide bound. For example, the observation that ADF (actin-depolymerizing factor) binds G- or F-actin containing bound ADP with an affinity 2 orders of magnitude higher than it binds G- or F-actin containing bound ATP or ADP-P, (15) may be most simply explained by a conformational exchange within actin between these two nucleotide states. Consistent with this expectation, one study reported that the DNase I-binding loop in subdomain 2 of actin, which is a β-strand in one crystal (3) and disordered in several other crystals (16), becomes an α-helix when ADP rather than ATP is bound to actin (17).

A more recent study has argued that there are no large conformational changes or domain shifts when ATP and ADP are bound to G-actin based upon the comparison of crystal structures for these two states (18). Because solution data from the same work show a very large difference in the subtilisin digestion rates between ADP-G-actin and ATP-G-actin (18), it is possible that crystal packing interactions favor a closed state of G-actin even though in solution the state of the cleft may be shifted by the bound nucleotide. This type of conclusion appeared in another recent actin crystal structure study (19), which showed that cleavage of the DNase I-binding loop introduces no significant change in the crystal structure of G-actin, whereas complementary solution studies suggested that the cleavage is associated with an opening of the nucleotide-binding cleft. An analysis of multiple Arp2 (actin-related protein-2), Arp3, and actin crystal structures with different bound nucleotides also demonstrated that there are “inconsistencies” between the structures observed and simple nucleotide-dependent states (20), suggesting that “alternative conformations have similar energies and are readily interconverted.” Such a possibility is also advanced by a recent molecular dynamics study of nucleotide-dependent states of G-actin, which favors the helix-coil transition in the DNase I-binding loop (21).

A comparison between actin and three actin-like molecules (Fig. 1) shows that although the nucleotide-binding cleft is relatively closed in all actin crystals (the opening that has been

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1 To whom correspondence should be addressed. E-mail: egelman@virginia.edu.
seen in one actin crystal (12) is still small compared with the opening seen in actin-like proteins) in archaeal hexokinase (22), ADP can be bound to only the second major domain when the cleft is wide open (Fig. 1, upper left), so the phosphates are not making any contact with the first domain. More recent results suggest that ATP can be bound to the archaeal hexokinase when the major domains are in the same wide-open conformation. A similar conclusion was reached about Arp2, for which it was shown that the second major domain can bind nucleotide in crystals even though the first major domain is disordered (20). The conclusion from this observation was that most of the binding energy for the nucleotide comes from the interaction with only the second major domain of Arp2. However, it would be expected for all proteins in this superfamily that ATP will be hydrolyzed only when the subunit is relatively closed, so the terminal phosphate will be in contact with the catalytic residues that are expected to be present in the first major domain. At this point, however, we have no detailed picture of the catalytic mechanism.

In a human hexokinase I crystal structure (23), the two domains are more closed, and the β-phosphate within ADP is bound to the first domain (Fig. 1, upper right). The two major domains are indicated by I and II (upper left and right and lower left), whereas in actin, the convention has been mainly to refer to the four subdomains, where the first major domain contains subdomains 1 and 2, and the second major domain contains subdomains 3 and 4 (lower right). ADP (upper left and right) and ATP (lower right) are shown in a red space-filling representation. The CHIMERA software system (69) was used to generate this figure.

What do these observations suggest? First, bacterial ParM cannot polymerize (24) under the conditions used for crystallization (no nucleotide or ADP), and these two conditions lead to very different crystal structures (8). ParM requires ATP or an ATP analog for polymerization. In contrast, actin crystal structures are remarkably similar whether ADP or ATP is present, and actin can polymerize under all of these conditions (with ADP, ATP, or no nucleotide) (25), albeit at different rates and with different critical concentrations for the polymerization. Concluding that G-actin appears to require only small conformational changes to polymerize, which we discuss below, it is possible that much larger conformational changes are associated with ParM binding of ATP, the ligand needed for polymerization to occur. An obvious question, which awaits an answer, is whether such small changes in G-actin, as reported from the crystal structures, can account for the nucleotide-dependent differences in actin affinity for actin-binding proteins.

Although ParM and hexokinase can exist in the absence of bound nucleotide, actin is quite unstable unless a nucleotide is bound (26). A model has been generated suggesting that the main role of the CCT chaperone needed for the proper folding of actin is to bring the two major domains together (27), and in this model, the stability of the molecule is then maintained after folding by the bound nucleotide.
The Structure of the Filament

Under conditions in which actin will crystallize (high protein and salt concentrations), it will also readily polymerize. The symmetry of an actin polymer (≈2.17 subunits/turn of a helix) is incompatible with any crystal space group (which can accommodate only helices with exactly two, three, four, or six subunits/turn), so G-actin has been either complexed with other proteins (such as profilin (28), DNase I (3), and gelsolin (16)) or drugs (such as latrunculin (29, 30), macrolides (31), and pectenotoxins (32)) or changed in various ways (such as by mutation (18) or chemical modification (17)) to prevent polymerization during crystallization. As a result, we have no atomic resolution structure for F-actin, only models. The most important model that has been generated used a rotational and translational search for a placement of the G-actin crystal structure into a helical filament so as to best match the observed x-ray fiber diffraction pattern from an oriented F-actin gel (33). The “Holmes model” for F-actin generated by this procedure contains the explicit assumption that no large scale conformational change is needed between a G-actin monomer and an F-actin protomer. The only change in actin structure that was introduced involved the movement of a hydrophobic loop from the body of the subunit to form a contact with subunits on the opposite long-pitch helical strand.

The absence of large conformational changes between the G-actin monomer and F-actin protomer has been confirmed by a number of low resolution electron microscopic (EM)3 studies (34, 35) as well as by cross-linking studies (36, 37). However, a puzzle has been why the nucleotide hydrolysis rate of actin is activated (38) by a factor of ≈40,000 after the transition from G- to F-actin in the absence of major conformational changes, suggesting that local changes must be taking place in the nucleotide-binding pocket (20).

The experimental evidence for the movement of the hydrophobic loop has been somewhat more complicated. Locking the loop in the parked position by disulfide cross-linking to the body of the actin monomer in a yeast actin mutant prevents filament formation (39). Thus, loop dynamics are essential to filament formation and stability. However, such a cross-linking, which prevents filament formation, can be carried out after polymerization in F-actin, but this causes filament disruption (40, 41). Systematic investigations using yeast actin cysteine mutants of this loop and EPR, cross-linking, and EM approaches (42) and fluorescence methods (43) provided further evidence for the hydrophobic loop equilibrating between parked and (less frequently) extended positions in both G- and F-actin. Thus, the loop cannot be simply considered to be parked in G-actin and extended in F-actin. Loop fluctuations lead to states of intrinsic filament instability, resulting (if trapped by cross-linking or one of the filament-destabilizing factors) in filament disruption (40).

Filament Dynamics

Extending the observations about the dynamics of the hydrophobic loop, there is a large body of data showing that the actin filament does not exist in a single state, but can be quite dynamic. Thus, asking “What is the structure of F-actin?” may be like asking “What is the color of middle C?”; these are category mistakes or ontological errors. We must instead ask what are the different structural states that F-actin can adopt. One of the most remarkable and unusual properties of F-actin is that although subunits have a very fixed axial rise in the filament (of ≈27.3 Å), the rotation between adjacent subunits can be quite variable (44). A high resolution cryo-EM reconstruction of an actin-scrub bundle (45) has shown that individual actin subunits are rotated on average by >10° away from their ideal positions. An actin-binding protein, cofilin, has been shown to change the average twist of subunits in F-actin by ~5°/subunit (46). But rather than imposing a new “twist” on the actin filament, an analysis of pure actin filaments has suggested that cofilin is stabilizing a state of F-actin that can be formed spontaneously in the absence of any other proteins (47). The slow kinetics for the initial binding of cofilin to F-actin were suggested to arise from a slow “breathing” within F-actin (48, 49), and the slow spontaneous change of twist (50) within an actin filament might be the rate-limiting isomerization. Similar slow kinetics for the binding of the peptide phalloidin (which stabilizes actin filaments) to F-actin have been observed (51, 52), suggesting that such isomerizations might impact the binding of a number of drugs and proteins to F-actin.

The breathing motions in F-actin and the dynamics of actin filaments are enhanced in the ADP state compared with the ATP and ADP-P1 states. Experimental evidence links these differences to a considerable weakening of subdomain 2 interactions with the adjacent longitudinal protomer in ADP-F-actin, as indicated by the proteolytic accessibility (53) and angular disorder (14) of subdomain 2 and the decreased thermal stability of F-actin (54). Thus, multiple conformational states of F-actin may originate from the dynamic rearrangements of subdomain 2 and its contacts. Such dynamic states of subdomain 2 and/or crystallization conditions and other factors may have precluded the atomic resolution of its contacts in the crystal structure of a longitudinal dimer (30).

A second dynamic mode within F-actin involves the ability of subunits to undergo a substantial tilt of ~30°. This was first observed within actin filaments being depolymerized by cofilin (47), but was subsequently observed within F-actin filaments in the absence of other proteins (55) as well as in the actin-scrub bundle (45). A crystal structure of actin complexed with the FH2 domain shows an arrangement of actin subunits similar to that in F-actin, except that subunits have been tilted by ~12° and rotated about the filament axis by ~14° away from their position in F-actin (56). However, because the actin-actin contacts within this crystal filament are so tenuous, the relation between the tilt of subunits in the crystal and in free actin filaments is either coincidental or imposed by the FH2 domain and therefore very interesting.

The observation that tilted actin subunits occur within filaments that are destabilized by various means as well as within rapidly polymerized filaments in vitro led to the suggestion that severing and depolymerizing agents such as cofilin (57–59) act by driving a time reversal of normal polymerization (40). When subunits are tilted within the free actin filaments, a substantial...

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3 The abbreviations used are: EM, electron microscopic; FH2, formin homology 2; AMP-PNP, adenosine 5’-(β,γ-imino)triphosphate.
opening of the nucleotide-binding cleft has been seen (55) within the actin protomers, so the subunit conformation is rather similar to that of the much more open hexokinase structures (Fig. 1). Interestingly, a cryo-EM reconstruction of bacterial ParM filaments (60) has shown that the ParM protomer is in a very similar open state to that found in both tilted actin and hexokinase (Fig. 1, upper left panel). Because these ParM filaments were reconstructed with the non-hydrolyzable ATP analog AMP-PNP bound, they provide additional evidence to the observations with Arp2 (20) and hexokinase (22, 61) that nucleotide can be bound only to the second major domain of proteins in this superfamily.

What are the functions of these different modes within F-actin? The many proteins that bind F-actin probably require and modulate these different modes, but this far, only cofilin has been seen to directly modulate one of these internal modes in F-actin (62). That such modes and transitions in F-actin are functionally important is also indicated by the reports that actin modifications, and in particular actin cross-linking, strongly inhibit force generation by actomyosin (63, 64). The observation with Arp2 (20) and hexokinase (Fig. 1, a very similar open state to that found in both tilted actin and hexokinase (22, 61) almost certainly indicates that the transitions among F-actin modes are indeed ideal positions. The exquisite degree of sequence conservation over the past 50 years, no such instrument is on the horizon. Nevertheless, with the more limited tools available to us, we think it likely that some of the most important questions about actin can still be addressed in the coming period. These questions will be answered by higher resolution structures of the actin filament, by spectroscopic and biochemical studies, and by new tools for the mutagenesis of actin (68).

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