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Water Molecules in the Nucleotide Binding Cleft of Actin: Effects on Subunit Conformation and Implications for ATP Hydrolysis

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In the monomeric actin crystal structure, the positions of a highly organized network of waters are clearly visible within the active site. However, the recently proposed models of filamentous actin (F-actin) did not extend to including these waters. Since the water network is important for ATP hydrolysis, information about water position is critical to understanding the increased rate of catalysis upon filament formation. Here, we show that waters in the active site are essential for intersubdomain rotational flexibility and that they organize the active-site structure. Including the crystal structure waters during simulation setup allows us to observe distinct changes in the active-site structure upon the flattening of the actin subunit, as proposed in the Oda model for F-actin. We identify changes in both protein position and water position relative to the phosphate tail that suggest a mechanism for accelerating the rate of nucleotide hydrolysis in F-actin by stabilizing charge on the β-phosphate and by facilitating deprotonation of catalytic water.

Introduction

Actin is a highly conserved protein that is found in large concentrations in eukaryotic cells. It is important to cytoskeletal organization and—in concert with accessory proteins—assembles into complex networks that allow cell motility, cell division, cytokinesis, muscle contraction, and a variety of other critical cellular functions.[1] These networks are dynamic; filaments grow and shrink as actin cycles between monomeric actin (G-actin) and filamentous actin (F-actin). The equilibrium between these two forms of actin appears to be regulated by a variety of actin binding proteins—capping proteins, formins, cofilin, profilin, and the Arp2/3 complex, to name a few.[2,3] In addition, the hydrolysis of the nucleotide bound in the central cleft of the actin molecule and the subsequent release of the cleaved inorganic phosphate appear to act as molecular timers, regulating filament dynamics and polarity of growth.[2] While ATP hydrolysis occurs in both forms of actin, its rate is accelerated by a factor of 10^4 in F-actin.[4] Not only does filament formation affect the rate of hydrolysis but filament properties also are regulated by the state of the nucleotide. ADP-actin filaments are less rigid than ATP-bound filaments, and actin binding proteins bind with different affinities based on the state of the nucleotide.[5,6]

Despite the importance of actin in the cell, there are still many important unresolved questions, including several questions related to nucleotide hydrolysis: neither the mechanism of catalysis in G-actin nor the mechanism by which filament formation accelerates hydrolysis has been fully

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Abbreviations used: F-actin, filamentous actin; G-actin, monomeric actin; SD, subdomain; cryo-EM, cryo-electron microscopy; MD, molecular dynamics; P-loop, phosphate-binding loop.

elucidated. These questions remain despite the fact that structural data on G-actin are plentiful. The first X-ray crystal structure for G-actin was published more than 20 years ago. This structure showed that actin is composed of four subdomains (SDs) arranged around a centrally bound nucleotide,[7] as shown in Fig. S1 of Supplementary Information. Since then, more than 25 structures of the actin monomer have been determined, and the structure of actin has been shown to be remarkably stable.

Actin crystal structures are all based on G-actin and cannot provide information on the conformational changes taking place to accelerate hydrolysis in F-actin; the lack of a high-resolution structure for F-actin is one of the primary factors still limiting our understanding of actin.[8] The development of new methods for determining structural information from lower-resolution experimental data, such as those from cryo-electron microscopy (cryo-EM) and X-ray fiber diffraction, has facilitated the development of several models of F-actin. The first of these models was the Holmes model, which was published at the same time as the first crystal structure for G-actin.[9] In this landmark study, the relative orientation and rotation of the protomer units were determined, but no details on polymerization-induced conformational changes were resolved. Almost 20 years later, refinements in the methods for obtaining structural information from fiber diffraction data led to the publication of an updated model (Oda model) for F-actin in which the primary conformational change upon polymerization was proposed to be a change in the propeller (or dihedral) angle between the four SDs of the actin protomer,[10] as pictured in Fig. S1. Two additional models have been proposed in the last year based on cryo-EM images: the Namba model[11] and the Wakabayashi model,[12] both incorporating an intersubunit propeller twist and additional SD motions. Most recently, a model referred to as the Holmes 2010 model,[13] which maintains the propeller twist of the previous models while minimizing perturbation to the internal stereochemistry of the G-actin structure, has been released.

One limitation to all these methods for obtaining structural information is that, because they are developed from low-resolution data, they do not have direct information about the atomic positions of residues important for catalysis or the atomic positions of the water molecules involved in ATP hydrolysis. This is important because one of the underlying reasons for the interest in the conformational change involved in filament formation is the need to understand how F-actin accelerates hydrolysis by 4 orders of magnitude. Previous studies have indicated that His161 and Gln137 are both important for ATP hydrolysis.[14–16] Based on the available crystal structures, Gln137 is hypothesized to be important in the stabilization of catalytic water, while His161 is believed to act as a catalytic base.[15,17] Several of the F-actin models and molecular dynamics (MD) simulations have suggested that the position of Gln137 changes upon flattening, but details of how this is coupled to catalysis are not fully elucidated.[10,13,18]

Electronic structure calculations have suggested that nucleotide hydrolysis occurs by a dissociative mechanism both in water and in the G-actin active site.[19–21] This suggests that stabilizing the leaving group would affect the barrier to the reaction at least as much as activating the nucleophile. G-actin crystal structures show only one basic residue (Lys18) close enough to stabilize the negative charge on the leaving group in this reaction, the β-phosphate.[15]

MD simulations have been used to better understand filament dynamics, although they seem to be hampered by the lack of a more detailed active-site structure. In two recent articles involving simulation of the actin filament, it has been observed that, during MD, the divalent cation changes its position relative to that seen in the monomeric crystal structure.[13,22] In the first of these articles, the cation moved to associate closely with negatively charged residues in the active site, occupying three distinct positions; this influenced the positioning of not only the divalent cation but also the nucleotide.[22] In a more recent article, the nucleotide tail bent and reoriented to coordinate three oxygen atoms with the divalent cation,[13] instead of the bidentate coordination seen in all monomeric crystal structures—an alteration that would have implications for the mechanisms of hydrolysis and phosphate release. Since experimental data have shown no sign of multiple stable sites for the cation or of a bent phosphate tail in the active site, these shifts are likely unrealistic.

In light of the remaining questions about the mechanism of hydrolysis in both the filament and the monomer, it seems critically important to understand the geometry of the active site, including the water molecules, and how polymerization changes this geometry. In order to investigate the effect of the water structure on the overall stability, structure, and dynamics of actin, we used two distinct methods for setting up MD simulations in the present work. In bulk water simulations, waters from outside the protein were allowed to diffuse in during a fixed preequilibration phase, and the dynamics of the protein based on this random initial water configuration were determined. To the best of our knowledge, this is the method by which all MD simulations of actin filaments have been performed to date. In the second method, ordered water simulations, water molecules were positioned based on the available crystal structures. These waters remained coordinated to the divalent cation