Structure *14*, 87–96, January 2006 DOI 10.1016/j.str.2005.09.013

The Crystal Structure of Murine Coronin-1: A Regulator of Actin Cytoskeletal Dynamics in Lymphocytes

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Summary

Mammalian coronin-1 is preferentially expressed in hematopoietic cells and plays a poorly understood role in the dynamic reorganization of the actin cytoskeleton. Sequence analysis of coronin-1 revealed five WD40 repeats that were predicted to form a β propeller. They are followed by a 130 residue extension and a 30 residue leucine zipper domain that is responsible for multimerization of the protein. Here, we present the crystal structure of murine coronin-1 without the leucine zipper at 1.75 Å resolution. Coronin-1 forms a seven-bladed β propeller composed of the five predicted WD40 repeats and two additional blades that lack any homology to the canonical WD40 motif. The C-terminal extension adopts an extended conformation, packs tightly against the bottom surface of the propeller, and is likely to be required for the structural stability of the propeller. Analysis of charged and conserved surface residues delineate possible binding sites for F-actin on the β propeller.

Introduction

Dynamic reorganization of the actin cytoskeleton is required for many cellular processes that involve movement of membranes or intracellular vesicles. In T lymphocytes, actin remodeling is required for cellular migration in response to chemokines and for the formation of the immunological synapse upon engagement with antigen presenting cells (reviewed in Miletic et al., 2003; Samstag et al., 2003). In addition to the rapid polymerization and depolymerization of individual actin filaments (F-actin), actin remodeling also entails the assembly and breakdown of individual filaments into a higher-ordered, branched network. These processes are driven by a complex array of signaling pathways and involve a number of actin binding proteins. Central to the control of this network is the Arp2/3 complex, which forms the nucleation point between mother and daughter actin filaments (Millard et al., 2004). The Arp2/3 complex is composed of seven subunits, including the two actin-related proteins (Arp2 and 3) (Robinson et al., 2001), that have been proposed to function as a pseudo actin dimer to promote the assembly of a new actin filament. In order to be fully active, the Arp2/3 complex requires several activation steps, including the binding to a nucleation-promoting factor (NPF) and the interaction with the side of the mother filament. One family of NPFs is the WASP (Wiskott-Aldrich syndrome protein) and related WAVE/SCAR proteins. They activate the Arp2/3 complex via their verprolin-homology/cofilin-homology/acidic (VCA) domain (reviewed in Millard et al., 2004). Like the Arp2/3 complex, WASP is maintained in an inactive state with its VCA domain trapped in an autoinhibited conformation. Binding of cdc42 GTPase to WASP results in the release of the VCA domain, which ultimately leads to WASP activation (Kim et al., 2000). In addition to positive signals to control the temporal and spatial activation of Arp2/3 within the cell, negative signals are required. However, while a detailed understanding of the processes that lead to Arp2/3 complex activation has emerged, little is known about factors that mediate the downregulation of the complex.

One class of proteins that are important for the downregulation of the Arp2/3 complex is the coronins (Humphries et al., 2002). Members of the coronin family are F-actin-associated proteins present throughout the eukaryotic kingdom. Some coronins are involved in the inhibition of the Arp2/3 complex, but their exact role in the remodeling of the actin cytoskeleton and intracellular membrane transport is poorly understood (de Hostos, 1999; Okumura et al., 1998; Rybakin and Clemen, 2005). While single-celled organisms typically express one coronin, mammals contain seven paralogs. In the slime mold *Dictyostelium discoideum*, deletion of *coronin* impairs several actin-related processes, including cytokinesis, cellular migration, and phagocytosis (de Hostos, 1999; de Hostos et al., 1993; Maniak et al., 1995). *S. cerevisiae* also express a single coronin gene product, Crn1p (Heil-Chapdelaine et al., 1998), and while *crn1* null mutants display no obvious phenotype, the overexpression of Crn1p disrupts actin organization and is lethal (Goode et al., 1999; Heil-Chapdelaine et al., 1998; Humphries et al., 2002). Crn1p binds directly to the Arp2/3 complex (Humphries et al., 2002; Rodal et al., 2005) and inhibits its activation by members of the WASP family (Humphries et al., 2002). Recent electron microscopy data show that the regulation of the Arp2/3 complex involves conformational changes of the complex and suggest that Crn1p traps the inhibited form of the complex (Rodal et al., 2005).

Mammalian coronin-1 (also known as p57 or TACO [tryptophane aspartate-containing protein]) is preferentially expressed in T lymphocytes and other hematopoietic cells (Ferrari et al., 1999; Nal et al., 2004; Okumura et al., 1998; Suzuki et al., 1995). While the exact relationship between coronin-1 and the actin cytoskeleton remains unknown, coronin-1 has been implicated in phagocytosis (Ferrari et al., 1999; Yan et al., 2005), activation of T lymphocytes (Nal et al., 2004), and integration of extracellular signals to the cytoskeleton (Gatfield et al., 2005). Similar to Crn1p in *S. cerevisiae*, coronin-1 associates with the Arp2/3 complex in neutrophils (Machesky et al., 1997), implying that mammalian coronin-1 has similar roles as *S. cerevisiae* Crn1p and regulates Arp2/3 complex activity. Recently, it was demonstrated that mammalian coronin-2 (also known as coronin-1B) also binds to the Arp2/3 complex (Cai et al., 2005).

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Based on their primary sequence, coronin proteins can be divided into two classes (Rybakin and Clemen, 2005). The first class (the "short" or conventional coronins) includes mammalian coronin-1, *S. cerevisiae* Crn1p, and *D. discoideum* coronin, all of which contain three distinct domains (de Hostos, 1999; Suzuki et al., 1995). The N-terminal domain of these coronins is composed of five WD40 repeats. These repeats are indicative of the "blades" found in β propeller proteins such as the β subunit of G proteins (Lambright et al., 1996; Wall et al., 1995). The N-terminal segment is followed by a less well-conserved variable or unique region, which differs in both sequence and length among the coronins and is predicted to be flexible and have little or no regular secondary structure. This variable extension connects the N-terminal β propeller with a moderately conserved C-terminal coiled-coil domain. Coronin-1 encodes a coiled-coil domain of the leucine zipper variety, which was recently shown to mediate the formation of homotrimeric complexes (Gatfield et al., 2005). The second class of coronins (the "long" coronins) includes mammalian coronin-7 and *C. elegans* POD-1; these coronins contain a duplication of the WD40 repeat region, but they lack the C-terminal coiled-coil region (Rybakin and Clemen, 2005).

Here, we describe the crystal structure of murine coronin-1 without the leucine zipper region at 1.75 Å resolution. The N-terminal domain of coronin-1 forms a seven-bladed β propeller and includes two blades that have no obvious WD40 fingerprint pattern. This domain is followed by a 50 residue C-terminal extension that lacks significant stretches of regular secondary structure, but packs tightly against the bottom face of the propeller. The first 40 residues of the extension are highly conserved and are most likely required for the structural stability of the β propeller domain. The analysis of the molecular surface of coronin-1 suggests regions that may serve as binding sites for F-actin.

Results and Discussion

Structure Determination

Coronin-1 was expressed with a baculovirus expression system with a vector encoding for the full-length protein attached to an N-terminal His_6 tag. During purification of recombinant coronin-1, the expressed protein appeared smaller than its predicted molecular weight. In addition, size exclusion chromatography revealed that the protein was monomeric, even though a homodimeric (Oku et al., 2005) or homotrimeric (Gatfield et al., 2005) protein was expected. Consistent with our gel filtration data, a construct spanning only the first 432 residues of coronin-1 was recently shown to be monomeric in solution (Gatfield et al., 2005); these authors also demonstrated that the leucine zipper motif is both necessary and sufficient for homotrimerization. N-terminal sequencing confirmed the expected sequence of our coronin-1 sample (data not shown); thus, we concluded that the C-terminal leucine zipper motif was proteolytically cleaved from our sample during expression or purification. However, as neither C-terminal sequencing nor mass spectrometry analysis proved to be successful for determination of the exact C terminus, we can only estimate that ~30–50 residues were proteolyzed from the C terminus of full-length coronin-1.

The C-terminally truncated coronin-1 crystallized in space group $P2_12_12_1$ with one molecule per asymmetric unit (Table 1). These crystals diffracted to 1.75 Å, and the structure was solved by multiwavelength anomalous dispersion (MAD) with data collected from a single selenomethionine-labeled coronin-1 crystal. The structure was refined to an R_{cryst} and R_{free} of 15.8% and 19.2%, respectively; the final model has good stereochemistry, with two residues located in the disallowed regions of the Ramachandran plot (Table 1). Two regions of coronin-1 are not defined in the electron density maps; these include residues 1–7 and all residues C-terminal of Arg402. However, as we could not accurately determine the C terminus of the crystallized protein construct, it is unclear whether Arg402 represents the true C terminus or if additional residues are present, but disordered, in the crystals. A second crystal form of coronin-1 was identified in space group $P2_1$ and was solved by molecular replacement with the refined coordinates of the $P2_12_12_1$ structure (Table 1). In this $P2_1$ structure, the first and last visible residues in the electron density are Ser9 and Asp394, respectively; both structures are very similar and superimpose with an rmsd of 0.3 Å. As there are no major differences between the two models, all figures and related discussions refer to the $P2_12_12_1$ structure.

Overall Structure of Coronin-1

The structural analysis reveals that coronin-1 contains an N-terminal β propeller (residues 8–352) followed by an extended C-terminal segment (residues 353–402) (Figure 1). The β propeller includes the predicted WD40 repeats and shares the same topology as the β subunit of G proteins (Lambright et al., 1996; Wall et al., 1995), the prototype of WD40-containing β propellers. The blades within the propeller are arranged in a circular fashion around a central axis and are numbered 1–7; most blades contain a four-stranded antiparallel β sheet. These four strands are designated strands A–D, with the strand closest to the central axis called strand A and strand D forming the edge of the propeller. By convention, each "WD repeat" begins with strand D of the preceding blade before forming strands A, B, and C, which are part of the next blade (Figure 2A). Thus, strand C forms the last β strand of the repeat and contains the sequence motif "WD" at its C terminus. Because of the circular arrangement of the blades, the N-terminal strand of coronin-1 forms strand D of the C-terminal blade, thus closing the propeller and stabilizing the overall structure (Figure 1). Like all β propellers, coronin-1 has a "top" face, which is formed by the loops connecting strand B with C and the loops connecting strand D of one blade with strand A of the following blade. The bottom face is formed by the AB and CD loops (Figure 1). The "top" face of the coronin-1 propeller is solvent exposed, while the "bottom" packs against the C-terminal extension of the protein. The C-terminal extension spans residues 353–402 of coronin-1 (Figures 1 and 2). This segment adopts a mostly extended conformation and spans the entire bottom surface of the propeller to form a short helix (residues 376–380) before crossing back over the funnel in the center of the barrel. In total, 2900 A^2 of solvent-exposed surface is buried at the interface between