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Structure of *Leishmania donovani* coronin coiled coil domain reveals an antiparallel 4 helix bundle with inherent asymmetry

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ABSTRACT

Coiled coils are ubiquitous structural motifs that serve as a platform for protein–protein interactions and play a central role in myriad physiological processes. Though the formation of a coiled coil requires only the presence of suitably spaced hydrophobic residues, sequence specificities have also been associated with specific oligomeric states. R*h*XX*h*E is one such sequence motif, associated with parallel trimers, found in coronins and other proteins. Coronin, present in all eukaryotes, is an actin-associated protein involved in regulating actin turnover. Most eukaryotic coronins possess the R*h*XX*h*E trimerization motif. However, a unique feature of parasitic kinetoplastid coronin is that the positions of R and E are swapped within their coiled coil domain, but were still expected to form trimers. To understand the role of swapped motif in oligomeric specificity, we determined the X-ray crystal structure of *Leishmania donovani* coronin coiled coil domain (*Ld*CoroCC) at 2.2 Å, which surprisingly, reveals an anti-parallel tetramer assembly. Small angle X-ray scattering studies and chemical crosslinking confirm the tetramer in solution and is consistent with the oligomerization observed in the full length protein. Structural analyses reveal that *Ld*CoroCC possesses an inherent asymmetry, in that one of the helices of the bundle is axially shifted with respect to the other three. The analysis also identifies steric reasons that cause this asymmetry. The bundle adapts an extended *a–d–e* core packing, the *e* residue being polar (with an exception) which results in a thermostable bundle with polar and apolar interfaces, unlike the existing *a–d–e* core antiparallel homotetramers with apolar core. Functional implications of the anti-parallel association in kinetoplastids are discussed.

1. Introduction

Coiled coils are self-assembly conferring structural motifs that are present usually as a part of larger proteins or independently (Lupas and Gruber, 2005), and are pivotal to a variety of biological processes (Vita et al., 2015, Burkhard et al., 2001). Formation of a coiled coil is structurally simple and involves suitably spaced hydrophobic residues that are mutually buried in a *knobs into holes* manner. Coronins, first identified in *Dictoseliyum* (de Hostos et al., 1991) and subsequently in other protists, fungi and higher eukaryotes, are multi functional proteins possessing distinct cytoskeleton dependent (de Hostos et al., 1993; Sahasrabuddhe et al., 2009; Cai et al., 2005; Maniak et al., 1995; Ferrari et al., 1999; Chen et al., 2014; Pieters et al., 2013) and independent functions (Jayachandran et al., 2014). Initially classified as long and short forms, a recent phylogenetic study (Eckert et al., 2011) classifies them into four Classes: Class-I and Class-II that are metazoan/-mammalian specific short coronins (400–650 residues), Class-III that are tandem coronin isoforms (1000 residues) and Class-IV, that represent the longest fused coronin isoform (1600–1700 residues). Sequence analysis of these classes indicates that all of them possess a conserved ~350 residue WD40 domain with Class-I/II having a C-terminal coiled coil domain (~30 residues), connected to the WD40 domain by a linker (65–80 residues) having a conserved region and a unique region (Morgan and Fernandez, 2008). The coiled coil domains of Class I and II coronins are primarily responsible for self-association, though alternative roles such as binding to F-actin (Chan et al., 2012; Gandhi et al., 2010) and other

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actin regulating proteins such as Arp2/3 and ADF/Cofilin have also been hypothesized. The sequences of the coiled coil domain of Classes I/II coronins largely contain four heptads with a conserved C-terminal trimerization sequence motif R*h*XX*h*E, *h* being a hydrophobic residue (Kammerer et al., 2005). This motif, observed in a large set of short coiled coil sequences is thought to confer a universal fold. The only coronin coiled coil domain crystal structure available in the literature is from mouse (Kammerer et al., 2005, PDB ID 2AKF) which is a parallel trimer, with the conserved Arg and Glu residues forming cyclic salt bridges.

Most eukaryotes contain at least two different classes of Coronins (humans have six Class-I/II coronins and one Class-III coronin), except for the parasitic apicomplexans and kinetoplastids which possess only a single coronin gene capable of carrying multiple functions (Eckert et al., 2011). Further, the actin cytoskeleton is also different in these parasites. Unlike the long filamentous actin structures (typically 500–1000 nm in length) observed in mammalian actins, these organisms exhibit actin predominantly in the form of unstable short filaments (25–100 nm), that primarily associates as peculiar bundles, patches or granule-like structures (Sahasrabuddhe et al., 2004; Skillman et al., 2013) which can polymerize to long filaments (up to 2000 nm) in *Leishmania*. *Leishmania donovani* coronin (*Ld*Coro: Uniprot ID: Q3T1U8), earlier annotated as CRN12, was first identified co-localizing with filamentous actin structures and led to the formation of higher order structures when overexpressed with actin (Nayak et al., 2005). Additionally, *Ld*Coro plays a significant role in cytokinesis, and has been shown to be involved in microtubule remodeling. During cell division, *Ld*Coro was observed co-distributed with actin at the distal tip of the cell body and mediated cell separation through microtubule binding via kinesin motor protein (K39), and as heterozygous *Ld*Coro gene knockouts displayed bipolar cell morphology defective in microtubule distribution while null mutants do not survive (Sahasrabuddhe et al., 2009). Phylogenetic analysis places *Ld*Coro in a distinct unclassified lineage intermediate to Classes I and II coronins and the sequence analysis identifies the presence of a ~350 residue N-terminal WD domain and a 53 residue coiled coil domain, connected by a ~100 residue linker. The linker in coronins consists of a region (~40 residues) conserved across taxa and a unique region of variable length. While the WD domain is similar to that found in other coronins, there are differences in the linker and the coiled coil domain. The unique region of the linker is longer in kinetoplastida (~70 residues) as is the coiled-coil domain, which is at least one heptad longer. The sequence identity of *Ld*Coro coiled coil domain (*Ld*CoroCC) with other Class-I/II mammalian coiled coil domains is comparably lower (<30%), and crucially lacks the C-terminal trimerization motif R*h*XX*h*E. A swapped version of this motif exists, though not at the C-terminus and it was proposed that the domain would still assemble as a trimer (Eckert et al., 2011). These altered sequence characteristics provide a need for structural characterization of the coiled-coil domain.

2. Results

2.1. Crystal structure LdCoroCC reveals a novel tetrameric assembly with a twisted ade core

The crystal structure of *Ld*CoroCC (53 residues, Fig. 1A) determined by SeMET-SAD phasing, at 2.2 Å resolution shows good stereochemical parameters (Table 1). The asymmetric unit contains 4 monomers, with each monomer forming an α-helix. Two monomers associate as an anti-parallel coiled coil, and the two coiled coils assemble as a tetramer (Fig. 1B). The oligomeric nature of the protein, though not entirely unexpected, is interesting, as it was expected to form parallel trimers, based on sequence analyses (Eckert et al., 2011). Each monomer of *Ld*CoroCC forms a single helix with their C-terminal regions (residues 475–510) involved in the formation of the four-helix bundle and their N-termini diverging. Accordingly, pair-wise superposition of the individual helices for the coiled coil regions result in lower root mean square displacement (RMSD) values, of ~1 Å (main chain atoms) compared to their N-termini, which superimpose with corresponding RMSDs of ~2 Å. The diameter of the four helix bundle measured as diagonal C_α to C_α distance, increases gradually from 8 to 10 Å at the ends, to 12–14 Å towards the central region. Buried surface area calculations with PISA server suggests the tetramer as the most probable oligomer of *Ld*CoroCC, with 7499 Å^2 of the available 12,368 Å^2 surface area buried on tetramerization, compared to dimer (AB/CD) assemblies with buried surface areas of 1863/1839 Å^2 respectively. Lack of contiguous head to tail lattice packing with symmetry related molecules, along the bundle axis, rules out any crystallization artifact and suggests that the tetramer of *Ld*CoroCC observed in the asymmetric unit likely represents a native conformation.

Heptad specific buried surface area calculation of the coiled coil region (475–507) resulted in residues *a*, *d* and *e* to be the most buried residues (out of the heptad residues) with 97%, 80% and 86% of buried area respectively, suggesting *Ld*CoroCC to be an *ade* core bundle (Fig. 1C). Anti-parallel four helix bundles have been classified into *ad*, *ade* and *adg* core designs based on the residues of their heptad register, buried in maximum upon tetramerization (Szczepaniak et al., 2014). Further, *Ld*CoroCC differed from buried surfaces of the canonical *ade* core bundles; Lac repressor (Lewis et al., 1996) and GCN4-pAeLV (Liu et al., 2007), which possess 100%, 86%, 98% and 100%, 94%, 91% of buried surfaces respectively for *a*, *d* and *e* residues. Moreover, at sequence level, the *e* residues in *Ld*CoroCC are considerably larger and are polar (unlike Lac repressor and GCN4-pAeLV which are apolar). The constituting helices of *Ld*CoroCC are axially rotated equivalent or more than the canonical *ade* core bundle axial rotation value (−26°) resulting in a dimer-of-dimer packing at AB and CD dimer interfaces, between hydrophobic *a* and *d* residues (Fig. 1D). Furthermore, the cross sections constructed at the *Ld*CoroCC region; layers; 0 to ±4 suggest a knobs-into-holes packing at AB, CD and AD dimers, unlike at the fourth interface i.e. BC interface where mutually facing side chains adjust locally through side chain torsion rearrangements. Significantly, the residue pairs in between the layers of AB and CD were observed a turn away from each other which can be seen in Fig. 1D, unlike other four helix bundles where in the equivalent residues of diagonal parallel helices are actually the same residue(s).

2.2. Tetramer formation in solution and thermostability

*Ld*CoroCC was further probed using SAXS to investigate if the tetrameric assembly observed in the crystal asymmetric unit is also preserved in solution. SAXS analyses of *Ld*CoroCC performed at a varying concentrations between 2 and 9 mg/ml reveals the following: (i) the experimental SAXS profile agrees better with the calculated profile based on tetramic model (χ = 0.43–0.85) than the dimer (χ = 0.83–1.7) or a trimer, based on the corresponding mouse structure (PDB ID: 2AKF) (χ = 0.63–1.58), (ii) the experimental radius of gyration R_g value from Guinier approximation (assuming rod shaped particles) of ~1 nm (Fig. 2A) is in agreement with the value calculated from the crystal structure using the web tool (Grigoryan and Degrado, 2011) favors the tetramer (calculated radius from crystal structure = 0.75 to 0.8 nm) over trimeric mouse CoroCC (calculated radius from crystal structure = 0.66 nm); Fig. 2C and (iii) the length of the bundle calculated from the distance distribution P(r) function of ~11 nm (Fig. 2B) is in agreement with the crystal structure length of ~9.5 nm, with the unstructured 20