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Crystal structure of the *Candida albicans* Kar3 kinesin motor domain fused to maltose-binding protein

Caroline Delorme, Monika Joshi, John S. Allingham *

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada K7L 3N6

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ABSTRACT

In the human fungal pathogen *Candida albicans*, the Kinesin-14 motor protein Kar3 (*Ca*Kar3) is critical for normal mitotic division, nuclear fusion during mating, and morphogenic transition from the commensal yeast form to the virulent hyphal form. As a first step towards detailed characterization of this motor of potential medical significance, we have crystallized and determined the X-ray structure of the motor domain of *Ca*Kar3 as a maltose-binding protein (MBP) fusion. The structure shows strong conservation of overall motor domain topology to other Kar3 kinesins, but with some prominent differences in one of the motifs that compose the nucleotide-binding pocket and the surface charge distribution. The MBP and Kar3 modules are arranged such that MBP interacts with the Kar3 motor domain core at the same site where the neck linker of conventional kinesins docks during the "ATP state" of the mechanochemical cycle. This site differs from the Kar3 neck–core interface in the recent structure of the *Sc*Kar3-Vik1 heterodimer. The position of MBP is also completely distinct from the Vik1 subunit in this complex. This may suggest that the site of MBP interaction on the *Ca*Kar3 motor domain provides an interface for the neck, or perhaps a partner subunit, at an intermediate state of its motile cycle that has not yet been observed for Kinesin-14 motors.

1. Introduction

Kinesin motor proteins transport intracellular cargos along microtubules (MTs) and influence the organization and dynamics of the microtubule (MT) cytoskeleton. They are typically composed of 4 domains; a motor domain, a neck or neck-linker, an α-helical coiled-coil forming region, and a cargo binding domain [1]. Of these domains, the motor domain is the most highly conserved (~40% shared identity) and is the key element that binds and hydrolyses ATP as part of the mechanochemical cycle responsible for kinesin movement [2,3]. During this cycle, conserved elements of the motor domain couple ATP binding, hydrolysis, and product release to conformational changes in the motor domain core that regulate MT affinity and guide re-orientation of the neck or neck-linker to produce direction-biased displacement of the kinesin and its cargo.

The kinesin superfamily is comprised of 14 different subfamilies, which are differentiated based on sequence, structure, function, and their directionality along MTs [1,4]. The main role of some kinesin families is to mediate proper cell division through their involvement in mitotic spindle assembly and stabilization, as well as chromosome movement [5]. The Kar3 kinesin from the *Saccharomyces cerevisiae* is a well-studied mitotic kinesin, and is designated a member of the Kinesin-14 family due to its C-terminal motor domain and minus-end-directed movement on MTs [6–8]. Like several other members of the Kinesin-14 family, Kar3 has a second MT-binding site located in the cargo binding domain, allowing it to cross-link and slide MTs of opposite polarity past each other via a power-stroke motility mechanism [7,9,10]. This functionality allows it to mediate mitotic spindle organization by generating inward forces that pull the spindles toward the mid-zone and bundle the MTs [11,12]. *S. cerevisiae* Kar3 is unique from most other kinesin isoforms because it interacts with two distinct kinesin-associated proteins (KAPs), named Cik1 and Vik1, which both lack the capacity to bind and hydrolyze ATP and yet can bind MTs and differentially influence the spatial and temporal localization of Kar3 [9,11,13,14]. Moreover, the *Sc*Kar3Cik1 and *Sc*Kar3Vik1 complexes play discrete and important roles during mitotic division, meiosis, and karyogamy [9,11,15–17].

Recent studies have shown that deleting the KAR3 gene in the related diploid ascomycete fungus *C. albicans* interfered with nor-

Abbreviations: E. coli, *Escherichia coli*; MD, motor domain; MT, microtubule; MTs, microtubules; MBP, maltose-binding protein; *Sc*, *Saccharomyces cerevisiae*; *Ca*, *Candida albicans*; KAPs, kinesin-associated proteins; MAPs, microtubule-associated proteins; MTT, maltotetrose.

* Corresponding author. Address: Department of Biomedical and Molecular Sciences, Queen's University, 18 Stuart St., Rm 652, Kingston, ON, Canada K7L 3N6. Fax: +1 613 533 2022.

E-mail address: allinghj@queensu.ca (J.S. Allingham).

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Table 1
Data collection and refinement statistics.

	MBP–CaKar3MD
Space group	$P2_1$
Cell dimensions	
a, b, c (Å)	49.46, 74.93, 98.67
α, β, γ (°)	90.00, 96.44, 90.00
Resolution (Å)[a]	20–2.15 (2.5–2.15)
R_{merge}[a]	8.1 (33.7)
I/I[a]	12.02 (3.83)
Completeness (%)[a]	98.8 (99.4)
Redundancy[a]	3.16 (3.15)
No. observed reflections	122,186
No. reflections used	36,680
R_{work}/R_{free}[b]	16.9/21.1
No. atoms	
Protein	5105
MgADP	28
MTT	45
Water	390
Average B-factors (Å^2)	
Protein	30.46
MgADP	38.47
MTT	31.09
Water	33.6
R.m.s deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.932
Favored	92.0
Allowed	7.3
Generously Allowed	0.7
Outliers	0.0

[a] Data in parentheses represent highest resolution shell.
[b] $R_{factor} = \Sigma||F_{(obs)} - F_{(calc)}||/\Sigma|F_{(obs)}|$, where R_{work} refers to the R_{factor} for the data utilized in the refinement and R_{free} refers to the R_{factor} for 5% of the data that were excluded from the refinement.

mal morphogenesis of mating projections and abrogated nuclear fusion during karyogamy [18]. KAR3 knockout strains also exhibited longer generation times and lower cell viability of mitotically dividing yeast, suggesting that the Kar3 kinesin protein plays an important role in mitosis and mating of *C. albicans*. Given that this commensal fungus can cause debilitating mucosal infections, as well as life-threatening systemic infections [19,20] in times of stress or in immune-compromised individuals, these insights highlight the potential for use of the *C. albicans* Kar3 kinesin as a novel target for antifungal drugs.

To begin building a molecular description of *C. albicans* Kar3, we have solved the X-ray crystal structure of its motor domain as a C-terminal fusion with maltose-binding protein (MBP). This is the first *C. albicans* motor protein structure to be determined and although its overall structure shows considerable homology with previously obtained structures of Kar3 from *S. cerevisiae* [10,21,22], and the filamentous fungus *Ashbya gossypii* [23], a number of unique features of the motor domain are apparent. Also, the close spatial arrangement of the MBP and *Ca*Kar3 motor domain (*Ca*Kar3MD) modules indicates the existence of an interface on *Ca*-Kar3 that may be involved in intramolecular or intermolecular interactions that are new to the Kinesin-14 family.

2. Materials and methods

2.1. Cloning, protein expression, and purification

cDNA for full-length *Ca*Kar3 was amplified from genomic DNA (ATCC number: 10231D-5), inserted into the TOPO cloning vector (Invitrogen), and sequenced to verify its identity. The *Ca*Kar3MD construct (Leu344–Lys687) was amplified by PCR and cloned into pET14d (Novagen) using NcoI and NotI restriction sites for untagged protein expression, or into pMal-MATa1 (Addgene) using HindIII and PstI for expression as a maltose-binding protein fusion [24]. Both vectors were expressed in the BL21-CodonPlus (DE3)-RIL *E. coli* cell line (Stratagene) in Luria–Bertani (LB) media supplemented with the appropriate antibiotics as previously described [23]. The untagged *Ca*Kar3MD construct was purified by ion-exchange chromatography (DEAE SP Sepharose Fast Flow, GE Healthcare) as described previously [25]. For the MBP–*Ca*Kar3MD construct, cells were re-suspended in Column Buffer (10 mM $NaPO_4$ pH 7.2, 200 mM NaCl, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 0.2 mM ATP, 5 mM β-mercaptoethanol and EDTA-free protease inhibitors (Sigma–Aldrich) and lysed on ice by sonication. Soluble protein was recovered by centrifugation at 21,000 rpm for 30 min in a Beckman JA-25.5 rotor, and the supernatant was loaded onto an amylose resin column (New England Biolabs) that had been equilibrated with Column Buffer. After thorough washing with Column Buffer, MBP–*Ca*Kar3MD was eluted with Column Buffer supplemented with 10 mM maltose. Peak fractions containing purified MBP–*Ca*Kar3MD were pooled and further purified by size exclusion chromatography in 20 mM Hepes, pH 7.2, 1 mM $MgCl_2$, 150 mM NaCl, 1 mM DTT, 0.1 mM ATP. For both protein constructs, final peak fractions were pooled and concentrated with Amicon Ultra concentrators (Millipore) and flash frozen in liquid nitrogen for storage at −80 °C.

2.2. Crystallization, data collection and processing, and structure determination

Crystals of the MBP–*Ca*Kar3MD fusion grew by hanging-drop vapor-diffusion at 4 °C after mixing protein (29 mg/ml) supplemented with 2 mM Mg-ATP in a 1:1 volume ratio with a solution of 0.1 M Hepes pH 7.5, 14% PEG 4000, 75 mM NaCl and 5% ethylene glycol. Plate-like crystals appeared after approximately 30 days and were flash-frozen in cyoprotectant comprised of the precipitant solution supplemented with 25% ethylene glycol. X-ray diffraction data were collected from a single frozen crystal on the GM/CA-CAT beam line; station 23 ID/B, at the Argonne National Laboratory (Argonne, IL), and were integrated and scaled with the program XDS [26]. The structure of MBP–*Ca*Kar3MD was solved by molecular replacement using coordinates for *Sc*Kar3MD (PDB: 3KAR) [21] and MBP (PDB: 1MH3) [24] as search models using AutoMR [27]. A series of manual building cycles using *Coot* [28,29] and iterative restrained refinement cycles using *Phenix_Refine* [30] were performed to generate the final model. Data collection and refinement statistics are summarized in Table 1. Coordinates and structure factors have been deposited in the Protein Data Bank with accession number 4H1G.

2.3. Microtubule-binding assay

The affinity of *Ca*Kar3MD and MBP–*Ca*Kar3MD for microtubules was determined as described previously [31], with the following modifications. Reactions of 100 µl microtubules (0–6 µM) were incubated with 4 µM kinesin and 2 mM MgAMPPNP for 15 min at room temperature in ATPase Buffer (20 mM HEPES, 5 mM magnesium acetate, 0.1 mM EGTA, 0.1 mM EDTA, 25 mM potassium acetate, 1 mM DTT, 40 µM Taxol, pH 7.2). Reaction mixtures were sedimented by centrifugation at 312,530×*g* in a TLA100 rotor for 15 min at 25 °C. Supernatant and pellet fractions were analyzed by SDS–PAGE and visualized with Coomassie Brilliant Blue R-250.

3. Results and discussion

3.1. Overall structure of the CaKar3 motor domain

To date, numerous kinesin motor domain structures, as well as structures of multi-subunit kinesin motor complexes, have been