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Crystal structure of WA352 provides insight into cytoplasmic male sterility in rice

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ABSTRACT

Plant cytoplasmic male sterility (CMS) is an important phenomenon and is widely utilized in hybrid crop breeding. The Wild Abortive CMS (CMS-WA), a well-known CMS type, has been successfully applied in the commercial production of hybrid rice seeds for more than 40 years. The CMS-WA causal gene WA352 encodes a novel transmenbrane protein and the interacts with the mitochondrial copper chaperone COX11, triggering reactive oxygen species production and resulting in male sterility in CMS-WA lines. However, the structure of WA352 is currently unknown, and the structural mechanism whereby WA352 perturbs COX11 function to cause CMS remains largely unknown. Here, we report the crystal structure of the C-terminal functional domain of WA352 at 1.3 Å resolution. This functional domain, consisting of five α helices, is spindle-shaped with a length of 42 Å, and a diameter of 28 Å. Notably, the absence of any structural similarity to a known protein structure suggests that the WA352 functional domain is a novel fold. In addition, surface conservation analysis and structural modeling of the WA352-COX11 complex revealed details about the WA352-COX11 interaction. Further structural analysis suggested that the WA352-COX11 interaction blocks the copper ion transportation activity of COX11, which is essential for the assembly of cytochrome c oxidase, resulting in male sterility in CMS-WA lines. Our study paves the way toward structural determination of the WA352-COX11 complex and provides new insight into the mechanism of plant CMS.

1. Introduction

Cytoplasmic male sterility (CMS) has been widely identified in higher plants, resulting from defects in mitochondrial function [1]. CMS is an important model system for studying the mitochondrial-nuclear interaction and plays a major role in hybrid crop breeding. Hybrid rice has been planted in approximately 40 countries and makes a notable contribution to global food production [2]. Several types of rice (*Oryza sativa L.*) CMS systems, including the Wild Abortive (CMS-WA) [3], Boro II (CMS-BT) [4], and HongLian (CMS-HL) [5], have been used for the commercial production of hybrid seeds thus far. The CMS-WA system has been the most widely utilized over the past 40 years and has had a great impact on modern agriculture [6]. In a long-term effort to identify the male sterile gene, *WA352* has been validated as the causal gene of CMS-WA, which is an ancient product of complex rearrangements in the mitochondrial genomes of the wild rice species [3,7].

Compared with known CMS proteins, WA352, consisting of 352 amino acids, has been shown to exhibit a distinct domain structure [3]. The N terminus of WA352 is anchored to the inner mitochondrial membrane by three transmembrane segments, and the soluble C-terminal region, which shows no similarity to the domains of any known functional mitochondrial proteins, is the functional domain causing CMS [3]. WA352 accumulates specifically in the anther tapetal cells of microspore mother cell stage, and interacts with COX11 of rice (OsCOX11) and disturb its function [3]. The deleterious interaction between WA352 and OsCOX11 triggers a reactive oxygen species (ROS) signal network in tapetum, resulting

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in cytochrome c release and premature programmed cell death, leading to defective pollen development and CMS [3,8]. Transgenic analysis with truncated WA352 structures encoding the C terminal of WA352 that interact with COX11 has clearly indicated that the soluble C-terminus of WA352 is essential and sufficient for inducing CMS [3]. However, due to the absence of sequence similarity between WA352 and known protein domains, it is difficult to generate an accurate model of the structure of WA352. Hence, no structure is currently available for WA352.

COX11, which is essential for cytochrome c oxidase assembly, is a highly conserved protein in all eukaryotes [9]. The N terminus of COX11 forms a single transmembrane helix anchored in the inner mitochondrial membrane, while the soluble C-terminal domain is located in the mitochondrial intermembrane space. COX11 is a copper-binding protein and primarily functions in receiving copper ions from COX17 and delivering copper ions to the Cu_B site of COX1, which is the catalytic core subunit of cytochrome c oxidase [10]. COX11 functions as a dimer with a stoichiometry of one copper ion per monomer [9,11,12]. Modeling and X-ray absorption spectroscopy analyses have shown that the COX11 dimer is composed of two antiparallel monomers, with two copper ions bound by the adjacent CFCF motifs at the dimer interface [11]. The COX11 dimer has been well characterized in the copper ion transport process. However, the structural mechanism by which WA352 perturbs COX11 function to cause CMS requires further investigation.

In the present study, through the combination of limited proteolysis and yeast two-hybrid assays, we identified the structural and functional domains of WA352. Furthermore, we determined the crystal structure of the C-terminal functional domain of WA352 at 1.3 Å resolution (Table 1). Structural analysis revealed that WA352 C-terminus exhibits a novel fold. Importantly, surface conservation analysis and structural modeling of the WA352-OsCOX11 complex revealed details about the WA352-OsCOX11 interaction. The superposition of our model with the COX11 dimer suggests that WA352 blocks the copper ion transportation activity of COX11 and causes CMS. Our study provides a new insight into the structural mechanism of CMS-causal factor in plant male sterility.

Table 1
Data collection and refinement statistics for the WA352 structure.

	Se-SAD	Native (5ZT3)
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	33.56, 34.96, 95.71	33.85, 35.44, 95.52
α, β, γ(°)	90,90,90	90,90,90
Resolution (Å)	45–1.97 (2.04–1.97)	45–1.30 (1.33–1.30)
R_{merge}	8.3(12.8)	8.2(83.8)
$I/\sigma(I)$	24.5(21.3)	12.1(2.4)
Completeness (%)	99.8(99.7)	98.4(94.2)
Redundancy	9.3(9.5)	6.8(6.7)
Refinement		
No. reflections		28,374
R_{work}/R_{free}		18.77/19.32
No. atoms		
Protein		922
Ligand/ion		0
Water		132
B-factors		
Protein		20.4
Ligand/ion		37.9
Water		43.1
R.m.s deviations		
Bond lengths (Å)		0.005
Bond angles (°)		0.735

2. Materials and methods

2.1. WA352 protein expression and purification

The full-length of *WA352* (GenBank Acc. JX131325.1) was used as a template. Expression constructs were subsequently cloned into a modified pET15b vector (Novagen), in which was attached with a 6 × His tag following a drICE protease cleavage site at the N-terminus. The fusion proteins were expressed in the *Escherichia coli* cell strain BL21 (DE3). The cells were cultured in lysogeny broth medium at 37 °C until OD_{600} approached 1.0. The temperature was then shifted to 16 °C, and the cells were induced by 0.2 mM isopropyl β-D-1-thiogalactopyranoside for 16 h. Next, the cells were harvested by centrifugation, resuspended in lysis buffer containing 25 mM Tris-HCl, pH 8.0, and 150 mM NaCl, and lysed by in a JN-02 homogenizer. The insoluble pellets were removed by centrifugation at 23,000 g for 1 h. The supernatant was subsequently loaded onto a Ni-NTA column pre-equilibrated in lysis buffer (Ni-NTA, Qiagen). Then the column was washed with lysis buffer supplemented with 15 mM imidazole. The fusion protein was eluted with buffer containing 25 mM Tris-HCl, pH 8.0, and 250 mM imidazole [13]. The recombinant protein was further fractionated using anion-exchange chromatography (Source 15Q, GE Healthcare). The relevant fractions were concentrated to approximately 20 mg ml^{-1} (Amicon, 10 kDa cutoff, Millipore), and the His tag was removed by drICE cleavage during concentration. Thereafter, the protein sample was subjected to size-exclusion chromatography (Superdex-200 10/300, GE Healthcare). The lysis buffer plus 5 mM dithiothreitol (DTT) was used for size-exclusion chromatography. For selenomethionine (SeMet) derivative protein, the cells were grown in medium from the Seleno Met™ Medium Base* (MD 12–501 Kit). After induction, the medium was supplemented with 50 mg l^{-1} selenomethionine. The SeMet-labeled protein was purified by the same method as described above.

2.2. Crystallization

Crystallization was performed using the hanging drop vapor diffusion method at 18 °C. Crystals of WA352 (218–345) were grown from the drops consisting of 1.4 μl of protein solution with an equal volume of the reservoir solution containing 100 mM Tris base/Hydrochloric acid pH 7.0, 200 mM Calcium acetate, and 20% (w/v) PEG 3000. All reagents were purchased from Sigma-Aldrich inc. After optimized, the crystals were collected after several days. The SeMet-labeled WA352 crystals were grown under the same conditions as the native WA352 crystals. The crystals were flash frozen in cryoprotectant made of the reservoir solution supplemented with 15% glycerol.

2.3. Data collection and structural determination

Native and anomalous diffraction data were collected at the Shanghai Synchrotron Radiation Facility (SSRF) on a beamline BL17U. All datasets were collected at 100 K and processed using the HKL2000 program suite and XDS packages [14]. Further processing was performed using programs from the CCP4 suite [15]. The WA352 structure was solved by a single-wavelength anomalous dispersion (SAD) method using Phenix [16]. The structure model of WA352 was manually built using Coot [17] and refined using Phenix. The structure refinement statistics and the quality of the final structure model are summarized in Table 1. All figures representing the structures were generated by PyMOL (PyMOL Molecular Graphics System, Schrödinger, Inc.).