Resonance assignments of the ORC2-WH domain of the human ORC protein

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Abstract
ORC2 is a small subunit of the origin recognition complex (ORC), which is important for gene replication. The ORC2 WH domain recognizes dsDNA sequences with its flexible β-sheet hairpins as anchors. Here, we report near-complete NMR backbone and side chain resonance assignments of the WH domain and study the backbone relaxation of the WH domain. These studies will contribute to further understanding of the structure–function relationship of the ORC protein.

Keywords ORC · ORC2 WH domain · Gene replication · Resonance assignment

Biological context
DNA replication plays an important role in the maintenance of genomic stability (Bell et al. 2002). The origin recognition complex (ORC) is required for the initiation of DNA replication during the cell cycle (Tsurimoto et al. 1990). The ORC has six domains, ORC1-5 each contain an AAA + type ATP domain and a C-terminal wing-helix domain (WHD), while ORC6 contains a transcription factor IIB (TFIIB) domain and a carboxy-terminal domain (Aparicio et al. 1997; Bell et al. 1993). ORC2 is the central component in ORC and is localized to centrosomes, which may function in establishing heterochromatin. ORC2 has been demonstrated to interact with ORC3, (Prasanth et al. 2004). The ORC2-ORC3 structure is also preserved in humans, as confirmed by the recent low-resolution cryo-electron microscopy discovery of human ORC2-ORC3 heterodimers (Tocilj et al. 2017). The ORC2 WH domain recognizes dsDNA sequences with its flexible β-sheet hairpins as anchors, while other proteins have different orientations in different species (Franziska et al. 2015; Zhang et al. 2018). Molecular mechanisms facilitate DNA site access by depolymerizing dsDNA and require the involvement of ATP.

To continue to study the biological significance of ORC2, chemical shift identification was carried out for the WH domain of ORC2. The chemical shift assignments will be useful for future studies. Here we report the backbone and sidechain assignment of the WH domain of ORC2, with > 95% of resonances in the WH domain assigned unambiguously.

Methods and experiments
The DNA fragment encoding the WH domain (residues 458–577) of ORC2 was obtained by PCR from Homo sapiens brain genomic DNA and cloned into the vector pET28A. The recombinant vector was then transformed into Escherichia coli strain BL21 (DE3). For uniform 13C- and 15 N-labeled recombinant WH domain, cells were cultured at 37 °C in LB minimal medium (containing 2.5 g/l 13C-glucose and 0.5 g/l 15NH4Cl as the sole carbon and nitrogen source, respectively) to an OD600 of 1.0, and induced with 1 mM IPTG. They were then incubated at 16 °C for 24 h. The induced cells were harvested by centrifugation at 5,000 g for 10 min, and resuspended in 35 ml Tris–NaCl buffer (10 mM Tris, 1 M NaCl, 1 mM DTT, pH 7.5). E. coli were lysed by high pressure and centrifuged at 12,000 rpm for 30 min at 4 °C. After centrifugation, the supernatant was purified with
Ni-NTA resin. The target protein was cleaved with TEV protease. After the tag was cut off, the solution was flowed through the Ni-NTA resin filled column (QIAGEN) again, to separate the His-tag from the target protein.

The flowthrough was further purified by Superdex 75 (GE Healthcare) in a buffer containing 10 mM Tris, pH 7.5, 1 mM DTT and 150 mM NaCl. Then, a single target protein component was collected through the UV absorption peak, and concentrated to ~20 mg/ml to collect the NMR signal, and the excess protein was frozen at -80°C. The final NMR buffer was 20 mM phosphate (pH 7.0), and 50 mM NaCl in 10:90% D₂O: H₂O.

All NMR experiments were recorded at 298 K on a Bruker AvanceIII 600 spectrometer equipped with a cryo-probe. The following spectra were recorded to obtain backbone and side chain resonance assignments: ¹H-¹⁵N HSQC, ¹H-¹³C HSQC, CBCA(CO)NH, CBCANH, HNCA, HN(CO)CA, HNCO, HN(CA)CO, H(CCO)NH-TOCSY, HBHA(CO)NH-TOCSY, (H)C(CO)NH-TOCSY, HCCH-COSY and HCCH-TOCSY. NMR data processing was achieved using NMRpipe and NMRDraw software (Delaglio et al. 1995), and then analyzed with Sparky3 (Goddard et al. 1993).

¹⁵N relaxation measurements were also performed on the Bruker AvanceIII 600 spectrometer using a ¹⁵N-labeled WH domain of ORC2 sample with a concentration of 0.6 mM at 298 K. The ¹H-¹⁵N heteronuclear NOE experiment was recorded in an interleaved fashion, alternately with and without proton presaturation in the recovery delay. The proton saturation time, D₁ was set to 5 s. ¹⁵N longitudinal relaxation times (T₁) and transverse relaxation times (T₂) were derived from eight spectra with different values for the relaxation delay (11, 61, 142, 243, 362, 523, 753, and 1147 ms) and seven relaxation delays (0, 17.6, 35.2, 52.8, 70.4, 105.6, and 140.8 ms), respectively. T₁ and T₂ values were extracted and fitted using a curve-fitting subroutine included in the program Sparky3 (Goddard et al. 1993).

Extent of assignment and data deposition

Almost all of residues of the WH domain were identified: in the backbone, 97% of NH and ¹⁵N, 100% of ¹³C, and 90% of ¹H chemical shifts were assigned; in the side chain, 96% of the aliphatic and 95% of the aromatic ¹H-¹³C nuclei were assigned. The ¹H-¹⁵N HSQC spectrum for the WH domain is shown in Fig. 1.

The residues in the conserved motif WH of ORC2 were well assigned. The datasets from the CBCANH, CBCA(CO)NH, (H)C(CO)NH-TOCSY, HCCH-COSY and HCCH-TOCSY spectra contained the ¹³C signals for the proline residues. The resonances of all four proline residues were identified. The consensus chemical shift index derived from the ¹Hα, ¹³Ca, ¹³Cβ, ¹³Cγ and ¹⁵N chemical shifts (Wishart et al.1994) is shown in Fig. 2. The bands below are the analytical structure of the crystal (PDB code: 5C8H) (Zhang et al. 2019). These data indicated the presence of four β-strands and six α-helices. The chemical shift data have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession number 27643.

Dynamic properties of the WH domain

The dynamic characteristics of the WH domain in solution were studied by measuring the relaxation time of the WH domain in the free state at T₁, T₂ and the steady state ¹H-¹⁵N NOE. The average values of T₁ (Fig. 3A), T₂ (Fig. 3B), and ¹H-¹⁵N NOE for positive values (Fig. 3C) of WH were 0.68 ± 0.02 s, 0.09 ± 0.002 s, and 0.74, respectively. The average ¹H-¹⁵N NOE value is 0.74, indicating that most of the regions of WH are relatively rigid, corresponding well to the narrow distribution of conformers in the calculated ensemble. Together, the experimental results show that the WH domain in solution is a structured protein with a regular backbone fold. The overall rotational correlation time (τc) of the ~15.2 kDa WH domain is 7.5 ns (as estimated from the average values of T₁/T₂), suggesting that the WH domain is mainly monomeric in solution.
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Author contributions  Jiahai Zhang and Xiaoming Tu gave the project ideas. Jiahai Zhang helped Lanlan Song identify protein chemical shifts and process data.

Data availability  Some or all data, models, or code that support the findings of this manuscript are available from the corresponding author upon reasonable request.

Declarations

Competing interests  The authors declare no competing interests.

Conflict of interest  We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the manuscript submitted.

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