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ARTICLE

Backbone and sidechain NMR assignments of residues 1–81 from yeast Sis1 in complex with an Hsp70 C-terminal EEVD peptide

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Abstract

Molecular chaperones aid proteins to fold and assemble without modifying their final structure, requiring, in several folding processes, the interplay between members of the Hsp70 and Hsp40 families. Here, we report the NMR chemical shift assignments for ^{1}H, ^{15}N, and ^{13}C nuclei of the backbone and side chains of the J-domain of the class B Hsp40 from *Saccharomyces cerevisiae*, Sis1, complexed with the C-terminal EEVD motif of Hsp70. The data revealed information on the structure and backbone dynamics that add significantly to the understanding of the J-domain-Hsp70-EEVD mechanism of interaction.

Keywords Sis1 · Hsp40 · J-domain · Hsp70

Biological context

Molecular chaperones play a central role in protein homeostasis, including assistance in macromolecular complex assembly, protein transport, aggregate dissociation and refolding of stress-denatured proteins, and targeting misfolded proteins for proteolytic degradation (O. Tiroli-Cepeda & H.I. Ramos, 2011). The Hsp70 (70 kDa heat shock protein) family is ubiquitous and participates in all of the biological processes mentioned above (Hartl 1996; Kim et al. 2013; O. Tiroli-Cepeda & H.I. Ramos, 2011). As a matter of fact, the proteostasis process depends on the functional interaction between Hsp70 and Hsp40 (Liu et al. 2020). Co-chaperones from the Hsp40 family (also named J-proteins) are characterized by the presence of the J-domain that is essential for the stimulation of the ATPase activity of Hsp70. Besides that, Hsp40s recognize and bind to unfolded or partially folded polypeptides and deliver them to Hsp70 (Kampinga et al. 2019; Pinheiro et al. 2019; Summers et al. 2009). Hsp70s have a conserved EEVD tetrapeptide at the C-terminus, which is involved in interacting with Hsp40s (Freeman et al. 1995; Yu et al. 2015).

Sis1, a class B Hsp40 from yeast *Saccharomyces cerevisiae*, binds the EEVD motif, while the class I (Ydj1) does not (Borges et al. 2012; Li et al. 2006; Yu et al. 2015), such that Sis1-EEVD interaction is required for in vitro protein refolding (Yu et al. 2015). However, the details of the interaction between EEVD motif and the J-domain remain to be understood. Here we describe the assignments of the backbone and sidechain of the J-domain (residues 1 to 81 and named $Sis1_{1-81}$) of Sis1 from *S. cerevisiae* in complex with the Hsp70 C-terminal EEVD motif. The results add significantly to the understanding of J-domain-Hsp70-EEVD mechanism of interaction.

Methods and experiments

Protein expression and purification were carried out as previously reported (Pinheiro et al. 2019). For isotopic labeling, M9 minimal medium was supplemented with ^{15}N ammonium chloride (1 g/L) and ^{13}C glucose (3 g/L) as the sole nitrogen and carbon sources.The octapeptide

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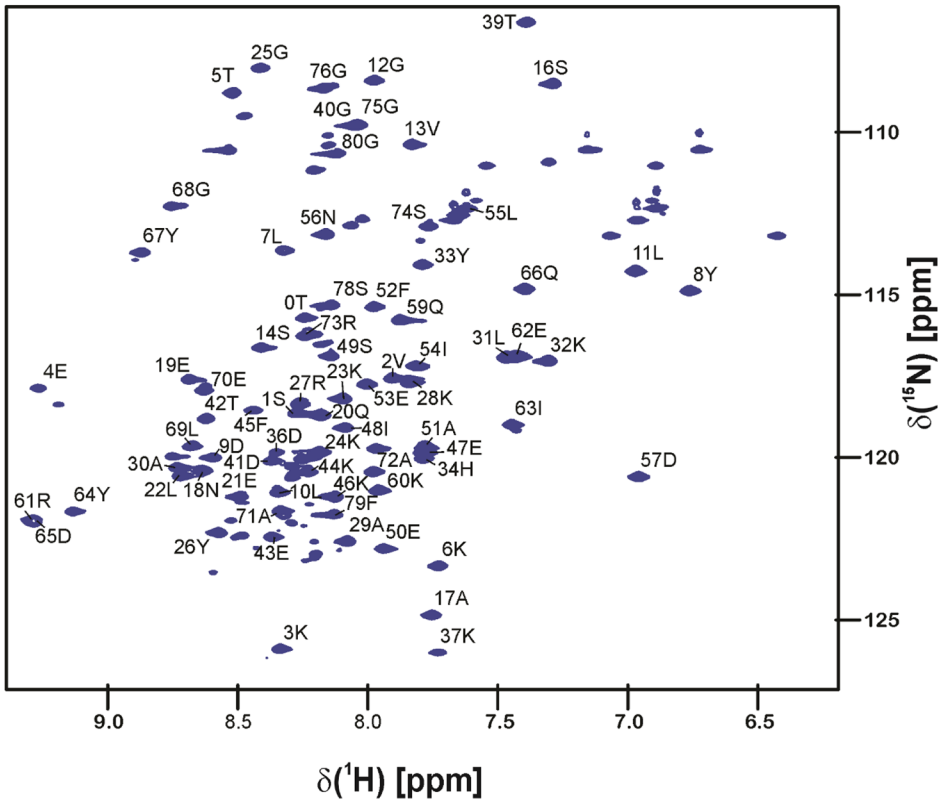

Fig. 1 The ^{1}H-^{15}N HSQC spectrum of Sis1$_{1-81}$:EEVD where each peak is labeled with its residue assignment

GPTIEEVD referring to the C-terminal tail of Hsp70 was synthesized and purified by GenOne Biotechnologies (Rio de Janeiro, Brazil). All NMR spectra were recorded in 25 mM sodium phosphate buffer (pH 7.5), 200 mM NaCl and 10% D_2O supplemented with 250 µM PMSF, 5 mM sodium azide, and 2 mM EDTA to improve protein stability and avoid degradation. The concentrations of Sis1$_{1-81}$ and EEVD-peptide used in the data collection were 1 mM and 4 mM, respectively.

NMR spectra were recorded on a Bruker Avance III HD 900 MHz spectrometer equipped with an inverse-detection triple resonance z-gradient TXI probe. All experiments were performed at 298 K. Resonance assignments for backbone were obtained from the following experiments: 2D [^{1}H,^{15}N] HSQC, 3D HNCO, 3D HNCA, 3D HNCACB, 3D CBCA(CO)NH and 3D HBHA(CO)NH (Gal et al. 2011; Grzesiek and Bax 1993; Ikura et al. 1990; Wittekind and Mueller 1993). To assign the aliphatic sidechain, 2D [^{1}H,^{13}C] HSQC, 3D (H)CCH-TOCSY, 3D H(C)CH-TOCSY, ^{15}N and ^{13}C-edited NOESY-HSQC (for both aliphatic and aromatic regions) experiments (Kay et al. 1993; Logan et al. 1992; Sattler 1999). NOE distance restraints obtained from ^{15}N- and ^{13}C-edited NOESY spectra were acquired with a mixing time of 100 ms. Triple-resonance experiments were achieved using non-uniform sampling (NUS), with sampling rates between 8 and 20%. 2D [^{1}H-^{15}N] HSQC spectra were acquired before and after each 3D experiment to confirm the stability of the protein sample. NMR data wereprocessed with NMRpipe (Delaglio et al. 1995) and analyzed with CcpNmr Analysis (Vranken et al. 2005) available on the NMRbox platform (Maciejewski et al. 2017).

Extent of assignments and data deposition

The Sis1$_{1-81}$:EEVD assigned backbone amide peaks are shown in the 2D [^{1}H-^{15}N] HSQC spectrum in Fig. 1 and refer to 100% of all possible amide H and amide N atoms (excluding the six prolines), 100% of all Cα atoms, 98.7% of all Cβ atoms and 91.6% of all CO atoms. At the end of the experiment, 96.4% of the backbone atoms were assigned. Considering all sidechain atoms, about 78.2% were assigned (77.5% of ^{13}C and 78.6% of ^{1}H). The chemical shift data is available at the Biological Magnetic Resonance Bank (https://www.bmrb.wisc.edu) and has the accession number 51,187. Note that there are unassigned minor peaks in the 2D [^{1}H-^{15}N] HSQC, possibly due to conformational exchange.

The order parameter (S^2) and the secondary structures were predicted from the ensemble of backbone chemical shifts (^{13}Cα, ^{13}Cβ, ^{13}CO, ^{15}N and ^{1}HN) of Sis1$_{1-81}$, both in the free and in the bound-state, Sis1$_{1-81}$:EEVD, using TALOS-N (Berjanskii and Wishart 2005; Shen and

Bax 2013), shown in Fig. 2. S^2 value is an indicator of flexibility and its analysis indicated that the bound state, $Sis1_{1-81}$:EEVD, had higher flexibility between residues 32 and 40 when compared to the free state (Fig. 2a). This loop contains the HSP70 interaction 34-HPD-36 motif. The decrease in S^2 correlates with a significant change in $^{13}C\alpha$, $^{13}C_b$ and ^{13}CO chemical shifts for this region in the bound state. The subtle change in helical propensity for residues K37 and P38 was not explained by the chemical shift changes. Secondary structure analysis indicated that five α-helices (α1 6–11, α2 19–33, α3 42–56, α4 58–66, and α5 69–74) were predicted with high confidence and that there is no evidence of any β-strand conformation in the protein (Fig. 2b,c). The difference in predicted secondary structure propensities was identified between free $Sis1_{1-81}$ and $Sis1_{1-81}$:EEVD, but they are small and mainly located at the first helix (residues 6–11). These data will enable us to characterize mechanistic details of the $Sis1_{1-81}$:EEVD interaction in future studies.

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Author contributions Data collection, analysis, and interpretation: COM, FCLA. Data analysis and interpretation and design of the work: COM, GMSP, CHIR.; FCLA. This work results from the collaboration of two research groups: Laboratory of Biochemistry of the Chaperome (PI CHIR) and Biomolecular NMR Laboratory (PI FCLA). All authors: drafted and critically reviewed the manuscript.

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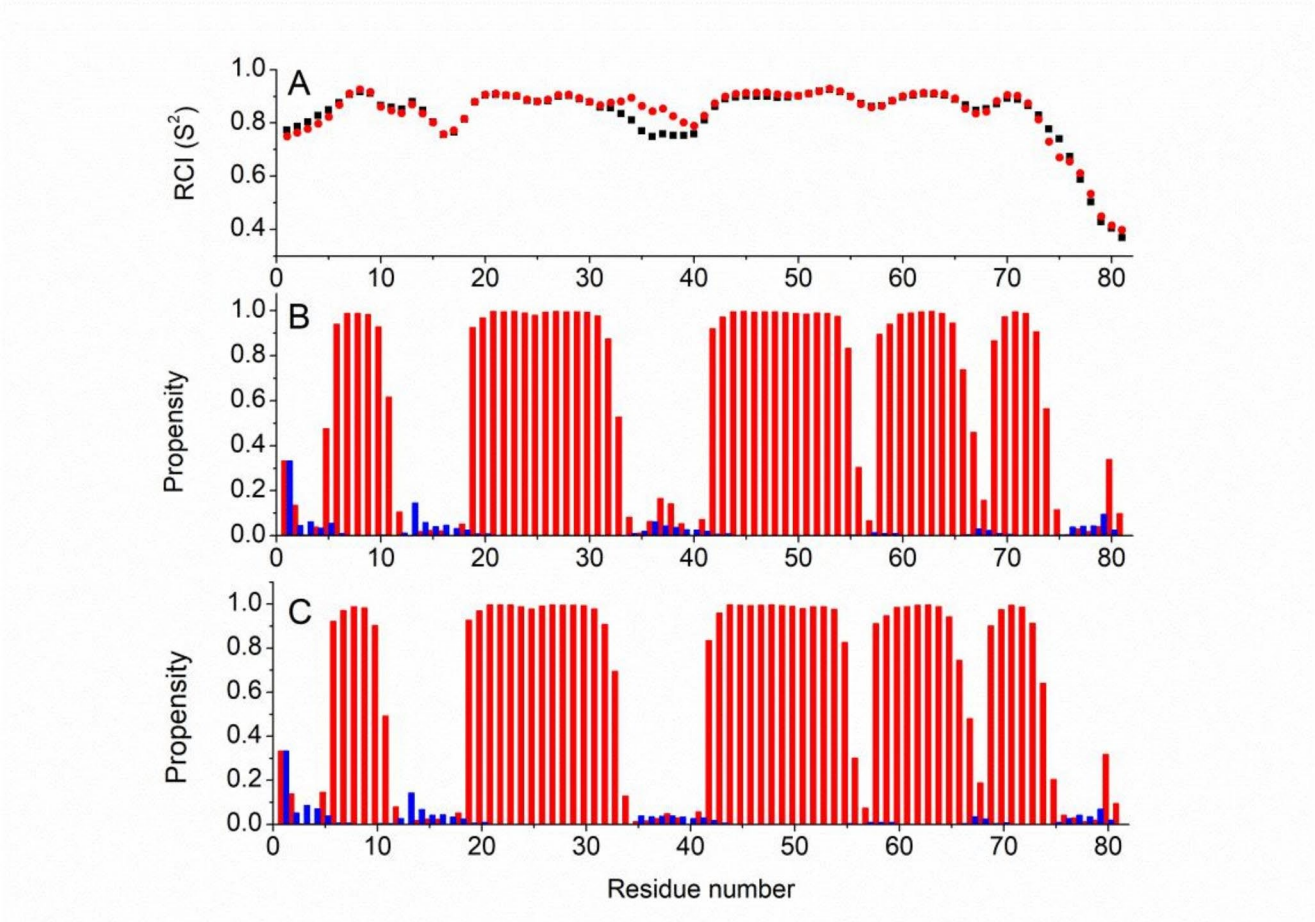

Fig. 2 Protein dynamics and secondary structure predictions. **a** Random-coil index (RCI) order parameter (S^2) as a function of the residue number for free $Sis1_{1-81}$ (red) and $Sis1_{1-81}$:EEVD (black). **b** Talos-N secondary structure prediction of free $Sis1_{1-81}$ as a function of residue number. **c** Talos-N secondary structure prediction of $Sis1_{1-81}$:EEVD as a function of residue number. Red, predicted probabilities for helix; blue, predicted probabilities for extended structure

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Data Availability The backbone assignments of $Sis1_{1-81}$:EEVD have been deposited in the Biological Magnetic Resonance Bank (https://www.bmrb.wisc.edu) under the accession number 51,187.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors have agreed to the publication of the manuscript.

Competing interests The authors declare that they have no conflicts of interest with the contents of this article.

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