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^1H , ^{13}C , and ^{15}N backbone and sidechain chemical shift assignments for the HEAT2 domain of human eIF4GI

Katherine A. Edmonds and Gerhard Wagner

Department of Biological Chemistry and Molecular, Pharmacology, Harvard Medical School, Boston, MA 02115, USA

Gerhard Wagner: gerhard_wagner@hms.harvard.edu

Abstract

The translation initiation factor eIF4G is required for the translation of many eukaryotic messenger RNAs. Its interaction with the ATP-dependent RNA helicase eIF4A plays an important role in the regulation of translation initiation. eIF4G in humans and other higher eukaryotes contains three HEAT domains, of which HEAT1 and HEAT2 contain binding sites for eIF4A. Here we report the near complete NMR resonance assignment of the 192-residue HEAT2 domain of the human translation initiation factor eIF4GI. The chemical shift data constitute the basis for NMR structural studies aimed at expanding understanding of the role of interactions between the initiation factor eIF4A and eIF4G in translation initiation.

Keywords

Translation initiation; Human eIF4G; NMR assignment

Biological context

Most eukaryotic messenger RNAs (mRNAs) require the initiation factor eIF4F for their translation. eIF4F is a heterotrimeric protein consisting of the cap-binding protein eIF4E which recognizes the 5' end of the mRNA, the DEAD-box RNA helicase eIF4A, and the large scaffolding protein eIF4G. The N-terminal region of eIF4G contains binding sites for the poly-A binding protein and eIF4E. In humans, the C-terminal part of eIF4G encompasses three HEAT domains. Of these, HEAT1 and HEAT2 both contain binding sites for eIF4A, and HEAT3 for the kinase mnk1. The HEAT1 domain is conserved in all eukaryotes, from yeast to humans. HEAT2 is conserved in most eukaryotes, except some fungi. In contrast, HEAT3 is not present in fungi or plants, but only in higher eukaryotes. The HEAT1 domain has been shown to enhance eIF4A ATPase and helicase activity, but the HEAT2 domain is less well understood. It appears to play a modulatory role, increasing affinity of eIF4G for eIF4A, but decreasing eIF4A affinity for ATP and RNA (Marintchev et al. 2009).

Methods and experiments

Sample preparation

eIF4G exists in two isoforms: eIF4GI and eIF4GII. Here we used the isoform I. The HEAT2 domain of human eIF4GI was expressed as two variants: an extended construct consisting of residues 1,220–1,437, and a more concise construct consisting of residues 1,236–1,427. Each variant was expressed as a fusion with an N-terminal protein G B1 domain, followed by a hexahistidine tag, and a tobacco etch virus (TEV) protease cleavage site. Both variants were grown in *Escherichia coli* BL21 (DE3) cells, and expressed overnight at 20 °C, in M9 minimal media containing $^{13}\text{C}_6$ glucose and $^{15}\text{NH}_4\text{Cl}$ as the sole carbon and nitrogen sources, respectively. ILV-methyl labeled samples were obtained using growth media described previously (Goto et al. 1999). Cells were lysed by sonication, followed by centrifugation. The fusion protein was purified from the soluble fraction by Ni^{2+} affinity chromatography (Qiagen). The GB1 tag was cleaved by overnight incubation with TEV protease, and removed by Ni^{2+} affinity chromatography, followed by size-exclusion chromatography. Typical sample conditions consisted of 2 mM protein in 20 mM Tris pH 7.0, 150 mM NaCl, 2 mM DTT, 1 mM EDTA, and 5 % D₂O.

NMR experiments

All NMR spectra were recorded at 298 K on Bruker 600, 800, or 900 MHz spectrometers equipped with cryogenic probes, or on a Varian Inova 600 MHz spectrometer equipped with a cryogenic probe, or on a Varian Inova 500 MHz spectrometer equipped with a room-temperature probe. Data were processed using NMRPipe (Delaglio et al. 1995), and analyzed using CARA (Keller 2004) and Sparky (Goddard and Kneller). Backbone chemical shifts were assigned for the extended construct of the HEAT2 domain using the following triple-resonance experiments: HNCACB, HNCOCACB, HNCA, HNCOCACB, HNCO, and HNCACO. Backbone chemical shifts for the shortened construct were assigned using HNCA and HNCACO spectra. Nitrogen shifts were obtained for proline residues using a 2D hCaN spectrum (Gal et al. 2011).

Sidechains were assigned using HCCcoNH, CCcoNH, HCCH-TOCSY, ^{15}N -separated NOESY-HSQC, and ^{13}C -separated NOESY-HSQC spectra; aromatic sidechains were assigned using (HB)CB(CGCD)HD and (HB)CB(CGCD CE)HE experiments (Yamazaki et al. 1993). Chemical shifts were referenced to internal 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS).

Assignments and data deposition

Complete backbone assignments were obtained for residues 1,236–1,427, as shown in the ^{15}N HSQC spectrum shown in Fig. 1. 89 % of all sidechain ^1H and ^{13}C resonances have been assigned. Complete assignments for methyls of isoleucine, leucine, and valine residues are shown in Fig. 2. ^{15}N relaxation experiments performed on the extended construct indicate that residues 1,220–1,239 and 1,428–1,438 are flexible and extremely mobile, leading to the design of the shorter construct (Fig. 3a). Chemical shifts were analyzed for indications of secondary structure using TALOS+ (Shen et al. 2009). The results, shown in Fig. 3b, are in good agreement with the previously published crystal structure (Bellsollell et

al. 2006), except for helix 1, which starts at residue 1,236 in the crystal, but at residue 1,240 in solution. A closer examination of the asymmetric unit in the crystal reveals that the extension of the N-terminal helix is likely to be a crystal-packing artifact as it extends into a groove on a neighboring molecule (Fig. 3c). All chemical shifts were deposited in the BioMagResBank (www.bmrb.wisc.edu) under accession number 19,842.

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Fig. 1. ^1H - ^{15}N HSQC of the HEAT2 domain of eIF4GI (residues 1236–1427). Tryptophan and glutamine sidechain resonances are indicated by “e”, and asparagine sidechain resonances are indicated by “d”. The *bottom panel* shows an expansion of the crowded center region for clarity

Fig. 2.
 ^1H - ^{13}C HSQC of ILV methyl-labeled eIF4GI HEAT2

Fig. 3.

a Backbone relaxation parameters for the extended construct of the HEAT2 domain of eIF4GI (residues 1220–1437) indicate that the tails are extremely mobile and flexible. **b** Backbone angles predicted by TALOS+ based on chemical shifts. Predicted phi angles are shown in black and psi in gray, for each residue. The lower panel shows the Random Coil index in black dots and the probability of alpha-helix formation in gray bars. Chemical shifts indicate that helix 1 begins at residue 1240. **c** The asymmetric unit of the crystal structure, 1 μg^3 in the PDB (Bellolell et al. 2006), shows an extended N-terminal helix packing into a groove on a neighboring molecule. This interaction site is circled