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Letter to the Editor: Backbone resonance assignment of human eukaryotic translation initiation factor 4E (eIF4E) in complex with 7-methylguanosine diphosphate (m^7GDP) and a 17-amino acid peptide derived from human eIF4GII

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Biological context

Eukaryotic initiation factor 4E (eIF4E) is a 25 kDa cytoplasmic protein involved in the mRNA cap-dependent translation initiation by the eIF4F complex (Scheper and Proud, 2002). The eIF4F complex consists of an eIF4E, a RNA helicase eIF4A, and a scaffolding protein eIF4G. The eIF4E plays an important role by binding both eIF4G and the 7-methyl GTP cap structure present at the 5′ end of all RNA polymerase II transcripts, thus facilitating the transfer of mRNAs to other initiation factors to form the 48S ribosomal complex. The cap binding of eIF4E is the rate-limiting step for the cap-dependent mRNA translation. The intracellular abundance of eIF4E is the lowest among the initiation factors. In addition, the availability of eIF4E is tightly regulated by the presence of eIF4E inhibitory proteins termed 4E-BPs that under the unphosphorylated state occupy the eIF4G binding site of eIF4E. Thus, under normal cellular conditions, the least competitive mRNAs, which include many potent growth regulatory molecules, would be translated less efficiently. In fact, artificial overexpression of eIF4E impairs cellular growth control, resulting in tumor formation, and this loss of growth regulation can be reversed by the co-expression of 4E-BPs. Furthermore, the strong correlation between aggressive tumors and eIF4E has been suggested by the high levels of eIF4E expression found in human breast, neck, and prostate cancers. Taken together, these lines of evidence suggest that eIF4E may be a good target for developing new anticancer agents. NMR is a well-established biophysical method used to identify a new class of small-molecule inhibitors and/or to validate the proposed binding site of designed compounds on macromolecules if three-dimensional structures of target proteins are available. Such structures have been reported for the m^7GDP-complexed form of eIF4E from yeast and murine, solved by NMR spectroscopy and by X-ray crystallography, respectively (Matsuo et al., 1997; Marcotrigiano et al., 1997). The starting point for any detailed NMR analysis is the backbone assignment of the target protein, on which we report here.

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Methods and experiments

The gene encoding full length of human eIF4E (1-217) was cloned into a pT7 expression vector. Recombinant eIF4E was overexpressed in *Escherichia coli* strain BL21(DE3) transformed with the expression plasmid. The cell cultures were grown at 30 °C in M9 minimal media in 99% ^2H_2O with [^{13}C]-glucose (2.5 g l^{-1}) and [^{15}N]-NH_4Cl (0.8 g l^{-1}) as the sole carbon and nitrogen sources, respectively. Protein expression was induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside at an optical density (600 nm) of 0.3, followed by 24 h of additional growth. The expressed protein was first immobilized on m^7GTP-sepharose 4B (Amersham Bioscience) column and the bound protein was eluted with 2 M KCl. The eluted fraction containing the labeled eIF4E was then applied to a Superdex75 (Amersham Bioscience) gel filtration column pre-equilibrated with a buffer (50 mM sodium phosphate, 200 mM NaCl, pH 6.8). Next, to ensure all slow-exchanging N^2H was exchanged back to NH,

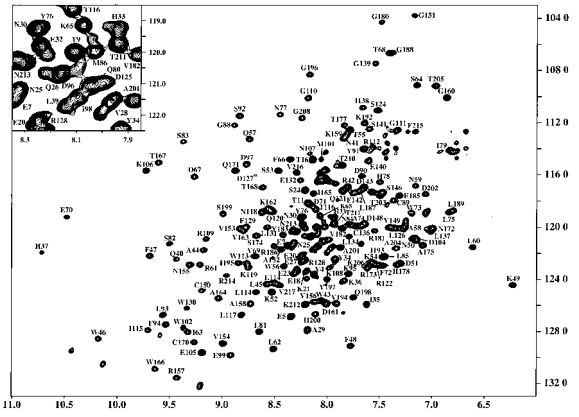

Figure 1. The assigned 2D ^{1}H-^{15}N TROSY spectrum of a 0.8 mM sample of the [^{2}H, ^{13}C, ^{15}N]-labeled human eIF4E in complex with 7-methylguanosine diphosphate and the eIF4GII derived-17-amino acid peptide. The experiment was performed at 298 K on a Bruker Avance 600 MHz spectrometer. For clarity several assignments have been omitted.

the protein was completely unfolded in 6 M guanidinium hydrochloride and subsequently refolded by a rapid 20-fold dilution into a buffer (50 mM Tris-HCl, pH 7.5, 200 mM NaCl, 1 mM dithiothreitol, 1 M 1-(3-sulfopropyl)pyridinium hydroxide) to give a final protein concentration of $\sim$100 μg ml^{-1}. The refolded eIF4E was collected by two-step column chromatography (m^{7}GTP-affnity and gel filtration) as described above.

The NMR sample of [^{2}H,^{13}C,^{15}N]-eIF4E consisted of a 1:1:1 ternary complex with m^{7}GDP, K_D of 0.048 μM (Niedzwiecka et al., 2002), and 17-amino acid peptide KKQYDREFLLDFQFMPA, $K_D = 0.15$ μM (Marcotrigiano et al., 1999) (Qiagen K.K., Tokyo, Japan) at a concentration of 0.8 mM in a 50 mM sodium phosphate buffer, pH 6.8, 200 mM NaCl, 1 mM deuterated dithiothreitol, 0.02% NaN_3 and 95% H_2O/5% $^{2}H_2O$. The peptide used is derived from the eIF4E recognition motif of one of the two human eIF4G isoforms, eIF4GII, and its presence was advantageous in keeping the protein stable in the solution for the time required for the spectral acquisition. In the absence of the peptide, both the apo- and the m^{7}GDP-complexed forms of the protein undergo a slow precipitation over a period of several weeks. All NMR spectra were acquired at 298 K on a Bruker Avance 600 spectrometer equipped with a triple-axis TXI probehead. All chemical shifts were referenced to internal DSS, either directly (^{1}H) or indirectly (^{13}C, ^{15}N) (Wishart et al., 1995). For the backbone resonance assignments, the following three-dimensional TROSY type spectra were recorded: HNCO, HNCA, HN(CO)CA, HNCACB, and HN(CO)CACB (Salzmann et al., 1998, 1999). In addition, 2D ^{1}H-^{15}N TROSY and 3D ^{15}N-edited NOESY-HSQC spectra were acquired. Data were processed with a software package, NMRPipe (Delaglio et al., 1995), and analyzed with XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

The extent of the assigned backbone resonances of human eIF4E is 96%. The N-terminal $\sim$35 residues are known to be flexible and unstructured, which indeed gave rise to intense crosspeaks in the central crowded region of the 2D ^{1}H,^{15}N-TROSY spectrum. Nevertheless, the high-resolution obtained for this part of the triple resonance spectra enabled us to assign all the residues except for Ala2, Thr3, and Glu19. In the core part of the protein, no crosspeaks were found in the spectra for Glu103, Arg123, Asp147, Ser207, and Ser209. Out of 206 C^{β} atoms of the side chains, 196 have been assigned. Figure 1 shows the labeled 2D ^{1}H-^{15}N-TROSY spectrum of [^{2}H,^{13}C,^{15}N]-human eIF4E. The obtained assignments have been deposited in the BioMagResBank (accession number 5712).

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