Journal of Biomolecular NMR, **26:** 189–190, 2003.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of the highly conserved 19 kDa C-terminal domain from human Elongation Factor 1Bγ

Sophie Vanwetswinkel[a], Jan Kriek[b], Gregers R. Andersen[c], Jan Dijk[b] & Gregg Siegal[a,*]
[a]*Leiden Institute of Chemistry, Einsteinweg 55, 2333 CC Leiden, The Netherlands;* [b]*Department of Molecular Cell Biology, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands;* [c]*Institute of Molecular and Structural Biology, University of Aarhus, Gustav Wieds Vej 10C, 8000 Aarhus, Denmark*

Received 1 January 2003; Accepted 6 March 2003

Key words: aromatic content, elongation factor-1, eukaryotic protein synthesis, tryptic fragment

Biological context

Eukaryotic elongation factor 1 (eEF1) consists of two functionally distinct parts that each play a central role in peptide elongation during the process of translation (reviewed in Merrick and Nyborg, 2000). eEF1A catalyses the GTP-dependent delivery of aminoacylated tRNAs to the acceptor site of the ribosome. eEF1B is essential for recycling the inactive eEF1A-GDP released from the ribosome to the active GTP state by stimulating the exchange of guanine nucleotides on eEF1A. In metazoans, eEF1B is formed by the association of three subunits, namely eEF1Bα, eEF1Bβ and eEF1Bγ. The eEF1Bα and eEF1Bβ moieties are the actual nucleotide exchange factors since both have been shown to catalyze *in vitro* exchange reactions through a homologous C-terminal catalytic domain (van Damme et al., 1990). On the other hand, scant information concerning the function of the third subunit eEF1Bγ is available. Indirect observations such as its over-expression in some gastric and oesophageal carcinomas (Chi et al., 1992; Mimori et al., 1995) as well as its co-immunoprecipitation with tubulin from the brine shrimp *Artemia* (Janssen and Möller, 1988) might suggest that eEF1Bγ is involved in some cellular regulatory process(es).

In a continuing effort to further extend our knowledge of the organization and mechanism of the entire eEF1 complex and shed light on the biological function of eEF1Bγ, we have focused on structural studies of this subunit. Recombinant human eEF1Bγ has been expressed in *E. coli* fused to an N-terminal His_{10}-tag and purified to homogeneity in high yield. Limited proteolysis using trypsin showed that this ∼50 kDa protein consists of two independent domains, a Glutathione S-transferase homologous N-terminal region (∼25 kDa) responsible for the interaction with eEF1Bα and a highly conserved, exceptionally protease resistant 162 residue C-terminal domain (eEF1Bγ[276-437]). Interestingly, BLAST searches against commonly available protein databases failed to reveal any significant hits for this latter domain other than orthologous proteins from other organisms. Its unusually high stability and solubility properties as well as the high quality of its 1D ^{1}H spectrum, prompted us to record NMR data for solution structure determination of the C-terminal domain. The essentially complete resonance assignment for this domain is reported here.

Methods and experiments

The gene coding for human eEF1Bγ was cloned into pET-16b (Novagen). The final plasmid contains a single point mutation in the eEF1Bγ coding sequence (V289A) that does not alter the protein's integrity or its binding properties. Recombinant full-length target protein was produced in *E. coli* BL21(DE3), purified by immobilized metal affinity chromatography on HiTrap Chelating resin (Amersham Biosciences) charged with Ni^{2+} ions and submitted to limited proteolysis using trypsin (A grade, Calbiochem; 1/20 w/w on ice). The C-terminal fragment of interest (eEF1Bγ[276-438]) was then isolated by ion-exchange chromatography on SourceQ (Amersham Biosciences). Isotopically labeled proteins were pre-

*To whom correspondence should be addressed. E-mail: g.siegal@chem.leidenuniv.nl

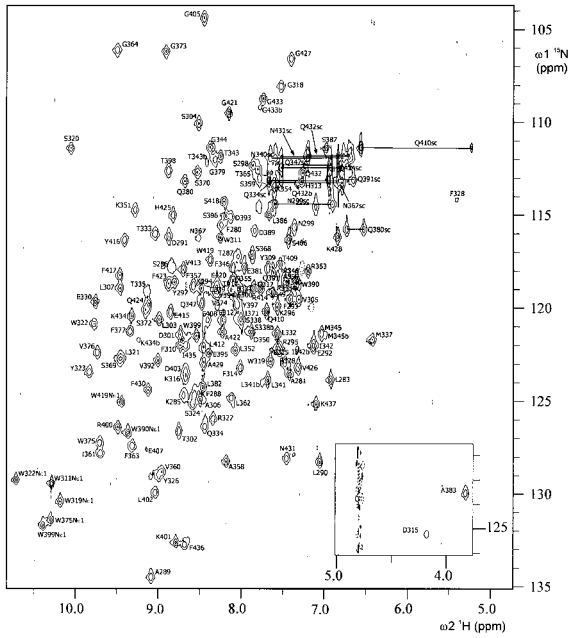

Figure 1. 2D [^{15}N,^{1}H]-HSQC spectrum of human eEF1Bγ[276-437] acquired at 600 MHz, 298°K and pH 7.5. Backbone amide, Trp Nε1 as well as Gln and Asn side chain NH_2 (marked with sc, peaks connected by lines) resonance assignments are indicated with residue numbers. The side chain R353Nε peak is folded from upfield in ω1. Note that for eight of the residues, a second backbone amide peak with intensity about 15% of the main peak (marked with a 'b') could be assigned, which probably corresponds to a minor conformer. The peaks from residues D315 and A383 are displayed in the inset.

pared from cells grown in M9-based minimal medium supplemented with $^{15}NH_4Cl$ as the sole nitrogen source, and either $^{13}C_6$-glucose, unlabeled glucose or a 1:10 mixture of each. The NMR samples contained approximately 1 mM eEF1Bγ[276-438] in 20 mM Tris-HCl pH 7.5, 75 mM KCl, 1 mM DTT, 0.02% NaN_3 (w/v) and 5% D_2O (v/v).

NMR data were acquired at 25 °C either on Bruker DMX600, Bruker AV750 or Varian Inova 800MHz spectrometers, processed using NMRPipe (Delaglio et al., 1995), and analyzed with Xeasy (Bartels et al., 1995). Most of the sequential assignments for the backbone were obtained using 3D HNCACB, CBCA(CO)NH and HBHA(CO)NH spectra. Aliphatic side-chain resonances were derived from 3D HCCH-TOCSY and CCH-TOCSY spectra. Additional data provided by 3D [^{15}N,^{1}H]NOESY-HSQC and [^{13}C,^{1}H]NOESY-HSQC experiments were used for further assignment as well as confirmation of the through-bond data. Aromatic ring proton and carbon resonances have been assigned using a 2D [^{13}C,^{1}H]CT-HMQC in combination with [^{13}C,^{1}H]NOESY-HSQC and HCCH-TOCSY spectra. Stereo-specific assignments of Val and Leu isopropyl groups were obtained by analysis of a 2D [^{13}C,^{1}H]CT-HSQC recorded on a 10% ^{13}C-labeled sample (Neri et al., 1989).

Extent of assignments and data deposition

We have assigned over 97% of the ^{1}H aliphatic and protonated aliphatic ^{13}C resonances. More than 96% of the amide ^{1}H and ^{15}N resonances of the backbone have been assigned with chemical shifts missing only for the following six amino-acids: K277, D278, H282, F336, N366 and F384. Protonated ^{15}N resonances have been found for all seven glutamine and tryptophan side chains while two out of six are missing for the asparagine side chains. Despite an unusually high aromatic content (about 20% of all residues), approximately 90% of the aromatic ^{1}H and ^{13}C side chain assignments have been obtained. The chemical shift values are available as supplementary material and have been deposited in the BioMagResBank database under accession number 5628.

A secondary structure prediction was performed based on the chemical shift index using ^{13}Cα, ^{13}Cβ and ^{1}Hα nuclei (CSI; Wishart and Sykes, 1994). The result indicates that the eEF1Bγ [276-437] domain probably consists of five α-helices and five extended strands.

Acknowledgements

We are grateful to Dr Geerten Vuister (University of Nijmegen) for recording data at 800 MHz. This work was supported in part by the EMBO through short-term fellowships granted to SV. GS acknowledges the Dutch Royal Academy of Sciences (KNAW) for fellowship support.

References

Bartels, C. et al. (1995) *J. Biol. NMR*, **5**, 1–10.
Delaglio, F. et al. (1995) *J. Biol. NMR*, **6**, 277–293.
Chi, K. et al. (1992) *Gastroenterology*, **103**, 98–102.
Mimori, K. et al. (1995) *Cancer*, **75**, 1448–1449.
Merrick, W.C. and Nyborg, J. (2000) *Translational control of gene expression*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 89–126.
Neri, D. et al. (1989) *Biochemistry*, **28**, 7510–7516.
Janssen, G.M.C. and Möller, W. (1988) *Eur. J. Biochem.*, **171**, 119–129.
van Damme, H.T.F. et al. (1990) *Biochim. Biophys. Acta*, **1050**, 241–247.
Wishart, D.S. and Sykes, B.D. (1994) *Meth. Enzymol.*, **239**, 363–392.