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^{1}H, ^{15}N and ^{13}C chemical shift assignment of the guanine nucleotide exchange domain of human Elongation Factor-one beta*

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Biological context

The transfer of aminoacyl tRNA (aa-tRNA) to the ribosomal A site, a necessary prerequisite for peptide elongation during protein translation, is catalyzed in eukaryotes by Elongation Factor-1 (EF-1) (Negrutskii et al., 1998). This factor is a protein complex consisting of 4 subunits. Binding of GTP to the alpha subunit (EF-1α) results in a conformational change that activates it for aa-tRNA binding. Thus EF-1α is a member of the G protein class of molecular switches. Association of the ternary complex of EF-1α · aa-tRNA·GTP with the ribosome induces a GTPase activity whereby EF-1α is released in the inactive, GDP bound form. Regulation of polypeptide elongation is accomplished through the recycling of inactive EF-1α · GDP to active EF-1α·GTP, which is catalyzed by either the EF-1β or EF-1δ subunit. A combination of biochemical experiments and crystallographic analysis of the bacterial homologues of EF-1α and EF-1β, EF-Tu and EF-Ts respectively, has begun to elucidate the mechanism of GDP/GTP exchange in prokaryotes (Kawashima et al., 1996). Interestingly, no obvious homology in primary sequence between bacterial EF-Ts and EF-1β (or EF-1δ) is found, raising the question to what extent the mechanism of GDP · GTP exchange is different in prokaryotes and eukaryotes (Maessen et al., 1986). In order to understand better the mechanism of nucleotide exchange and its role in eukaryotic regulation of translation, we have set out to solve the solution structure of a guanine nucleotide exchange domain from EF-1.

Sequence alignment of EF-1β and EF-1δ demonstrates that the C-terminal portion of these proteins is highly conserved. Proteolytic studies on EF-1β have demonstrated that most of the guanine nucleotide exchange activity resides in the C-terminal domain. Accordingly, a 91 amino acid residues-construct from the C-terminus of human EF-1β was cloned and expressed in *E. coli*, yielding a protein with high in vitro guanine nucleotide exchange activity (Pérez et al., 1998). Here we report the essentially complete resonance assignment for this domain.

Methods and results

The sequence of hEF-1β(134–224) (91 amino acids, 10.4 kDa) is as follows:

MLVAKSSILL DVKPWDDETD MAKLEECVRS IQADGLVWGS SKLVPVGYGI KKLQIQCVVE DDKVGTDMLE EQITAFEDYV QSMDVAAFNK I

[U-^{15}N,^{13}C] hEF-1β(134-224) was produced by bacterial growth on 'Bioexpress 1000' media (Cambridge Isotopes). The purification, analysis of activity and sample preparation was performed using the strategy described previously (Pérez et al., 1998). The NMR samples contained ∼1 mM hEF-1β(134–224) in 10 mM sodium phosphate pH 6.9, 1 mM DTT, 100 mM NaCl and 0.02% NaN_3.

*These data have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4117.
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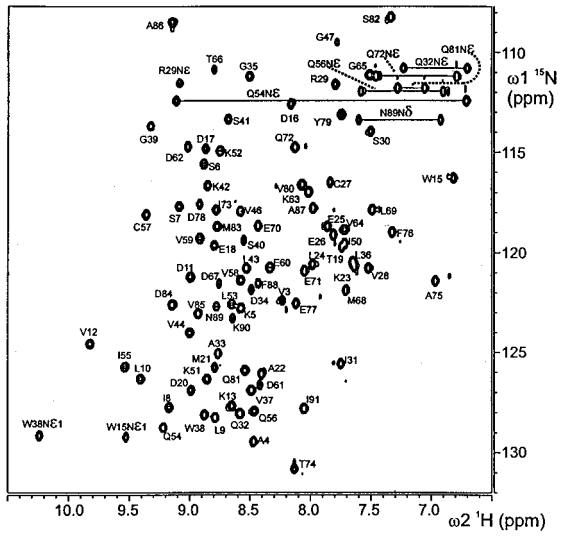

Figure 1. 2D [^{15}N,^{1}H]-HSQC specrum of human EF-1β(134-224). The letter:number labels indicate the assignment at 303 K and pH 6.9. Also shown are the side chain Nϵ1 resonances of the two tryptophans and the side chain NH_2 resonances (peaks connected by lines) for glutamines and asparagines. The resonance of A86 is folded from downfield while that of T74 is folded from upfield in ω1. The peak marked R29Nϵ derives from the sidechain Nϵ-Hϵ correlation for R29 and is also folded from upfield.

All NMR experiments used for the assignment were performed at 30 °C using a Bruker DMX-600 spectrometer equipped with a z-axis pulsed field gradient accessory and a triple resonance probe. 3D ^{15}N-edited NOESY-HSQC (150 ms) and TOCSY-HSQC (45 ms) spectra acquired with (768×192×96) complex points yielded ∼64% of the sequential connectivities. The remaining backbone assignments were obtained with the following heteronuclear experiments: CT-HNCA (1024×84×64 complex points), CT-HN (CO)CA (1024×84×72), CT-HNCO (1024×88×64) (Grzesiek and Bax, 1992a), HN(CA)CO (1024×48×48) and CBCA(CO)NH (1024×114×78) (Grzesiek and Bax, 1992b). Time domain data were processed with either X-WINNMR (Bruker) or NMRPipe (Delaglio et al., 1995). The ^{1}H, ^{15}N and ^{13}C chemical shifts were referenced according to the method of Wishart and Sykes (1994), using external DSS.

Side chain assignments were derived from both a 3D HCCH-TOCSY (Bax et al., 1990) and a series of 2D [^{1}H ^{1}H]-TOCSY (11 ms, 39 ms and 58 ms TOCSY mixing times) spectra of a sample dissolved in 95% H_2O/5% $^{2}H_2O$. The sequence specific aromatic resonance assignments were derived from [^{1}H, ^{1}H]-DQF-COSY, TOCSY and NOESY spectra recorded using a solvent of 99.95% $^{2}H_2O$.

A 2D [^{1}H,^{15}N]-HSQC spectrum of hEF-1β(134–224) showing the backbone amide resonances is shown in Figure 1. Of the 89 expected ^{1}H/^{15}N correlations (91 minus two prolines) 85 are visible.

Extent of assignments and data deposition

We have assigned 97.8% of the non-labile ^{1}H resonances, 96.7% of the protonated ^{15}N resonances of the backbone, all protonated ^{15}N resonances from the asparagine, glutamine and tryptophan side chains and 96.7% of the ^{13}C resonances from the protonated and carbonyl carbons (the aromatic carbons are not included). The chemical shift assignments are available as supplementary material and have been deposited in the BioMagResBank (accession number 4117).

The secondary structure elements of hEF-1β(134–224) were identified from a qualitative analysis of short and long range NOEs, NH exchange rates, $^{3}J_{HNH\alpha}$ coupling constants (Kay and Bax, 1990) and the chemical shift index using ^{13}Cα,^{13}Cβ, ^{13}CO and Hα nuclei (CSI; Wishart and Sykes, 1994). The data indicate that the secondary structure of hEF-1β(134–224) consists of a four-stranded, antiparallel β-sheet and two α-helices. The β-strands span residues 4–12, 38–45, 50–61, 82–89 and the helices span 23–30 and 67–74.

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