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Sequence-specific ^{1}H, ^{13}C and ^{15}N assignment of the EH1 domain of mouse Eps15

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Biological context

Polypeptide growth factors play an essential role in the regulation of cell migration, cell differentiation and cell proliferation. Binding of growth factors to specific transmembrane receptors results in activation of the intrinsic tyrosine kinase, and in internalisation of the receptors via receptor-mediated endocytosis. Recently, a new family of proteins has been found which is involved in the internalisation and intracellular trafficking of growth-factor receptors (Benmerah et al., 1995, van Delft et al., 1997). These proteins are characterised by the presence of an Eps15 Homology (EH) domain of approximately 100 amino acids (Wong et al., 1995). EH domains are frequently present in multiple copies and include EF-hand calcium-binding domains. Here, we present assignments for the EH1 domain of mouse Eps15, the first EH domain NMR assignments to be reported.

Methods and results

Escherichia coli K10 cells, transformed with the pGex-2T expression vector (Pharmacia, Uppsala, Sweden) containing the EH1 domain from mouse Eps15 (residues 1–120, Fazioli et al., 1993) were grown at 30 °C. Purification of the EH1, which was expressed as a GST-fusion protein, involved binding to glutathione-agarose beads (Sigma, St. Louis, MO) and cleavage of the fusion protein with thrombin (Sigma). The yield was 5 mg L^{-1} in minimal media.

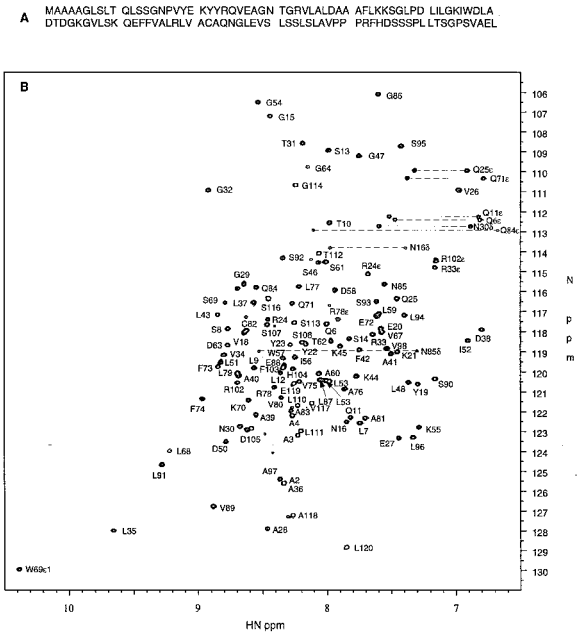

Figure 1. A. Primary sequence of the EH1 domain (residues 1-120) from mouse Eps15 (Fazioli et al., 1993). B. ^{1}H-^{15}N 2D HSQC spectrum of the EH1 domain acquired on a ^{15}N-labelled EH1 sample (1 mM, pH 5.2, 25 °C).

Initial studies indicated that the protein is soluble in the 1–2 mM range only at concentrations of 100 mM NaCl. Phosphate-buffered saline solutions (100 mM KH_2PO_4, 100 mM NaCl, pH 5.2, 90% H_2O/10% D_2O) also containing 1 mM dithiothreitol, 1 mg/ml Pefabloc (Boehringer-Mannheim) and 1 mM azide were used. Additionally, samples contained 10 mM $CaCl_2$. However, subsequent studies indicated a very

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low affinity for Ca^{2+} at pH 5.2. Sample volumes of 500 µl were used for all NMR studies.

For assignments of the backbone resonances, 3D HNCO, 3D HNCA, 3D CBCA(CO)NH and 3D ^{15}N-separated TOCSY- and NOESY-HSQC experiments were used. Pulsed field gradient versions of the experiments, with water flip-back pulses and sensitivity enhancement in the ^{15}N-dimensions (Kay et al., 1994; Muhandiram and Kay, 1994) were acquired at 25 °C on Varian Unity *Inova* 500 and Varian Unity *Inova* 750 spectrometers equipped with z-gradient triple-resonance probes. The program NMRPipe (Delaglio et al., 1995) was used for transformation of all data.

111 of the expected 112 backbone resonances were observed in a 2D ^{1}H/^{15}N HSQC spectrum (cf. Figure 1), enabling the production of a list of ^{1}H/^{15}N shift pairs for analysis of the 3D spectra in the program XEASY (Bartels et al., 1996). Using the commonly followed protocol, these shift pairs were connected by means of the HNCA, CBCA(CO)NH and ^{15}N-separated NOESY-HSQC data. Sequential assignment was then obtained by mapping unique connected fragments onto the primary sequence by means of the C^{α} and C^{β} chemical shift information in conjunction with the ^{1}H spin-system topologies. Seven additional peaks were observed in the ^{15}N HSQC spectrum which are attributed to proline trans/cis isomerization.

Assignments of the non-aromatic sidechains were obtained using 3D HC(C)H- and (H)CCH-TOCSY and H(CCO)NH experiments. Stereospecific assignments for the methyl groups of Val and Leu residues were obtained through analysis of a 2D CT-^{1}H/^{13}C HSQC spectrum acquired on a 10% biosynthetically directed fractionally ^{13}C-labelled sample (Szyperski et al., 1992). Aromatic spin systems were assigned using an ^{15}N-filtered 2D ^{1}H-TOCSY (Whitehead et al., 1997) acquired on an ^{15}N-labelled sample in conjunction with a 2D constant-time ^{1}H/^{13}C HSQC optimised for the aromatic resonances. Sequence-specific assignments for the aromatic spin systems were based on NOEs with $C^{\beta}H_2$ in a 2D NOESY spectrum. Sequence specific assignments of Arg $N^{\epsilon}H$ groups were made using the $N^{\epsilon}H$ - C^{δ} correlations observed in the 3D HNCA. NH_2 groups of Asn and Gln were assigned using the correlations to the $C^{\alpha/\beta/\gamma}$ observed in a 3D CBCA(CO)NH spectrum acquired with $\kappa = 4$ ms to allow observation of NH_2 resonances.

Extent of assignments and data deposition

All ^{1}H, ^{15}N and ^{13}C backbone resonances were assigned except Met^{1} NH_2, C′ of the C-terminus and Pro-preceding residues and Lys^{65} NH. Assignments were made for all non-aromatic sidechains with the exception of the $C^{\gamma}H$ of five of the twenty Leu residues. The EH1 sequence contains 7 Pro residues including Pro^{99}, Pro^{100}and Pro^{101}. No residue-specific assignments were made for Pro^{99} and Pro^{100} owing to lack of sequential connectivities in the amide-based triple resonance data, although the spin-systems were identified in the HCCH-TOCSY spectra. All aromatic protons and carbons were assigned with the exception of the ζ ^{1}H and ^{13}C resonances of Phe^{42} and the ^{13}C resonances of Phe^{73} for which no correlations in the CT-^{1}H/^{13}C-HSQC were observed. Of the labile sidechain protons, only $N^{\delta 1}H$ of His^{104} remains unassigned. The assignments for EH1 domain from mouse Eps15 at pH 5.2 and 25 °C have been deposited in the BiomagResbank (accession number 4140).

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