Journal of Biomolecular NMR **29:** 443–444, 2004.

Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N resonance assignments of human Notch-1 calcium binding EGF domains 11-13

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Received 10 December 2003; Accepted 23 January 2004

Key words: cbEGF, hNotch-1, ligand binding region, triple module construct

Biological context

One of the fundamental processes underlying development is the determination of cell fate. Notch and its homologues are cell surface receptors that are involved in cell fate decision-making in a wide variety of tissues and organisms. Dysfunction of the Notch signalling pathway can result in defective cellular differentiation, which may in turn lead to human disease states including anomalous embryonic development and cancer (reviewed in, Artavanis-Tsakonis et al., 1999; Nam et al., 2002). While some molecules involved in regulating Notch activity have been identified, little is understood regarding the molecular recognition processes governing Notch function.

The ligand binding region of *Drosophila* Notch has been mapped to a single pair of calcium binding epidermal growth factor-like (cbEGF) domains, 11 and 12 (Rebay et al., 1991). We are interested in elucidating the structural basis of Notch-ligand interactions and understanding how these protein-protein interactions have such profound influences on development and disease. Here we report on chemical shift assignments for the calcium saturated form of a construct comprising human Notch-1 cbEGF domains 11-13, a first step towards the structure determination of the ligand binding region.

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Methods and experiments

Human Notch-1 cbEGF domains 11-13 (residues 411-526) were expressed recombinantly using plasmid pQE30 (Qiagen) in *E. coli* using previously described methods (Knott et al., 1996). This results in an additional SerAla being appended to the N-terminus together with a FXa cleavage site. Isotopically enriched cbEGF11-13 was prepared by preparing BL21 cells transformed with the plasmid in M9 minimal media with ^{15}N-ammonium chloride as the sole nitrogen source with or without ^{13}C-glucose as the carbon source. The protein was initially purified using Ni^{2+} chelating Sepharose (Pharmacia). The protein was reduced with DTT and purified by reverse phase HPLC prior to *in vitro* refolding using an oxido-reduction buffer containing 3 mM L-cysteine and 0.3 mM L-cystine as described previously. Final purification steps included additional reverse phase and anion exchange chromatography and removal of the hexahistidine tag using FXa. NMR samples were prepared at 0.5–1.0 mM protein in 90%H_2O/10%$^{2}H_2O$ containing 15 mM $CaCl_2$ and 0.02% NaN_3, at pH 6.1. This concentration of calcium was deemed saturating based on titration monitored by ^{1}H-^{15}N HSQC and previously reported calcium dissociation constants (Rand et al., 1997).

All NMR data were acquired at 25 °C on GE-Omega/home-built spectrometers with triple resonance triaxial gradient probes operating at 600 or 750 MHz, with the exception of the ^{13}C-edited NOESY experiment which was acquired on a Bruker

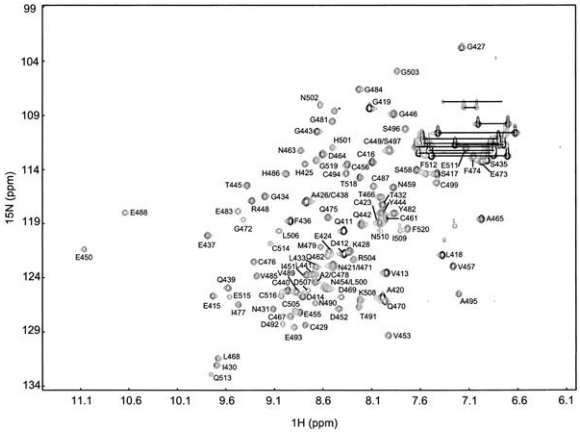

Figure 1. ^{1}H-^{15}N HSQC spectrum with assignments of human Notch-1 cbEGF domains 11-13. The spectrum was acquired on a sample containing 15 mM $CaCl_2$ and 0.02% NaN_3 at pH 6.1 and 25 °C. An asterisk highlights a single crosspeak that could not be assigned. Assigned side chain NH_2 cross peaks from Asn and Gln residues are connected by horizontal, straight lines. Residues are numbered according to the intact human Notch-1 sequence with the exception of A2, which is a cloning artefact.

DRX800 and the HNCACB experiment which was acquired on a Varian UnityInova 500. Data were processed using Felix97 (Accelrys) and assigned using NMRView 5.0 (Johnson and Blevins, 1994). Backbone sequential assignments were made using 3D 1H-15N TOCSY-HSQC and NOESY-HSQC, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH and HNCO experiments (see, Cavanagh et al., 1996). Side chain assignments were extended with 3D HCCH-TOCSY and ^{13}C-edited NOESY data.

Extent of assignments and data deposition

As shown in Figure 1, all of the backbone NH signals were sequentially assigned for residues 411-520. A single unassigned cross peak, which most likely corresponds to one of the unassigned residues in the C-terminal tail (521-526), is highlighted by an asterisk. The cross peaks for cbEGF domains 11 and 12 are of higher intensity than those for cbEGF13, which are exchange broadened. Cross peaks for residues 411-420 are relatively sharp, suggesting some fast timescale motion for the N-terminal region of the construct. Backbone $^{13}C'$, $^{13}C^{\alpha}$ and ^{15}N chemical shifts are $>91\%$ complete for residues 411-520, and backbone ^{1}H chemical shifts for NH and $C^{\alpha}H$ atoms are $>94\%$ complete. Side chain ^{1}H and ^{13}C chemical shifts are more than 87% complete. Thirteen of the fifteen side chain NH_2 groups from Asn and Gln residues appeared in the spectra and were assigned sequence specifically. The chemical shift assignments were formatted using the CCPN format converter (http://www.ccpn.ac.uk), and they have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 6031.

Acknowledgements

The authors thank Dr Daniel Nietlispach, Prof Ernest Laue and Dr Mark Wormald for their assistance with data acquisition. AM was supported by a postdoctoral grant from the Wenner-Gren Foundations. SH held an MRC Clinical Training Fellowship. VK and PAH thank the MRC for grant support (G000164). AKD is a Wellcome Trust Senior Research Fellow (ref: 057725/Z/99/Z/CH/MB).

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