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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N resonance assignment of the vascular endothelial growth factor receptor-binding domain in complex with a receptor-blocking peptide

Borlan Pan & Wayne J. Fairbrother*
Department of Protein Engineering, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, U.S.A.

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Biological context

Vascular endothelial growth factor (VEGF) is a homodimeric member of the cystine-knot family of growth factors (Muller et al., 1997) that functions as an endothelial cell-specific mitogen and is a primary modulator of physiological angiogenesis (Ferrara, 2001). VEGF is also an important mediator of pathological angiogenesis in a variety of disorders including cancer, proliferative retinopathies, rheumatoid arthritis, age-related macular degeneration, and psoriasis (Folkman, 1995); antagonists of VEGF therefore have therapeutic potential. Six different isoforms of VEGF share a common N-terminal receptor-binding domain of 115-residues/monomer. VEGF functions by binding to and dimerizing its tyrosine kinase receptors, KDR and Flt-1, using a pair of identical binding sites localized at the poles of the dimeric receptor-binding domain.

Three classes of disulfide-constrained peptides that block binding of VEGF to its receptors were identified recently using phage-display methods (Fairbrother et al., 1998). Characterizing the interaction between the peptide antagonists and the VEGF receptor-binding domain could lead potentially to the design of novel, small-molecule antagonists of VEGF. A crystal structure of one of the peptide antagonists in complex with $VEGF_{8-109}$ revealed it to be a poor candidate for epitope transfer to a small-molecule scaffold (Wiesmann et al., 1998). Unfortunately, the highest-affinity class of peptides has not proved amenable to crystallographic analysis. As a first step in characterizing the complex between VEGF and this class of phage-derived peptide antagonists by NMR, we report here nearly complete assignments for $VEGF_{11-109}$ in complex with peptide v107. Comparison with backbone assignments reported previously for free $VEGF_{11-109}$ (Fairbrother et al., 1997) allows for identification of the peptide-binding site via quantitative chemical shift mapping.

*To whom correspondence should be addressed. E-mail: fairbro@gene.com

Methods and experiments

The receptor-binding domain of human VEGF ($VEGF_{11-109}$) was overexpressed in *Escherichia coli* as a His-tagged protein, purified, refolded and cleaved specifically as described previously (Fairbrother et al., 1997). Samples of the VEGF/v107 complex for NMR studies contained 1.0 mM $^{13}C/^{15}N$-labeled $VEGF_{11-109}$ dimer and 2.25 mM synthetic v107 in 0.5 ml of 50 mM NaCl, 20 mM NaH_2PO_4 (pH 7.0), 0.002% sodium azide, and either 90% H_2O/10% D_2O or 100% D_2O, as appropriate.

Spectra were acquired at 45 °C on either a Bruker DRX-600 or DRX-800 spectrometer equipped with 5-mm inverse triple-resonance probes with three-axis gradient coils. Backbone $^{1}H^{N}$, ^{13}C, and ^{15}N resonances were assigned sequentially using the following experiments (Cavanagh et al., 1995): 2D ^{15}N-HSQC, and ^{13}C-HSQC, and 3D HNCO, (HCA)CONH, HNCA, and CBCA(CO)NH. The backbone resonance assignments were verified by observation of sequential correlations in a 3D ^{15}N-edited NOESY-HSQC spectrum (mixing time, 80 ms). Aliphatic side-chain resonance assignments were obtained from analysis of 3D HBHA(CO)NH, HCC(CO)NH, and HCCH-TOCSY spectra. Aromatic resonance assignments were obtained from a 3D ^{13}C-edited NOESY-HSQC spectrum

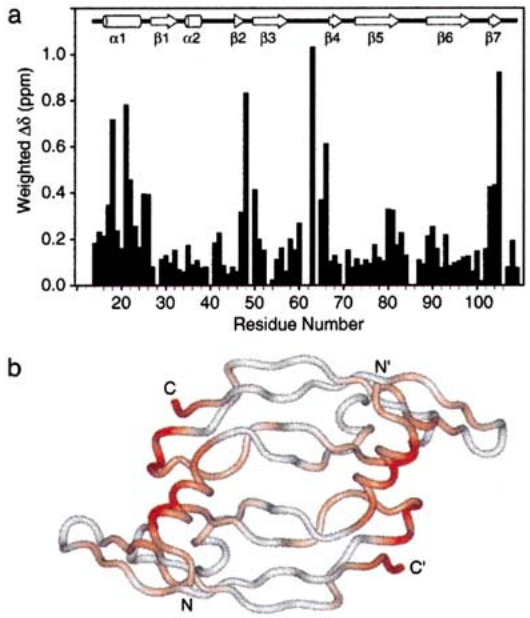

Figure 1. (a) Plot of weighted net change in $VEGF_{11-109}$ chemical shifts for $^{1}H^{N}$, $^{13}C'$, and ^{15}N resonances of free and v107-bound protein. (b) Mapping of the quantitative chemical shift perturbations on the crystal structure of VEGF. Red colors indicate greater chemical shift perturbations.

(mixing time, 110 ms) centered in the aromatic region. Assignments for the Asn and Gln side-chain amide groups were determined from the ^{15}N-edited NOESY-HSQC spectrum. Stereospecific assignments of β-methylenes were determined by analysis of HNHB, ^{13}C-edited NOESY-HSQC, and ^{15}N-edited NOESY-HSQC spectra. Spectra were processed using FELIX (Accelrys, Inc.) and analyzed using FELIX and XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

Backbone assignments for $VEGF_{11-109}$ in complex with v107 are essentially complete, except for ^{15}N of all 7 prolines, ^{15}N, $^{1}H^{N}$, and $^{13}C'$ of the two N-terminal residues, H11 and H12, and ^{15}N, and $^{1}H^{N}$ of E13 and N62. All expected intraresidue and sequential $^{13}C^{\alpha}$ and $^{13}C'$ correlations were observed (where expectation is based on the observed amide resonances). The side-chain assignments are also complete except for $^{1}H^{\delta 2}/^{13}C^{\delta 2}$ and $^{1}H^{\varepsilon 1}/^{13}C^{\varepsilon 1}$ of histidines and $^{1}H^{\varepsilon}/^{15}N^{\varepsilon}$ of arginines. Stereospecific assignments were established for 17 of 52 β-methylenes having nondegenerate proton resonances. The ^{1}H, ^{13}C, and ^{15}N resonance assignments have been deposited in the BioMagResBank (BMRB; http://www.bmrb.wisc.edu) under accession number 5185.

Broadening of the backbone amide resonances of N62 in the VEGF/v107 complex is consistent with similar broadening observed for C61, N62, E64, and G65 in spectra of free $VEGF_{11-109}$ (Fairbrother et al., 1997), and suggests conformational averaging in this region. Note that new high-field data have lead to revision of some tentative assignments reported for free $VEGF_{11-109}$; the revised backbone assignments have been deposited also in the BMRB under accession number 5186. V107-induced chemical shift perturbations of $VEGF_{11-109}$ were assessed quantitatively; the weighted net change in $VEGF_{11-109}$ chemical shifts for backbone $^{1}H^{N}$, $^{13}C'$, and ^{15}N resonances (Meininger et al., 2000) of free and v107-bound $VEGF_{11-109}$ are plotted in Figure 1a. The chemical shift perturbations are localized to the pole regions of the VEGF dimer (Figure 1b), corresponding closely to the previously determined binding sites for the VEGF receptors KDR and Flt-1 (Muller et al., 1997; Wiesmann et al., 1997).

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