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Letter to the Editor: Backbone ^{1}H, ^{15}N, and ^{13}C resonance assignments of the repeated domain of human βig-h3 protein

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Biological context

βig-h3, also called RGD-CAP or keratoeithelin, is an extracellular matrix protein that is induced by transforming growth factor-β in the human cornea, skin, and matrix of many connective tissues. Previous studies showed that βig-h3 is a cell adhesion protein that is involved in tissue development, repair, and remodeling (Rawe et al., 1997; Dieudonne et al., 1999; Ohno et al., 1999). βig-h3 is composed of 683 amino acids containing C-terminal RGD motif and four repeated internal domains of 140 amino acids (designated fas-1 domain), which are observed in other homologous proteins such as human osteoblast specific factor 2 and insect fasciclin-1. Recently, it was reported that the RGD motif was not necessary for mediating cell spreading and the 4th repeated domain alone was sufficient to mediate cell adhesion via interacting with $\alpha_3\beta_1$ integrin (Kim et al., 2000). Yet the molecular basis of interaction between βig-h3 and integrin at the atomic level is not clarified and the structure of the homologous domain of any other protein is not known. We have started NMR structure determination of the 4th domain of βig-h3 in order to know the structure-function relationship of βig-h3. Here we report backbone assignments for the 4th domain of βig-h3.

Methods and experiments

The fragment of βig-h3 cDNA encoding amino acid 498-637, the 4th repeated domain, was generated by polymerase chain reaction and cloned into a pET-29b (Novagen). The recombinant βig-h3(498-637) was expressed in *E. coli* BL21 (DE3) with a cleavable N-terminal S-tag and a C-terminal His_6-tag. [^{15}N]-labeled βig-h3(498-637) was prepared from cultures grown in M9 media containing ^{15}N-NH_4Cl, and [90% ^{2}H, ^{15}N/^{13}C]-labeled sample was produced in 90% D_2O/10% H_2O M9 media with ^{15}N-NH_4Cl and ^{13}C-d-glucose. Each sample was purified by S-protein agarose (Novagen) column chromatography and nickel charged Chelating Sepharose (Pharmacia) column chromatography, and the N-terminal S-tag was cleaved by thrombin (Novagen) during the purification steps. Two NMR samples, [^{15}N]- and [90% ^{2}H, ^{15}N/^{13}C]-βig-h3(498-637) were prepared at concentration of 1 mM in 20 mM sodium phosphate buffer (90% H_2O/10% D_2O, pH 6.0) containing 1 mM NaN_3. All NMR spectra were acquired at 298 K on a Bruker DRX 600 spectrometer. [^{15}N]-labeled sample was used to collect HSQC, NOESY-HSQC, TOCSY-HSQC, and HNHA spectra. [90% ^{2}H, ^{15}N/^{13}C]-labeled sample was used to acquire ct-HNCACB, ct-HN(CO)CACB (Shan et al., 1996) and the following TROSY-based spectra: HNCA, HN(CO)CA, HNCO, and HN(CA)CO (Salzmann et al., 1998, 1999). HNHA, TOCSY-HSQC, and NOESY-HSQC were used to obtain ^{1}Hα resonances and the other spectra were used to obtain ^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ, and ^{13}C′ resonances. Proton chemical shifts were referenced to the methyl signal of 2, 2-dimethylsilapentane-5-sulfonic acid (DSS) externally. ^{13}C and ^{15}N chemical shifts were referenced indirectly to DSS. All NMR spectra were processed using NMRPipe/NMRDraw

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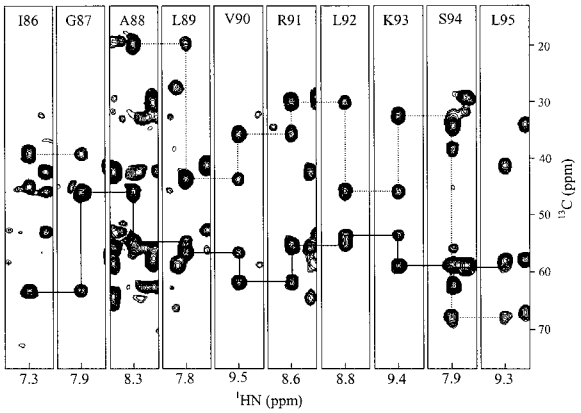

Figure 1. Strip plot from the HNCACB spectrum of βig-h3(498-637). Sequential ^{13}Cα and ^{13}Cβ connectivities for residues Ile86-Leu95 are shown with solid and dashed lines, respectively.

(Delaglio et al., 1995) and analyzed using NMRView (Johnson and Blevins, 1994).

Extent of assignments and data deposition

The final product includes 6 and 7 extra residues at the N- and C-termini, respectively, which are excluded from the assignments. Residues 1–140 reported here correspond to the residues 498–637 of βig-h3. Backbone ^{1}H/^{15}N resonances were assigned except for 9 prolines (Pro3, Pro4, Pro45, Pro54, Pro55, Pro115, Pro119, Pro137, and Pro138). Cα, Cβ, and C′ resonances were completely assigned except for 3 prolines (Pro3, Pro54, and Pro137) that precede other prolines and 101 Hα resonances were unambiguously assigned. The ^{1}H, ^{15}N, and ^{13}C resonances of βig-h3(498-637) have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 5522.

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