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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments and secondary structure of the N-terminal domain of human tissue inhibitor of metalloproteinases-1

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Biological context

Matrix metalloproteinases (MMPs) hydrolyze diverse protein components of the extracellular matrix during the course of tissue remodeling in reproduction, development, wound healing and diseases including cancer, arthritis, atherosclerosis and macular degeneration. Tissue inhibitors of metalloproteinases (TIMPs) appear to regulate such processes by high affinity noncovalent binding of MMPs. Sensitivity to inhibition by TIMPs distinguishes MMPs from other methionine-turn-containing zinc endopeptidases. TIMPs-1, 2, 3 and 4 tightly bind 1:1 the activated forms of most MMPs with Ki values less than 1 nM (Murphy and Willenbrock, 1995). TIMPs are secreted by many cultured cell lines and are found in body fluids and tissue extracts (Murphy and Willenbrock, 1995).

TIMPs can inhibit MMP-mediated aspects of disease progression, such as angiogenesis. Delivery of TIMPs in vivo causes significantly fewer and smaller metastatic tumors to form in animal hosts (Khokha, 1994). TIMPs may slow tumor growth by preventing MMP-mediated establishment of the blood supply, i.e. angiogenesis. TIMP-1 inhibits new blood vessel formation in assays in vivo (Johnson et al., 1994).

The N-terminal domain of 126 residues, two-thirds of the full length, of recombinant human TIMP-1 (N-TIMP-1) inhibits MMPs-1, -2, -3 and $-3(\Delta C)$ with Ki values of less than 2 nM (Huang et al., 1996). This domain, with three disulfide bridges, can be refolded from *E. coli* inclusion bodies much more readily than can full-length TIMPs which contain six disulfide bridges. Engineered forms of N-TIMP-1 may become suitable for use in gene therapy. Here we report the ^{1}H, ^{13}C and ^{15}N resonance assignments and secondary structure of the N-terminal inhibitory domain of human tissue inhibitor of metalloproteinases-1, N-TIMP-1.

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Methods and results

N-TIMP-1 was purified as reported (Huang et al., 1996). The *E. coli* host strain was grown in Celtone CN medium for preparation of ^{13}C/^{15}N-labeled N-TIMP-1 and Martek 9 N medium for preparation of ^{15}N-labeled N-TIMP-1 (Martek Biosciences Co., Columbia, MD). NMR samples of 0.5 mM ^{15}N-labeled and 0.9 mM ^{13}C/^{15}N-labeled N-TIMP-1 were exchanged with a buffer containing 20 mM sodium acetate-d_3 at pH 6.0 with 150 mM NaCl, 1 mM NaN_3, and 7% D_2O. For NMR, 650 μl of each sample was placed in an 8 mm microcell.

The NMR experiments were carried out at 20 °C with a Bruker DRX-500 NMR spectrometer equipped with an 8 mm probe tuned to ^{15}N, ^{13}C and ^{1}H frequencies and fitted with a shielded z-gradient coil (Nalorac Corp., Martinez, CA). HSQC-type spectra employed the fast HSQC method to minimize saturation of the water resonance (Mori et al., 1995). SYBYL TRIAD (Tripos Inc., St. Louis, MO) was used for processing and interpreting NMR spectra.

Sequence-specific assignments for the backbone were obtained using HNCA, HNCO, (HA)CA(CO)NH, HA(CACO)NH, HN(CA)HA, and ^{15}N-NOESY-HSQC spectra with identification of aliphatic carbon side

chain spin systems provided by CBCA(CO)NH and CCA(CO)NH spectra (Bax and Grzesiek, 1993). Aliphatic and aromatic side-chain assignments relied on 3D HCCH-TOCSY spectra in H_2O (Kay et al., 1993) and ^{13}C-edited FSCT-HSMQC-NOESY (Van Doren and Zuiderweg, 1994). Stereospecific valine and leucine methyl assignments were obtained as described (Neri et al., 1989).

Figure 1 summarizes sequential and medium-range NOEs, $^3J_{HNH\alpha}$ coupling constants and $^{13}C\alpha$ and $^{13}C\beta$ secondary chemical shifts which reveal the secondary structure of N-TIMP-1. The sequential and medium-range NOEs were obtained from 3D ^{15}N- and ^{13}C-separated NOESY spectra, each recorded with a mixing time of 80 ms. N-TIMP-1 consists of an N-terminal helix (His^7-Ser^{15}), C-terminal helix (Ser^{109}-Thr^{119}), as well as four long β-strands and two short β-strands: Leu^{17}-Asn^{30} (A), Tyr^{35}-Tyr^{46} (B), Phe^{60}-Ala^{65} (C), Phe^{83}-Gln^{90} (D), Leu^{93}-Ile^{96} (E) and Val^{102}-Pro^{104} (F). The β-sheet wraps to form a sheared β-barrel (strands A–E). The patterns of NOEs, $^3J_{HNH\alpha}$ coupling constants and $^{13}C\alpha$ secondary chemical shifts are in nearly complete agreement with the crystallographic observations for full-length TIMP-1 bound to MMP-3 at 2.8 Å resolution (Gomis-Rüth et al., 1997). N-TIMP-1, in solution with the C-terminal truncation, may form a transient turn of 3_{10} helix from Tyr^{120}-Cys^{124}. The X-ray structure of full-length TIMP-1 contains a third α-helix from Thr^{119}-Cys^{124}.

Extent of assignments and data deposition

Most residues were fully assigned, apart from Glu^{67} and Ser^{68} where no assignments were obtained. For Thr^2 and Val^{69}, assignments of amide groups are missing. Aromatic protons and carbons were assigned with the exception of the Hζ and Cζ resonances of Phe. Three of four Asn and four of six Gln side-chain NH_2 resonances have been assigned. 95.3% of all ^{1}H, ^{15}N, and ^{13}C backbone nuclei and 94.3% of all side chain nuclei have been assigned.

The ^{1}H, ^{13}C and ^{15}N chemical shifts for N-TIMP-1 at T = 293 K in a buffer containing 20 mM sodium acetate-d_3 at pH 6.0 with 150 mM NaCl, 1 mM NaN_3 and 7% D_2O have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4327.

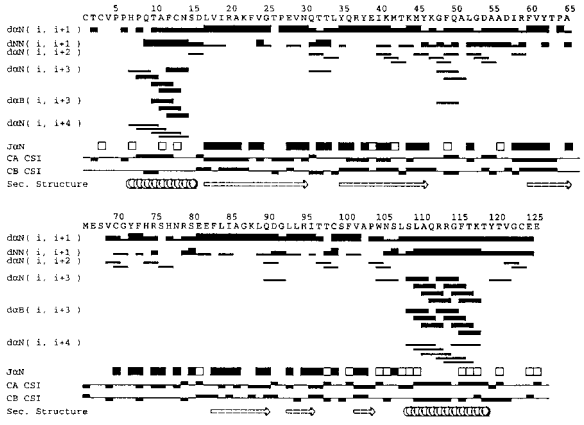

Figure 1. Backbone NOEs, J couplings and ^{13}C chemical shifts revealing the sequence placement of the elements of secondary structure in human N-TIMP-1. For sequential and medium range NOEs: tall, medium and short boxes indicate strong, medium and weak NOEs with upper distance bounds of 3.0, 4.0 and 5.0 Å, respectively. For $^3J_{H\alpha HN}$: filled squares indicate scalar couplings of at least 8 Hz; open squares indicate scalar couplings of less than 6 Hz; and no square is drawn where the scalar coupling lies between 6 and 8 Hz or is unclear or not observed. A coiled symbol marks the location of helix and dashed arrows mark the location of β strands.

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References

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 132–138.
Gomis-Rüth, F.-X., Maskos, K., Betz, M., Bergner, A., Huber, R., Suzuki, K., Yoshida, N., Nagase, H., Brew, K., Bourenkov, G.P., Bartunik, H. and Bode, W. (1997) *Nature*, **389**, 77–81.
Huang, W., Suzuki, K., Nagase, H., Arumugam, S., Van Doren, S.R. and Brew, K. (1996) *FEBS Lett.*, **384**, 155–161.
Johnson, M.D., Kim, H.-R. C., Chesler, L., Tsao-Wu, G., Bouck, N. and Polverini, P.J. (1994) *J. Cell. Physiol.*, **160**, 194–202.
Kay, L.E., Xu, G.-Y., Singer, A.U., Muhandiram, D.R. and Forman-Kay, J.D. (1993) *J. Magn. Reson.*, **B101**, 333–337.
Khokha, R. (1994) *J. Natl. Cancer Inst.*, **86**, 299–304.
Mori, S., Abeygunawardana, C., O'Neil Johnson, M. and Van Zijl, C.M.P. (1995) *J. Magn. Reson.*, **B108**, 94–98.
Murphy, G. and Willenbrock, F. (1995) *Methods Enzymol.*, **248**, 496–510.
Neri, D., Szyperski, T., Otting, G., Senn, H. and Wüthrich, K. (1989) *Biochemistry*, **28**, 7510–7516.
Van Doren, S.R. and Zuiderweg, E.R.P. (1994) *J. Magn. Reson.*, **B104**, 193–198.