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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignments of matrilysin (MMP7) complexed with a sulfonamide hydroxamate-type inhibitor

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Biological context

Matrilysin, or Matrix Metallo-Proteinase-7 (MMP7) is an important member of the MMPs family, which is a group of zinc- and calcium-dependent enzymes collectively responsible for remodelling of connective tissue (Parks and Mechem, 1998). As a smallest one, matrilysin is unique in that the gene encodes only the minimal domains required for activity. Unlike most other MMPs, MMP7 lacks C-terminal domain, which is similar to hemopexin (a plasma heme binding protein) and has been thought to have a role in defining the substrate specificity. This suggests that the structure of matrilysin is most likely functionally distinctive (Wilson et al., 1996). Its specific proteolytic targets have expanded to many other extracellular proteins. These substrates include an array of other proteinases, chemotactic molecules, latent growth factors, growth factor binding proteins, cell surface receptors, and cell-cell and cell-matrix adhesion molecules. Excess MMP activation and expression have been implicated in the accelerated breakdown of connective tissue seen in pathological conditions such as arthritis, multiple sclerosis and Alzheimer's disease. Therefore, MMPs are viable targets for drug design (Wittaker et al., 1999).

Although the structure of MMP7 was determined by X-ray crystallography in 1995 (Browner et al., 1995), no NMR resonance assignments have been published so far on this protein. This prompted us to study the structural features of MMP7 in solution by NMR spectroscopy. Recently, an MMP7 homologue (Y100-K272) with an extended C-terminal of 7 residues as compared with that in above crystal structure has been cloned and expressed in this laboratory. This homologue demonstrated definitely hydrolytic activity against thioester type of substrates. Furthermore, it formed stable complex with a sulfonamide hydroxamate-type inhibitor, which represented as the first example of the complex of MMP7 and sulfonamide hydroxamate-type inhibitor. We report here the NMR backbone assignments of MMP7 homologue in the complex. These assignments provide a basis for further structural and dynamic studies of MMP7, especially for the studies on the interaction of MMP7 with signal molecules.

Methods and experiments

A synthetic gene was designed to encode the known amino acid sequence (Y100-K272) of MMP7 using optimized *E. coli* codons. This is highly desirable for the production of proteins in *E. coli*, it offers the advantages of optimizing expression through codon usage, diminishing the effects of undesirable regulatory DNA sequences. The gene of MMP7 was synthesized in this laboratory and was cloned into pET11c vector. The construct was transformed for overexpression in *E. coli* strain BL21 (DE3). The uniformly ^{15}N- and

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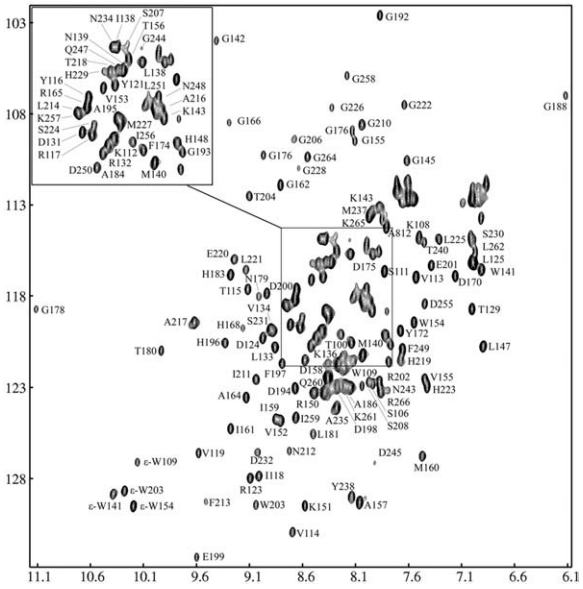
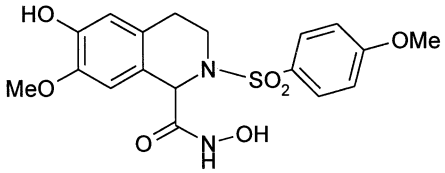


Figure 1. ^{1}H-^{15}N HSQC spectrum of matrilysin recorded at 37 °C. The assignment of peaks is indicated with their one-letter amino acid and number. The top left corner of the HSQC spectrum is an enlarged view of a central region.

^{13}C-labeled human matrilysin (MMP7) was obtained by growing cells in M9 medium containing $^{15}NH_4Cl$ and ^{13}C6-glucose as the sole nitrogen and carbon source. Protein expression was induced by 0.4 mM IPTG. The cells was then harvested and sonicated in lysis buffer. The protein was purified and refolded according to the published procedures (Pathak et al., 1998) with a minor modification.

The NMR samples contained 0.6 mM of MMP7 in an equimolar complex with the inhibitor in a buffer containing 10 mM deuterated Tris-Base, 100 mM NaCl, 10 mM $CaCl_2$, 0.1 mM $ZnCl_2$, 2 mM NaN_3, in 90% H_2O/10% D_2O at pH 6.9. All experiments were recorded at 37 °C on a Varian unity INOVA 600 MHz spectrometer equipped with a triple-resonance, Z-gradient probe. All experiments were achieved using Varian BioPack pulse sequences. The assignments of the ^{1}H, ^{15}N, ^{13}CO, and ^{13}C backbone resonances were based on the following experiments: CBCA(CO)NH, HNCACB, HNCO, HN(CO)CA, HNCA, HCACO, HCCH-COSY and HCCH-TOCSY (Cavanagh, et al., 1996). The spectra were processed with NMRPipe (Delagio et al., 1995) and analyzed with NMRView (Johnson and Blevins, 1994). The assignments were further confirmed by sequential NOEs in the ^{15}N-edited NOESY-HSQC spectra.



Scheme 1. Structure of the hydroxamic acid inhibitor

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum of matrilysin is shown in Figure 1. The ^{1}H-^{15}N resonances appeared in the HSQC spectrum have been nearly completely assigned. In total, 91% of all possible backbone shifts were obtained. 91% of ^{1}HN and 85% ^{15}N, 99% of $^{13}C_\alpha$ and 96% $^{13}C_\beta$, 95% H_α and 84% of ^{13}C chemical shifts were assigned. The missing assignments were mainly located on the dynamic loop region. The rich of Pro residues (∼6% of total residues) was another reason for the incomplete assignment.

The chemical shifts for backbone resonances have been deposited in the BioMagResBank under accession number BMRB- 6014 (http://www.bmrb.wisc.edu).

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