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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments for a truncated and inhibited catalytic domain of matrix metalloproteinase-2

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Biological context

Matrix metalloproteinase-2 (MMP-2), or gelatinase A, belongs to a family of at least 20 zinc-dependent enzymes that degrade the major components of the extracellular matrix (see Parks and Mecham, 1998). These play an important role in normal physiology, such as tissue and joint remodeling, trophoblast implantation, embryogenesis, uterine involution and wound repair. Dysregulated MMPs have been implicated in pathological diseases including arthritis, cancer invasion and metastasis, periodontitis, and heart failure, making them important targets for developing drugs. Understanding the structural differences among the MMPs offers an opportunity to design inhibitors that are selective for one or a small subset of the MMPs.

We have initiated a multidimensional heteronuclear NMR study of the MMP-2 catalytic domain to provide structural guidance for the design of selective inhibitors. Most extracellular MMPs comprise the following domains: a propeptide, a catalytic domain, and a hemopexin-like domain. Two members of the family, i.e., MMP-2 and MMP-9, contain an additional insertion within their catalytic domains which has three fibronectin-like gelatin-binding modules. To facilitate the structure determination, the NMR study focused on a complex of an active catalytic domain of MMP-2 (MMP-2C) with a short peptide linker replacing the fibronectin-like modules, and a hydroxamate inhibitor in the presence of Ca^{2+} and Zn^{2+}. Here we report the sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of this engineered MMP-2 catalytic domain.

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Methods and results

The MMP-2C DNA construct encodes residues 110–214 and 397–446 of the full-length MMP-2 with a seven-residue linker NTSANYS, which resembles the MMP-8 sequence. The protein product contains 163 amino acid residues, including an N-terminal Met with a molecular weight of 18.5 kDa. The gene for MMP-2C was constructed by PCR amplification using human full-length MMP-2 as the template and was cloned into an *E. coli* expression vector containing the RecA promoter, then transformed into *E. coli* strain W3110-II5 for expression.

MMP-2C was expressed by induction with 50 μg/mL nalidixic acid at 37 °C for 4 h in M9 minimal media containing 18 mM $^{15}NH_4Cl$ and 33 mM ^{13}C-glucose. Inclusion bodies were prepared from harvested cells and solubilized in 20 mM Tris · HCl, pH 8.0, 6 M urea, and 10 mM DTT at room temperature for 1 h, then centrifuged at 20 000 g for 20 min at 4 °C. The supernatant was loaded onto a Q Sepharose High Performance column and the protein was eluted with a 0–250 mM NaCl gradient. The pooled protein was refolded by dialysis at 4 °C in 20 mM Tris · HCl, pH 8.0. After refolding, the protein was centrifuged, chromatographed on a Q Sepharose High performance column and stored at −80 °C. NMR samples were prepared by adding 10–20% excess unlabelled inhibitor dissolved in DMSO to a dilute protein solution and concentrating the mixture to 0.5–0.6 ml followed by dialysis in a buffer containing 20 mM Tris-d_{11} · HCl,

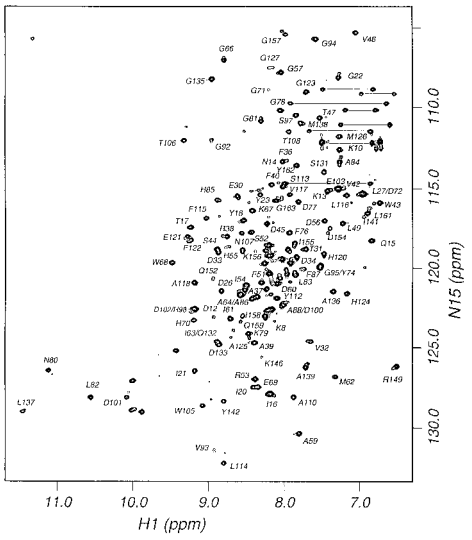

Figure 1. 2D ^{1}H,^{15}N-HSQC spectrum of 0.4 mM [U-^{15}N] MMP-2C at pH 7.3–7.4, 30 °C. The backbone resonance assignments for the majority of the residues are indicated; several are omitted for clarity. Side-chain NH_2 resonances are connected by horizontal bars.

5 mM $CaCl_2$, 10 μM $ZnCl_2$, 20 μM unlabelled inhibitor, and 0.02–0.03% sodium azide, pH 7.3–7.5. The final protein concentration was 0.3–0.4 mM.

All NMR experiments were performed at 30 °C on a Varian INOVA 600 MHz spectrometer using a 5 mM triple resonance probe with Z-gradient. The main-chain and the aliphatic side-chain resonances were assigned using the gradient version of 3D HNCO (Muhandiram and Kay, 1994), HNCACB (Muhandiram and Kay, 1994), ^{15}N-edited TOCSY-HSQC (Zhang et al., 1994), CBCA(CO)NH (Muhandiram and Kay, 1994), HBHA(CO)NH (based on Grzesiek and Bax, 1993), HN(CA)CO (based on Clubb et al., 1992), H(CCO)NH (Grzesiek et al., 1993), C(CO)NH (Grzesiek et al., 1993), HCCH-COSY (based on Bax et al., 1990), and HCCH-TOCSY (Kay et al., 1993). The aromatic resonances were assigned using 2D $(H_\beta)C_\beta(C_\gamma C_\delta)H_\delta$ and $(H_\beta)C_\beta(C_\gamma C_\delta C_\epsilon)H_\epsilon$ (Yamazaki et al., 1993) and a 3D ^{13}C-edited NOESY spectrum.

Extent of assignments and data deposition

Figure 1 shows an annotated 2D ^{1}H-^{15}N HSQC spectrum of ^{15}N-labeled MMP-2C. The backbone ^{1}H–^{15}N resonances have been assigned for all residues except 1–5, 58, 65, 73, 109, 143, 147–148. The absence of the ^{1}H-^{15}N cross peaks for these residues is likely to be due to either unfavorable dynamics and/or the relatively high pH. All backbone carbonyl resonances have been assigned except for residues 1–5, 14, 24, 55, 70, 88, 111, and 147. Aliphatic side-chain assignments are complete for most residues with the exceptions of the Asn or Gln side-chain NH_2 groups, the Arg guanidinium groups, the Met methyl groups, and residues Lys-8, Lys-79, Lys-146, and Asn-147. The majority of the aromatic side-chain resonances are only partially assigned. No stereospecific assignments have been made. Several residues, e.g., Tyr-142, Asn-80, and Ala-125, appear to have satellite cross peaks in the ^{1}H-^{15}N HSQC spectrum. These satellite cross peaks are present in fresh samples and their relative intensities remain the same over a long period of time. The presence of free enzyme is unlikely because the inhibitor, with its nanomolar affinity, should saturate all the enzyme and free enzyme would be degraded rapidly in the presence of the cations. Since the protein sample appeared to be homogeneous by electrospray mass spectrometry and SDS-PAGE, it is unlikely that the satellite peaks represent gross heterogeneity or degradation, although subtle chemical heterogeneity cannot be ruled out. It is possible that a minor conformation is present in the vicinity of the inhibitor binding site. The assignments have been deposited with the BioMagResBank (http://www.bmrb.wisc.edu) database (accession code 4565).

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