

## Letter to the Editor: $^1\text{H}$ , $^{15}\text{N}$ , $^{13}\text{C}$ , and $^{13}\text{CO}$ assignments and secondary structure determination of collagenase-3 (MMP-13) complexed with a hydroxamic acid inhibitor

Franklin J. Moy<sup>a</sup>, Pranab K. Chanda<sup>b</sup>, Scott Cosmi<sup>b</sup>, Wade Edris<sup>b</sup>, Jeremy I. Levin<sup>c</sup> & Robert Powers<sup>a,\*</sup>

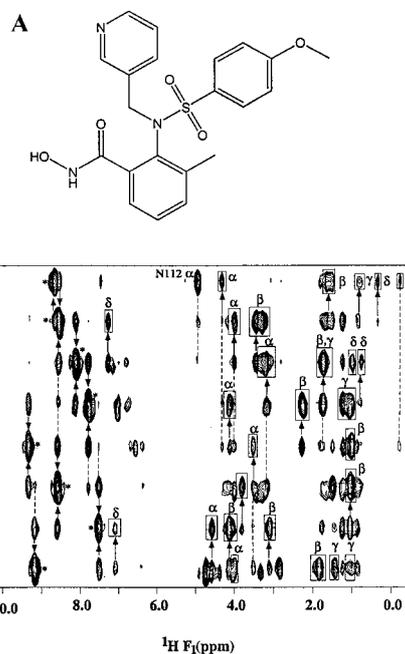
<sup>a</sup>Department of Structural Biology, <sup>b</sup>Department of Core Biotechnology and <sup>c</sup>Department of Chemical Sciences, Wyeth Research, 85 Bolton Street, Cambridge, MA 02140, U.S.A.

Received 3 March 2000; Accepted 25 April 2000

**Key words:** collagenase-3, hydroxamic acid, matrix metalloproteinase, MMP-13, resonance assignments, secondary structure

### Biological context

Human collagenase-3 (MMP-13) is a member of the matrix metalloproteinase (MMP) family which is a highly active set of targets for the design of therapeutic agents for the disease areas of arthritis and oncology (for a review see Zask et al., 1996). The MMPs are involved in the remodeling and degradation of extracellular matrix proteins and are highly regulated. The apparent loss in this regulation results in the pathological destruction of connective tissue and the ensuing disease state. MMP-13 was recently identified on the basis of differential expression in normal breast tissues and in breast carcinoma. There have been a number of X-ray and NMR structures solved for the catalytic domain of MMPs complexed with a variety of inhibitors, but the X-ray structure of MMP-13 was only recently determined during the preparation of this manuscript (Lovejoy et al., 1999). Here we report the near complete  $^1\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{CO}$ , and  $^{13}\text{C}$  NMR assignments and secondary structure of MMP-13 complexed with a hydroxamic acid inhibitor (WAY-151693) (Figure 1A). These data provide a basis for determining the solution structure of the MMP-13:WAY-151693 complex and for further investigation in the design of novel MMP-13 inhibitors.



**Figure 1.** (A) Illustration of the hydroxamic acid inhibitor (WAY-151693). (B) Composite of amide strips taken from the 3D  $^{15}\text{N}$ -edited NOESY-HMQC spectrum for the stretch of residues from Leu-113 to Glu-120 corresponding to part of  $\alpha_{\text{B}}$ . The diagonal peaks are indicated by an asterisk, NH(i+1) NOEs are indicated by solid arrows and NH(i+2,3,4) NOEs are indicated by dashed lines. Intrasidue NOEs are boxed and labeled.

### Methods and results

The uniform  $^{15}\text{N}$ - and  $^{13}\text{C}$ -labeled 165 amino acid catalytic fragment of human collagenase-3 (MMP-13)

\*To whom correspondence should be addressed. E-mail: powersr@war.wyeth.com

was expressed in *E. coli* strain BL21(DE3) containing the plasmid pProMMP-13 according to a published method (Freije et al., 1994). MMP-13 was purified as previously described by Moy et al. (1997) with minor modifications. N-terminal amino acid sequencing was performed to confirm the protein's identity while the uniform  $^{15}\text{N}$  and  $^{13}\text{C}$  labeling of MMP-13 was confirmed by MALDI-TOF mass spectrometry (PerSeptive Biosystems).

The sulfonamide derivative of the hydroxamic acid compound was prepared from 2-amino-3-methylbenzoic acid methyl ester and p-methoxybenzenesulfonyl chloride followed by alkylation with 3-picolyl chloride, hydrolysis (LiOH/THF) to afford the carboxylic acid and conversion to the hydroxamic acid (oxalyl chloride/DMF/ $\text{NH}_2\text{OH}$ ). Formation of the HCl salt yielded WAY-151693.

The NMR samples contained 1 mM of MMP-13 in an equimolar complex with WAY-151693 in a buffer containing 10 mM deuterated Tris-Base, 100 mM NaCl, 5 mM  $\text{CaCl}_2$ , 0.1 mM  $\text{ZnCl}_2$ , 2 mM  $\text{NaN}_3$ , 10 mM deuterated DTT, in either 90%  $\text{H}_2\text{O}/10\%$   $\text{D}_2\text{O}$  or 100%  $\text{D}_2\text{O}$  at pH 6.5. All NMR spectra were recorded at 35 °C on a Bruker AMX-2 600 spectrometer equipped with a triple-resonance gradient probe. Spectra were processed using the NMRPipe software package (Delaglio et al., 1995) and analyzed with PIPP (Garrett et al., 1991), NMRPipe and PEAK-SORT, an in-house package. The assignments of the  $^1\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{CO}$ , and  $^{13}\text{C}$  resonances were based on the following experiments: CBCA(CO)NH, CBCANH, C(CO)NH, HC(CO)NH, HBHA(CO)NH, HNCO, HCACO, HNHA, HNCA, HCCH-COSY and HCCH-TOCSY (for reviews see Bax et al. (1994); Clore and Gronenborn (1994)). The accuracy of the MMP-13 NMR assignments was further confirmed by sequential NOEs in the  $^{15}\text{N}$ -edited NOESY-HSQC spectra.

Our prior analysis of the MMP-1 NMR structure (Moy et al., 1997) established buffer conditions where the enzyme was still active and stable for 1–2 months and the rate of self-cleavage of the enzyme had been diminished. Under these conditions MMP-13 rapidly degraded within a few hours, requiring the use of an inhibitor to assign the MMP-13 NMR resonances.

The secondary structure of the MMP-13:WAY-151693 complex (for reviews see Wishart and Sykes (1994); Wüthrich (1986)) was determined to be composed of three  $\alpha$ -helices corresponding to residues 28–44 ( $\alpha_A$ ), 112–123 ( $\alpha_B$ ) and 153–163 ( $\alpha_C$ ) and a mixed parallel and anti-parallel  $\beta$ -sheet consisting of

five strands corresponding to residues 83–86 ( $\beta_1$ ), 95–100 ( $\beta_2$ ), 59–66 ( $\beta_3$ ), 14–20 ( $\beta_4$ ) and 49–53 ( $\beta_5$ ). This is essentially identical to the secondary structure observed for other MMP structures.

### Extent of assignments and data deposition

There are three distinct regions in the MMP-13:WAY-151693 spectra where the resonance assignments are incomplete. Residues T144–H148 correspond to part of the dynamic loop region previously seen in the MMP-1 structure (Moy et al., 1997). This suggests a similar dynamic profile for this region in the MMP-13 structure, even in the presence of a high-affinity inhibitor ( $\text{IC}_{50} = 33$  nM). Residues P87 to N91 contain a cluster of prolines that disrupt the sequential assignment process because of the missing NH. Residues G70 to Y73 correspond to a loop region in the vicinity of the structural zinc that was readily assigned in the MMP-1 structure. The backbone and side-chain  $^1\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$ , and  $^{13}\text{CO}$  assignments are essentially complete for the remainder of the protein. Figure 1B shows representative strips from the 3D  $^{15}\text{N}$ -edited NOESY-HMQC spectra for residues L113 to E120, corresponding to part of  $\alpha$ -helix B, indicating both sequential and intraresidue assignments. A table of chemical shifts has been deposited in the BioMagRes-Bank Database (accession code 4679).

### References

- Bax, A., Vuister, G.W., Grzesiek, S. and Delaglio, F. (1994) *Methods Enzymol.*, **239**, 79–105.
- Clore, G.M. and Gronenborn, A.M. (1994) *Methods Enzymol.*, **239**, 349–362.
- Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.
- Freije, J.M.P., Diez-Itza, I., Balbin, M., Sanchez, L.M., Blasco, R., Tolivia, J. and Lopez-Otin, C. (1994) *J. Biol. Chem.*, **269**, 16766–16773.
- Garrett, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.
- Lovejoy, B., Welch, A.R., Carr, S., Luong, C., Broka, C., Hendricks, R.T., Campbell, J.A., Walker, K.A.M., Martin, R., Van Wart, H. and Browner, M.F. (1999) *Nat. Struct. Biol.*, **6**, 217–221.
- Moy, F.J., Pisano, M.R., Chanda, P.K., Urbano, C., Killar, L.M., Sung, M.-L. and Powers, R. (1997) *J. Biomol. NMR*, **10**, 9–19.
- Wishart, D.S. and Sykes, B.D. (1994) *Methods Enzymol.*, **239**, 363–392.
- Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York, NY.
- Zask, A., Levin, J.I., Killar, L.M. and Skotnicki, J.S. (1996) *Curr. Pharm. Des.*, **2**, 624–661.