Journal of Biomolecular NMR (2005) 31: 265–266
DOI 10.1007/s10858-005-0500-6

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of the alkaline proteinase inhibitor APRin from *Pseudomonas aeruginosa*

Sengodagounder Arumugam[a], Robert D. Gray[b] & Andrew N. Lane[a,*]
[a]*J.G. Brown Cancer Center, University of Louisville, KY 40202, U.S.A;* [b]*Department of Biochemistry and Molecular Biology, University of Louisville, KY 40202, U.S.A.*

Received 26 October 2004; Accepted 14 December 2004

Key words: APRin, metalloproteinase inhibitor, NMR assignments

Biological context

The alkaline proteinase inhibitor (APRin) from *Pseudomonas aeruginosa* is an 11.5-kDa, high affinity, high specificity inhibitor of the serralysin class of zinc-dependent proteinases secreted by several Gram-negative bacteria (Feltzer et al., 2000). Serralysins are capable of degrading a variety of host proteins and thereby enhance the pathogenicity of these organisms (Morihara and Homma, 1985). The X-ray structure of the proteinase-inhibitor complex reveals that five N-terminal inhibitor residues occupy the extended substrate binding site of the enzyme and that the terminal amino group coordinates to the catalytic zinc of the enzyme (Hege et al., 2001). However, there is no known structure of the free inhibitor, and the conformational properties of the N-terminal region of the protein are unclear (Feltzer et al., 2003). Metalloproteinase inhibitors are of considerable biological interest as they are involved in regulating Zn metalloproteinase activity in numerous contexts including tissue destruction in pseudomonal infections, tumor metastases, and autoimmune diseases such as rheumatoid arthritis. Knowledge of the mechanism of action of these inhibitors could lead to new approaches to the preparation of therapeutically useful compounds for the treatment of these and other metalloproteinase-dependent diseases.

Here we report the NMR resonance assignments for the uncomplexed APRin and a preliminary evaluation of its secondary structure content.

*To whom correspondence should be addressed. E-mail: anlane01@louisville.edu

Methods and results

APRin was expressed as a fusion protein with the pelB signal peptide of *E. coli* using the IPTG-inducible plasmid pET22b+ (Novagen, Madison, WI) and purified from the periplasm of *E. coli* BL21(DE3) cells as previously described (Feltzer et al., 2000). Clean removal of the signal peptide was verified by electrospray mass spectrometry. For ^{15}N-labeled and $^{15}N/^{13}C$-labeled protein, the method of Marley et al. (2001) was used. The growth medium was replaced by M9 minimal medium supplemented with $(^{15}NH_4)_2SO_4$ (1 g l^{-1}) and ^{12}C or $^{13}C_6$ D-glucose (4 g l^{-1}) as the sole nitrogen and carbon sources.

For NMR, protein samples were prepared in 20 mM Na phosphate, 0.1 M KCl, pH 6.5. All NMR spectra were recorded in 5 mm Shigemi tubes at 25 °C on 14.1 or 18.8 T Varian Inova spectrometers. For the $^{15}N/^{12}C$ protein the concentration was 0.8 mM, and for the $^{15}N/^{13}C$ protein, the concentration was 1.7 mM.

All spectra were acquired in phase sensitive mode using the method of States et al. (1982) with solvent suppression by Watergate (Piotto et al., 1992). NMR spectra were processed using the program NMRPIPE (Delaglio et al., 1995). Prior to Fourier transformation the data were zero filled to increase resolution and a shifted sine-squared function was applied in all dimensions. Forward linear prediction was used to extend the time domain data in the ^{15}N and ^{13}C dimensions. Spectra were analyzed on screen using the Sparky

(T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco).

The heteronuclear spectra recorded for APRin were: $^{15}N/^{1}H$ 2D HSQC, 3D TOCSY-HSQC, with an isotropic mixing time of 50 ms, NOESY-HSQC, using NOE mixing times of 100 and 150 ms, $^{13}C/^{1}H$ 2D HSQC, 3D HNCA, HN(CO)CA, C(CO)NH, H(CCO)NH and CBCA (CO)NH, HNCO, HCACO, HNHA (Cavanagh et al., 1996, and references therein). A typical HSQC spectrum of APRin is shown in Figure 1.

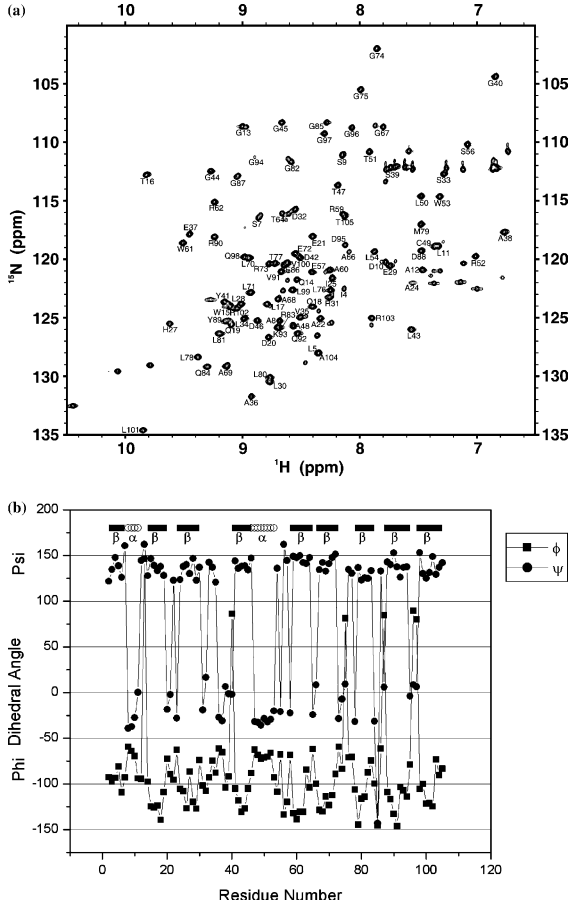

Figure 1. (a) 2D $^{15}N/^{1}H$ HSQC spectrum of APRin recorded at 18.8 T and 25 °C. The spectrum was recorded with 150 increments in t_1 and with an acquisition time of 0.1 s in t_2. (b) Secondary structure of APRin based on TALOS analysis (Cornilescu et al., 1999).

Extent of assignments and data deposition

The ^{15}N and $^{1}H_N$ resonances for 97 of a possible 100 non-proline residues backbone amides were assigned. Resonances for the remaining residues were either not detected or there were no unambiguous connectivities in the triple resonance experiments. About 98% of the Cα and Cβ signals were assigned from the triple resonance experiments. A total of 94% of the Hα resonances in the protein were unambiguously assigned. 86% of the remaining side-chain carbon atoms and 80% of the protons including six glutamine NH_2 side-chain signals have been assigned. Ser1, Pro106 and aromatic ring resonances were not assigned. Based on the chemical shift data (Cornilescu et al., 1999) and characteristic NOEs, the secondary structure (Figure 1b) was determined to be similar to that observed in the crystal state in its complex with metalloproteinase (Hege et al., 2001).

All available ^{15}N, ^{13}C and ^{1}H chemical shifts of backbone as well as the side-chain atoms of APRin have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number: 6292.

Acknowledgements

This work was supported by the Kentucky Challenge for Excellence. NMR spectra were recorded at the JG Brown Cancer Center NMR Facility.

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