Journal of Biomolecular NMR, **15:** 265–266, 1999.
KLUWER/ESCOM

Letter to the Editor: ^{1}H and ^{15}N sequential assignment and solution secondary structure of ^{15}N labelled human pancreatic ribonuclease

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Received 3 August 1999; Accepted 17 September 1999

Key words: antitumor potential–structure relationship, cytotoxins, ribonuclease superfamily

Biological context

Several members of the RNase A (bovine pancreatic ribonuclease) superfamily exhibit anticancer activity. Among the mammalian members of the superfamily, most of the antitumour activity studies have been carried out with a dimeric RNase from bovine seminal vesicles (BS-RNase) (Youle and D'Alessio, 1997). These studies show that dimer formation is crucial for cytotoxicity. Investigations are underway to transfer by protein engineering the structural determinants responsible for the antitumour activity of BS-RNase to a human immunocompatible backbone (Piccoli et al., 1999). Knowledge of the 3D structures of the involved proteins is central to rationally fulfil this objective. As a first step, human pancreatic ribonuclease (HP-RNase), a 127-residue monomeric protein (Beintema et al., 1984) was constructed (Russo et al., 1993). The expressed recombinant protein was undistinguishable from the natural product isolated from human pancreas (Weickmann et al., 1981). Here, we present the assignment of practically all of its ^{1}H and ^{15}N spectral resonances, as well as its secondary structure in aqueous solution. The cytotoxic activity of ribonucleases has been related to their ability to evade the cytosolic ribonuclease inhibitor (RI) (Murthy and Sirdeshmukh, 1992). The structure of HP-RNase will be useful to introduce changes in it in order to increase its resistance to RI.

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Methods and results

The cDNA coding for HP-RNase (Russo et al., 1993) after appropriate mutagenesis was cloned into the prokaryotic vector pET-22b(+) and expressed in *E. coli* after IPTG induction. The recombinant protein was solubilized from inclusion bodies, reoxidized and refolded in a GSH/GSSG buffer. The ^{15}N-labelled protein was obtained with the same procedure from cells grown in minimal medium containing a ^{15}N-labelled protein hydrolysate from *E. coli*. Samples were measured in either H_2O/D_2O (90%/10%, v/v) or D_2O on a Bruker AMX 600 NMR spectrometer with *z*-axis pulsed gradients. The uncorrected pH was 4.5. The following pulse sequences: COSY, TOCSY (60 ms mixing time), NOESY (100 ms mixing time) (Wüthrich, 1986), ^{15}N-HSQC (Bodenhausen and Ruben, 1980), ^{15}N-HSQC-TOCSY and ^{15}N-HSQC-NOESY (Grzesiek and Bax, 1992) were used to obtain the ^{1}H and ^{15}N assignments. TSP was used as a direct vs. indirect reference for ^{1}H and ^{15}N chemical shifts.

Strips from the ^{15}N-HSQC-NOESY experiment at 298 K for residues 5 to 11 and a summary of the NMR data used to determine the solution secondary structure are given in Figure 1. The linkage between sequential amide proton resonances in this helical segment of the protein illustrates the sequence-specific method followed in the assignment. The intensities of the main-chain sequential NOEs, the observed short-range NOEs (Wüthrich, 1986) and the conformational chemical shifts of the $^{1}H_{\alpha}$ resonances indicate that the secondary structure in solution of HP-RNase con-

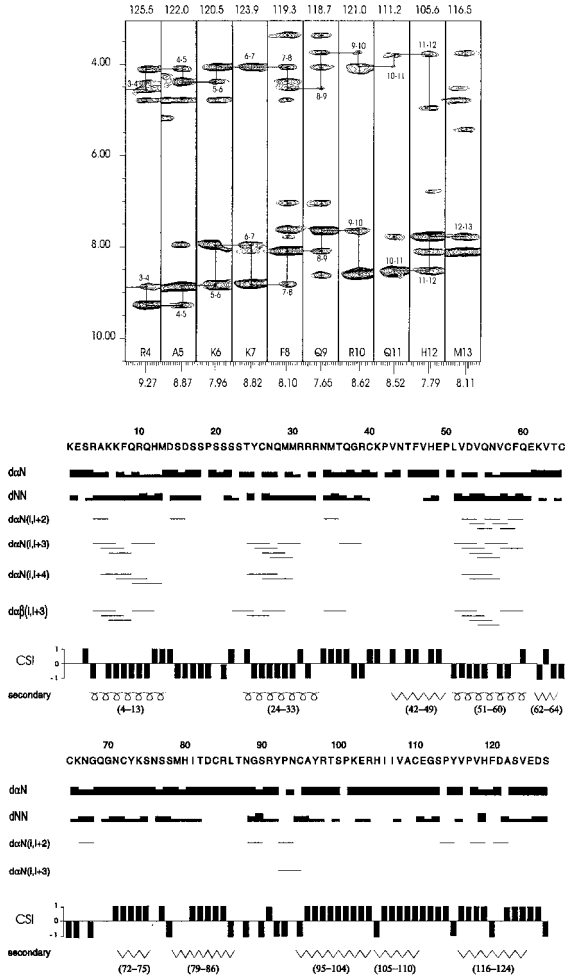

Figure 1. Top: Strips from distinct ^{15}N planes of the HSQC-NOESY spectrum, giving the backbone sequential connectivity between residues 4–13 of HP-RNase. The ^{15}N, H_α and HN chemical shifts (ppm) are given by the top x-axis, y-axis, and bottom x-axis, respectively. Bottom: Sequential dipolar correlations of HP-RNase indicative of secondary structure, drawn as lines whose thickness reflects the NOE intensity. Short range NOEs and chemical shift indices are shown. The indicated secondary structure (α-helix, spiral; β-strands, wavy lines) is depicted.

sists of three α-helices spanning residues 4–13, 24–33, and 51–60, and seven β-strands spanning residues 42–48, 62–64, 72–75, 79–85, 95–104, 105–110, and 116–124. The intensity of the NOE cross correlation between the H_α of the residue preceding the Pro and the HN of the residue following it provides evidence for a *cis* arrangement of the bonds Tyr92–Pro93 and Ser 113–Pro114. In all other Pro residues (19, 42, 50, 101, and 117), the peptide bond preceding Pro is found to be *trans*. The exchange of the hydroxylic protons of Ser 75 and Tyr 97 with those of the solvent is sufficiently slow to give rise to narrow signals that are easily detectable in the NOESY spectrum. In addition, four distinct resonances were found for the ring protons of Tyr 97, indicating that this ring flips slowly on the NMR time scale. Most of these structural features are common to RNase A, with which it shares a sequence identity of 80%. Interestingly, the largest differences in H_α conformational chemical shifts of the two proteins are found in the so-called hinge peptide constituted by residues 20–24, which plays a major role in the BS-RNase dimer formation, suggesting a conformational difference in this region.

Extent of assignments and data deposition

The assignments of backbone amide and H_α protons is complete. Some distal side chain protons in a few arginines and lysines have been left unassigned. Assignments of backbone amide ^{15}N are complete, except for the proline residues. Seven $^{15}N_\epsilon$ out of the 10 arginines and 13 side-chain ^{15}N out of 16 asparagines and glutamines have been unambiguously assigned. The ^{1}H and ^{15}N chemical shifts for HP-RNase have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4370.

Acknowledgements

This work was supported by the European Commission under the INCO-Copernicus Project No. IC15 CT 96-0903. The assistance of the Ministerio de Asuntos Exteriores (Spain) and OMFB (Hungary) (project E26/97) is gratefully acknowledged.

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