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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments and secondary structure determination of the RC-RNase 2 from oocytes of bullfrog *Rana catesbeiana*

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Biological context

Five newly identified ribonucleases, namely, RC-RNase 2, RC-RNase 3, RC-RNase 4, RC-RNase 5 and RC-RNase 6, have been isolated from oocytes of bullfrog *Rana catesbeiana* (Liao et al., 2000). These frog ribonucleases share some common features with the previously reported bullfrog RC-RNase (Liao, 1992) in that they all have a pyroglutamate residue at the N-terminus and contain four disulfide bridges. These novel enzymes possess different specific activities, base specificities, and optimal pH values for their activities. These ribonucleases also possess cytotoxicity to tumor cells. It is interesting to know the possible residues involved in ribonuclease activity and/or cytotoxicity. In addition, the availability of the newly identified bullfrog ribonucleases would be helpful to the investigation of these ribonucleases as potential agents for tumor therapy. To date, only a few 3D structures of frog ribonucleases have been reported. For example, onconase, a ribonuclease isolated from oocytes of *Rana pipiens*, has reached phase III clinical trials as antitumor drug (Juan et al., 1998), and its structure has been solved by X-ray crystallography (Mosimann et al., 1994). The solution structure of RC-RNase has been determined using 2D ^{1}H NMR experiments (Chang et al., 1998). RC-RNase 2, one of the newly identified frog ribonucleases, contains 105 amino acid residues and shares 42.8% sequence identity with RC-RNase. The catalytic activity of RC-RNase 2 is 1000-fold less than that of RC-RNase, but its cytotoxicity toward HeLa cells is only 3 times less than that of RC-RNase. The RC-RNase 2 gene was cloned and successfully expressed in *E. coli*. Here we report the resonance assignments of the recombinant RC-RNase 2 which contains an additional methionine residue at its N-terminus.

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Methods and experiments

The uniformly ^{15}N- and/or ^{13}C-labeled RC-RNase 2 with an additional methionine at its N-terminus was expressed in *E. coli* BL21 (DE3) by the vector pET11d. After IPTG induction, the recombinant proteins in inclusion bodies were collected and renatured (Boix et al., 1996). The purity and molecular mass of the recombinant RC-RNase 2 were checked by SDS-PAGE and ES/MS spectrometry, respectively.

The NMR samples were prepared with 50 mM phosphate buffer at pH 3.5 in 90% H_2O/10% D_2O and contained 0.35 ml of 1.5–2 mM protein in a Shigemi NMR tube. All NMR experiments were recorded at 310 K on a Bruker AVANCE 600 spectrometer equipped with a triple resonance probe. All heteronuclear NMR experiments were performed as described in review articles (Bax et al., 1994; Kay, 1995). The sequential connectivities were identified using CBCA(CO)NH, HNCACB, HNCO, HNCACO and C(CO)NH. The ^{1}H resonances were assigned using TOCSY-HSQC, HAHB(CO)NH, and HCCH-TOCSY. Side-chain ^{13}C resonances were obtained from C(CO)NH and HCCH-TOCSY. Side-chain NH_2 protons of Asn and Gln were identified using 2D ^{1}H-^{15}N HSQC and 3D NOESY-HSQC. Aromatic ^{13}C

and ^{1}H resonances were assigned using 2D ^{1}H-^{13}C HSQC, 2D NOESY, and 2D TOCSY data. Of the four proline residues in the sequence, three (Pro^{41}, Pro^{74}, and Pro^{95}) were assigned by sequential connectivities. The unassigned proline (Pro^{105}), the last residue in the sequence, was identified in the expected $^{13}C^{\delta}$ chemical-shift region of proline residues using HCCH-TOCSY. All spectra were processed by XWIN-NMR and analysed using AURELIA on an SGI O_2 workstation. Linear prediction was applied in the ^{15}N and ^{13}C dimensions to improve the digital resolution. 2,2-Dimethyl-2-silapentane-5-sulfonate (DSS) was used as an external chemical shift standard at 0.00 ppm. The ^{15}N and ^{13}C chemical shifts were indirectly referenced using the consensus Ξ ratios of the zero-point frequencies at 310 K (Wishart et al., 1995).

Extent of assignments and data deposition

A 2D ^{1}H-^{15}N HSQC spectrum, shown in Figure 1A, demonstrates that RC-RNase 2 gives superb NMR data. We have assigned the NMR resonances of backbone ^{15}N, $^{1}H^{N}$, $^{1}H^{\alpha}$, $^{13}C^{\alpha}$ and $^{13}C'$ for all residues with the exception of Met^{-1}. The amide protons of Gln^{1} and Phe^{98} were not observed, presumably due to the fast exchange rate with H_2O. We have also assigned over 95% of side-chain ^{1}H, ^{15}N and ^{13}C resonances. The side-chain NH_2 protons of Asn^{34} were found to have unusual upfield chemical shifts at 3.55 and 6.36 ppm. The consensus chemical shift index (CSI) (Wishart and Sykes, 1994) generated using $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ chemical shifts is shown in Figure 1B, revealing that RC-RNase 2 contains three α-helices and five β-strands. The chemical shifts of ^{1}H, ^{15}N and ^{13}C at pH = 3.5 and T = 310 K have been deposited in the BioMagResBank under accession number BMRB-4825.

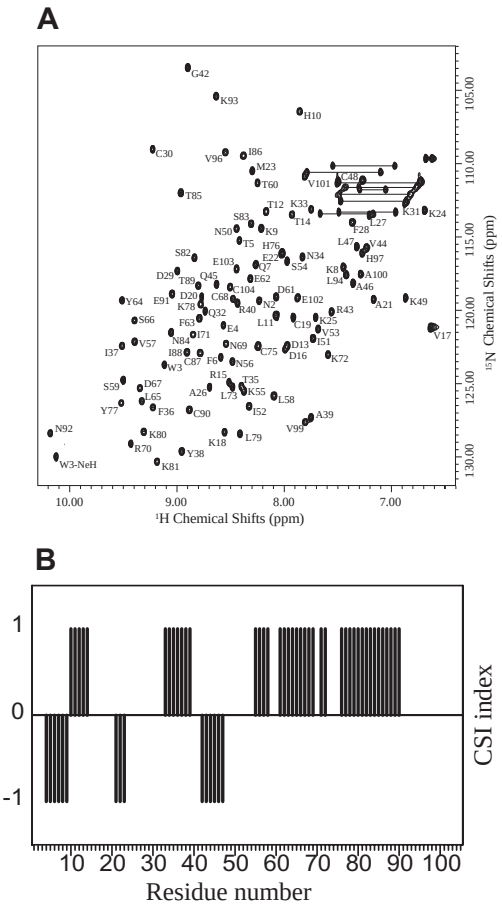

Figure 1. (A) A 600 MHz 2D ^{1}H-^{15}N HSQC spectrum of recombinant RC-RNase 2 obtained at 310 K. The NMR sample contained about 1.5 mM RC-RNase 2 in 50 mM phosphate buffer, pH 3.5. The resonance assignments are indicated with the one-letter amino acid code and residue number. Side-chain amide protons of Asn and Gln are indicated by horizontal lines. (B) CSI consensus plot for recombinant RC-RNase 2, generated using $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ chemical shifts.

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