Letter to the Editor: $^1\text{H}$, $^{13}\text{C}$ and $^{15}\text{N}$ resonance assignments and secondary structure of the cytotoxic protein RNase 4 from bullfrog *Rana catesbeiana* oocytes

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Biological context

Cytotoxic ribonucleases with antitumor activity are mainly found in the oocytes and early embryos of frogs, and share sequence similarity with each other. They all belong to the RNase A superfamily but possess different cytotoxicities, base specificities and ribonucleaseolytic activities. RC-RNase 4, one of the five novel ribonucleases isolated from the oocytes of *Rana catesbeiana*, is a 106 amino acid protein with a pyroglutamate residue at the N-terminus (Liao et al., 2000). Phylogenetic analysis of RNase A superfamily reveals that RC-RNase 4 is very similar to onconase, a cytotoxic ribonuclease isolated from the oocytes of bullfrog *Rana pipiens*, which is now undergoing phase III clinical trials for cancer treatment (Juan et al., 1998). RC-RNase 4 shares 66.0% sequence identity with and its cytotoxicity resembles that of onconase. However, RC-RNase 4 has the base specificity of CpG whereas onconase has that of UpG. Thus, it is interesting to investigate the possibility of RC-RNase 4 as a potential agent for tumor therapy and to study its structural–functional relationship. In addition, among the newly identified frog ribonucleases, only RC-RNase 4 shows distinct CD data, which display two negative ellipticities at 212 and 229 nm when compared to one minimum (~ 211 nm) observed for the other frog ribonucleases. The melting studies also reveal that RC-RNase 4 possesses higher thermostability. To elucidate RC-RNase 4’s similarities to onconase and its unique properties, we applied NMR techniques to determine the 3D solution structure of RC-RNase 4. Here we report the resonance assignments of a recombinant RC-RNase 4 which contains an additional methionine residue at its N-terminus.

Methods and experiments

The cDNA coding for rRC-RNase 4 [M(−1),Q1] was subcloned into the pET11d expression vector and expressed in *E. coli* BL21(DE3) with IPTG as induction agent (Boix et al., 1996). The uniformly $^{15}\text{N}$- and/or $^{13}\text{C}$-labeled proteins were purified by cation exchanger (CM52) and FPLC (Mono S). The authenticity of the RC-RNase 4 sample was verified by SDS-PAGE and ES/MS analysis. Approximately 20 mg of the recombinant RC-RNase 4 was purified from one liter of culture. All NMR experiments were performed on a Bruker AVANCE 600 spectrometer equipped with a triple resonance ($^1\text{H}$, $^{13}\text{C}$ and $^{15}\text{N}$) probe including shielded z-gradient. NMR samples were prepared in 50 mM phosphate buffer in 90% H$_2$O/10% D$_2$O at pH 3.5 and contained 0.35 ml of 1.5 mM protein in a Shigemi NMR tube. For the native RC-RNase 4, 2D $^1\text{H}$ NMR spectra (COSY, TOCSY and NOESY) were collected. All heteronuclear NMR experiments were carried out as described in review articles (Clore and Gronenborn, 1994; Kay, 1995). All spectra were processed using XWIN-NMR and analyzed using AURELIA on SGI O2 workstations. Linear prediction was used in the $^{13}\text{C}$ and $^{15}\text{N}$ dimensions to improve the digital resolution. Sequence-specific assignment of the backbone atoms

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was achieved by the independent connectivity analysis of CBCA(CO)NH, HNCA, HN(CA)CO and C(CO)NH. The $^1$H resonances were assigned using TOCSY-HSQC, HAHB(CO)NH, HCCH-TOCSY and HC(CO)NH. Combined information from 2D $^1$H-$^15$N HSQC and 3D NOESY-HSQC experiments yielded assignments for side-chain amide resonances of the Asn and Gln residues. Aromatic resonances were assigned using 2D $^1$H-$^13$C HSQC, 2D NOESY and 2D TOCSY data.

**Extent of assignments and data deposition**

A well-resolved 2D $^1$H-$^15$N HSQC spectrum, shown in Figure 1A, indicates that RC-RNase 4 is suitable for NMR structural study. We have assigned all resonances of the backbone nuclei ($^1$H$^N$, $^{15}$N, $^{13}$C$^\alpha$, $^1$H$^\alpha$ and $^{13}$C$^\beta$) and $^{13}$C$^\gamma$ except for the $^{15}$N of Met$^{-1}$ and $^{13}$C$^\gamma$ of Pro$^{106}$. The backbone amide protons of Gln$^1$ and Phe$^{99}$ were observed in RC-RNase 4 but not in RC-RNase 2 (Hsu et al., 2001). In addition, over 97% of the $^1$H, $^{13}$C and $^{15}$N resonances of the side chains have also been assigned. The labile protons of the hydroxy groups of Ser$^{3}$ and Ser$^{59}$ were detected and assigned at 6.35 and 5.88 ppm, respectively. Four distinct resonances were found for the aromatic protons of Tyr$^{78}$. The consensus chemical shift index (CSI) plot (Wishart and Sykes, 1994) shown in Figure 1B reveals that RC-RNase 4 consists of three (or two) $\alpha$-helices and five $\beta$-strands, similar to RC-RNase 2 (Hsu et al., 2001). However, RC-RNase 2 possesses a longer $\beta$4 strand (Asp$^{61}$ to Lys$^{72}$) than RC-RNase 4 (Tyr$^{64}$ to Asn$^{69}$) and their $\alpha$2 helices are located differently. Furthermore, the residues located in the $\alpha$3 region show slower exchange rates for RC-RNase 4 compared to those for RC-RNase 2, indicating that the $\alpha$3 helix in RC-RNase 4 is more stable and rigid. The $^1$H, $^{13}$C and $^{15}$N chemical shifts at 310 K have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-4893.

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**References**


