Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments and secondary structure of the liver ribonuclease from bullfrog *Rana catesbeiana*

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Biological context

The major ribonuclease derived from the liver of bullfrog *Rana catesbeiana* (RCL) was first purified along with three minor ribonucleases by Tomita et al., and it was suggested that the major liver ribonuclease might play a key role in the metamorphic climax of tadpole liver (Tomita et al., 1979). The primary structure of RCL was shown to have 65.8 and 71.2% sequence identity to the two lectins (RCE and RJE) derived from the eggs of *R. catesbeiana* and *R. japonica*, respectively (Nitta et al., 1989). These three enzymes possess high similarity of base specificity and thermostability. However, unlike RCL, both RCE and RJE possess agglutination ability to tumor cells. The lack of lectin activity in RCL hints that the agglutination site differs from that of RCE and RJE when in contact with sialic acids (Okabe et al., 1991). Therefore, it is interesting to investigate the structural differences that result in the variation of the biological properties among these ribonucleases. Compared to RCL, the liver ribonuclease, RC-RNase L1, isolated from local bullfrog *R. catesbeiana* reveals a single amino acid difference (Arg^{12} → Thr^{12}), and it was found that both RCL and RC-RNase L1 possess similar biochemical activity and cytotoxicity (Liao et al., unpublished data). When compared to the six ribonucleases found from oocytes of *R. catesbeiana*, RC-RNase L1 shares the highest sequence identity of 65.8% to RC-RNase (RCE), and it has been grouped into the same subset with RC-RNase in a phylogenetic study (Liao et al., 2000). NMR structural studies of RC-RNase along with other frog oocyte ribonucleases have been reported (Chang et al., 1998; Hsu et al., 2001), but structural studies of ribonucleases from frog liver have not yet been performed. The isotopically labeled recombinant RC-RNase L1 containing 112 amino acid residues with an extra methionine at the −1 position was expressed for the NMR experiments. Here the resonance assignments of the ^{1}H, ^{15}N and ^{13}C nuclei are reported.

Methods and experiments

The uniformly ^{15}N- and ^{13}C-labeled RC-RNase L1 with an additional methionine at the N-terminus was expressed in *E. coli* BL21 (DE3) containing the constructed vector [rRC-RNase L1(M-1)/pET11d] by IPTG induction (Boix et al., 1996). Most of the recombinant proteins residing in the inclusion bodies were collected and resolved in 6 M guanidine-HCl, and refolded by the addition of the GSH/GSSG-containing solution. The recombinant proteins were extracted on CM52 and FPLC mono S columns. The purity of the recombinant RC-RNase L1 was checked by SDS-PAGE and ES/MS spectrometry.

The purified RC-RNase L1 (1.6 mM, pH 3.5) was prepared in H$_2$O/D$_2$O (90%/10%, v/v) as the NMR sample. All NMR experiments were performed on a Bruker AVANCE 600 spectrometer equipped with a triple (^{1}H, ^{15}N and ^{13}C) resonance probe including shielded z-gradient and recorded at 310 K. All heteronuclear NMR experiments for the recombinant RC-RNase L1 were carried out as reviewed by Kay (1995). Sequence-specific assignment of the backbone atoms

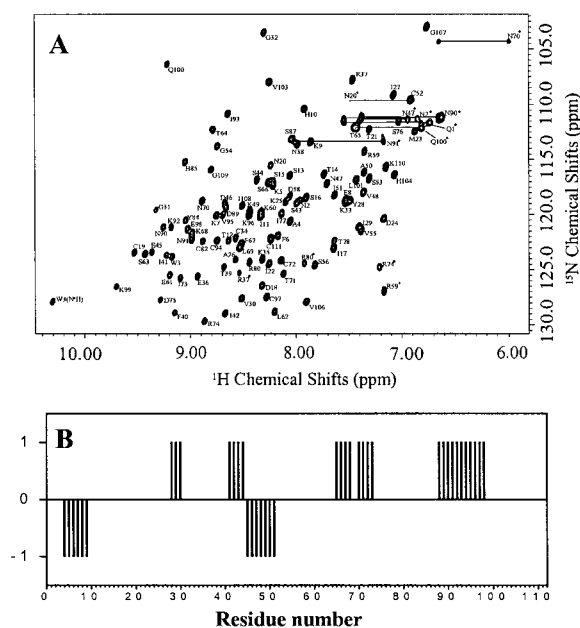*To whom correspondence should be addressed. E-mail: bmchinp@ccvax.sinica.edu.tw

Figure 1. (A) The 600 MHz 2D ^{1}H-^{15}N HSQC spectrum of RC-RNase L1 recorded at 310 K and pH 3.5. The resonance assignments are indicated with one-letter amino acid code and residue number. The residues marked by an asterisk (*) indicate the folded side-chain N$^\varepsilon$H protons of Arg, and the side-chain NH$_2$ resonances of Asn and Gln residues. Side-chain NH$_2$ resonances of Asn and Gln are also connected by horizontal lines. (B) The CSI consensus plot for the recombinant RC-RNase L1, generated using ^{1}H$^\alpha$, ^{13}C$^\alpha$, ^{13}C$^\beta$, and ^{13}C' chemical shifts (Wishart and Sykes, 1994).

was achieved by the independent connectivity analysis of 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. Combined information from 2D ^{1}H-^{15}N HSQC and 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 and 3D ^{13}C-edited NOESY-HSQC experiments.

Extent of assignments and data deposition

Figure 1A shows the 2D ^{1}H-^{15}N HSQC spectrum of RC-RNase L1 acquired at 310 K, pH 3.5. The resonances of the backbone nuclei are all assigned with the exception of Met^{-1}. In total, over 95% of the ^{1}H, ^{15}N and ^{13}C resonances of the side chains have been assigned. The N$^\varepsilon$ and C$^\gamma$ resonances of Arg[59] have downfield shifts at 87.03 ppm and 37.83 ppm, respectively. The side-chain amide protons of Asn[38] are

found to possess unusual upfield shifts, as observed for the corresponding residue Asn[34] in RC-RNase 2 (Hsu et al., 2001). Four distinct ^{1}H and ^{13}C resonances are assigned for the aromatic ring of Tyr[84]. Figure 1B shows the consensus chemical shift index (CSI) plot of RC-RNase L1. CSI analysis of RC-RNase L1 suggests that the difference between RC-RNase L1 and the reported oocyte ribonucleases (RC-RNase and RC-RNase 2) is the absence of secondary structure in three regions: Ile[11]-Thr[14], Arg[59]-Glu[61] and Cys[19]-Asn[20]. As the corresponding regions in RC-RNase and RC-RNase 2, the first two have β structures and the last one has weak α helix contents. However, RC-RNase L1 and RC-RNase (RCE) do share some common features that cannot be detected in other oocyte ribonucleases. The presence of β structure in the region of Tyr[28]-Val[30] is unique for these two proteins. In addition, the alternative presence of proline residues of RC-RNase L1 in the region of Pro[79]-Pro[88] might be the reason for the shortening β structure contents. The ^{1}H, ^{15}N and ^{13}C chemical shifts at 310 K have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4942.

Acknowledgements

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