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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of recombinant onconase/P-30 protein

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Biological context

Onconase, or P-30, is a 104 amino acid protein initially purified from the extracts of *Rana pipiens* oocytes and early embryos based on its anticancer activity both in vitro and in vivo (Darzynkiewicz et al., 1988). Sequence analysis and structural comparison showed that it was highly homologous to pancreatic RNase A and two frog lectins found in the eggs of *Rana catesbeiana* and *Rana japonica* (Youle and D'Alessio, 1997; Chang et al., 1998; Irie et al., 1998). Onconase displays cytotoxic and cytostatic activity against numerous mammalian cell lines in vitro. Although onconase is a poorer ribonuclease, at about one-hundredth the specific activity of RNase A, it is up to 5000 times more toxic to animals than RNase A and is resistant to RNase inhibitors, placental ribonuclease inhibitor and Inhibit-Ace^{TM}. Furthermore, onconase also displays anti-tumor activity in vivo and has been found to specifically inhibit HIV-1 replication in infected H9 leukemia cells at non-cytotoxic concentrations. It is presently undergoing phase III clinical trials for cancer treatment. The X-ray crystal structure of onconase has been solved (Mosimann et al., 1994). Several genetically engineered onconase mutants and chimeric ribonucleases involving onconase, RNase A, eosinophil-derived neurotoxin and human pancreatic ribonuclease have been constructed (Boix et al., 1996; Newton et al., 1998). The biochemical properties of these variants have been examined for exploring the structural basis of the functional diversity and possible pharmaceutical applications. As part of our effort in elucidating the structural basis of the functions of frog ribonucleases we have under taken the structure determination of onconase. Here we report the resonance assignments of an engineered onconase, rOnc (Q1, M23L), with Met at the −1 position and with Met23 mutated to Leu23. This 105 amino acid, 12 kDa enzyme has reduced enzymatic activity and cytotoxicity (Boix et al., 1996). Cleavage of the N-terminal Met−1 and cyclization of the Gln1 residue to reform the pyroglutamate N-terminal reconstituted the cytotoxicity and enzymatic activity. The N-terminus glutamine of onconase has not been cyclized for this study.

Methods and results

Synthetic onconase gene, rOnc[(M(−1),Q1,M23L)], was cloned in pET-11d plasmid and expressed in *E. coli* BL21 (DE3) strain with IPTG as the inducing agent. Protein was isolated as inclusion bodies. After vigorous washing, the protein was denatured in 6 M guanidine-HCl containing 100 mM reduced glutathione, and incubated at room temperature under nitrogen for 2 h. The proteins were then renatured by rapid dilution into a Tris-acetate buffer containing 0.5 M L-arginine and 8 mM oxidized glutathione and incubated at 0 °C for 24 h and purified, as described previously (Boix et al., 1996). The purity of the proteins was checked by 10 and 27% SDS-polyacrylamide gel electrophoresis and was found to be greater than 95%. Uniform ^{13}C- and/or ^{15}N-labeled

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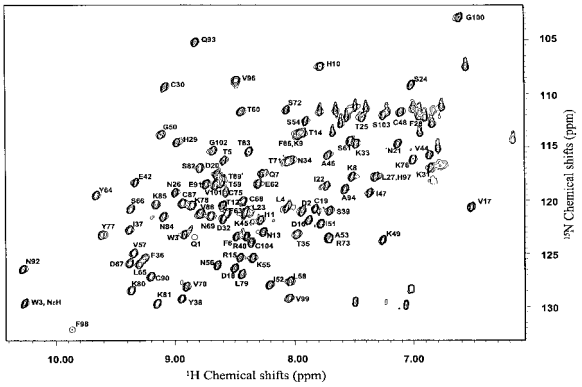

Figure 1. 600 MHz ^{15}N-HSQC spectrum of (U-^{15}N)[(M(−1),Q1, M23L)] onconase obtained at 310 K. NMR samples were sealed in Shigemi tubes and contain 0.35 ml of 2 mM protein in 50 mM phosphate, 10% D_2O at pH 3.5. The resonance assignments are listed next to the resonances.

protein samples were purified from *E. coli* cells grown in M9 medium, supplemented with 2 g/l of U-^{13}C glucose and/or 1 g/l of $^{15}NH_4Cl$ (Cambridge Isotopes, Andover, MA, U.S.A.).

Samples for NMR experiments were sealed in Shigemi (Tokyo, Japan) tubes and contain about 0.35 ml of 2 mM protein in 50 mM phosphate, 10% D_2O at pH 3.5. The pHs were measured with a JENCO microelectronic pH-vision model 6071 pH meter equipped with a 4 mm electrode. All NMR experiments were performed on a Bruker AVANCE600 NMR spectrometer with the probe temperature set at 310 K. NMR parameters for all heteronuclear NMR experiments are as described previously (Lin et al., 1998). Spectra were processed using XWIN-NMR (Bruker AG, Karlsruhe, Germany) and analyzed using AURELIA on SGI computers. Linear prediction was used in the ^{13}C and ^{15}N dimensions to improve the digital resolution. Chemical shifts were referenced to DSS (2,2-dimethyl-2-silapentane-5-sulfonate) at 0 ppm (Markley et al., 1998). The backbone resonance assignments were accomplished from analysis of the following heteronuclear 3D spectra: HNCO, HN(CA)CO, CBCANH, CBCA(CO)NH, HBHA(CO)NH. Side chain assignments were obtained from analysis of ^{15}N-TOCSY-HSQC, HCCH-COSY and HCCH-TOCSY spectra.

Extent of assignments and data deposition

$^{1}H^{N}$, ^{15}N, $^{1}H^{\alpha}$, $^{1}H^{\beta}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and C′ assignments were obtained for all but the N-terminal Met residue. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of (U-^{15}N)-labeled rOnc (Q1, M23L). Assigned resonances are labeled on the spectrum. In addition, most of the side chain resonances have been assigned. The $^{1}H^{\alpha}$, $^{1}H^{\beta}$, $^{1}H^{N}$, ^{13}C, and ^{15}N chemical shifts described herein have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB number 4371.

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References

Biox, E., Wu, Y., Vasandani, V.M., Saxena, S.K., Ardelt, W., Ladner, J. and Youle, R.J. (1996) *J. Mol. Biol.*, **257**, 992–1007.

Chang, C.F., Chen, C.P., Chen, Y.C., Hom, K., Huang, R.F. and Huang, T.H. (1998) *J. Mol. Biol.*, **283**, 231–244.

Darzynkiewicz, Z., Carter, S.P., Mikulski, S.M., Ardelt, W.J. and Shogen, K. (1988) *Cell Tissue Kinet.*, **21**, 169–182.

Iries, M., Nitta, K. and Nonaka, T. (1998) *Cell Mol. Life Sci.*, **54**, 775–784.

Lin, T.H., Chen, C.P., Huang, R.F., Lee, Y.L., Shaw, J.F. and Huang, T.H. (1998) *J. Biomol. NMR*, **11**, 363–380.

Markley, J.L., Bax, A., Arata, Y., Hilbers, C.W., Kaptein, R., Sykes, B., Wright, P.E. and Wüthrich, K. (1998) *J. Biomol. NMR*, **12**, 1–23.

Mosimann, S.C., Ardelt, W. and James, M.N.G. (1994) *J. Mol. Biol.*, **236**, 1141–1153.

Newton, D.L., Boque, L., Wlodawer, A., Huang, C.Y. and Rybak, S.M. (1998) *Biochemistry*, **37**, 5173–5183.

Youle, R.J. and D'Alessio, G. (1997) In *Ribonucleases – Structures and Functions* (Eds, D'Alessio and Riodan), Academic Press, San Diego, CA, pp. 491–552.