Journal of Biomolecular NMR, **20:** 289–290, 2001.
KLUWER/ESCOM

Letter to the Editor: ^{1}H and ^{15}N sequential assignment and secondary structure of the monomeric N67D mutant of bovine seminal ribonuclease

Orlando Crescenzi[a], Alfonso Carotenuto[b], Anna M. D'Ursi[b], Teodorico Tancredi[c], Giuseppe D'Alessio[d], Francesca Avitabile[e] & Delia Picone[e,*]
[a]*Dipartimento di Chimica Org. e Biochimica, Università Federico II di Napoli, I-80126 Napoli, Italy;* [b]*Dipartimento di Scienze Farmaceutiche, Università di Salerno, I-84084 Fisciano, Italy;* [c]*Istituto Chimica MIB del CNR, I-80072 Arco Felice, Italy;* [d]*Dipartimento di Chimica Biologica, Università Federico II di Napoli, I-80134 Napoli, Italy;* [e]*Dipartimento di Chimica, Università Federico II di Napoli, Via Cintia, I-80126 Napoli, Italy*

Received 2 April 2001; Accepted 12 April 2001

Key words: antitumor activity, domain swapping, ribonuclease

Biological context

Bovine seminal ribonuclease (BS-RNase) is the only dimeric protein of the ribonuclease family, with two identical subunits linked to each other through two disulfide bridges between two adjacent cysteines. Each subunit is homologous (80% identity) to bovine pancreatic RNase A, with all the amino acid residues of the active site present at the same sequence position in both enzymes.

The 3D structure of BS-RNase is very peculiar, since it is the only known dimeric enzyme characterized, in solution, by an equilibrium between two different quaternary structures (Piccoli et al., 1992). In the form indicated as M×M the N-terminal arm is swapped between the subunits, whereas in the form indicated as M=M no swapping occurs.

Beside the ribonuclease activity, BS-RNase is endowed with several peculiar biological activities, such as cytotoxicity toward malignant cells, immunosuppression and antispermatogenesis (D'Alessio et al., 1997). The structure of M×M has been solved by X-ray at 1.9 Å resolution (Mazzarella et al., 1993), whereas no structural data are yet available for the M=M form. Insight into the structure of this form can be gained by studying the monomeric derivative of BS-RNase, which is an obligatory intermediate in the folding process leading to the dimer. The monomer can be obtained from the native protein by selective reduction of the interchain disulfide bridges, followed by blocking of the sulfhydryl groups. It displays a ribonuclease activity higher than that of the parent dimeric protein, but does not retain any of the special biological functions (Piccoli et al., 1998). A preliminary NMR study performed in our laboratory on a monomeric sample obtained from the native protein, i.e. based exclusively on homonuclear data, confirmed that the global fold of this protein is similar to that of all RNase A-like proteins (D'Ursi et al., 1995). A more detailed structural investigation was precluded by the lack of heteronuclear spectra and by the heterogeneity of the protein sample, arising from the deamidation of Asn67 which occurs spontaneously in native BS-RNase with formation of a mixture of Asp and isoAsp (Di Donato and D'Alessio, 1981). To avoid this complication we decided to express a mutant derivative of BS-RNase containing an Asp residue at position 67, also in view of the fact that this mutation does not affect the biological activity (A. Di Donato, personal communication).

A detailed structural comparison of this protein with other monomeric ribonucleases can constitute the basis to understand the requirements which induce the N-terminal domain swapping of BS-RNase.

Methods and experiments

E. coli strain BL21(DE3) was transformed with a recombinant pET-22b+ plasmid cDNA coding for the N67D mutant of BS-RNase. The plasmid was a gift of

*To whom correspondence should be addressed. E-mail: picone@chemistry.unina.it

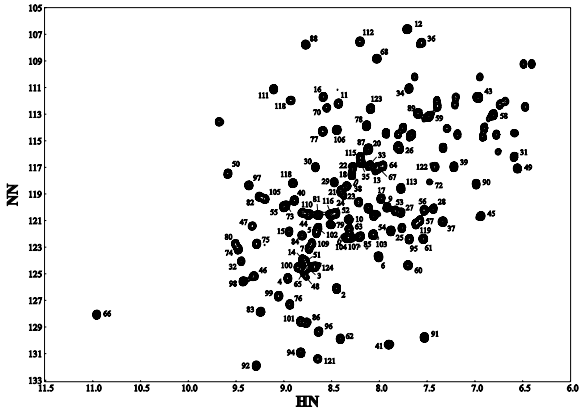

Figure 1. ^{1}H-^{15}N HSQC spectrum of the N67D mutant of mBS-RNase. Backbone NH resonances are labelled with the residue number.

Prof. A. Di Donato. Protein production was induced by addition of 0.4 mM IPTG. The labelled sample was obtained by growing the cells on a M9 minimal medium containing 1 g/L of $^{15}NH_4Cl$.

Monomeric BS-RNase (mBS-RNase) was solubilized from inclusion bodies in fully reduced and denatured form. Following renaturation and reoxidation in the presence of a buffer containing oxidised/reduced glutathione, gel filtration on Sephadex G-75 yielded pure mBS-RNase (about 15 mg/L of LB medium and 1 mg/L of M9 medium). 2D and 3D NMR spectra (COSY, TOCSY, NOESY, double quantum COSY, ^{15}N-HSQC,^{15}N-TOCSY-HSQC, ^{15}N-NOESY-HSQC, HNHA and HNHB) were collected at 500 or 600 MHz on Bruker DRX instruments. NMR samples (2 mM) were prepared by dissolving the appropriate amount of protein in either 95% H_2O-5% D_2O or in D_2O, at pH 5.65 and 300 K. Spectra were referenced to the water signal at 4.70 ppm. For heteronuclear experiments small volume tubes were utilised (New Era Enterprises).

NMR data were processed with NMRPipe (Delaglio et al., 1995) and analyzed with NMRView (Johnson and Blevins, 1994). Apart from the differences originating from the N67D mutation, the 2D NMR spectrum of recombinant mBS-RNase was indistinguishable from that of the monomer obtained from the natural protein. Almost complete assignment of proton and ^{15}N resonances has been achieved by a combination of 2D and 3D experiments. The wide dispersion of amide resonances is illustrated by the ^{1}H-^{15}N HSQC spectrum recorded at 300 K (Figure 1).

Extent of assignments and data deposition

Out of 125 residues (taking into account the N-terminal Met0), 122 spin systems were partially or completely assigned. Unassigned backbone resonances correspond to Met0, Lys1 and Phe120. Identification of hydrogen bonded NHs, obtained by a combination of H/D exchange data and NH resonance temperature coefficients, and secondary structure elements, determined on the basis of short- and medium-range NOEs, confirmed that the secondary structure of mBS-RNase is very similar to that of bovine pancreatic ribonuclease (RNase A), the main differences being concentrated in the hinge region (residues 16–22), i.e. in the region primarily involved in the swapping of the N-terminal domains between the two subunits.

The chemical shift values of the proton and nitrogen resonances have been deposited in the BioMagResBank (http://bmrb.wisc.edu) under accession number 4980.

Acknowledgements

This work was supported by grants of the Italian MURST (COFIN.MURST).

References

D'Alessio, G., Di Donato, A., Mazzarella, L. and Piccoli, R. (1997) In *Ribonucleases: Structures and Functions* (D'Alessio, G. and Riordan, J.F. , Eds.), Academic Press, New York, NY, pp. 383–423.

Delaglio, F., Grzesiek, S., Vuister, G., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Di Donato, A. and D'Alessio, G. (1981) *Biochemistry*, **20**, 7232–7237.

D'Ursi, A., Oschkinat, H., Cieslar, C., Picone, D., D'Alessio, G., Amodeo, P. and Temussi, P.A. (1995) *Eur. J. Biochem.*, **229**, 494–502.

Johnson, B. and Blevins, R. (1994) *J. Biomol. NMR*, **4**, 603–614.

Mazzarella, L., Capasso, S., Demasi, D., Di Lorenzo, G., Mattia, C.A. and Zagari, A. (1993) *Acta Crystallogr.*, **D49**, 389–402.

Piccoli, R., Di Donato, A. and D'Alessio, G. (1988) *Biochem. J.*, **253**, 329–336.

Piccoli, R., Tamburrini, M., Piccialli, G., Di Donato, A., Parente, A. and D'Alessio, G. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 1870–1874.