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Letter to the Editor: Sequential assignment and solution secondary structure of doubly labelled ribonuclease Sa

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Biological context

Ribonuclease Sa, a very small (96 residues) enzyme excreted by *Streptomyces aureofaciens*, is of biophysical interest as a model system for studying protein stability and folding. The conformational stability of RNase Sa has been well defined (Pace et al. 1998) and the production of variants lacking groups which make hydrogen bonds or hydrophobic interactions in the wild type protein is being used to quantify the contribution of such interactions to protein stability. Here, we present the ^{13}C backbone, ^{1}H and ^{15}N NMR assignments and the solution secondary structure of RNase Sa. Future NMR experiments, including relaxation measurements, hydrogen exchange and pKa determinations can further define the dynamics and stability of RNase Sa in solution and quantify the contribution of salt bridges to the protein's stability.

RNase Sa is homologous to the larger, highly specific ribonuclease, α-sarcin, which effectively inhibits protein synthesis in some tumor cell lines (Wool, 1997), and whose structure was recently determined (Campos-Olivas et al., 1996). Calculation of RNase Sa's 3D solution structure is currently in progress. A comparative study of the α-sarcin and RNase Sa solution structures could aid our understanding of α-sarcin's ability to preferentially enter tumor cells prior to selectively cleaving the large rRNA.

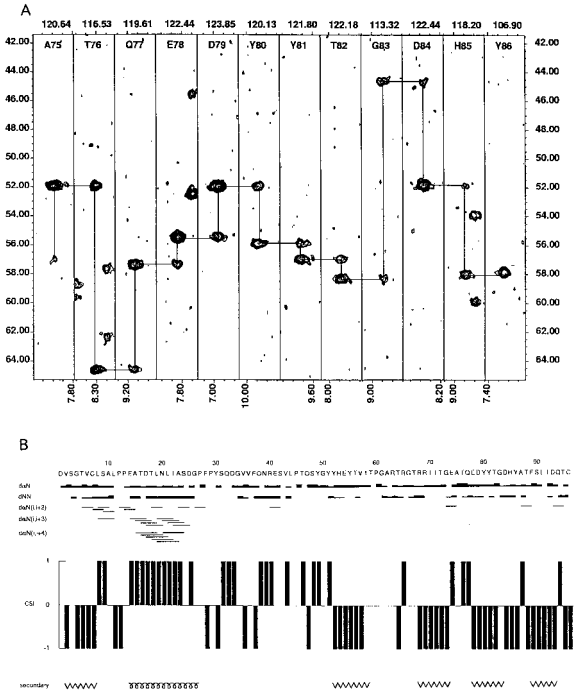

Figure 1. (A) Strips from distinct ^{15}N planes of the HNCA spectrum giving the backbone connectivity between residues 75 and 86 of RNase Sa. The ^{15}N,^{1}H and ^{13}C chemical shifts (in ppm) are given by the top x-axis, bottom x-axis and y-axis, respectively. (B) Dipolar connectivities of RNase Sa indicative of secondary structure are drawn as lines whose thickness reflects the NOE intensity. The consensus CSI for RNase Sa is shown. The indicated secondary structure (α-helix, spiral; β-strands, wavy lines) is depicted.

Methods and results

RNase Sa was expressed in *Escherichia coli* (strain RY 1988) harboring the expression plasmid pEH100.

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^{15}N- and ^{15}N-, ^{13}C-labelled proteins were produced by growing cells in M9 minimal media to 1.2–1.5 OD, collecting the cells by centrifugation and then resuspending them in media containing ^{15}N-NH_4Cl or ^{15}N-NH_4Cl and ^{13}C-glucose with 0.1 mM IPTG to induce protein production. RNase Sa was purified as described by Hebert et al. (1997) and the yield was 20–25 mg/L. All samples were measured at 303 K in either H_2O/D_2O (90%/10%, v/v) or D_2O, on a Bruker AMX600 spectrometer with z-axis pulsed gradients. The pH (uncorrected for deuterium isotope effect) was 5.5. A battery of spectra, including the conventional homonuclear pulse sequences: COSY, TOCSY (60 ms mixing time), NOESY (80 ms mixing time) (Wüthrich, 1986); and heteronuclear pulse sequences ^{15}N-HSQC (Bodenhausen and Ruben, 1980), ^{15}N-HSQC-TOCSY, ^{15}N-HSQC-NOESY; HNCA, HN(CO)CA, and HNCO (Grzesiek and Bax, 1992), were used to obtain the ^{1}H, ^{15}N and backbone ^{13}C assignments. TSP was used to directly reference the ^{1}H chemical shifts and to indirectly reference the ^{15}N and ^{13}C chemical shifts. The spectra were assigned within the ANSIG computer program (Kraulis et al., 1994).

Strips from the HNCA experiment for residues 75 to 86 and a summary of the NMR data used to determine the solution secondary structure are shown in Figure 1. The linkage of the backbone chain resonances provided by the HNCA and HN(CO)CA experiments was complete except for one break at Pro12–Pro13. The intensities of the main-chain NOEs (Wüthrich, 1986), and the composite ($^{13}C_\alpha$, $^{13}C'$, $^{1}H_\alpha$) chemical shift index (Wishart and Sykes, 1994) indicate that the solution secondary structure of RNase Sa consists of an α-helix (residues 14–26) and 5 β-strands (residues 2–7, 52–58, 67–73, 78–83 and 89–93). The intensities of the (i) $^{1}H_\alpha$, Pro (i+1) $^{1}H_\delta$ and (i) $^{1}H_\alpha$, Pro (i+1) $^{1}H_\alpha$ NOEs provide evidence that the Gly 26–Pro 27 peptide bond is *cis* and that the other bonds are *trans* (Wüthrich, 1986). All these findings are in general agreement with RNase Sa crystal structures (Sevcik et al., 1996). Four distinct resonances were found for the ring protons of both Tyr 52 and Tyr 55, indicating that these rings flip slowly. The observation of phenolic protons of Tyr 52 and Tyr 81 in NOESY spectra in H_2O solution strongly suggests that these protons form hydrogen bonds.

Extent of assignments and data deposition

The ^{1}H assignments, with the exception of rapidly exchanging protons, are almost complete. Only Ser 31's H_βs, which likely overlap with its H_α, and Tyr 51's H_ϵs, whose peaks may be very broad due to slow ring flipping, are unassigned. The Pro 12 $^{13}C_\alpha$ and the $^{13}C'$s of the residues preceding the six prolines, Gly 73, and the C-terminal Cys 96 are unassigned. The Gly 73 $^{13}C'$ is not observed as the Glu 74 ^{15}NH lies near the water line. All other $^{13}C_\alpha$ and $^{13}C'$ atoms are assigned. The ^{15}N amide assignments are complete except for the proline residues, and the ^{15}N of Asp 1 at the N-terminus.

The ^{1}H, ^{15}N and ^{13}C chemical shifts for RNase Sa have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4259.

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