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Letter to the Editor: Assignments of ^{1}H, ^{13}C, and ^{15}N resonances of intramolecular dimer antifreeze protein RD3

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Biological context

Antifreeze protein (AFP) exists in body fluids of polar fish and insect larva living in cold environments, where the temperature is subzero. AFP has a unique ability to adsorb to a hexagonal ice crystal to inhibit its growth, which results in a depression of the freezing point of water, leading to a protection of tissues from freezing injury. We have examined a new Type III AFP intramolecular dimer named RD3 by NMR spectroscopy. RD3 comprises N- (residues Asn^{1}-Glu^{64}) and C- (residues Ser^{74}-Glu^{134}) terminal domains connected by a nine-residue linker (D^{65}GTTSPGLK^{73}) (Wang et al., 1995). Each domain has over 80% homology in primary structure with the ordinary Type III AFP monomer, and both domains have 80% homology with each other. RD3 is the only species of intramolecular dimer among all known types and isoforms of AFPs, and possesses about 1.9 times higher activity compared with the ordinary Type III AFP on a molar basis. Although the structure of the N-domain plus linker portion (residues 1–76) was clarified recently (Miura et al., 1999), the overall structure of intact RD3 is not determined yet. We have succeeded in expressing non-labeled, ^{15}N-labeled and ^{13}C-/^{15}N-labeled recombinant protein of RD3, for which the antifreeze activity was identified. Here we report the 2D- and 3D-NMR-based assignments of the ^{1}H, ^{13}C and ^{15}N resonances of RD3.

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Methods and results

RD3 protein was isolated from the *E. coli* expression system constructed in our group. A DNA encoding RD3 was designed and synthesized according to the amino acid sequence of RD3. The synthesized DNA was ligated with pKK223-3UC vector. *E. coli* JM105 was transformed with the expression plasmid. The transformant was cultured in 2× YT medium. RD3 protein was overexpressed in the transformant and purified by sequential column chromatography. The ^{15}N- and ^{13}C/^{15}N-labeled proteins were obtained from the transformant cultured in M10 minimal medium containing ^{15}N-labeled NH_4Cl and ^{15}N-labeled NH_4Cl/^{13}C-labeled glucose, respectively.

For 2D- and 3D-NMR experiments performed in H_2O solution, 8 mg of the lyophilized non-labeled and labeled RD3 samples was dissolved in 0.5 ml of H_2O (H_2O : D_2O = 9 : 1) containing 25 mM of KCl and 1 mM of NaN_3 to give a final concentration of 1 mM (pH 6.8). For the experiments performed in D_2O solution, 8 mg of the non-labeled sample was dissolved in 100% D_2O containing 25 mM of KCl and 1 mM of NaN_3 to give a final concentration of 1 mM (pH 6.8).

The NMR experiments were performed on JEOL JNM-Alpha and Varian Unity-500 spectrometers at 4 °C. The following sets of 2D- and 3D-NMR data were acquired for the spectral assignments of the ^{1}H-, ^{13}C- and ^{15}N-resonances: (1) NOESY in D_2O (70 ms, 512 × 256); (2) {^{15}N-^{1}H}-HSQC (512 × 256); (3) {^{13}C-^{1}H}-HSQC (512 × 256); (4) ^{15}N-edited

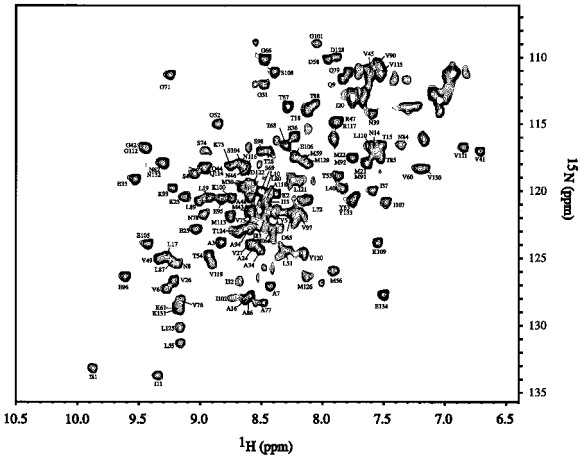

Figure 1. ^{1}H-^{15}N-HSQC spectrum of RD3 obtained at 4 °C. ^{15}N-labeled RD3 sample was dissolved in 0.5 ml of H_2O ($H_2O : D_2O = 9 : 1$) containing 25 mM of KCl and 1 mM of NaN_3 to give a final concentration of 1 mM (pH 6.8).

NOESY (100 ms, 512 × 128 × 32); (5) ^{15}N-edited TOCSY (100 ms, 512 × 128 × 32); (6) HNCA (512 × 32 × 32); (7) HNCACB (512 × 128 × 32); (8) CBCA(CO)NH (512 × 40 × 26); (9) C(CO)NH (512 × 96 × 32); (10) HC(CO)NH (512 × 96 × 32); (11) HCCH-TOCSY (512 × 64 × 96). The 3D-HNHA (512 × 160 × 96) experiments were performed to obtain the ^{3}J-coupling constants between NH and C$^{\alpha}$H protons.

In the 2D and 3D experiments, the ^{1}H chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS). The ^{13}C and ^{15}N chemical shifts were referenced using internal DSS and the frequency ratios Ξ (^{15}N/^{1}H) = 0.101329118, (^{13}C/^{1}H) = 0.251449519 (Wishart et al., 1995). All of the NMR data were processed and analyzed on an SGI Power Indigo2 workstation (Silicon Graphics, Mountain View, CA) using NMRPipe (Delaglio et al., 1995) and PIPP (Garrett et al., 1991) software.

The resonance assignments of RD3 were performed successfully by employing the 2D- and 3D-NMR spectra using the standard strategy (Wüthrich, 1986; Evans, 1995). Figure 1 shows a well-separated ^{1}H-^{15}N-HSQC spectrum of RD3, in which the assignments are indicated for each cross peak. For a small overlapping region of Figure 1, the resonance assignments were performed from a set of ^{13}C-edited 3D-NMR spectra. We could identify all resonances originating from the nine residues of the linker portion of RD3.

Extent of assignments and data deposition

All of the ^{1}HN-, ^{1}H-, ^{15}N-, ^{13}C$^{\alpha}$-, and ^{13}C$^{\beta}$-assignments were completed except for the ^{13}C$^{\beta}$-resonances of Ile^{32} and Pro^{57}. We compared the chemical shifts of all the assigned resonances originating from the N-terminal globular domain (Ser^{4}-Glu^{64}) with those from the C-terminal globular domain (Ser^{74}-Glu^{134}). It was found that the chemical shifts of the C$^{\alpha}$-resonances of the N-domain are almost identical to those of the C-domain within +/−2.0 ppm for the conserved residues. However, the chemical shifts of the conserved residues are not perfectly coincident for the ^{15}N, ^{1}HN, and ^{13}CαH-resonances between the two domains. A total of 80 $^{3}J_{NH-H\alpha}$ coupling constants out of 134 were identified without signal overlapping. The chemical shift assignments and the $^{3}J_{NH-H\alpha}$ coupling constants have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 4449.

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