

Letter to the Editor: ^1H , ^{13}C and ^{15}N resonance assignments of the antifreeze protein cfAFP-501 from spruce budworm at different temperatures

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Biological context

Antifreeze proteins (AFPs) have been studied for more than three decades since the first reported antifreeze glycoproteins (AFGPs) in Antarctic fishes (Fletcher et al., 2001). AFPs have the ability of lowering the freezing point of aqueous solutions and inhibiting ice recrystallization and help many organisms to survive in subzero surroundings. Previous investigations suggested that the AFPs depress the freezing point non-colligatively by binding to ice and inhibiting the growth of ice, a property known as thermal hysteresis (TH) activity (Davies et al., 1997). There are many hypotheses regard to the antifreeze mechanism, while it is still not clear exactly how AFPs bind and inhibit ice growth (Jia et al., 2002). Early studies suggested that the hydrogen bonds between the AFPs and ice surface play an important role, which causes the AFPs irreversible binding to the ice surface. Recent results show that the van der Waals and hydrophobic interactions make a significant contribution to the adsorption (Graether et al., 1999). The crystal structure of cfAFP-501 (121 amino acids with a molecular weight of 12.5 kDa) from spruce budworm or *Choristoneura fumiferana* (isoform 501) shows the similar β -helical structure to that of the shorter 9 kDa isoform of cfAFP-337 with additional two loops, while its thermal hysteresis (TH) activity is about 2 times higher than that of the cfAFP-337 (Leinala et al., 2002). In order to investigate the dynamic properties at different temperatures and thereafter the mechanism of the enhanced thermal hysteresis (TH) activity, we here report the nearly

complete sequence-specific backbone and side chain resonance assignments at 290 K and the backbone assignments at 270 K of the cfAFP-501 protein.

Methods and experiments

The *cfAFP-501* gene was cloned into pET20(b) vector (Novagen) for recombination expression in *Escherichia coli* strain BL21(DE3)/pLysS cells (Leinala et al., 2002). Uniformly ^{15}N -labeled and $^{15}\text{N}/^{13}\text{C}$ -labeled cfAFP-501 samples were obtained by growing the bacteria containing the expression plasmid in the M9 minimal media. The cfAFP-501 protein presented in inclusion body and the refolding and purification procedure was similar to that of cfAFP-337 (Gauthier et al., 1999).

NMR samples were prepared in 50 mM sodium-phosphate buffer at pH 5.7 with 50 mM sodium chloride, 90% $\text{H}_2\text{O}/10\%$ D_2O , and 2 μl 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) was added as the chemical shift reference. All NMR data were recorded at temperatures of 270 K and 290 K on the Bruker Avance 500 MHz (with a cryoprobe) and 600 MHz spectrometers, both equipped with four RF channels and triple-resonance probes with pulsed field gradients. The NMR spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed by using NMRView (Johnson and Blevins, 1994). The 2D ^{15}N -edited HSQC, 3D triple-resonance spectra of HNCA, HNCACB, CBCA(CO)NH, HBHA(CO)NH and HNCO were used to obtain the backbone resonance assignments. The 3D spectra of H(CCO)NH-TOCSY,

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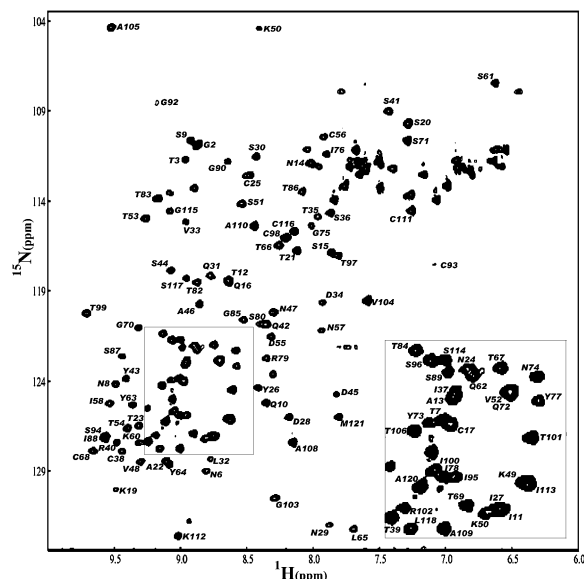


Figure 1. ^{15}N -edited 2D-HSQC spectrum of the uniformly ^{15}N labeled cfAFP-501 protein at 270K and pH 5.7. The sample was dissolved in 90% $\text{H}_2\text{O}/10\%$ D_2O containing 50 mM of phosphate and 50 mM NaCl. The assignments are annotated by the resonance peaks with the one-letter amino acid code and the sequence number.

(H)C(CO)NH-TOCSY, HCCH-TOCSY, HCCH-COSY (mixing time 17 ms), and ^{15}N -edited TOCSY-HSQC (mixing time 80ms) were used for the side chain resonance assignments. The assignments were confirmed with ^{15}N -edited 3D NOESY-HSQC (mixing time 100 ms) and 2D ^{13}C -edited HSQC spectra.

Extent of assignments and data deposition

Figure 1 shows the 2D ^{15}N -edited HSQC spectrum of the cfAFP-501 at 270 K (spectrum at 290 K is not shown here). Slightly shifts were observed for most peaks between the spectrum at 270 K and 290 K. We should note that overlapping resonance peaks of N8/Y43 and C116/G75 at 290 K were resolved at 270 K, while the resolved resonance peaks of T12/Q16, S94/T88 and K49/K113 at 290 K became overlapped at 270 K. Nearly complete backbone resonance assignments of $^1\text{H}^{\text{N}}$, ^{15}N , $^{13}\text{C}^{\alpha}$, $^1\text{H}^{\alpha}$ atoms have been obtained except for residues D1, C4, V5,

V18 at both temperatures. A total of 80 slow exchange amide protons were identified from a series of H/D exchange experiments. The side chain ^1H and ^{13}C resonance assignments were obtained for over 90% of the residues at 290 K. While the side chain assignments at 270 K were not performed due to the poor quality of the spectra. The relaxation data indicates that the cfAFP-501 protein forms into oligomer at both temperatures (Graether et al., 2003). Deviations of the ^1H and ^{13}C chemical shifts from random coil values (Wishart et al., 1995), combined with sequential and medium range NOEs indicate that the cfAFP-501 is a β -helical protein. A table of the ^1H , ^{13}C , ^{15}N chemical shifts assignments of the cfAFP-501 at 270 K and 290 K has been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu/>) under the accession number 6111.

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