Stress Responsibility of Antifreeze Protein from Antarctic Marine Diatom

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The antifreeze protein (AFP) has the ability to bind ice crystals and AFP causes the thermal hysteresis (TH) activity that is, lowering freezing temperature below the melting temperature. The AFP genes (*Cn-AFP* and *Cn-isoAFP*) from the Antarctic marine diatom, *Chaetoceros neogracile* were cloned and characterized. The *Cn-AFP* and *Cn-isoAFP* ORF are 849 bp and 855 bp, respectively. The Cn-AFP protein consists of 282 amino acid (29.2 kDa), and the Cn-isoAFP protein was composed of 284 amino acid (29.2 kDa). The similarity of Cn-AFP and Cn-isoAFP was 74.6% and Cn-AFPs showed a significant homology with the AFPs or IBPs from other psychrophilic organisms. Recombinant Cn-AFP was exhibited to display antifreeze activities based on measuring the TH activity and modified morphology of single ice crystals. Recombinant mature Cn-AFPs showed much higher TH activity than that of pre-mature Cn-AFPs at the same protein concentration. The ice crystal shapes changed to an elongated hexagonal shape in the presence of the recombinant mature Cn-AFP, while single ice crystal showed a circular disk shape in an absence of Cn-AFP. Northern blot showed a dramatic increase of *Cn-AFP* and *Cn-isoAFP* transcripts when the cells were placed under freezing, 10℃ thermal and high light stress condition for few hours. Also, the amount of Cn-AFPs protein was increased by the same environmental stress condition. To examine the stress responsibility of Cn-AFPs, we are under performing heterologous transformation of Cn-AFPs to *Arabidopsis thaliana*.

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