

Extraction, separation and measurement of the thermal hysteresis activity of antifreeze proteins from *Ammopiptanthus nanus*

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Abstract: Antifreeze protein (AFP) is one of the proteins that have the antifreeze ability during the biology adapts the low temperature. It is very important to increase the resisting and coercing ability at low temperature. DE-52 ion-exchange cellulose was used to extract and separate the antifreeze proteins from *Ammopiptanthus nanus* leaves. Differential scanning calorimetry (DSC) was used to measure the thermal hysteresis activity (THA). The results reveal that when the concentration of the protein is 20mg/ml, the THA of the AFP is 0.46°C. Its molecular weight is about 119.24kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Extraction:

Plant material was collected from mature *Ammopiptanthus nanus* leaves which grown outside in Turpan Eremophytes Botanic Garden in the winter. AFPs were extracted by grinding in liquid nitrogen using a pug mill, and dissolving in extraction buffer (12 mmol/L Tris-HCl, 0.1 mol/L NaCl, 0.1 mmol/L EDTA, 1 mmol/L PMSF, pH 8.6). Centrifugation at 25,000g for 30 min at 4°C, the AFPs was retained in the supernatant.

Thermal hysteresis activity:

Thermal hysteresis activity defined as the temperature difference between the melting point and the non-equilibrium hysteresis freezing point of a solution. The sample solution was measured by differential scanning calorimetry (DSC) using the Q100 DSC (TA, USA). The negative control is ddH₂O (5 l) and BSA (5 l, 20 mg/mL) (Fig 2). When the content of ice crystal is 0.36%, the THA is 0.46°C. And the content of ice crystal is decreasing while the THA is increasing (Table 1).

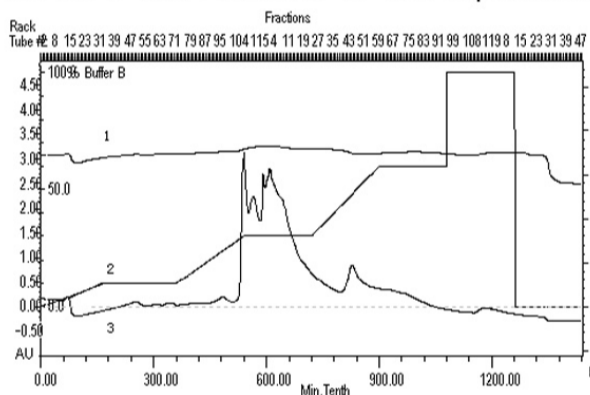


Fig. 1 DE-52 ion-exchange chromatography of antifreeze proteins
Note: curve 1—the change of pH; curve 2—the change of NaCl%; curve 3—the separation of the proteins

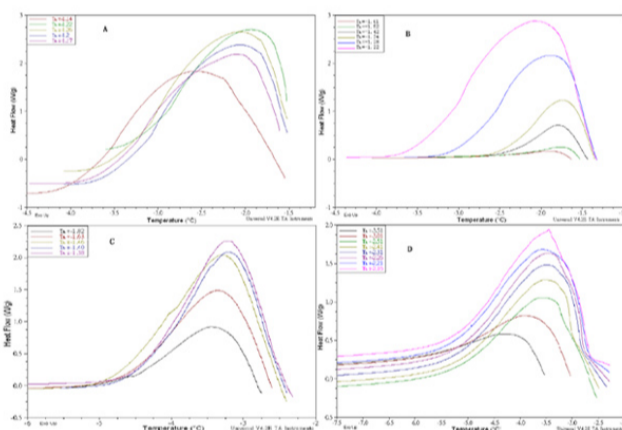


Fig 2 The DSC curve. A—DSC curve of ddH₂O devoid of AFP, B—DSC curve of BSA solution devoid of AFP, C—DSC curve of protein solution devoid of AFP, D—DSC curve of the AFP isolated from *Ammopiptanthus nanus*.

Purification:

The AFP purify was determined by SDS-PAGE using the Bio-Rad mini-system. Sample was run on 10% acrylamide gels, using PRO-STAIN™ Marker I (SBS). Gel was stained with Coomassie brilliant blue. The molecular weight of the AFP is about 119.24kDa (Fig 3).

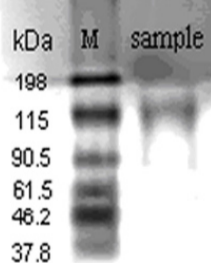


Fig 3 SDS-PAGE of AFP of *Ammopiptanthus nanus*
M—PRO-STAIN™ Marker I, sample—the AFP of *Ammopiptanthus nanus*

Table 1 The hold temperature (Th), onset temperature (To), percent ice nuclei and THA of AFP solution

Th/°	To/°	THA/°	Nuclei%
-4.51	-4.55	0.04	85.71
-4.01	-4.04	0.03	79.49
-3.51	-3.55	0.04	71.12
-3.01	-3.05	0.04	58.3
-2.51	-2.68	0.17	31.06
-2.46	-2.75	0.29	16.64
-2.41	-2.76	0.35	12.54
-2.36	-2.72	0.36	8.7
-2.31	-2.67	0.36	5.09
-2.26	-2.68	0.42	2.41
-2.21	-2.62	0.41	0.85
-2.16	-2.62	0.46	0.36

Conclusions:

THA is an important characteristic to determine the AFPs. Data from this study indicates that when the concentration of AFP is 20mg/ml, the THA is 0.46°C and the content of ice crystal is only 0.36%. So we report here that there is AFP in *Ammopiptanthus nanus* leaves and it has the ability of preventing growth of ice crystal increases substantially when the content of the crystal is diminished. Its molecular weight is about 119.24kDa by SDS-PAGE. The content of ice crystal is decreasing while the THA is increasing. And there is a curve regression relationship between the THA and the content of ice crystal, and hence, the content of crystal has to be taken into consideration when the THA is to be quantified. The present results indicate that the AFP has a great potential to protect the *Ammopiptanthus nanus* during winter.

