Effect of antifreeze activity and lipid interaction of tryptophan scanning of 11-residue lysine homopeptide

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ABSTRACT

Antifreeze peptides and proteins play crucial roles in the survival of organisms that thrive in cold environments, such as polar fish [1]. These compounds serve diverse functions, including cryopreservation, which is essential for organisms facing freezing conditions. Peptides exhibit varied primary and secondary structures, offering a range of functionalities in cold adaptation.

In our study, we focused on an 11-residue lysine homopeptide, K11, where each position was substituted with Trp using a scanning technique. Peptides were synthesized using the Fmoc/tBu strategy [2], followed by purification and characterization via RP-HPLC and mass spectrometry.

Antifreeze activity of peptide was evaluated using differential scanning calorimetry (DSC) and quantifying ice recrystallization inhibition, known as thermal hysteresis (THA). Results showed a slightly increased antifreeze activity with fewer remaining nucleation points across all peptides in the scanning sequence. Substituting internal residues whit Trp, enhanced ice recrystallization inhibition, highlighting the significance of peptide structure.

However, Trp substitution disrupted the secondary structure of K11, as obtained by circular dichroism, notably affecting the type II polyproline helix of K11 and resulting in decreased antifreeze activity compared to the K11 homopeptide. This highlights the intricate relationship between peptide structure and functionality in antifreeze mechanisms.

INTRODUCTION

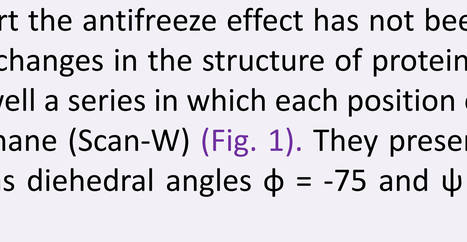
Antifreeze proteins (AFPs) are expressed in fish that inhabit the polar zones, allowing them to survive in environments at temperatures below zero. This physical property is considered of enormous importance in the cryopreservation of cells, tissues and organisms. There are different types of secondary structure for AFPs. The exact mechanism by which these molecules exert the antifreeze effect has not been fully clarified at present. In order to appreciate changes in the structure of proteins, a study of lysine peptide (K11) is carried out as well a series in which each position of the peptide K11 chain was replaced by tryptophane (Scan-W) (Fig. 1). They present secondary polyproline II structure (PPII) that has diehedral angles ϕ = -75 and ψ = +145 and ϕ = -75 and ψ +160 for PPI (Fig. 2A).

METHODOLOGY

- All lysine peptides were synthesized by Fmoc solid phase synthesis (Fig. 1) [3].
- The peptides were purified at 95% purity and analyzed by high performance liquid chromatography (HPLC).
- The molecular mass of the peptides was confirmed by mass spectroscopy MALDI-TOF and HPLC-MS [4].
- The secondary structure of the peptides was determined by circular dichroism spectroscopy (CD), Jasco J-815 equipment.
- The effects of thermal hysteresis of all the peptides synthesized was evaluated in a DSC1 METTLER-TOLEDO calorimeter, the antifreeze activity was quantified with reference to a water control with the STARe 10.0 program (Fig. 2B).

0	KKKKKKKKKKK	K11
1	WKKKKKKKKKK	W1
2	KWKKKKKKKKK	W2
3	KKWKKKKKKKK	W3
4	KKKWKKKKKKK	W4
5	KKKKWKKKKKK	W5
6	KKKKKWKKKKK	W6
7	KKKKKKWKKKK	W7
8	KKKKKKKWKKK	W8
9	KKKKKKKKWKK	W9
10	KKKKKKKKKWK	W10
11	KKKKKKKKKKW	W11

Figure 1: Synthesized peptides of Scan-W of K11 homopeptide.



Temperature program for measurements of DSC. **(B)**

- ✓ Preliminary method (below).
- ✓ Inhibition of recrystallization (on the right).

Step	T° Start (°C)	T° End (°C)	Rate (°C/min)	Time (min)
1	10	-25	2	17,5
2	-25	10	1	35,0

Step	T° Start (°C)	T° End (°C)	Rate (°C/min)	Time (min)
1	10	-25	3	11,7
2	-25	-10	3	35,0
3	-10	THo	1	
4	THo	THo	0	10,0
5	THo	-10	1	
6	-10	-25	3	5,0
7	-25	10	3	11,7

Figure 2: (A) Poliproline structures type I and II. (B) Temperature program for DSC measurements.

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RESULTS

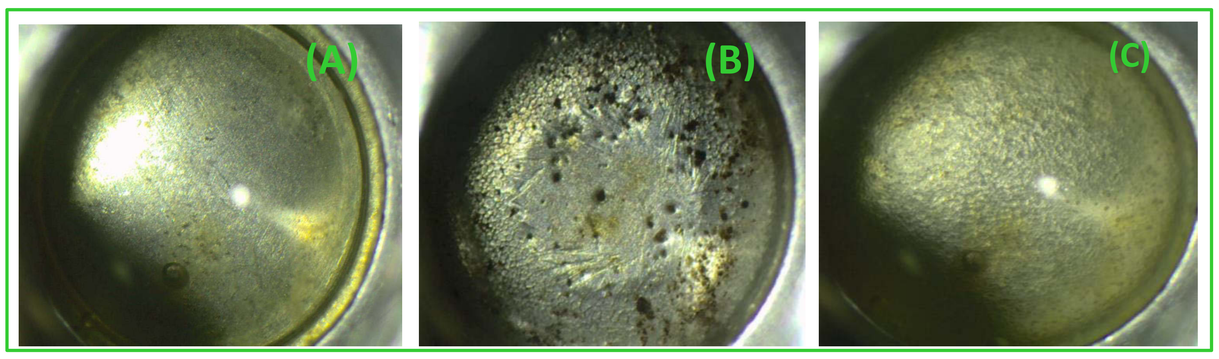

Figure 3: (A) Liquid phase sample, (B) Spike crystallization, (C) Recrystallization of partially fused sample on DSC

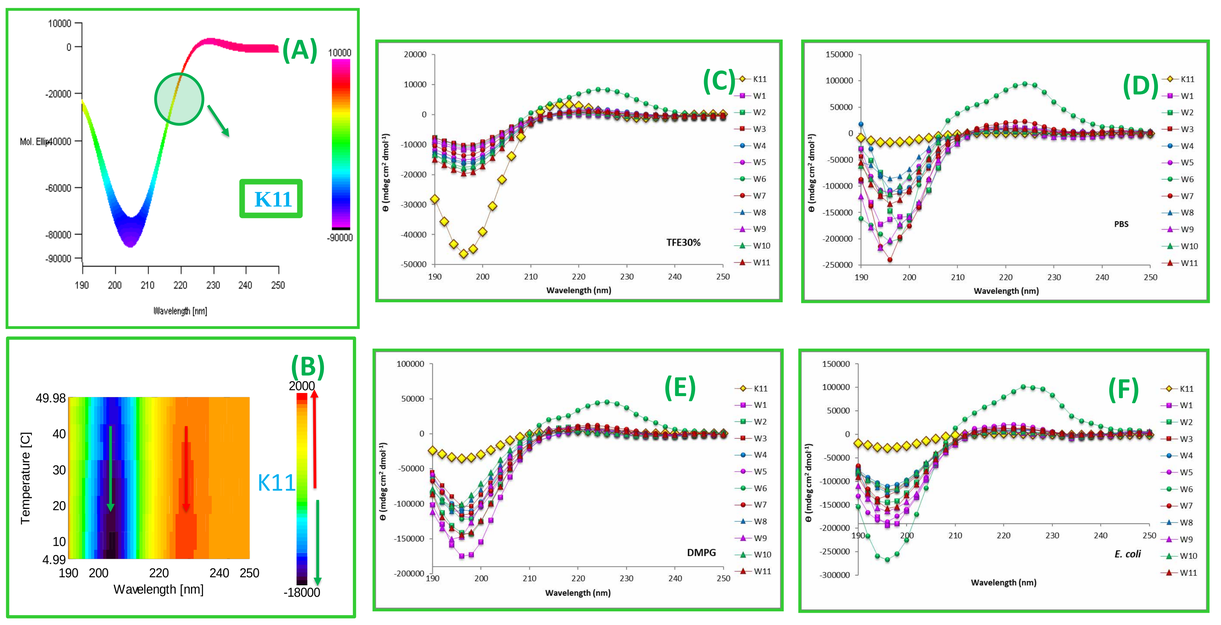

Figure 4 : (A, B) Peptide K11 CD spectrum between 5 ° C and 50 ° C, (C) Scan-W series CD results in 30% Trifluoroethanol, (D) Scan-W series CD results in PBS, (E) results Scan-W series in DMPG and (F) results Scan-W series in lipidic extract from *E.Coli*

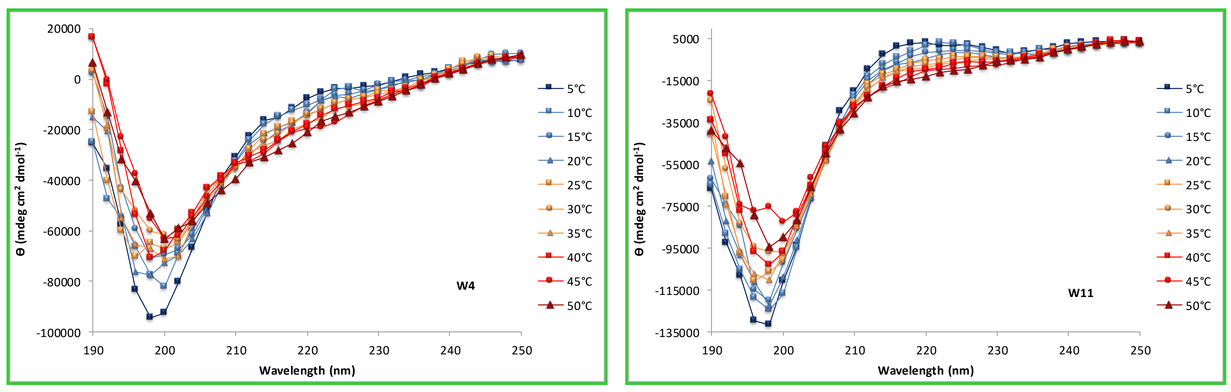

Figure 5 : (A) Peptide K11W4 CD spectrum between 5 ° C and 50 ° C, (B) Peptide K11W11 CD spectrum between 5 ° C and 50 ° C.

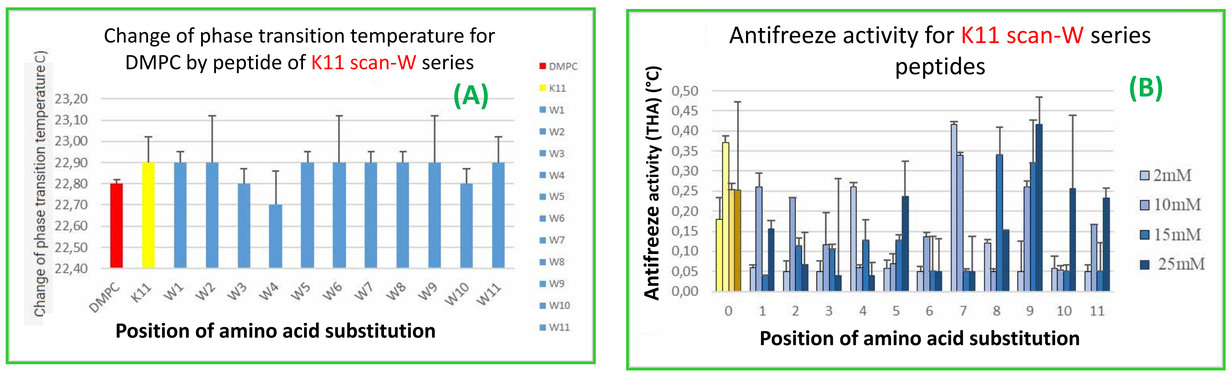

Figure 6 : **(A)** Change of the transition temperature of DMPC due to the peptide, (B) inhibition of recrystallization of ice for peptides at 2,10,15 & 25 mM of 11 residues measured by differential scanning calorimetry

CONCLUSIONS

✓The presence of peptides in the solution produces a morphological change of the ice crystals. (Fig. 3A-C)

✓The results obtained by circular dichroism show that the series of peptides have a more defined structure when the substitution by tryptophane (Scan-W) than homopeptide K11 (Fig. 4 A-B), in different medium (Fig. 4 C-F).

✓ When comparing the effect of temperature on the definition of the secondary structure type Polyproline II, a common isodichroic point is observed between all temperatures. (Fig. 4A-B) for K11, but not in Scan-W.

✓In the peptides studied, the antifreeze activity is increased by substituting lysine for tryptophane at positions other than the amino and carboxyl ends of the peptide (Fig. 6B).

✓The interaction with ice is not directly related to the interaction with lipid model of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) that slightly increased the phase transition because of peptide (Fig. 5A).

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