Effects of a Type I Antifreeze Protein on the Melting of AFP and AFP plus Solute Aqueous Solutions Studied through NMR Microimaging Experiments

Yong Ba, Yougang Mao, Luiz Galdino and Zorigoo Günsen

Department of Chemistry and Biochemistry, California State University Los Angeles, Los Angeles, California 90032. E-mail: yba@calstatela.edu.

Antifreeze proteins (AFPs) provide protection for organisms living through sub-zero winters from freezing damage. The antifreeze mechanism was attributed to AFPs' ability to inhibit the growth of seed-ice crystals through binding to specific ice surfaces. So far, antifreeze activity studies have been focused on the effects of AFPs on the behaviors of individual micro-size ice crystals in super-cooled AFP aqueous solutions. However, AFPs' effect on the bulk melting of frozen AFP solutions has not been paid attention. To learn the lacked information, we have carried out NMR microimaging experiments to observe the effect of a type I AFP, HPLC6 isoform, on the bulk melting of frozen AFP and AFP plus solute aqueous solutions. The solutes studied include sodium chloride, alanine, threonine, arginine and aspartic amino acids and glucose (Table 1). All the solutions were frozen with liquid nitrogen and then placed in a -20 ^{0}C freezer over night before the NMR microimaging experiments. The temperatures of the samples during the experiments were increased gradually from -30 ^{0}C to higher temperature by temperature-controlled cold nitrogen gas. The results are summarized in Table 1. Note that the ranges of the melting points represent the temperatures from the initial detectable water phases to the disappearance of the ice phases. The exact ratios of the coexisting two phases are not given. We observed that the AFP is able to lower the bulk melting points of the frozen AFP solutions (compared to pure ice) and can also inhibit the melting of ice or maintain the ice in super-heated state in the frozen AFP plus solute solutions (compared to the corresponding solute samples). Together with the already-known behaviors of micro-size ice crystals in super-cooled AFP solutions, we hope to understand the collective effect of AFPs on the survivals of AFP-expressing organisms living through freezing and thawing procedures.

Table 1. Melting points of the frozen aqueous solutions containing the AFP, solutes and AFP plus solutes.

Substances	Concentrations	Concentrations	Concentrations
AFP	1.0 mg/ml	2.0 mg/ml	5.0 mg/ml
Melting Point	-0.4 ^{0}C	-0.6 ^{0}C	-0.8 ^{0}C
AFP+NaCl	1.0 mg/ml + 1.0 mg/ml	2.0 mg/ml + 5.0 mg/ml	
Melting point	-1.0 ~ -0.3 ^{0}C	-1.0 ~ -0.7 ^{0}C	
NaCl	1.0 mg/ml	5.0 mg/ml	
Melting point	-3.0 ~ -1.0 ^{0}C	-6.0 ~ -1.0 ^{0}C	
AFP+Ala	2.0 mg/ml + 2.5 mg/ml		
Melting point	-0.4 ~ -0.1 ^{0}C		
Ala	2.5 mg.ml		
Melting point	-0.4 ~ -0.2 ^{0}C		
AFP+Thr	2.0 mg/ml + 3.34 mg/ml		
Melting point	-0.4 ~ -0.1 ^{0}C		
Thr	3.34 mg.ml		
Melting point	-0.4 ~ -0.2 ^{0}C		
AFP+Arg	2.0 mg/ml + 4.97mg/ml		
Melting point	-0.4 ~ -0.1 ^{0}C		
Arg	4.97 mg.ml		
Melting point	-0.4 ~ -0.2 ^{0}C		
AFP+Asp	2.0 mg/ml + 3.6 mg/ml		

Melting point	-0.4 ~ -0.1 ^{0}C		
Asp	3.6 mg.ml		
Melting point	-0.4 ~ -0.1 ^{0}C		
AFP+glucose	2.0 mg/ml + 5.13 mg/ml		
Melting point	-0.4 ~ -0.2 ^{0}C		
glucose	5.13 mg/ml		
Melting point	-0.4 ~ -0.1 ^{0}C		

Acknowledgements: Thanks to grant NIH 1SC1 GM083826; LG thanks CSULA MBRS-RISE Program under the grant number GM61331.