Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science
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Title of Doctoral Dissertation

Analysis of Ice Recrystallization Inhibition Activities among Different Antifreeze Proteins
（異なる不凍タンパク質の間の氷再結晶抑制活性の解析）

Ice recrystallization (IR) is a phenomenon where large ice crystals are formed at the expense of small ice crystals. In the recrystallization process, ice crystals grow with time (*t*) in different ways: (i) growing uniformly, (ii) shrinking by melting or disappearing, or (iii) merging to become larger ice grains. Antifreeze proteins (AFPs) are able to inhibit such events by binding onto specific ice crystal plane to inhibit further ice growth. The current research focused on analyzing ice recrystallization inhibition (IRI) efficiency and FIPA pattern (fluorescence-based ice plane affinity pattern: a pattern demonstrating AFP-bound ice plane) for the wild-type AFPI, II , III, a defective mutant of AFPIII (A20L)　and a fungal AFP (Tis8). For the evaluation of ice-recrystallization rate, only the specific ice grains exhibiting uniform growth were monitored. The rate was then evaluated by employing Ostwald-ripening principle which is represented as $r^3 = r_0^3 + kt$, where r_0 is the radius of an averaged ice grain size at t=0 and k is the recrystallization rate ($\mu m^3 \cdot min^{-1}$). The cube of ice grains` average radius (r) was plotted against time (t) which provided the slope representing the recrystallization rate (k). The k values obtained at different concentrations for each type of AFP were plotted to make semi-log plots which were ultimately represented in sigmoidal curves. The inflection point of the curves provided a unique concentration of AFP (C_i) at which a turnover from diffusion-limited growth to liquid-to-ice-transfer-limited growth occurs. The C_i value was used to represent the IRI efficiency of each AFP species. The following C_i values were estimated for the analyzed AFPs: 0.27 μM (Tis8), 0.60 μM (AFPII), 3.00 μM (AFPIII), 4.69 μM (AFPI), and 7.69 μM (AFPIII A20L). Additionally, the FIPA analysis showed that AFPIII A20L has affinity to least area on ice hemisphere whereas Tis8 has affinity towards a whole set of ice planes. The AFPII has less affinity on ice planes than Tis8. The AFPI and AFPIII have almost same amount of affinity on ice planes although their ice bound planes are slightly different. Consequently, the following sequence was found by arranging the AFP samples in decreasing order of adsorption area: Tis8 > AFPII > AFPIII ≥ AFPI > AFPIII A20L. The same ranking was obtained when we compared their IRI efficiency. All these suggest that IRI efficiency is increased if AFPs bind to multiple ice planes. It can also be assumed that introducing mutation in the ice binding site of an AFP that can improve the affinity of AFP on ice hemisphere can increase "IRI Efficiency".