## 2 004년도 한국조류학회 정기총회 및 학술발표회

The Korean Society of Phycology
The 18th Annual Meeting
Programme & Abstracts

일시 : 2004. 5. 28 ~ 5. 29

장소 : 상명대학교 주최 : 한국조류학회

http://bric.postech.ac.kr/phycology/

후원 : 한국과학기술단체총연합회

## Reduction of freeze-thaw-induced hemolysis of red blood cells by an algal ice-binding protein

Jae-Shin Kang\*, James Raymond¹ and Sung-Ho Kang

Korea Polar Research Institute, KORDI

Department of Biological Sciences, University of Nevada, Las Vegas

Attempts to use natural antifreeze proteins to protect cells and tissues from freezing damage have had mixed results. Some success has been achieved in preventing hemolysis of red blood cells and maintaining sperm motility. There is some evidence that the protective effect of the antifreeze proteins is due to their ability to inhibit the recrystallization of ice. Ice-binding proteins (IBPs; formerly called ice-active subtances, or IASs) from an Antarctic sea ice diatom and other Antarctic photosynthetic organisms can inhibit the recrystallization of ice at concentrations in the microgram  $\mathrm{ml}^{-1}$  range. At natural concentrations in seawater, the IBPs cause pitting and other changes in the habit of ice crystals, which are an indication that they adsorb to the ice surface. Other evidence of ice-binding by IBPs is their preferential incorporation in the ice phase of partially frozen solutions and incorporation in ice hemispheres. Unlike antifreeze proteins, the IBPs do not significantly lower the freezing point. Rather their function appears to be prevention of damage in the frozen state as they have been shown to increase survival of diatoms subjected to a freeze-thaw cycle . These results encouraged us to evaluate the ability of an algal IBP to reduce freezing damage to another cell type, red blood cells.

IBP was semi-purified from cells of the cryophilic diatom *Navicula glaciei* Vanheurck obtained from Antarctic sea-ice. Cell-free supernatant was frozen, centrifuged to remove brine and non-ice-binding impurities, melted, concentrated with ion exchange (Sephadex) chromatography, concentrated again by vacuum-concentration and dialyzed. Protein concentration was 179 g ml<sup>-1</sup> (Pierce BCA assay). An assay of IBP activity indicated that the IBP solution was very active. Human blood was collected from healthy volunteers and immediately heparinized. Blood samples were immersed in liquid nitrogen for 10 min and thawed on ice unless stated otherwise. Freezing for a longer period (overnight) did not change the results. Significance of regression lines was tested with the t-statistic. P values less than 0.05 were considered to indicate significance.

Navicula IBP reduced hemolysis in a concentration—dependent manner, achieving an approximately 50% reduction at a concentration of 77 g ml<sup>-1</sup>. To examine how hemolysis was affected by the rate of thawing, samples were thawed on ice  $(0^{\circ})$ , in air at room

## 2004 International Meeting of the Federation of Korean Microbiological Societies



Sponsored by
Korea Research Foundation
The Korean Federation of Science and Technology Societies
Korea Polar Research Institute, KORDI

Program

한국해양학회 2004년도

추계학술발표대회 및 심포지엄 안내문

일 시: 2004년 11월 4일(목)-11월 5일(금)

장 소 : 인하대학교