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Supercooling enhancement by adding antifreeze protein and ions to water in a narrow space

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ABSTRACT

The control of ice crystal growth has been an important issue in various fields, such as the quality maintenance and improvement of food texture in frozen food, and the storages of tissues and organs in hospitals. The adding of antifreeze protein to food or organs has been focused on recently because it is appropriate for controlling ice growth. However, the effects of ions, which are originally contained in the substances being preserved, on the activities of antifreeze protein have not yet been clarified in a thermal non-equilibrium state. We therefore conducted experiments on the unidirectional freezing of dilute aqueous solutions of winter flounder antifreeze protein, sodium chloride or sodium permanganate in a narrow space between two cover glasses. The temperatures at ice/solution interfaces were measured with a small thermocouple. The concentration of antifreeze protein was estimated by fluorescence microscopy, and the concentration of permanganate ion was measured from the intensity of transmitted light. It was found that the depression of interface temperature (defined as the difference between the interface temperature of the solution and that of pure water) of the mixed solution was much lower than the sum of the depression of the interface temperatures of two solutions of single solute. In addition, we found that the protein concentration increases with time, while the ion concentration decreases slightly in the front regions of the most advanced points of serrated interfaces in the mixed solution. The diffusion of ions to the ice growth direction enhances the protein diffusion to the front-edge regions. This is possibly the reason for the low value of the interface temperature of the mixed solution. Simultaneously, high concentration regions of the protein in the bottom-edge region deteriorate the diffusion of ions. This is reason for the deterioration of the depression of interface temperature due to an increase in the interface velocity in the cases of mixed solutions.

1. Introduction

The enhancement of supercooling of water has been an important issue in various fields, such as the quality maintenance and improvement of food texture in food preservation, the storages of tissues and organs in hospitals, and the control of the ice packing factor of ice slurry to be used in heat exchangers or in the preservation of fish and fruits. The use of antifreeze protein (AFP) or antifreeze glycoprotein (AFGP) has been focused on recently. This is because AFP and AFGP lower the freezing point, while they retain the melting point in a quasi-equilibrium condition in osmometers [1]. Supercooling is maintained at any temperature between the depressed freezing point and the unchanged melting point. In contrast, supercooling is not enhanced when salts are added to water because both the freezing point and melting point of the solution drop. It should is noted that these freezing and melting points were determined using the osmometer as follows: firstly, an AFP solution in oil is rapidly frozen. Secondly, the frozen solution is gradually warmed until only a single crystal remains. Thirdly, the solution with the crystal is either cooled or warmed. The temperatures at which this seed crystal starts to grow and shrink are defined as the freezing and melting points respectively [2].

There are many experimental and computational studies concerning the functional elucidation of AFP. Peculiar ice crystals were observed in the experimental studies, e.g. small ice crystals in the shape of hexagonal bi-pyramids were usually seen in the solutions of winter flounder AFP (HPLC6) in the osmometers [3–5]. The adsorption of AFP to ice surfaces was predicted by molecular dynamics simulations, e.g. the hydrogen atoms of four threonine residues in HPLC6 were bonded to the oxygen atoms on the pyramidal facets in the small crystals [4,5]. Some other studies dealt with bigger ice crystals: Grandum et al. [6] observed HPLC6 solutions, which were frozen rapidly, using a scanning tunneling

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Nomenclature

B	brightness
C	salt concentration (wt%)
C_{max}	maximum salt concentration (wt%)
c	AFP concentration (mg/ml)
c_{max}	maximum AFP concentration (mg/ml)
G_C	gradient of salt concentration (wt%/μm)
G_T	temperature gradient (K/mm)
R	ice growth rate in a certain direction (μm/s)
u_i	interface velocity in the negative X-direction (μm/s)
T_i	temperature at ice/solution interface (°C)
T_0	temperature at ice/water interface (°C)
t	reference time (s)
X, Y, Z	Cartesian coordinate (μm)

Greek symbols

ΔT	depression of interface temperature [$= T_i - T_0$] (°C)
θ	angle between the a-axis of ice crystals and the temperature gradient direction (°)

electron microscope and showed the footprint of the adsorbed HPLC6 molecules to a {20–21} crystal face. But in gentle cooling which arises in the applications mentioned at the beginning of this introduction, it is not proved whether HPLC6 shows the same tendency as it does in the case of rapid freezing. Graether et al. [7] kept AFP solution in a large space and observed the ice crystals in the state of gentle cooling. They found that the crystals grew to the c-axis direction. But it was extremely difficult to measure the distribution of AFP in the solutions. Furukawa et al. [8] observed the interface of the concavo–convex form which consisted of prism planes using AFGP solution. Furthermore, they labeled the fluorescence molecule to AFGP and obtained the concentration of AFGP near the interface. However, the influence of the protein near the interface on freezing point depression in the case of non-equilibrium state has not yet been clarified.

In almost all the previous experimental studies, the effects of ionic compounds dissolved in AFP solutions on the supercooling were not discussed even if a buffer was used to promote the dissolution of AFP into water. As far as the present authors know, Evans and coworkers were the first to report experimental results on the effects of ions on the freezing point depression of AFP solutions using the osmometer [9]. They clarified the cooperative effects, in which the freezing point depression for the mixture of salt and AFP is significantly more than the sum of the freezing point depression of the salt and that of AFP solution. Kristiansen et al. observed a similar effect for the mixed solutions of insect AFP and various salts [10]. Hagiwara and Yamamoto [11] obtained remarkable drops of the interface temperature in the case of unidirectional freezing for the aqueous solutions of HPLC6 and sodium chloride. Despite Hagiwara and Yamamoto's efforts, the local concentrations of ions and HPLC6 were not measured, and thus the interaction among the ions, AFP and ice surfaces has not yet been discussed.

In the present study, we investigate the local effects of ions and HPLC6 on the freezing of solutions in a narrow space. We measure the local interface velocity and the local interface temperature. Moreover, we conduct the measurements of local concentrations of permanganate ion and HPLC6. We then discuss the relationship among these local values.

2. Apparatus

The apparatus consists of an inverted biological microscope (Nikon ECLIPSE Ti-E), a monochrome CCD video camera, a digital multi-meter (Yokogawa 7561) and a pulse generator. The light sources were a halogen lamp and a laser. The apparatus was set up in a temperature-controlled room kept at 8 °C.

Fig. 1 shows the details of the cooling section, mounted on the bench of the microscope. A dilute aqueous solution of HPLC6, sodium chloride or sodium permanganate, or a mixed solution of HPLC6 and either sodium chloride or sodium permanganate, was introduced into a narrow space of $25 \times 22 \times 0.02$ mm^3 between parallel cover glasses by means of the capillary action of the liquid. The solution volume was 11 mm^3, which is lower than those used in similar experiments on unidirectional freezing [8,12–16] (see Table 1). Also, the solution thickness was the lowest among these experiments.

The gap of 0.02 mm between the cover glasses was created using a screen which was printed on the lower side of the upper cover glass. (The screen printed on the cover-glass contributes to controlling the growth direction of the ice crystal.) The lower cover-glass was in contact with the edge of the copper plate. This plate was cooled by a Peltier device with a coolant flowing through the device. The cooling rate of the device was controlled with a controller (Sensor Control Inc., FC3510). This controller automatically controlled the Peltier device from the temperature measured with a thermocouple inside the Peltier device and a predetermined temperature-drop rate.

The overall temperature gradient in the ice growth direction, G_T, was estimated to be between the temperature gradient in the HPLC6 solution, $G_{T\mathrm{sol}}$, and the temperature gradient in the ice, $G_{T\mathrm{ice}}$ [17]. The values of $G_{T\mathrm{sol}}$ and $G_{T\mathrm{ice}}$ near the front edge of the serrated interface were 0.20 and 0.80 K/mm respectively [14]. These values are lower than the values of other unidirectional freezing experiments shown in Table 1. To realize such a low temperature gradient, we operated the controller of the Peltier device at its lowest temperature-drop rate of 0.0125 K/s.

Ice was formed naturally, and without any seed, from the cooler edge of the solution layer.

3. Measurement methods

3.1. Interface velocity

Serrated interfaces were observed in all the solutions. The interface configuration did not change noticeably during the period of image capturing in each solution except for a small part of the front edge of the serrated interface. The interface velocity was defined using the following two images and the time difference for the two instants: an image in which the most advanced point of the serrated interface had just appeared and another image in which this point reached the center of the thermocouple junction (see Fig. 2). The margin of error for the interface velocity was 1.7%. Also, the velocity may vary during the two instants because of the change in the front edge around the most advanced point of the serrated interface. The image-capturing conditions are shown in Table 2.

3.2. Interface temperature

A K-type thermocouple, whose element wires were 13 μm in diameter (ANBE SMT Co., KFT-13), was used for the temperature