Recrystallization behavior of ice crystals in sucrose solution in the presence of AFP Type I
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ABSTRACT
Recrystallization rate of ice crystals in 33% sucrose solutions, a model frozen dessert, was measured in the presence of antifreeze protein Type I (AFP type I) at -10°C, which is relevant to practical storage or distribution temperature of frozen dessert. The concentrations of AFP type I were set to 0.01, 0.1, and 1μg/ml, respectively. Ice crystals during recrystallization process were observed by using a light microscope equipped with cold stage as follows. 2μl sample solution enclosed between two coverslips was placed on the sample stage and cooled to -30°C at 90°C/min. After 10min, sample temperature was elevated to -10°C and recrystallization of ice crystals was observed for about 15 hours. Using the ice crystal images obtained by microscopic digital camera, number-averaged ice crystal size was evaluated by the technique of image analysis. Based on the theory of Ostwald's ripening, recrystallization rate of ice crystals was obtained by analysis of time course of the number-averaged ice crystals size, and effect of addition of AFP type I upon recrystallization rate was evaluated quantitatively. The recrystallization rate of the sample containing 1μg/ml AFP type I was about 13% of that of sample without AFP. However, no significant reduction of recrystallization rate was observed for the samples containing 0.1μg/ml or 0.01μg/ml AFP type I. These results suggest the following; first, 1μg/ml AFP type I has strong suppression effect on recrystallization of ice crystals in frozen sucrose at one of practical storage or distribution conditions. Secondly, less than 0.1μg/ml AFP type I is no longer effective for suppressing recrystallization of ice crystals.

Keywords: antifreeze protein; AFP; recrystallization; ice crystal

INTRODUCTION
Recrystallization of ice crystals in frozen foods is characterized by an increased average size of ice crystals after completion of water solidification [1,2]. It is a main cause for physical deterioration of frozen foods during storage and distribution. For example, the increased size of ice crystals in ice cream brings about a coarse, grainy, and icy texture, destroying its preferred creamy and smooth texture. Recrystallization of ice crystals in frozen meat damages the cell structure, causing drip loss after thawing.

Recently, many antifreeze proteins (AFP) have been extracted in variety of sources [3-5]. They are now expected as additives for suppressing this recrystallization process. However, there have been few researches investigating recrystallization behavior of ice crystals at practical storage or distribution conditions of frozen foods. In order to put AFP into practical use as a recrystallization suppressor, evaluation of their suppression ability is essential.

Our objective is to provide reliable information related to the suppression ability of AFP type I, a typical antifreeze protein from fish, in frozen sucrose solution having relevance to frozen desserts near practical storage or distribution temperature of frozen foods. In particular, effect of AFP concentration upon the suppression ability was investigated in this study.

MATERIALS & METHODS
The freeze-dried powder of AFP type I was purchased from A/F Protein Inc (Waltham, MA, USA). The sample solutions were 33% sucrose solutions containing AFP type I. The concentrations of AFP type I were set to 0.01, 0.1, and 1μg/ml, respectively. A procedure to observe the recrystallization process was similar to that of previous studies [6-7] as follows. 2μl sample solution enclosed between two coverslips (diameter 16mm) was placed on cold stage (type HFS91; Linkam Scientific Instruments, Surrey UK) and cooled from 30°C to -30°C at 90°C/min. After 10min, sample temperature was elevated to -10°C. Finally after reaching -10°C, ice crystal images were photographed periodically for about 15 hours as a digital data by the microscopic digital camera (DS-5M-L1; Nikon Corp., Tokyo, Japan) connected to a light microscope.
and saved in digital format. Using the ice crystal images, the size of each crystal was calculated as the radius of a circle having the equivalent projected area of the crystal. From the data set of each crystal size, the number-averaged crystal radius, \( r \), was calculated. For these procedures, commercial image analysis software PopImaging 4.00 (Digital being Kids Corp., Yokohama, Japan) and image handling software Photoshop CS3 (Adobe Systems Incorporated, USA) were used. To evaluate recrystallization rate, the theory based on the Ostwalds ripening principle [8,9] was used, as in previous studies [5,6,10]. According to the theory, the recrystallization process at constant temperature can be described by:

\[
r^3 = r_0^3 + k t
\]

where \( r \) is the number-based mean crystal radius, \( r_0 \) is the number-based mean crystal radius at time \( t=0 \), and \( k \) is the recrystallization rate. The recrystallization rate \( k \) is evaluated as the slope of the cube of the mean radius versus time.

Using the value of \( k \), effect of addition of AFP type I upon recrystallization rate was evaluated quantitatively.

**RESULTS & DISCUSSION**

Figure 1 shows typical ice crystal images at different time for the sample of (a) control (without AFP), (b) 0.01, (c) 0.1, and (d) 1 \( \mu \)g/ml AFP, respectively. For control, 0.01\( \mu \)g/ml, and 0.1\( \mu \)g/ml, the ice crystals grew in size extensively with increasing storage time. On the other hands, the sample with 1\( \mu \)g/ml AFP had significant smaller ice crystals, suggesting suppression of recrystallization of ice crystals.

![Figure 1. Typical ice crystal images at different times. Sample: (a) control, (b) 0.01\( \mu \)g/ml, (c) 0.1 \( \mu \)g/ml, (d) 1\( \mu \)g/ml.](image)
Figure 2 shows typical plots of cube of the number-averaged equivalent radius $r$ against time. The solid lines in the plots were results of the fitting by eq (1). From the slopes of the fitting results, recrystallization rates $k$ were evaluated.

![Figure 2. Typical Plots of the cube of the mean equivalent radius as a function of time. The solid lines represent results of fitting by eq. (1).](image)

Table 1 summarizes the recrystallization rate $k$ for the samples examined in this study. The recrystallization rate of the sample containing 1 µg/ml AFP type I was about 13% of that of sample without AFP (control). No significant reduction of recrystallization rate was observed for the samples containing 0.1µg/ml or 0.01µg/ml AFP type I. These results indicate the followings; first, 1 µg/ml AFP type I has strong suppression effect on recrystallization of ice crystals in frozen sucrose at one of practical storage or distribution conditions. Secondly, less than 0.1µg/ml AFP type I is no longer effective for suppressing recrystallization of ice crystals. In commercial ice creams, stabilizers such as polysaccharides (ex. Locust bean gum) or gelatin are often added. It was reported that the addition of 3-10mg/ml locust bean gum to fructose solutions reduced recrystallization rate $k$ to 40-70% of control [11]. Considering these, AFP type I has much stronger suppression ability with much less concentration.

<table>
<thead>
<tr>
<th>Recrystallization rate $k$ (µm$^3$/h)</th>
<th>Control</th>
<th>0.01µg/ml</th>
<th>0.1µg/ml</th>
<th>1µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>186±54</td>
<td>200±58</td>
<td>173±25</td>
<td>24±8</td>
</tr>
</tbody>
</table>

The physical meaning of 1µg/ml is unclear now. Further study to investigate the physical meaning of the concentration would be needed. It has been hypothesized that AFP molecules bind to ice surface and inhibit ice crystal growth [12, 13]. According to the hypothesis, minimum amount of AFP type I molecules to suppress recrystallization of ice crystals could be estimated from the total surface area of ice crystals, which might be a hint for discussing the physical meaning of 1µg/ml.

CONCLUSION

1µg/ml AFP type I has strong suppression effect on recrystallization of ice crystals in frozen sucrose near practical frozen storage or distribution conditions (-10°C). Less than 0.1µg/ml AFP I is no longer effective for suppressing recrystallization of ice crystals. These results would contribute to determination of additive amount of AFP for efficient suppression of recrystallization of ice crystals in frozen dessert. Further study to investigate the physical meaning of these concentrations would be needed.
REFERENCES


