A mixed solution of saline and HPLC6 and its effects on the interface with ice in unidirectional freezing

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1. Introduction

In the experiments using a Clifton nanoliter osmometers [1, 2], HPLC6, a winter flounder ice-binding protein, is found to lower the hysteresis freezing point (HFP), while it does not alter the melting point (MP). The thermal hysteresis (TH), defined by the difference between MP and HFP, increases in a non-colligative way as the protein concentration increases.

Recent experimental results [3] show that TH of a mixed solution of HPLC6 and salt is more pronounced than the sum of TH of the HPLC6 solution and TH of the salt solution. A similar effect has been measured for the mixture of insect protein and various salts [2]. This synergetic enhancement of TH is very interesting. However, it may be difficult to extend the discussion of the relationship between the cooling that occurs in nature and the cooling in the experiments using the osmometers. This is because the cooling rate in the osmometers is lower than the cooling rate in nature. In the case of flounders in winter, seawater with ice crystals is taken in through the flounders' mouths, then some of the seawater flows between the gills in the capillary vessels and the remainder is ingested into the intestinal tract [4]. The blood and intestinal tract are cooled quickly by the seawater. Because of this difference between the rate which occurs in nature and that in the osmometers, experimental results with higher cooling rates are necessary to elucidate the activities of HPLC6.

In the present study, we carry out experiments on the unidirectional freezing of a mixture of dilute, aqueous solution of HPLC6 and a physiological saline solution in a narrow space between two cover glasses. This narrow gap models on the capillary vessels and the membrane of intestinal tract of a winter flounder. The velocity of ice growth in these experiments is at least 15 times higher than that used for the evaluation of HFP. The particular focus of these experiments is on measuring the temperature at the interface and the concentration of HPLC6.

2. Experimental methods

2.1. Apparatus

The apparatus consisted of an inverted microscope, a CCD video camera, a digital multi-meter, a pulse generator and a bench-top cooling section. The apparatus was placed in a temperature-controlled room, kept at 8°C. The observation area was 0.39×0.30 mm^2. The pixel resolution of the camera was 0.29×0.29μm^2.

The details of the cooling section in the apparatus are shown in Fig. 1. The mixed solutions of HPLC6 and saline were introduced into the space of $50 \times 20 \times 0.02$ mm^3 between the parallel cover glasses by means of the capillary action of the liquid. The gap of 0.02 mm between the cover glasses was created by using a screen, printed on the lower side of the upper cover glass. The lower cover glass was in contact with the edge of the copper plates, and these plates were cooled by electric cooling devices with coolant flowing through the devices. The total cooling rate of the devices was controlled by the input electric current to the devices and the flow rate of the coolant. The cooling section was covered with a transparent box filled with nitrogen gas to prevent condensation. The gas supply was cut off before starting each measurement, so that convection heat transfer could not occur due to the gas flow.

2.2. Temperature measurement

A thermocouple, whose element wires were 0.013 mm in diameter, was used for the temperature measurement. It was inserted into the space between the cover glasses before the space was filled with the liquid. The electromotive force of the thermocouple was converted to digital signals with the multi-meter. The recording of the output signal from the multi-meter into the memory on a personal computer was synchronized with the recording of the images obtained with the CCD camera. We defined the interface temperature as the temperature measured when the interface reached the centre of the thermocouple junction. When the interface was serrated, the interface temperature was evaluated when the front edge of interface (the advanced points in the interface in the ice growth direction) reached the center of the junction.

2.3. Concentration measurement

We utilized fluorescence microscopy to measure the local concentration of HPLC6. For this purpose, the amino-base side chain of lysine residue and that of the N-terminus in HPLC6 were tagged with fluorescein isothiocyanate (FITC). The concentration of protein was set at 0.25 and 0.125 mg/ml, while the concentration of saline was 0.9wt% and 0.45wt%.

3. Results and Discussion

3.1. Interface configuration

Figures 2(a) and 2(b) show typical images of interfaces in the cases of physiological saline solution and the mixed solution of HPLC6 and saline, respectively. The interface in Fig. 2(a) seems like the blades of scissors, while the interface in Fig. 2(b) has a zigzag shape. In addition, the interface shape similar to Fig. 2(a) can be seen occasionally in the case of the mixed solution.

3.2. Interface temperature

In the case of the mixed solution (concentrations: 0.125mg/ml for HPLC6 and 0.45wt% for saline), the ensemble-averaged value of interface temperature was –0.55°C. The sum of the ensemble-averaged value of interface temperature for the HPLC6 solution of 0.125mg/ml and that for the physiological saline solution of 0.45wt% is –0.41[= –0.035+(–0.375)]°C. Thus, the ensemble-averaged value of interface temperature for the mixed solution is 34% more pronounced than the sum of the ensemble-averaged values. This is consistent with TH enhanced by salts.

3.3. The effect of interface velocity

It was found that the interface temperature does not depend on the interface velocity in the case of saline solution. On the other hand, as shown in Fig. 3, the interface temperature clearly decreases with a decrease in the interface velocity in the case of the mixed solution. This result clearly indicates that the interaction between ice and the ions or HPLC6 becomes more noticeable as the interface moves slower. This suggests that it takes time for ions and HPLC6 to modify structures, such as the hydrophobic hydration or aggregation of protein and the solvation of ions [5].

3.4. Concentration of protein

Figure 4 shows a typical color contour map of the concentration of HPLC6 in the case of mixed solution. The concentration is uniform in a deep, narrow region of solution in the ice. This is different from the concentration distribution in the case of HPLC6 solution where the concentration becomes higher with time near the bottom edges of the serrated interface (the innermost parts in the ice growth direction). This low concentration is evidence of modification of protein structure due to the ions.

Acknowledgments

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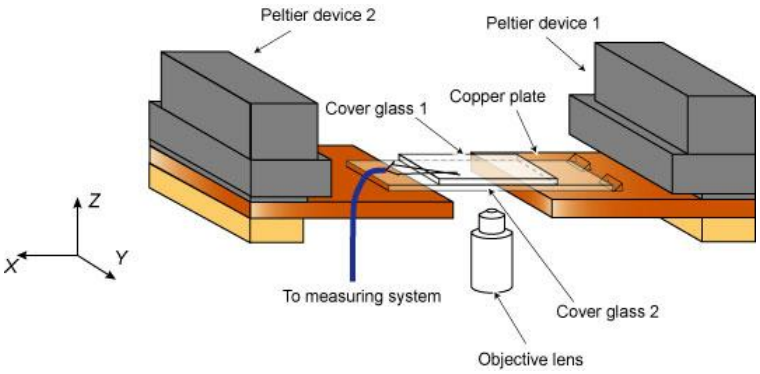
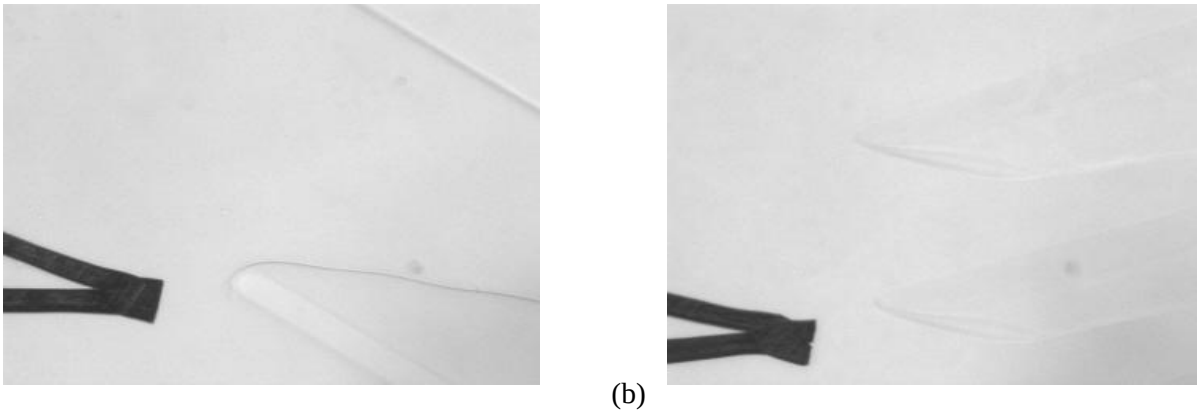


Fig. 1 Details of cooling section



(a) (b)

Fig.2 Images of interfaces: (a) physiological saline solution, (b) mixed solution

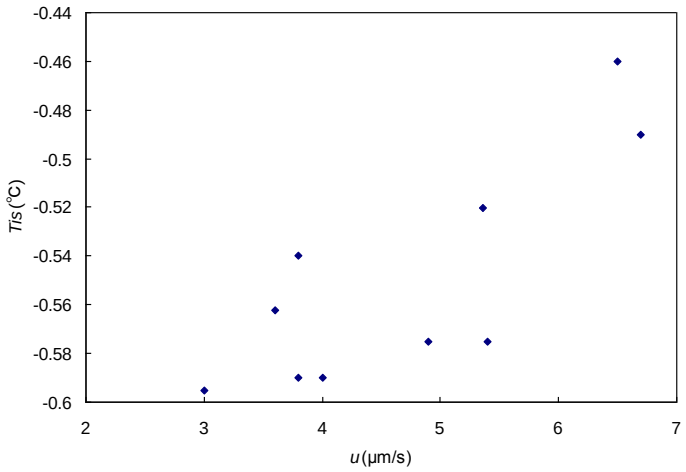


Fig.3 Interface temperature as a function of interface velocity in the case of mixed solution

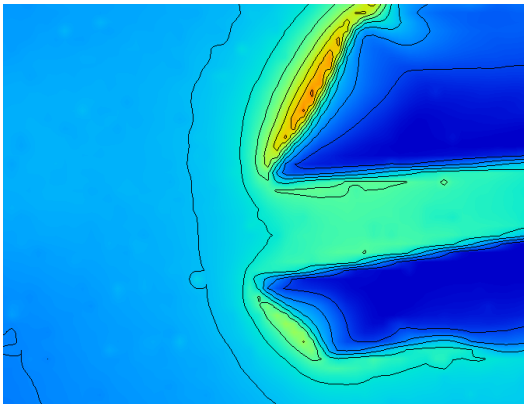


Fig.4 Color contour map of the concentration of HPLC6 in the mixed solution