Original Paper

Change of Polymer Network by the Freezing of Food Hydrogel

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The freeze concentration processes of agarose gels with or without sucrose were observed using a Cryo-SEM and a light microscope for the purpose of understanding the behavior of a polymer network and a sucrose solution in its gel during the freezing processes. Furthermore, whether the polymer network and a sucrose solution in the frozen gels are in a crystalline state or a glassy state was investigated using differential scanning calorimetry (DSC). As a result, it was suggested that the polymer network during freezing is assembled, like a membrane, on the overall surface of ice crystals. However, the diffusion of water is thought to be not very inhibited by the membranes because the ice crystals grew very fast and the form of ice crystals became polygonal. Such behavior of the polymer network itself was independent of the presence of sucrose. The sucrose molecules added to the gel affected the ice crystal growth in the gel in the same way as in the case of a sucrose solution. The results of the DSC measurement indicated that only the sucrose solution in the gel turns into a glassy state.

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Introduction

Food hydrogels such as pudding and terrine are often preserved in the form of a frozen gel. When a low concentration hydrogel is frozen, the structure of the polymer network changes as ice crystals grow in the gel. Thawing of this frozen gel causes undesirable quality changes such as syneresis, resulting in a spongy state. It is well known that these quality changes can be empirically controlled to a certain extent by adding saccharides or triglycerides[1),2)]. This effect due to the saccharides and triglycerides has been explained in terms of the freezing point depression and the decrease in water mobility due to the viscosity increase in the freeze concentrated solution. However, the mechanism of the quality changes and the controlling mechanism using additives are still uncertain. Especially with regard to the behavior of a polymer network during freezing, it seems that the following consideration has not been recognized; the polymers forming the network can not diffuse in the same manner as non-gelling polymers or small molecules. If the polymers excluded from the ice crystals do not diffuse away from the surface of then rapidly, then they may form a membrane assembled on the surface of the ice crystals, and may act as a physical obstruction of the further growth of ice crystals. Such a process, however, has not been actually observed yet.

The purpose of this work is to understand the behavior of the polymer network during freezing and the effect of a saccharide on it, First, the

〔Key words: Gel, Polymer network, Membrane, Ice crystal growth, Glass transition〕

morphology of the polymer network in the frozen gel state was observed using a Cryo-SEM. As the second step, by means of dyeing the gel, the dynamic behavior of the polymer network and the saccharide solution during freezing was observed using a light microscope with the low temperature stage. Furthermore, the state of the saccharide and the polymer network in the frozen gel, i.e., whether it is in a crystalline state or a glassy state, was investigated using differential scanning calorimetry (DSC).

Materials & Methods

We used agarose gel as the typical hydrogel. The agarose purchased from Sigma Chemical, Co. is a Type I-A, low gelling type. The saccharide added to the agarose gels was sucrose from the Kokusan Chemical Co. The dye used for the light microscopic observations was methylene blue (Wako Pure Chemical Industry, Ltd., 1B 429, Med. Puriss).

Observation by a Cryo-SEM

Two types of gel samples, agarose gels without additives and agarose gels containing sucrose, were prepared. The concentration of each gel is listed in Table 1. The agarose powder and the mixed powder of agarose+sucrose were suspended in distilled water, and dissolved by heating to 80℃. The solutions were poured into 1 ml polyethylene capsules having a 7 mm diameter. They were allowed to gel by cooling to room temperature. After 24 hours, they were frozen in a deep-freezer at -50℃ for 24 hours. The frozen gels were taken out of each capsule without thawing and set on the stage of a Cryo-SEM (Hitachi Co., S-4000). The internal surfaces of the frozen gels were observed under high vacuum from 200 to 500 magnifications at -80℃ with an accelerating voltage of 10 kV.

Observation by a light microscope

Agarose solutions with or without sucrose, whose concentrations are shown in Table 1, were prepared in the same way as that for the Cryo-SEM observation. The solutions were dyed by adding a drop of a 0.82×10^{-2} g/ml methylene blue solution, and then dropped on slide glasses before covering with a cover glass. The thickness of the samples was 0.1～0.3 mm. After leaving at room temperature for 1 hour to allow the solutions to gel, the samples were put on the Cryo-stage as shown in Fig.1. The samples were slowly cooled by heat conduction through a slide glass which is in contact with the stages cooled down to -30℃. The freeze concentration processes of these samples and the

Table 1. Samples used for this study

Cryo-SEM observation
1%* agarose gel
1%* agarose gel with 20%** sucrose
light microscope observation
2%* agarose gel
2%* agarose gel with 20%** sucrose
0.82×10^{-2} g/ml Methylene blue solution
DSC measurement
2%* agarose gel
2%* agarose gel with 20%** sucrose
20%** sucrose solution

*: weight% of agarose to agarose-water solution

**: weight% of sucrose to sucrose-water solution

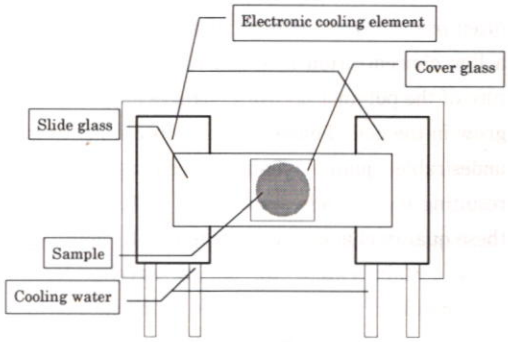

Fig. 1. A detail of Cryo-stage used for light microscope observation. The electronic cooling elements are cooled down to about -30℃, and the center of sample is cooled down to -10℃ by heat conduction through a slide glass. It was observed from a top by a light microscope.

0.82×10^{-2} g/ml methylene blue solution as a reference were observed at 10×63 magnifications using a light microscope (Nikon, SMZ-2T) attached to a video camera system (Kyoshin Electric Co., XC-711 camera and Matsushita Electric Industrial Co., Ltd. NV-SX10 recorder). The recorded video image was printed with a Sony UP-5000 color video printer.

DSC measurements

Agarose solutions with or without sucrose were prepared in the same way as for the Cryo-SEM observation. The components are listed in Table 1. An approximately 46 mg portion of the solutions was poured and sealed in the DSC aluminum pans, then allowed to gel in each pan. After maintaining the samples at room temperature for 24 hours, DSC measurements were carried out using a DSC-50 (Shimadzu Co.). For comparison, a 20% sucrose solution was also examined. In these DSC experiments, all samples were cooled from room temperature to -80℃ at -2℃/min, held for 20 minutes, and then heated at 2℃/min.

Results

Observation by a Cryo-SEM

Figs. 2 and 3 show the Cryo-SEM photographs of a frozen agarose gel and a frozen agarose gel with 20% sucrose, respectively. The internal surfaces, which appeared by cutting the frozen gels, can be observed in both photographs. The black areas in the photographs represent a trace of sublimed ice crystals (○). From a comparison of Figs. 2 and 3, it can be seen that the ice crystal size of the sucrose free gels was larger than that of gels added with sucrose. The distribution of the ice crystal size in both gels is shown in Table 2. In both gels, it was appreciated that the membranous structure, which is thought to be a concentrate of the polymer network, might be formed on the overall surface of the ice crystals. These membranes might construct compartments surrounding the ice crystals. The remarkable difference between the two gels is as follows ; in gels containing sucrose, the trace of ice crystals had a rounded morphology as shown in Fig. 3, and the existence of the gap (arrow) between the compartments were observed, whereas

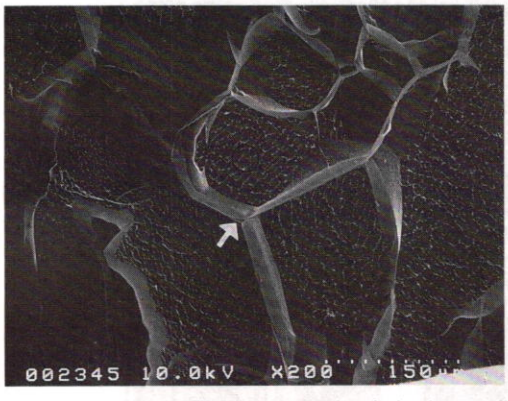

Fig. 2. A Cryo-SEM photograph of the internal surface of a 1% agarose gel at -80℃, at 200 magnifications. The sample has never been thawed. The white arrow indicates the deltoid void among the compartments ; ○, the trace of sublimed ice crystal.

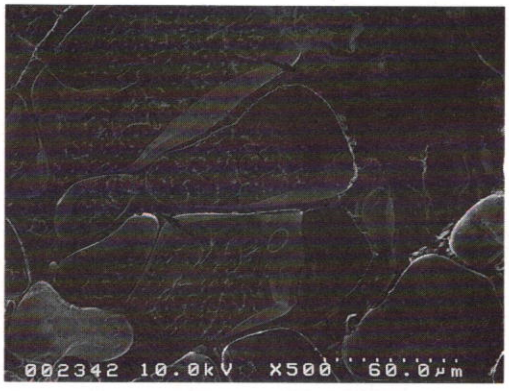

Fig. 3. A Cryo-SEM photograph of the internal surface of a 1% agarose gel with 20% sucrose at -80℃ observed, at 500 magnifications. The sample has never been thawed. The arrow indicates the gap between the compartments ; ○, the trace of sublimed ice crystal.

Table 2. Distribution of ice crystal size

sample	ice crystal size(μm)
1% agarose gel	50～500
1% agarose gel with 20% sucrose	10～150

in the sucrose-free gel, the trace of ice crystals showed a polygonal form without a large gap as shown in Fig. 2. Especially, it was found that in the sucrose-free gel a small deltoid void was formed among the compartments as indicated by the white arrow in Fig.2.

Observation using a light microscope

Fig. 4 shows a series of photographs during the freeze concentration processes of a methylene blue solution. Ice crystals in the methylene blue solution grew as dendrites, and the residual solution was concentrated at the edges of the ice crystals as they grew. Thus, no compartment was observed.

The freeze concentration process in a sucrose -free agarose gel showed a different behavior from that in the case of the methylene blue solution; the compartments were formed as shown in Figs. 5 (a) and (b). However, the ice crystal growth process could not be recorded as a dynamic image because each ice crystal was small and the growing rate was very high. It was noted that the compartments formed by freezing were maintained to some extent after thawing as shown in Figs. 5 (c) through (d).

In an agarose gel with sucrose, the course of the freeze concentration could be observed since the ice crystal growth was retarded by the addition of sucrose. Fig. 6 shows the ice crystal growing process. Although no heterogeneity in the colored concentration could be observed just before freezing, the growth of ice crystals was observed accompanying the progress of freeze concentration. The rounded ice crystals were formed, and these surfaces became clearer as the freezing progressed. Fig. 7 is an enlarged photograph of (i) in Fig. 6 which represents the final stage of freezing. Fig. 7 indicates that the form of ice crystals (○) were round and the gap (arrow) between the ice crystals became blue, which is the same results as those obtained by the Cryo-SEM observation. After thawing, the compartments were still observed to some extent as shown in Figs. 8 (a) through (d). Though the photograph (d) printed by the video printer was not clear, we could recognize the com-

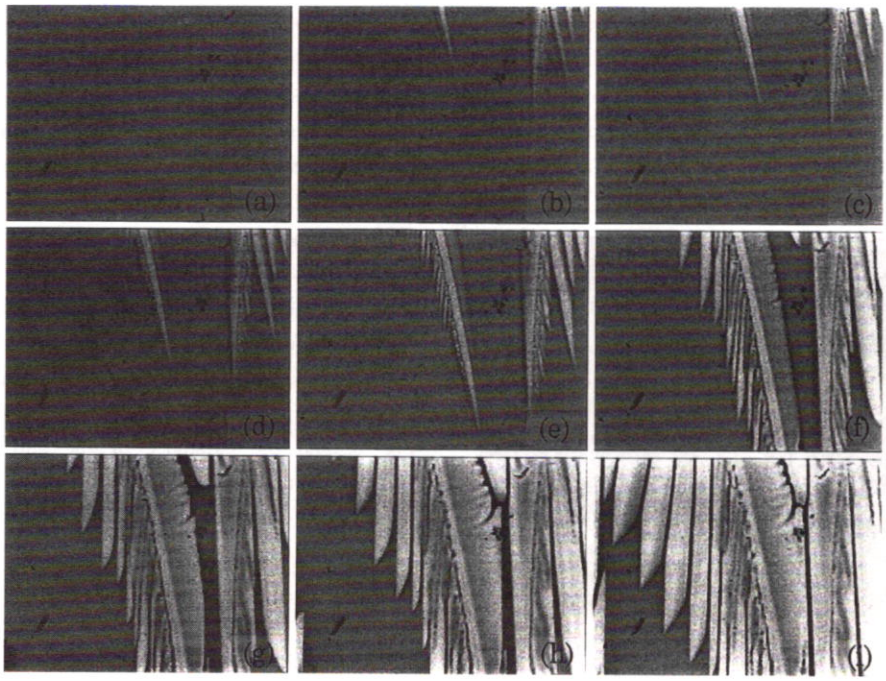

Fig. 4. Series of photographs during the freeze concentration process of a methylene blue solution. (a) before cooling ; (b) beginning freezing ; →(c)→(d)→(e)→(f)→(g)→(h)→(i) (-10℃～-15℃)

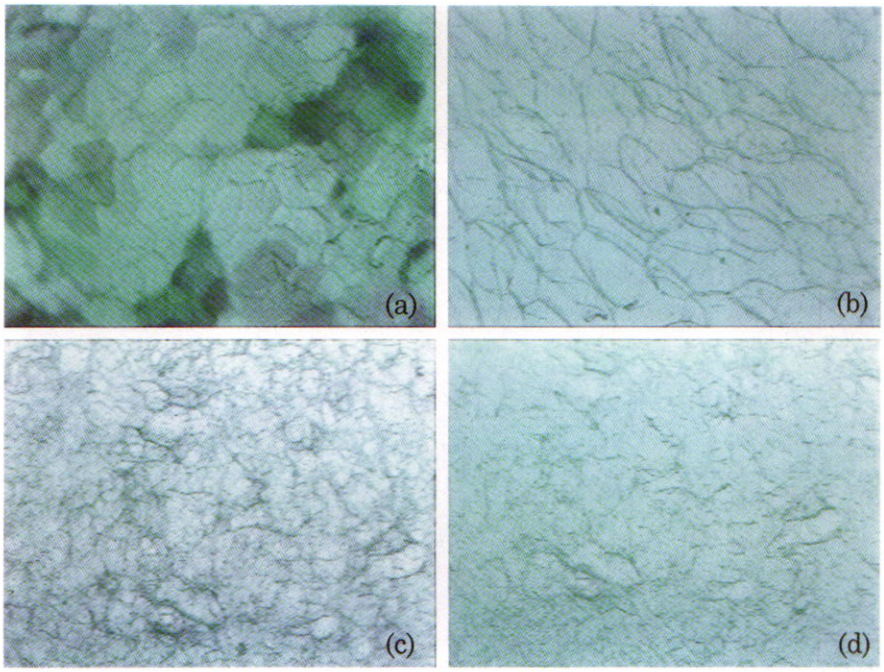

Fig. 5 A frozen 2% agarose gel. (a) using polarized light ; (b),(c),(d)normal ; (a), (b), (c) before thawing at -10℃～-15℃ ; (d) after 30minutes kept at room temperature after thawing.

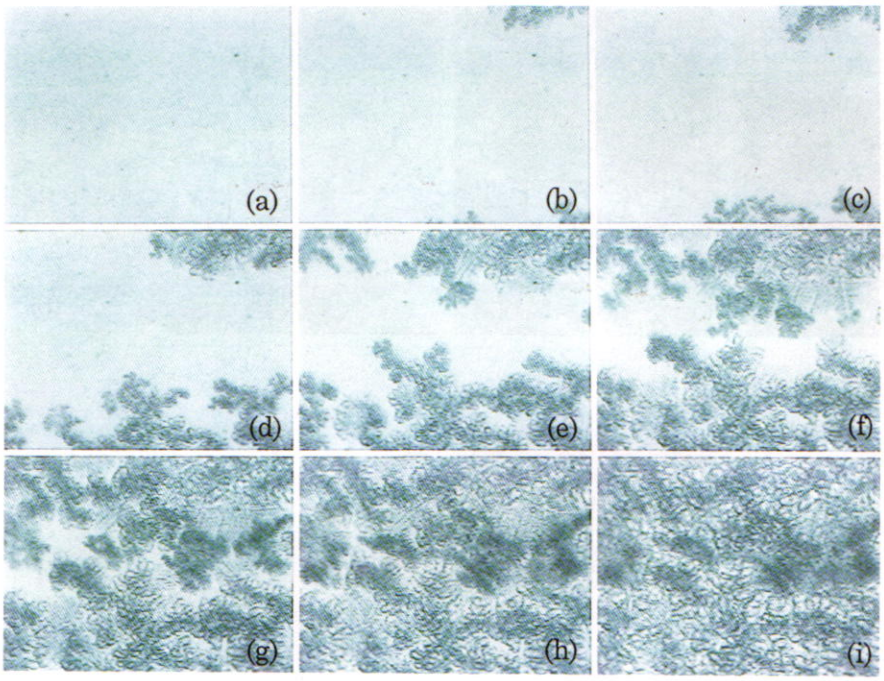

Fig. 6. Series of photographs during the freeze concentration process of a 2% agarose gel with 20% sucrose. (a) before cooling ; (b) beginning freezing ; →(c)→(d)→(e)→(f)→(g)→(h)→(i) (-10℃～-15℃)

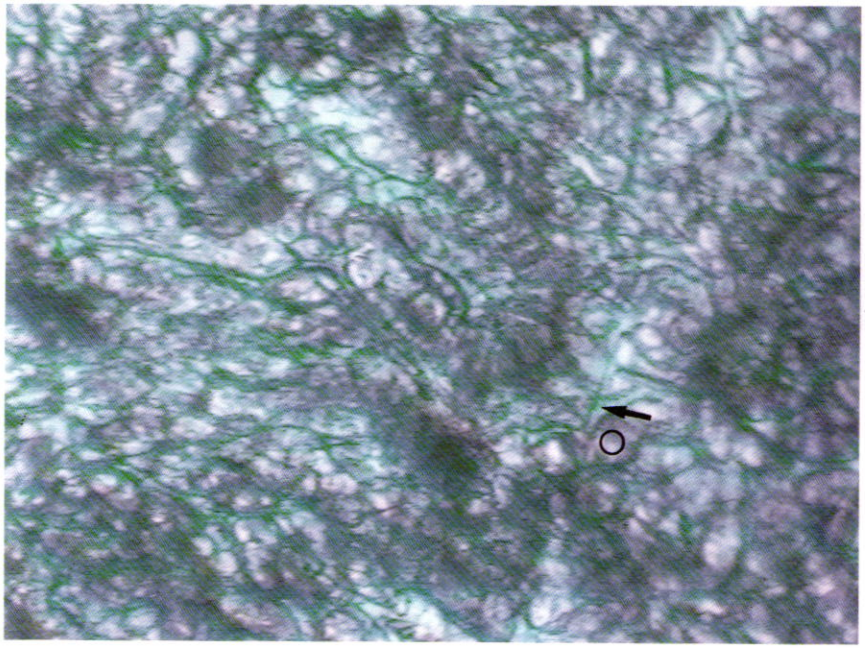

Fig. 7. An enlarged photograph of (i) in Fig.6. The color tone is controlled to provide a clear contrast. The arrow indicates the gap between the compartments ; ○, rounded ice crystal.

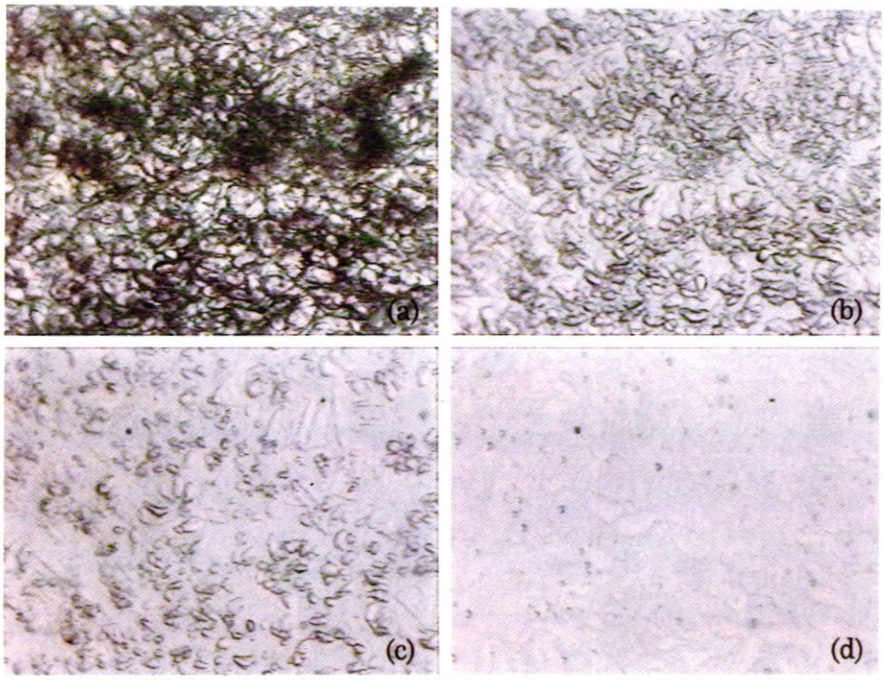

Fig. 8. Series of photographs during the thawing concentration process of a 2% agarose gel with 20% sucrose. (a) before thawing ; →(b)→(c)→(d) after 30minutes kept at room temperature after thawing.

partments under the microscope.

DSC measurements

Fig. 9 shows the rewarming DSC curves of agarose gels with or without sucrose. As a reference, the result of the sucrose solution is also shown in Fig. 9. The DSC curve of an agarose gel without sucrose showed no step-like shift representing a glass transition. On the other hand, the DSC curves of an agarose gel with sucrose and the sucrose solution showed an appreciable step-like shift to an endothermic direction, i.e., a glass-rubber transition. The glass transition temperatures, which were taken at the midpoint, were -37℃ for the agarose gel with sucrose and -33℃ for the sucrose solution.

Discussion

Freezing behavior of a pure agarose gel

The freeze concentration process of the polymer network as described in the results could not be followed using a light microscope due to the rapid ice crystal growth. However, the observations of the final frozen state, using both a light microscope and a Cryo-SEM, revealed the formation of the compartments by freezing. Particularly, the existence of the small deltoid void observed in the Cryo-SEM photograph (Fig. 2) implies that the void was formed by the membranes approaching from three sides. This shows that the walls of the compartment are made of two membranes. After all, it was thought that the freeze concentration of the polymer network proceeds as follows; as the ice crystals grow larger than the mesh size of the polymer network (nm order), the undiffusible polymer network is assembled in succession on the overall surface of the ice crystals, and so the assembled polymer network forms membranes covering overall surface of ice crystals. The growth of the ice crystals, however, continued until the membranes collided with each other because of the high permeability of water in the membrane. Finally, the polygonal compartments are formed. This freeze concentration process of the polymer network is significantly different from that of a small molecule. Generally, polymers added to a solution are thought to inhibit the ice crystal growth during the freezing process[3]. However, our results suggest that the ice crystal growth was not inhibited so much by the polymer network excluded from the ice crystal during the freezing process.

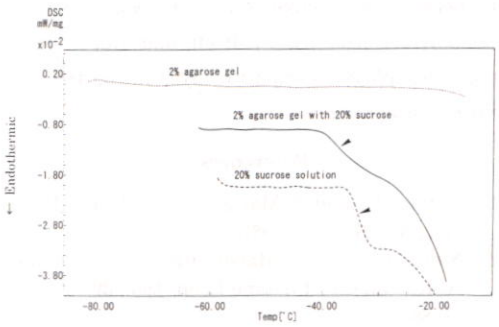

Fig. 9. Rewarming DSC curves of agarose gels with or without sucrose and a sucrose solution as a reference. The curves are shifted along the ordinate. Cooling rate : −2℃/min, ; heating rate : 2℃/min.

Furthermore, the result that the compartments were maintained to some extent even after thawing implies an irreversible interaction among the agarose polymers.

The DSC measurements were carried out for the purpose of understanding the state of the walls of the compartments. As shown in Fig. 9, the appreciable step-like shift except for the melting peak of the ice, could not be obtained by the rewarming of the pure agarose gel. It was reported that in the case of high concentration hydrogels, the step-like shift representing a glass transition is appreciated[4,5]. However, this kind of transition was not reported for low concentration gels. In the present study, even if the walls of the compartments become a glassy state, the step-like shift may not clearly appear in the DSC curve since the heat capacity increment due to a glass transition is very small because of the low concentration of agarose. Alternatively, the walls of the compartments may only be the concentrates. Anyhow, the state of the compartment walls will be the subject of our next study.

Effect of saccharides on the ice crystal growth in the gel

The results of the Cryo-SEM showed that in the case of frozen gels with sucrose, gaps were formed between the compartments. Also, from the dynamic image observation, it was found that the color within the gap did not change very much while that of the membrane turned deep blue. The methylene blue molecules,which were excluded from the ice crystals, may not diffuse in the residual solution but the dye was adsorbed on the membranes made by the polymer network with polar groups. Because of this adsorption, the walls of the compartments could be observed after thawing.

For the sucrose-containing gel system, the slow movement of the ice crystal growth front implies that the added sucrose plays a role of an inhibitor to ice crystal growth. The sucrose molecules, which are self-diffusible, are excluded from the ice crystals. Some of the excluded sucrose molecules may then be trapped in the agarose membranes, while others diffuse into the residual solution. Consequently, the viscosity of the residual solution gradually increases in the progress of the concentration. Therefore, the mobility of water decreases in the residual solution, so that the ice crystal growth is slower than in the sucrose free gel. Finally, the concentrated sucrose solution may turn into a glassy state at Tg' which is defined for the sucrose-water system[6]. In this condition, the membranes could not collide with each other as shown in the Cryo-SEM photograph (Fig. 3). On the other hand, the undiffusible polymer network would not significantly affect the ice crystal growth as the polymer network in a pure agarose gel, because the polymer network can not freely diffuse by itself even in the case of this gel with sucrose added.

The above view is also supported by the results of the DSC measurement which showed the glass to rubber transition in the rewarming curve of the sucrose-containing agarose gel. It can be said that the existence of the polymer network in the sucrose containing agarose gel is independent of the glass transition of the system, since the glass transition temperature of the sucrose containing gel was close to the Tg' of the sucrose-water system, even though a slight difference was appreciable. With respect to the state of the compartment walls in the frozen gel containing sucrose, we could not recognize any glass transition from our DSC experiments. However, it has been reported that many hydrogels show the glass-rubber transition at low temperatures[5,7]. The present time, this discrepancy is not fully understood. It may be necessary to take the DSC measurement conditions, i.e., the scan rate and the sample volume into consideration.

Fig. 10 shows a summarized flow chart which represents the behavior of the polymer network and sucrose solution in the gel during freezing. It was elucidated that although the polymer network is assembled during freezing on the overall surface of the ice crystals, the diffusion of water is not inhibited so much by the membrane made of the polymer network, and that such behavior of the polymer network is independent of the existence of sucrose. However, sucrose molecules added to the gel have an effect on the ice crystal growth in the same way as in the case of the sucrose solution. In other words, the behavior of the greatest part of sucrose molecules in the gel might be irrespective of the polymer network. From these suggestions, it should be recognized that the difference in mobility, between immovable agarose molecules forming the polymer network by itself and free sucrose molecules, plays an important role in the freezing process of hydrogels.

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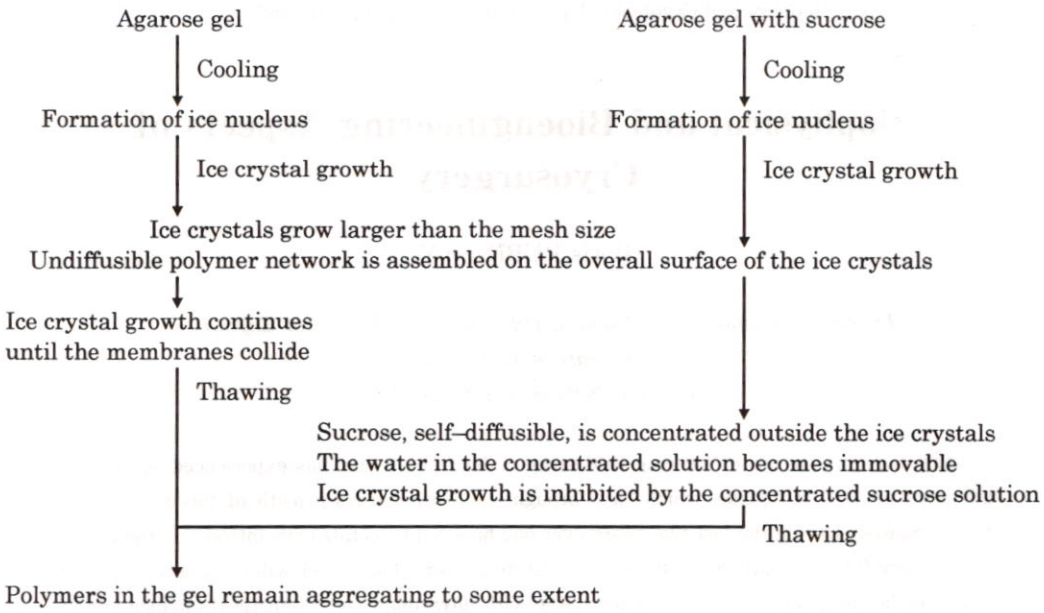

Fig. 10. A flow chart shows the freezing behavior of the polymer network and the sucrose solution in the gel.

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