Non-segregated crystalline state of dilute glycerol aqueous solution

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ABSTRACT

When a dilute aqueous solution freezes at 1 atm, it is segregated into water-rich ice Ih and solute-rich freeze-concentrated glassy solution. A similar segregation is observed at the crystallization of homogeneous glassy aqueous solutions by heating. The influence of solutes on the nucleation of solvent water and the solute discharge process from the crystalline ice are not clear. In this study, I made a homogeneous dilute glassy glycerol aqueous solution (0.07 mol fraction) using pressure liquid cooling vitrification (PLCV), measured the specific volume and the sample temperature during the compression and decompression processes, and examined the polyamorphic and crystallization behaviors. It is found that the sample crystallized slightly above the crystallization temperature is amorphized homogeneously under pressure, and that the amorphized sample is equivalent to the homogeneous glassy sample made by PLCV. This indicates that glycerol solutes in the crystalline sample are dispersed homogeneously and the crystalline sample does not segregate. These experimental results suggest that nucleation does not involve segregation and that crystal growth induces segregation. The discovery of the non-segregated crystalline state has an implication in not only the understanding of crystallization of glassy ice in meteorology and planetary physics but also the application to cell thawing techniques in cryobiology and food engineering.

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I. INTRODUCTION

In cryopreservation of cells, crystallization of water inside and outside the cells and crystal growth damage the cells. In order to reduce the damage, vitrification or microcrystallization of water inside and outside the cell is attempted. Glycerol (GL) is used as one of the cryoprotectants to avoid crystallization of water. In the case of a glycerol aqueous solution (GLaq solution), the homogeneous nucleation temperature $(T_{\rm H})$ decreases as the solute concentration increases.^{1–4} The GLaq solution of the larger concentration than the eutectic concentration (x_e) relatively easily forms a homogeneous glass without crystallization. Since the nucleation of ice is a kinetic phenomenon, $T_{\rm H}$ and $x_{\rm e}$ depend on the cooling rate. When the usual aqueous solution below x_e , for example, electrolyte, alcohol, and sugar aqueous solutions, freezes slowly at 1 atm, a part of solvent water crystallizes to hexagonal ice (ice Ih) and the solutes are excluded from ice Ih. As a result, the aqueous solution is segregated into water-rich ice Ih and solute-rich freeze-concentrated glassy solution (FCS). This indicates that the solute molecules hardly

dispersed homogeneously in ice Ih and that the spatial distribution of solute concentration is non-uniform in the segregated sample.

When the homogeneous glassy aqueous solution near x_e is heated at 1 atm, the solvent water crystallizes near or above the glass transition temperature (T_g) and separates into the water-rich ice Ih and the solute-rich FCS. For example, when the homogeneous glassy GLaq solution near x_e is heated at 1 atm, a part of glassy solvent water is crystallized and the GLaq solution segregates into the waterrich crystalline part and the GL-rich FCS part.² ⁷ This indicates that even if the water around the cells can be successfully vitrified by mixing the cryoprotectant such as glycerol, the crystallization of solvent water occurs during the thawing process and it may damage the cells. Therefore, in order to establish a thawing technique for reducing the damage of frozen cells, it is necessary to fully understand the crystallization and crystal growth of glassy aqueous solution. However, there are few research studies relating to the crystallization of a glassy aqueous solution. The reason is that it is difficult to vitrify the aqueous solution considerably below x_e without segregation.

Therefore, experiments relating to crystallization of a homogeneous glassy "dilute" aqueous solution have rarely been performed.^{8,9}

There are several reports on crystallization of a glassy aqueous solution near and above x_e by heating at 1 atm.^{2,5-7} It is confirmed that the structure of cubic ice (ice Ic) or stacking disorder ice (ice Isd) is formed in the crystallized aqueous solution.^{5,6,10} On the other hand, Inaba *et al.* proposed that when the homogeneous glassy GLaq solution at 55 wt.% (~0.19 mol fraction) crystallizes, a two-dimensional ordered ice relating to ice Ic is formed in the crystalline sample slightly above T_x .^{2,7} The crystallization process of solvent water in the glassy GLaq solution is similar to that of low density amorphous (LDA) ice of pure water. The LDA transforms to ice Ic (or ice Isd) between 140 K and 160 K by heating at 1 atm.¹¹⁻¹⁴ The T_x of LDA depends on the heating rate¹⁵ and the stable state of LDA.¹⁶

Understanding the relation between the structure of solute and the polyamorphic behavior of solvent water from a viewpoint of water polyamorphism is significant. Previously several experiments^{4,8,9,17-34} and computer simulations³⁵⁻⁴¹ relating to the polyamorphic behaviors of an aqueous solution have been reported. The existence of solute changes the polyamorphic states of solvent water and affects the liquid–liquid transition of the aqueous solution.^{9,24,25,39,41} Moreover, the polyamorphic change in solvent water affects the conformation of solute²⁸ and the molecular vibration of solute.²³ The segregation of electrolyte aqueous solutions is suggested to also relate to the separation of solvent water into low-density liquid (LDL) and high-density liquid (HDL).^{8,26,35} The behavior of the aqueous solution seems to relate to water polymorphism. However, the study on the polyamorphism of an aqueous solution has only just begun and the findings are still unclear in the present stage.

Previously, in order to investigate the water polyamorphism of the aqueous solution system, we prepared a homogeneous glassy GLaq solution in the concentration region below the x_e (=~0.13) by cooling the aqueous solution under $\ensure^{42,43}$ and examined the polyamorphic transition under pressure.^{4,9,23,24} By compressing the GLaq solution to 0.3 GPa at room temperature and then by cooling it to 77 K at a cooling rate of 40 K/min, we succeeded in making a homogeneous glassy GLaq solution. For readability, I name the method of vitrification by cooling liquid sample under pressure as "pressure liquid cooling vitrification (PLCV)" in this paper. The solvent water in the glassy GLaq solution made by PLCV is related to high-density amorphous (HDA) ice of pure water. The glassy GLaq solution transforms to a low-density glassy GLaq solution with LDAlike solvent water in the low pressure region, indicating that the transition is a polyamorphic transition relating to the LDA-HDA transition of pure water.4

The polyamorphic transition of the GLaq solution is reversible against the change in pressure.^{4,9,24} The pressure at which the polymorphic transition occurs depends on the temperature and the solute concentration. The width of the hysteresis of the polyamorphic transition becomes narrower as the temperature increases. In temperature regions below the T_x , the polyamorphic transition does not depend on the temperature history.²⁵ This suggests that the polyamorphic transition is a metastable equilibrium transition. Furthermore, the polyamorphic transition of the glassy GLaq solution is reproduced well at the same temperature. The good reproducibility suggests that the GL molecules disperse

homogeneously in both LDA-like and HDA-like solvent waters. If aggregation of GL molecules relating to segregation occurs during polyamorphic transition, polyamorphic transition will not be reproduced against the repetitive pressure change at the same temperature because polyamorphic transition depends strongly on solute concentration.^{4,9,24}

The PLCV has enabled homogeneous vitrification of dilute aqueous solutions. In this study, I prepared the homogeneous glassy GLaq solution of x = 0.07 mol fraction that is remarkably lower than the x_e (=~0.13) by using PLCV and examined the polyamorphic transition and crystallization of the glassy GLaq solution. Particularly, I will discuss a possibility that the crystalline state slightly above the T_x is the non-segregated crystalline state.

II. SAMPLE AND EXPERIMENTAL METHODS

A. Preparation of solution sample

The GLaq solution of x = 0.07 using in this study was prepared by mixing GL and D₂O which were purchased from Wako Pure Chemical Industries, Ltd. Here, *x* stands for solute molar fraction which is a ratio of solute molar number to the sum of solute molar number and water molar number. The solute concentration of GLaq solution in this study (x = 0.07) is below x_e (=~0.13) at 1 atm.^{4.24} D₂O was used instead of H₂O to avoid the duplication of OH stretching vibration modes between the GL molecule and the H₂O molecule in the Raman spectroscopic measurement. There are no strong vibration modes of GL molecule in the region of 2200–2700 cm⁻¹ where the OD-stretching vibration modes of D₂O appear.⁹

B. Pressure liquid cooling vitrification method (PLCV)

In order to make the homogeneous glassy dilute GLaq solution, the PLCV was used in this study. First, the liquid sample was sealed in an indium container at 1 atm and the indium container was set in a piston-cylinder pressure device. The piston-cylinder device was placed in a hydraulically controlled press apparatus, and a pressure of 0.3 GPa was applied to the sample at room temperature. Subsequently, the sample was cooled to 77 K with liquid nitrogen at a cooling rate of ~40 K/min. A temperature–pressure protocol of preparation is shown by gray dashed lines in Fig. 1(a). I have confirmed in the previous Raman spectroscopic studies that the GLaq solution of x = 0.07 vitrified by PLCV is a homogeneous high-density glass without crystalline phases.⁹ The high-density glassy GLas solution (HDGS) marked by A in Fig. 1(a) is used as a starting sample in this study.

C. Volume and temperature measurements under pressure

The change in piston displacement (d) and the change in sample temperature (T_{samp}) during the compression and decompression were measured simultaneously using a special indium container with a thermocouple described in Ref. 4. T_{samp} is measured by the thermocouple in direct contact with the sample. In the simultaneous measurement, the change in d corresponds to the relative change in sample volume and it is difficult to calculate the absolute value of volume.



FIG. 1. The temperature–pressure protocols for the preparation of sample and the measurements of *d* and *T*_{samp}. (a) The preparation paths of HDGS (sample A) are shown by gray dashed lines. The first measurement series for sample A is represented by solid lines. The decompression and compression processes are colored by blue and red, respectively. Samples B, C, and D are the LDGS, NSCS, and the glassy sample made by PIA of NSCS, respectively. The schematic illustrations of the solution state of samples A, B, and C are shown as inset. Black dots in the inset are GL solutes. Pale red, pale blue, and pale green backgrounds represent HDA-like, LDA-like, and crystalline solvent waters, respectively. (b) The second measurement series for sample D is represented by dashed lines. (c) The SCS (sample E) is made by annealing the sample C at ~200 K at 0.01 GPa. The green two-dot chain line is the measurement path for the sample E. The schematic illustration represents the solution state of SCS. Red and green regions in the inset represent FCS and ice Ih, respectively.

In order to measure the exact specific volume of sample, a different type of indium container was used. The weight of indium container was adjusted to be 13.000 ± 0.0002 g, and the compression curve of *d* for only indium metal of 13.000 ± 0.0002 g was measured in advance. The weight of indium container with the sample was measured before the measurement of specific volume. The compression curve of *d* for only the indium metal was subtracted from the compression curve of *d* for the indium container with the sample and the compression curve of the specific volume of sample was calculated. Using the same method, the decompression curve of specific volume was calculated.

The effect of friction between the piston and the cylinder on the pressure value is corrected so that the compression and decompression curves of only the indium metal become the same curve. The detailed correction method has been described in Ref. 9.

The measurement temperature (T_{cyl}) is measured by a thermocouple in direct contact with the cylinder. T_{cyl} is controlled by the balance between the heater attached to the cylinder and the surrounding cold nitrogen gas. Since the volume of cylinder is extremely larger than the sample volume, there is little influence of the change in T_{samp} due to the transformation of sample on the stability of T_{cyl} . The stability of T_{cyl} is within $\pm \sim 0.2$ K.

D. Raman spectroscopic study

In order to identify the state of solvent water (D₂O), the Raman profile of OD-stretching modes in the range of 2200–2700 cm⁻¹ with the resolution of 0.2 cm^{-1} was measured at ~30 K. The sample recovered at 1 atm at 77 K was mounted in a sample holder for a cryostat, and the sample holder was placed in the cryostat. In order to clarify the difference of the Raman profile between two polyamorphic states, all the Raman spectra were recorded at 30 K.

The Raman spectroscopic measurement was performed using a microscopic Raman spectrometer system (Jobin Yvon, Inc. T-64000). The exciting light source was an argon ion gas laser operating at 488 nm with a power of 400 mW. The laser light via the optical system was attenuated, and the laser power in front of the sample was ~20 mW. Therefore, we can ignore the elevation of sample temperature caused by the laser irradiation.

III. RESULT AND DISCUSSION

The decompression and compression curves of d and the corresponding T_{samp} for HDGS made by PLCV are shown in Figs. 2(a) and 2(b), respectively. The simultaneous measurement of d and T_{samp} was performed twice each at $T_{\text{cyl}} = 150$ K and 155 K as shown in Fig. 1(a). The increase in d and the elevation of T_{samp} as shown by vertical arrows in Fig. 2 are observed in the decompression process. This indicates that the HDGS transforms to the lowdensity glassy sample (LDGS) with LDA-like solvent water. Since the polyamorphic transition is slower than the decompression rate, the heat generation survives after the decompression process finishes at 0.01 GPa. Similarly, the stepwise decrease in d and the rapid elevation of T_{samp} are observed at the low-density to high-density polyamorphic transition in the compression process. As T_{cyl} increases from 150 K to 155 K, the pressure of the high-density to low-density polyamorphic transition increases, the pressure of the low-density to high-density polyamorphic transition decreases, and the width of hysteresis becomes narrow. It has been reported in the previous studies^{4,9,24} that as the solute concentration decreases, the stepwise change in specific volume becomes more drastic and the narrowing of hysteresis becomes clearer.

When the HDGS is decompressed at T_{cyl} = 160 K, it transforms to LDGS at ~0.1 GPa and then crystallizes. A large exothermic event



FIG. 2. The simultaneous measurements of d and T_{samp} against the change in pressure. The compression and decompression curves of d and the corresponding T_{samp} are shown in (a) and (b), respectively. d are shifted vertically for clarity. The red and blue curves stand for the compression and decompression processes, respectively. The first measurement series of Fig. 1(a) and the second measurement series of Fig. are represented by solid and dashed lines, respectively. The third measurement series of Fig. 1(c) is represented by green two-dot chain lines. Vertical arrows stand for the onset pressure of high-density to low-density polyamorphic transition in the decompression process.

relating to the crystallization is observed as shown in Fig. 2(b). The sample marked by C in Fig. 1(a) (sample C) was recovered at 1 atm at 77 K, and the Raman spectrum was measured at ~30 K (C in Fig. 3). The central frequency of the largest peak in the OD-stretching vibration modes is ~2286 cm⁻¹ that coincides with that of crystalline ice I (ice Ih or ice Ic). This indicates that the sample C is crystal relating to ice Ih or ice Ic. The Raman profile of sample C is broader than that of ice I. This suggests that the hydrogen-bonded network is distorted.

Next, when the sample C was compressed at $T_{cyl} = 160$ K, no polyamorphic transition occurred around 0.4 GPa, and the rapid decrease in *d* and the large exothermic event occurred at ~0.65 GPa [Figs. 2(a) and 2(b)]. The densified sample marked by D in Fig. 1(a) (sample D) was cooled to 77 K at 0.8 GPa, recovered at 1 atm, and then the Raman spectrum was recorded at 30 K. The OD-stretching Raman profile of the densified sample (D in Fig. 3) is almost the same as that of HDGS made by PLCV (A in Fig. 3). This similarity between Raman profiles indicates that the crystalline sample C amorphizes under pressure and transforms to the high-density glassy sample. The pressure-induced amorphization (PIA) of the sample C relates to the PIA of ice Ih to HDA.⁴⁴

Moreover, the sample D at $T_{cyl} = 150$ K and 155 K undergoes the polyamorphic transition against the change in pressure as shown by dashed lines in Figs. 2(a) and 2(b). The polyamorphic behavior is almost the same as that of HDGS made by PLCV. In addition, when the sample D is decompressed at $T_{cyl} = 160$, it crystallizes at the low pressure [Figs. 2(a) and 2(b)]. The crystallization behavior is also the same as that of HDGS made by PLCV. When the recrystallized sample is compressed at $T_{cyl} = 160$, it amorphizes at the same pressure as the amorphization pressure of the sample C. The PIA behavior is also the same as that of sample C.

The good agreement of transformation behavior between sample D and sample A (HDGS) indicates that the GL molecules in sample D are dispersed homogeneously. This suggests also that the GL molecules in the crystalline sample C before PIA are dispersed homogeneously. This indicates that although the solvent water in the sample C is a crystal-like ice I, the sample C does not segregate into ice Ih and FCS. If the segregation occurs in the sample C, the polyamorphic behavior of the glassy sample made by PIA should be different from that of HDGS made by PLCV. The reason is that the polyamorphic transition depends on the solute concentration.^{4,9,24} Actually, I further heated the sample C to 200 K at 1 atm, made the crystalline GLaq solution that is segregated intentionally [sample E in Fig. 1(c)], and then examined the transformation of the sample E at 150 K under pressure [green two-dot chain lines in Fig. 2(a)]. The transformation of sample E is quite different from that of sample C. Rather, it seems to be similar to the transformation of ice Ih.

For readability, I hereafter name the crystalline GLaq solution without segregation (sample C) as "the non-segregated crystalline sample (NSCS)" and the intentionally segregated crystalline GLaq solution made by heating to 200 K as "the segregated crystalline sample (SCS)."

Figure 4 shows the compression curves of the specific volume for LDGS at T_{cyl} = 155 K, NSCS at 164 K, and SCS at 164 K. The LDGS is made by the decompression of the starting HDGS at T_{cyl} = 155 K. The NSCS is made by the decompression of the starting HDGS at T_{cyl} = 164 K. Because of the usage of different containers, the measurement temperature, T_{cyl} , is different between the specific volume measurement and the simultaneous measurement of *d* and T_{samp} . In the case of the specific volume measurement, the crystallization of HDGS occurred at T_{cyl} = 164 K. The compression curves of specific volume for the NSCS, the SCS and ice Ih (D₂O) are measured in the temperature range between 77 K and 164 K and are shown in the supplementary material (Figs. S1–S3).

The LDGS at $T_{cyl} = 155$ K undergoes the low-density to highdensity polyamorphic transition at ~0.25 GPa. This polyamorphic behavior is consistent with the result in the simultaneous measurement of *d* and T_{samp} . The compression curve for the NSCS



FIG. 3. The OD-stretching Raman spectra of HDGS, LDGS, NSCS, and SCS. The Raman spectrum of the HDGS made by PLCV is drawn by blue (a). Here, in order to correct the effect of structural relaxation by pressure (Ref. 49), the HDGS is annealed at 150 K at 0.8 GPa before the Raman measurement. The Raman spectrum of NSCS is represented by green (c). The Raman spectrum of sample D made by PIA of NSCS is drawn by a red spectrum (d). After the HDGS made by PIA of the NSCS was annealed at 160 K, 170 K and 190 K at 1 atm, the Raman measurements were carried out. The sample annealed at 160 K, 170 K, and 190 K corresponds to LDGS, NSCS, and SCS, respectively. The vertical dashed line represents a peak position (\sim 2286 cm $^{-1}$) of the largest OD-stretching mode for the crystalline ice lh (lc).



FIG. 4. Compression curves of the specific volume for LDGS (a blue solid curve), NSCS (a black one-dot chain curve), and SCS (a red two-dot chain curve). A green dot curve is the compression curve of the sample made by the decompression of HDGS at 160 K.

shows that the PIA occurs at ~0.6 GPa without the polyamorphic transition around 0.25 GPa. On the other hand, when the sample made by the decompression of HDGS at 160 K nearly equal to T_x is compressed, two stepwise decreases in specific volume appear at ~0.2 GPa and ~0.6 GPa. The former relates to the polyamorphic transition and the later relates to the PIA. This indicates that the local parts of LDGS crystallize during the HDGS to LDGS transformation in the decompression process. As the result, the metastable LDGS and NSCS exist independently in the same sample. The appearance of two transformations indicates that the intermedium state between the low-density glassy state and the non-segregated crystalline state does not exist. This consists with the previous result that the LDA of pure water is different from the crystalline ice such as polycrystalline ice and nano-size crystalline ice.¹⁵

The PIA of the SCS at 164 K occurs in the pressure region between 0.7 GPa and 0.8 GPa (Fig. 4). The pressure of PIA, P_{PIA} , for the NSCS is about 0.1 GPa lower than P_{PIA} for the SCS, and P_{PIA} for the SCS is similar to P_{PIA} for ice Ih (D₂O) (see Fig. S4 of the supplementary material). The lower P_{PIA} for the NSCS suggests that the grain size of crystalline ice in the NSCS is smaller.⁴⁵

The specific volume of LDGS at 155 K at 0.01 GPa, NSCS at 164 K at 0.01 GPa and SCS at 164 K at 0.01 GPa are 0.852 \pm 0.005 cm³/g, 0.879 \pm 0.005 cm³/g, and 0.903 \pm 0.005 cm³/g, respectively. The specific volume of LDGS is about 3.2% smaller than that of NSCS, and the specific volume of NSCS is 2.7% smaller than that of SCS.

Figure 5 shows the temperature dependences of the specific volume for NSCS, SCS, and ice Ih (D_2O) at 0.01 GPa. The specific volume of NSCS little increases as the temperature increases and the temperature dependences seem to be small. On the other hand, the specific volume of SCS and ice Ih increase with the increase in temperature and their slopes are almost the same.

The isothermal compressibility, $\kappa_{\rm T} \left[= -\frac{1}{V} \left(\frac{\partial V(T,P)}{\partial P} \right)_T \right]$, at 0.01 GPa is estimated from the slope of compression curve, where



FIG. 5. Temperature dependences of specific volume at 0.01 GPa. The specific volume for NSCS, SCS, and ice Ih (D_2O) is represented by blue open circles, green open triangles, and red open squares, respectively. The dashed lines are drawn by guide of eyes.

V is the specific volume. $\kappa_{\rm T}$ of NSCS at 164 K is 116 TPa⁻¹ and is larger than that of SCS at 164 K (88 TPa⁻¹). $\kappa_{\rm T}$ of NSCS starts to increase rapidly around 140 K as shown in Fig. 6. The temperature dependence of $\kappa_{\rm T}$ near T_x is different from those for SCS and ice Ih. This suggests that the crystalline structure of NSCS seems to become unstable at high temperatures. The crystalline structure of NSCS may have many lattice imperfections and large deformations.

The OD-stretching Raman profile, the temperature dependence of $\kappa_{\rm T}$ and the temperature dependence of $P_{\rm PIA}$ for SCS are similar to those for ice Ih. The similarity of physical properties suggests that the crystalline solvent water in SCS is qualitatively equivalent to bulky ice Ih although a little solute is doped in crystalline solvent water.⁴⁶

On the other hand, the small specific volume, the broad ODstretching Raman profile, the large $\kappa_{\rm T}$ near $T_{\rm x}$, and the low $P_{\rm PIA}$ for NSCS show that the crystalline state of solvent water in NSCS is different in nature from the state of ice Ih although the crystalline structure of NSCS is similar to crystalline ice Ih. These results suggest that the NSCS is more unstable than ice Ih.

Next, I measured the changes in *d* and T_{samp} for HDGS of x = 0.07 during the heating at 0.01 GPa and examined the transformation process. The HDGS was heated from $T_{cyl} = 77$ K to 250 K at 0.01 GPa at the heating rate of ~18 K/min and *d* and T_{samp} were measured simultaneously. $T_{cyl}-T_{samp}$ vs T_{cyl} and *d* vs T_{cyl} are shown by the red lines in Figs. 7(a) and 7(b), respectively. The position of *d* corresponding to the specific volume of LDGS (0.86 cm³/g), NSCS (0.88 cm³/g), and SCS (0.91 cm³/g) was estimated from the independent measurement of the specific volume described in the supplementary material (Fig. S5).

A small endothermic event appears at $T_{cyl} = \sim 132$ K as shown in an inset of Fig. 7(a). This endothermic event is caused by the glass transition of HDGS. T_g is higher than T_g of HDA (~110 K).¹⁶

The large increase in *d* relating to the polyamorphic transition from HDGS to LDGS occurs between 141 K and 156 K (between α and β). However, the large exothermic event is not observed between α and β . When the specific volume becomes



FIG. 6. Temperature dependences of κ_T at 0.01 GPa. κ_T for NSCS, SCS, and ice lh (D₂O) are represented by blue filled circles, green filled triangles, and red filled squares, respectively. The dashed lines are drawn by guide of eyes.



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FIG. 7. The changes in $T_{cyl}-T_{samp}$ and the corresponding *d* for HDGS during the heating. Vertical dashed lines of α , β , γ , δ , ε , ζ , and η stand for the position of 141 K, 156 K, 161 K, 175 K, 188 K, ~205 K, and ~220 K, respectively. (a) The $T_{cyl}-T_{samp}$ curve (red) for HDGS is plotted as the function of T_{cyl} . The negative slope stands for the exothermic event. The expanded $T_{cyl}-T_{samp}$ curve between 120 K and 155 K is shown in an inset. The dashed line in the inset is drawn by guide of eyes. (b) The *d* curve (red) for HDGS is plotted as the function of T_{cyl} . The horizontal gray lines stand for the positions of the specific volume of 0.86 cm³/g, 0.88 cm³/g, and 0.91 cm³/g which are the specific volume of LDGS, NSCS, and SCS at 0.01 GPa, respectively. Blue curves are the changes in $T_{cyl}-T_{samp}$ and the corresponding *d* for the segregated glassy GLaq solution made by PIA of SCS at 77 K.

0.86 cm³/g (point β), the increase in volume suspends temporarily. This indicates that the polyamorphic transition completes. Subsequently, the large exothermic event occurs and the specific volume starts to increase again. When the exothermic event completes at ~160 K (point γ), the specific volume reaches ~0.88 cm³/g. This indicates that LDGS transforms to NSCS. I infer that the exothermic event between α and β is caused by both the polymorphic transition and the crystallization of LDGS. The delayed appearance of heat generation caused by the polyamorphic transition is presumably caused by canceling of the heat absorption due to the adiabatic expansion and the heat generation has been observed in the previous studies for the polyamorphic transitions of dilute glassy GLaq solutions^{4,9} and of glassy lithium chloride aqueous solutions.^{21,22}

The specific volume increases gradually between 161 K and 175 K (between γ and δ in Fig. 7) and reaches ~0.91 cm³/g which corresponds to the specific volume for SCS. This indicates the occurrence of the transformation from NSCS to SCS. A small exothermic event appears between 175 K and ~188 K (between δ and ϵ) in Fig. 7(a), and the slope of the volume change becomes larger. This may be heat generation caused by the crystal growth. It is difficult to discriminate the existence of heat generation relating to the

transformation from NSCS to SCS because of the large heat generation caused by the crystallization at point β .

Moreover, a tiny exothermic event is observed between ~205 K and ~220 K (between ζ and η) in Fig. 7(a). The temperature region between ζ and η roughly agrees with that of the transformation from ice Isd to ice Ih.^{10,14,47,48} Kuhs *et al.*¹⁰ and Handa *et al.*⁴⁸ proposed that the Isd(Ic)-to-Ih transformation region is 180–200 K and 176–216 K, respectively.

In the crystallization process of the homogeneous glassy GLaq solution by heating, a two-step of the volume change was observed between β and ϵ in Fig. 7(b). The first step is the crystallization to NSCS between β and δ . This may relate to the nucleation because the physical properties differ from those of bulky ice Ih. The second step is the transformation accompanying to the segregation between δ and ζ . This may relate to the crystal growth.

This SCS including the bulky ice Ih was cooled at 77 K again and then was compressed to 1.5 GPa at 77 K. The bulky ice Ih in the SCS was amorphized to HDA under pressure, and the segregated glassy sample that consists of water-rich HDA and FCS was formed. The two-step crystallization was not observed in the transformation process of the segregated glassy sample by heating with the same heating rate (a blue line in Fig. 7).

IV. CONCLUSION

The crystallization of a homogeneous glassy GLaq solution of x = 0.07 and the crystalline state were examined. When the sample crystallized slightly above T_x is compressed, it transforms to the homogeneous glass that is the same as the HDGS made by PLCV. This indicates that the crystalline sample slightly above T_x does not segregate as shown in Fig. 8 although the OD-stretching Raman spectrum of NSCS suggests that the crystal structure is identified as crystalline ice I. Moreover, the physical properties of NSCS, for example, the specific volume, κ_T , and P_{PIA} , are different from those of crystalline ice Ih. This suggests that the NSCS is mechanically more unstable than the state of ice Ih.

The instability of NSCS suggests the grain size of crystal in NSCS is small.⁴⁵ This suggestion is consistent with other experimental findings suggested from the broader OD-stretching Raman profile, the lower P_{PIA} , and the smaller specific volume. Therefore, I infer that the solvent water in NSCS is composed of nano-sized nuclei before constructing the three-dimensional framework of ice Ih (or ice Isd). The nucleation occurs at T_x and then the presence of glycerol molecules may inhibit the crystal growth of solvent water. The segregation does not occur at the stage of nucleation and seems to occur during the crystal growing process to three-dimensional crystalline ice.

Inaba *et al.* proposed that the ice structure in the crystalline sample made by heating the glassy GLaq solution relates to the 2D-ordered ice similar to ice Ic.^{2,7} The structure of NSCS in this study may be similar to the 2D-ordered ice. However, the solute concentration of the GLaq solution examined by Inaba *et al.* is x = ~0.19 (55 wt. %), which is considerably higher than that of the GLaq solution examined in this study (x = 0.07). The solvent water in Inaba's sample will be interfacial water in the narrow regions between GL molecules. On the other hand, the solvent water in this study, it is difficult to identify the microscopic crystalline structure of solvent water in the NSCS. Further research on the microscopic structure of NSCS is required, for example, x-ray diffraction study.

Previous experiments^{\$,26,29,31} and computer simulations^{35,38-41} of aqueous solutions have pointed out the relationship between the polymorphic behavior of solvent water and the segregation phenomenon of aqueous solutions. The interactions between solute and LDL/HDL, for example, the solubility of solute in LDL/HDL, may relate closely to the segregation.^{8,26,35,39} Understanding the relationship between water polyamorphism and the segregation in the aqueous solution system from the viewpoint of the results in this study is important for understanding the dynamics of aqueous solutions.

In this study, I show for the first time that the non-segregated crystalline state differing from the state of bulk ice Ih is able to exist in the aqueous solution system in the low concentration region below x_e . This novel state of aqueous solution may be applied not



FIG. 8. Schematic description of the segregation processes of HDGS by heating and of dilute GLaq solution by slow cooling. The black dots stand for GL molecules. When the homogeneous HDGS with HDA-like solvent water (pale red) is heated at 1 atm, it transforms to the homogeneous LDGS with LDA-like solvent water (pale blue). By heating further, the LDGS transforms to NSCS in which the solutes disperse homogeneously. The solvent water in the NSCS may be composed by nuclei of ice Ic (pale green). By heating further, the NSCS segregates into the water-rich ice Ih (Isd) (green) and the solute-rich FCS (red). The solvent water in FCS (red) is HDA-like (Refs. 9 and 24). When the dilute GLaq solution at room temperature (white) is cooled slowly at 1 atm, it segregates into ice Ih and FCS. If the dilute GLaq solution at 1 atm is hyper-quenched with $\sim 10^6$ K/s (Ref. 50), it may form LDGS (Ref. 9).

only to a thawing technique for cryopreserved cells but also to a reaction field in synthetic chemistry and pharmacy. Moreover, the crystalline state of NSCS is interesting for the general understandings of the nucleation of glassy water^{10–12} and of the Ic-Isd-Ih transformation.^{10,14}

SUPPLEMENTARY MATERIAL

See the supplementary material for the compression curves of the specific volume for NSCS, SCS, and crystalline ice Ih measured at various T_{cyl} , which are shown in Figs. S1–S3, respectively. The temperature dependences of $P_{\rm PIA}$ for the NSCS, SCS, and crystalline ice Ih are shown in Fig. S4. The change in specific volume for the homogeneous high-density glassy GLaq solution by heating at 0.01 GPa is shown in Fig. S5.

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