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**Note:** The content of the table is in Japanese.
Transformation between Inverse Bicontinuous Cubic Phases of a Lipid from Diamond to Primitive

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ABSTRACT: I studied the transformation between inverse bicontinuous cubic phases (QII) from diamond (QII D) to primitive (QII P) in a single-crystal region of monoolein. X-ray diffraction data reveal that the crystallographic orientation of QII D rotates 55° around the [011] axis from QII P. This indicates that one direction of the four-branched water channels in the QII D phase is preserved in the six-branched water channels of the QII P phase. I therefore built a transformation model that would keep the direction of the water channels fixed in both phases and cause the water channels along other direction in QII D to shrink and disappear.

INTRODUCTION

A triply periodic minimal surface (TPMS) has an infinitely extending structure and separates space into two interwoven regions1,2 (Figure 1). Three TPMSs, viz., diamond (D), primitive (P), and gyroid (G), which have cubic symmetry with space groups Pn̅3m, Im̅3m, and Ia3d, respectively, are frequently encountered in real systems, such as lyotropic liquid crystals,3,4 diblock copolymers,5 prolamellar bodies of plants,6 and organized smooth endoplasmic reticulum.7 Inverse bicontinuous cubic (QII) phases formed by lipid–water systems are typical examples. QII phases having a diamond (QII D), primitive (QII P), or gyroid (QII G) structure have been observed.8

The three TPMSs can be transformed into each other mathematically by Bonnet transformation,8 but the transformation requires that parts of the minimal surfaces pass through each other. The transformation also requires tearing the membrane of the physical system, thus the Bonnet transformation between QII phases is physically unrealistic.3,9 Another mechanism has been proposed that simplifies the transformations as the merging and separation of junctions of a water network labyrinth.10,11 This mechanism indicated that transformation without tearing is feasible. Moreover, it has been shown that transformations among all three TPMS structures can preserve both the topology and zero mean curvature throughout,12 and corresponding intermediate structures have been proposed.12–14 In the transformation between D and P, an intermediate with rhombohedral symmetry has been proposed. This transformation between the D and P surfaces is obtained by pushing or pulling along one of the (111) directions.12 The resulting changes in surface structure have been illustrated.13 Thus, it has been predicted that the crystallographic orientation would rotate 60° around one of the ⟨111⟩ axes during transformation in a real system.9

Exploring the orientational (or epitaxial) relationships in transformations between the QII phases requires a technique to control the crystal orientation. Several such techniques have been reported15–18 and employed to study the phase transition between QII G and QII D.16,19 However, only one direction of the crystal orientation can be aligned through these techniques. Thus, it seems difficult to elucidate orientational relationships in transformations between QII phases. My group, therefore, has developed a method to make a single crystal domain of the QII D phase,20 which clearly exhibited a crystal orientation of the QII D phase. Using this method, I studied the QII D→QII P phase transition in the single-crystal region.

MATERIALS AND METHODS

A single-crystal region was prepared according to a procedure reported previously20 except that the additive was replaced by t-butyl alcohol (tBA). A 80:20 v/v solution of water/tBA (Wako Pure Chemical Industries, Osaka, Japan) was prepared. The solution was mixed with dried monoolein (1-oleoyl-rac-glycerol) (Sigma Chemical Co., St. Louis, MO, USA) in a 60:40 w/w solvent/monoolein ratio. The sample was loaded into a polyimide capillary (PIT-S, Furukawa Electric Co., Tokyo, Japan) whose inner diameter was 0.5 mm. The length of the loaded sample was about 30 mm. One end of the sample was sealed with a tube-sealing compound (Chaseal, Chase Scientific Glass, TN, USA). The other end of the sample was soaked in water at 25 °C. After about 1 month, the sample in the capillary separates into two parts: a powder region and a single-crystal region of the QII D phase.20 The powder region was cut off, and the remaining portion was transferred into a 20 mM L-arginine (Arg) (Wako Pure Chemical Industries, Osaka, Japan).
The phase of the sample in the X-ray-exposed area was QII regions. In the structure of the QII phases of the lipid, the positions of two di regions was not aligned with the capillary axis. The sample was rotated from 0 to 180°. The capillary axis was adjusted to the rotating axis and perpendicular to the X-ray direction. The crystallographic orientation of the single-crystal gradually. (f, g) X-ray diffraction patterns of the sample in (e) were measured from just after the Arg soak to about 1 day in roughly 1 to 2 h intervals. The capillary axis was adjusted to the rotating axis and perpendicular to the X-ray direction. The crystallographic orientation of the single-crystal region was not aligned with the capillary axis. The sample was rotated from 0 to 180° in increment steps of 2° and X-rays were used for 10 s at each step. To measure the phase transition at the same sample position, measurements were repeated at intervals of 1 or 2 h. The results of the diffraction data analysis were almost the same as those reported previously. I determined the peak positions of the diffraction spots with display software ALBULA (Dectris, Baden, Switzerland). Using these peak positions, rotational matrices of the crystal orientations of QII^D (R_D) and QII^P (R_P) were calculated from the base coordinate. Of the data for the QII^P phase, diffraction spots from the {110}, {111}, {200}, {211}, {220}, {221}, and {222} planes were used in the calculation. In the case of the QII^P phase, spots from {110}, {200}, {211}, and {222} planes were used in the calculation. Diffraction spots from the {220} planes of the QII^P phase, which were not included in the extinction rules, could not be observed. Some of the spots listed above were not observed because they were located outside the detection area or at a blind angle with respect to the horseshoe-shaped frame. Rotational matrices R_D and R_P were used to calculate the peak positions of unobservable diffraction spots in the extinction rules, and then the phase of the sample was identified. The rotational matrix from QII^D to QII^P, R_{DP}, was calculated via R_{DP} = R_D R_P. A rotational matrix is defined by a rotational axis and a rotational angle. The rotational axis of R_{DP} is an eigenvector of the matrix. The rotational angle, θ, around the rotational axis was calculated from the trace of R_{DP} via 1 + 2 cos θ = TrR_{DP}. The detailed calculations are in the Supporting Information.

I constructed the model of a hypothetical intermediate in Figure 5 as follows. I aligned orientations of two-four-way junctions of the QII^D phase and one six-way junction of the QII^P phase. Next, I calculated the midpoint of the corresponding junctions in the QII^D and QII^P phases as the hypothetical intermediate. Then, I calculated the periodical structure of the intermediate using the periodicity of the QII^D and QII^P phases.

■ RESULTS AND DISCUSSION

Figure 2 illustrates the sample preparation process. To obtain a long single-crystal region of monoolein in a capillary, I used a 20 mL-arginine (Arg). Arg diffused slowly into the capillary from the open edge, and the single-crystal region of QII^p was formed far from the open edge. The polycrystal region was cut off; the remaining part consisted of only the single-crystal region. The single-crystal region was soaked in 20 mM L-arginine (Arg). Arg diffused into the capillary from the open edge, and the single-crystal region of QII^p was formed far from the open edge. A single-crystal region of QII^p was formed far from the open edge. The polycrystal region was cut off; the remaining part consisted of only the single-crystal region.
method reported previously by my group, with the modification of using TBA instead of 1,4-butanediol. The length of the single-crystal region was 5 mm. Arg solution is known to convert the QII D phase of monoolein into QII P. The open end of the capillary sample was soaked in 20 mM Arg to change the phase of the single crystal. Thus, the slow penetration of Arg solution led to a gradual QII D–QII P phase transition from the open end. Figure 3 shows the time evolution of the X-ray diffraction patterns of the sample 3.5 mm from the edge immersed in 20 mM Arg solution. The images are cumulative data of rotational angles (a–c) from 30 to 40° and (d–f) from 110 to 120°. Only the right halves of the images are shown because the original images are centro-symmetric. (a, d) After 7.5 h of soaking in the Arg solution. (b, e) After 12 h. (c, f) After 16 h. Diffraction spots of the QII D phase are seen in (a) and (d), and those of the QII P phase are in (c) and (f). Diffraction spots from both phases can be seen in (b) and (e). Some strong peaks from the QII D and QII P phases are surrounded by dashed circles of red and blue, respectively. Some Miller indices of the QII P phase are assigned in (a) and (d), and those of the QII D phase are assigned in (c) and (f).

Figure 3. X-ray diffraction images of the capillary sample, 3.5 mm from the edge immersed in 20 mM Arg solution. The images are cumulative data of rotational angles (a–c) from 30 to 40° and (d–f) from 110 to 120°. Only the right halves of the images are shown because the original images are centro-symmetric. (a, d) After 7.5 h of soaking in the Arg solution. (b, e) After 12 h. (c, f) After 16 h. Diffraction spots of the QII D phase are seen in (a) and (d), and those of the QII P phase are in (c) and (f). Diffraction spots from both phases can be seen in (b) and (e). Some strong peaks from the QII D and QII P phases are surrounded by dashed circles of red and blue, respectively. Some Miller indices of the QII P phase are assigned in (a) and (d), and those of the QII D phase are assigned in (c) and (f).

immediately to the left of the (01̅1) plane of QII D, which was assigned to the (01̅1) plane of QII P phase. In Figure 3b, a diffraction spot from the (111) plane of QII D adjoins one from the (200) plane of QII P.

From the positions of the diffraction spots in the two-dimensional images, I could calculate the positions of the spots in reciprocal space (Figure 4). Figure 4a is the view along the [0̅1̅1̅] direction of the QII D phase, which almost corresponds to the [0̅1̅1̅] direction of the QII P phase. Figure 4b is the view along the [111] direction of the QII D phase, which almost corresponds to the [200] direction of the QII P phase. I calculated rotational matrix $R_{DP}$ from QII D to QII P. A rotational matrix is defined by a rotational axis and a rotational angle. The normalized rotational axis of rotational matrix $R_{DP}$ was $[0.143 \pm 0.658 0.740]$, and the calculated rotational angle of the matrix was 54°. Visual inspection in reciprocal space indicated that the [0̅1̅1̅] directions of the two phases were nearly the same, as were the [111] direction of QII D and the [200] direction of QII P (Figure 4). The rotational matrix satisfying these relationships must have $[0̅1̅1]$, its rotational axis and a rotational angle of 55°. The rotational axis calculated from rotational matrix $R_{DP}$ was close to the [0̅1̅1] direction, and the angle difference was 9°. The difference between the rotational angles is very small. I therefore concluded that the crystallographic orientation rotated 55° around the [0̅1̅1] axis in the phase transition from QII D to QII P.

Using this orientational relation, I propose a model for the phase transition from QII D to QII P. Figure 5 shows water channels of QII D, a hypothetical intermediate, and QII P, viewed along the [0̅1̅1] direction. The lipid surface changes with the water channels. However, to simplify the model, I treat only the
Figure 5. Model of the transition pathway from the QII\textsuperscript{D} phase to the QII\textsuperscript{P} phase. Pipes indicate the centers of the water channels. Different tones of pipes indicate two different water channels. Models are viewed along the [01\overline{1}] direction of QII\textsuperscript{D} or QII\textsuperscript{P}. (a) The QII\textsuperscript{D} phase. The [1\overline{1}1] direction of the water channels is fixed during the phase transition. Water channels in the [1\overline{1}1] direction begin to shrink (red dashed arrows) at the start of the phase transition. (b) Hypothetical intermediate. Water channels in one direction (the [1\overline{1}1] direction in the QII\textsuperscript{D} phase) are short. (c) The QII\textsuperscript{P} phase of the corresponding orientation. The [1\overline{1}1] direction of water channels of the QII\textsuperscript{D} phase (green dotted line) is fixed and corresponds to the [200] direction of water channels of the QII\textsuperscript{P} phase. All directions marked with arrows or lines in the figures are perpendicular to the [01\overline{1}] viewing direction. The angle between the [200] and [1\overline{1}1] (or [1\overline{1}1]) directions is 55°, which is the rotational angle around the [01\overline{1}] direction in the transformation. The water channels of the [1\overline{1}1] direction in the QII\textsuperscript{D} phase (a) (red dashed line) shrink (b) and disappear (c) during the transformation.

The transformation of water channels explicitly. The phase transition starts from the QII\textsuperscript{D} phase (Figure 5a). The QII\textsuperscript{D} phase has simplified water channels along four directions: [1\overline{1}1], [1\overline{1}1], [1\overline{1}1], and [1\overline{1}1]. At the start of the transition, water channels along the [1\overline{1}1] direction start to shrink. In the hypothetical intermediate, water channels along the [1\overline{1}1] direction in the QII\textsuperscript{D} phase shorten (Figure 5b), but one direction of the water channels is fixed, namely, the [1\overline{1}1] direction of the water channels in the QII\textsuperscript{D} phase. Then, the [1\overline{1}1] direction of water channels in the QII\textsuperscript{D} phase disappears, and the QII\textsuperscript{P} phase is formed (Figure 5c). The [1\overline{1}1] direction of water channels in the QII\textsuperscript{D} phase is still fixed in the QII\textsuperscript{P} phase, but the Miller indices of the direction become [200] because the crystallographic orientation changes during the transition.

The transformation from D to P has been studied theoretically,\textsuperscript{10–14} but the orientational relationships observed in a single crystal during the D–P transformation remain poorly studied. Squire et al. predicted that only one of the (111) axes in the crystallographic orientation of both phases was fixed during the phase transition according to the rhombohedral pathway model.\textsuperscript{15} I expected that the direction of the water channels of the QII\textsuperscript{D} phase, which disappear in the phase transition, would be fixed, but my data showed that the [01\overline{1}] axis is fixed as the rotational axis of crystallographic orientation and one other direction of the water channels is conserved. On the other hand, my model seems to agree well with the theoretical model for the surface transformation. The concept of merging junctions developed by Sadoc and Charvolin\textsuperscript{16} was used in my transformation model. Hyde and coworkers have reported a rhombohedral pathway model that preserves the topology and zero mean curvature throughout.\textsuperscript{12–14} The hypothetical intermediate in Figure 5b has rhombohedral symmetry. Thus, my transition model seems to agree well with the rhombohedral pathway model. Because the rhombohedral pathway model was developed on the basis of a unit cell, the model tolerates the rotation of the unit cell. Moreover, the transformation model of Squire et al. is in agreement with my model, except for the orientational relationship. Thus, my model and previous theoretical models by other groups differ only in terms of the direction preserved during the transformation.

The QII\textsuperscript{P}–QII\textsuperscript{D} transformation occurs at the interface between the two phases in the single-crystal region. Thus, the newly formed QII\textsuperscript{D} phase is in contact with the existing QII\textsuperscript{P} phase. This contact presumably affects the crystallographic orientation in the phase transition. Thus, having a membrane-forming adequate connection between the QII\textsuperscript{P} and QII\textsuperscript{D} phases is important in decreasing the transition energy and hence preserving the direction of the water channels. The preservation, therefore, would be energetically favorable in a macroscopic phase transition. Epitaxial relationships involving the type I cubic phase also seem to minimize the movement of the lipid and water during phase transition.\textsuperscript{23} Further experimental and theoretical studies are required in which the effect of the interface between the QII\textsuperscript{D} and QII\textsuperscript{P} phases is considered.

In the rhombohedral pathway model, the rPD surface was considered to be a pathway for the transformation from D to P surfaces.\textsuperscript{12,13} In the transformation, the c-axis length of the rhombohedral unit cell of the rPD surface decreases by 36% and the a-axis length increases by 28%.\textsuperscript{24} This indicates that the length of the [1\overline{1}1] direction of D shrinks by 36% in P whereas the length of the direction perpendicular to [1\overline{1}1] expands. My model in Figure 5 also shows the disappearance of the water channel in the [1\overline{1}1] direction in the QII\textsuperscript{D} phase. The single crystal of the QII\textsuperscript{D} phase is a liquid crystal, and thus the membrane surface and water channels are movable during the phase transition. However, a large length change in one direction would damage the single crystal. Indeed, X-ray diffraction reveals a deterioration of the single crystal during the phase transition.

I concluded that one direction of the water channels is preserved: the [1\overline{1}1] direction of the QII\textsuperscript{D} phase corresponds to [200] of QII\textsuperscript{P}. But there are eight choices because the set of (111) axes includes eight directions. Thus, there is reason to restrict the crystallographic orientation in the transition. In this experiment, Arg solution was used to induce a phase transition in the lipid in the capillary. Thus, the phase transition advanced in one direction along the capillary axis from the open end of the capillary located at the bottom. This would affect the choice of axis. Indeed, my data indicates that the preserved directions of the water channels were almost perpendicular to the capillary axis. The direction in which the phase transition advances might restrict the preserved directions of the water channels.

The phase of monoolein is QII\textsuperscript{D} under excess water conditions. In this report, I used Arg to obtain the QII\textsuperscript{D} phase.
from the Q\textsubscript{II}\textsuperscript{P} phase.\textsuperscript{22} However, the phase-transition mechanism is unclear. Yamazaki and coworkers have reported that the phase of monoolein with charged lipids such as dioleoylphosphatidyl acid depended on the surface charge, and they observed the Q\textsubscript{II}\textsuperscript{P} phase.\textsuperscript{4,25} Thus, Arg might affect the membrane surface and stabilize the Q\textsubscript{II}\textsuperscript{P} phase.

The X-ray powder diffraction technique has been widely used in the analysis of Q\textsubscript{II} phase transitions.\textsuperscript{9,26,27} However, data obtained by this technique does not include information on sample orientation. By contrast, the single-crystallization method\textsuperscript{20} and alignment technique\textsuperscript{15–18} of the Q\textsubscript{II} phase allow us to study the orientation of the phases during the transition. These techniques promise to yield high-quality structural information on the Q\textsubscript{II} phases.

## CONCLUSIONS

By using the single-crystal region of the Q\textsubscript{II} phase of monoolein, I revealed orientational relationships in the Q\textsubscript{II}\textsuperscript{D}–Q\textsubscript{II}\textsuperscript{P} phase transition. I also proposed a model for the transition in which one direction of the water channels was preserved while the other direction shrank and disappeared. The change in crystallographic orientation in my model did not agree with those reported in previous theoretical works, but in all other respects, my model did agree well with previous models. This result is one example of the benefits produced by the single-crystallization technique of the Q\textsubscript{II} phase. Moreover, the techniques used in this article would help us to acquire further information about the transformation between Q\textsubscript{II} phases.

### ASSOCIATED CONTENT

1. Supporting Information

Sequential X-ray diffraction images for Figure 3 and two movies for Figure 5. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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### REFERENCES


