Observation of the Transient Rotator Phase of *n*-Hexadecane in Emulsified Droplets with Time-Resolved Two-Dimensional Small- and Wide-Angle X-Ray Scattering

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Crystallization of *n*-hexadecane in emulsion droplets was studied using time-resolved two-dimensional small- and wide-angle x-ray scattering with differential scanning calorimetry (2D-SAXS-WAXS-*in situ* DSC) which provides information about both nano- and subnanoscale structural change. *n*-hexadecane in droplets reproducibly crystallized into the stable triclinic phase via a transient-rotator phase. This is in contrast with previous results that the rotator phase of *n*-hexadecane was observed only occasionally for bulk samples. Thus we confirmed the existence of rotator phase in *n*-hexadecane, which is important for the study of crystallization of soft materials. We suggest that the rotator phase at the interface of oil and water plays a precursor role for bulk crystallization. This study demonstrates that 2D-SAXS-WAXS-*in situ* DSC is a powerful tool for the study of a transient phase.

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Normal alkanes (C_nH_{2n+2}, *n*-alkanes) are among the most basic components of soft materials such as surfactants, lipids, and liquid crystals [1]. It is, therefore, important to study the crystallization kinetics of *n*-alkanes for further understanding of soft materials. Recently, two characteristic phenomena have attracted attention in the study of alkane crystallization [2–7]. One is that *n*-alkanes crystallize into a stable phase via a metastable ''rotator'' phase, which is a lamellar crystal exhibiting long-range order in the molecular axis orientation and the center-of-mass position but lacks long-range order in the rotational degree of freedom of the molecules about their long axis [3,4,8]. The concepts and principles of such a metastable phase are considered to be important for further understanding of polymer phase behavior [9,10]. The second phenomenon is surface freezing, where a single crystalline monolayer is formed at the surface of the isotropic liquid bulk at temperatures up to 3 °C above the bulk freezing temperature [5–7]. Most materials exhibit surface melting—the surface melts at a lower temperature than the bulk. Thus surface freezing in *n*-alkanes is rather peculiar. Because the surface monolayer is in a rotator phase for $n < 44$ and is considered to be an ideal nucleation site for the bulk rotator phase, the relationship between these two phenomena is key to understanding the crystallization of *n*-alkanes. In spite of numerous studies, the precise mechanism of crystallization of *n*-alkanes, especially *n*-hexadecane (alkane number = 16, hereafter C_{16}), has not yet been clarified. Although Sirota and Herhold first observed the transient-rotator phase of C_{16} in bulk samples in 1999 [2,11], it was detected in only 9 out of 85 measurements. For *n*-octadecane (C_{18}), the existence of a transient phase itself is robust [11], and the existence of a transient-rotator phase of C_{16} seems almost decisive. However, there still exists controversy because it was not detected by other groups [12].

The emulsification technique has been widely used for the study of crystallization of *n*-alkanes [13–15]. It enables us to vary the distribution and importance of catalytic impurities by controlling the particle size of emulsion, as well as to separate the process of nucleation and crystal growth [16]. Crystallization in emulsion droplets is not, however, as simple as often presumed [17]. If the hydrophobic portion of the surfactant has a similar molecular structure to the alkanes, it can increase the nucleation rate [18,19]. Recently, crystallization in droplets has been considered as a tool for controlling polymorphism by interfacial heterogeneous crystallization [20]. These phenomena may be related to the crystallization process in the surface freezing and the rotator phase described above. Therefore, it is important to study these characteristic crystallization phenomena of *n*-alkanes in the crystallization of emulsions. In the present study, we performed time-resolved small-angle x-ray scattering (SAXS) and wide-angle x-ray scattering (WAXS) measurements of C_{16} in emulsion droplets to observe the initial stages of crystallization. Combined measurement of SAXS and WAXS is a powerful tool for studying structures over a wide range of scales simultaneously. The use of synchrotron radiation (SR) enables us to perform time-resolved SAXS-WAXS experiments due to its high brilliance and intensity. The recent development of two-dimensional (2D) detectors such as x-ray CCD detectors [21] allows us to perform *in situ* observations of phase transitions which provide much greater information than that with a one-dimensional detector. The combination of SR with 2D detection makes it possible to observe the weak and anisotropic scattering at the initial stage of crystallization, which would otherwise be difficult to detect.

The sample used was droplets of C_{16} in an oil-in-water (O/W) emulsion. Samples of C_{16} with >99% purity were purchased from Sigma Chemicals and no further purification steps were taken. We added 1 wt. % concentration of emulsifier (polyoxyethylene sorbitan monolaurate, Tween 20, Sigma Chemicals) to the water before emulsification. The O/W emulsion droplets were made by a novel method using a microfabricated channel array, called microchannel emulsification [22]. This technique allows us to prepare large emulsion droplets of nearly monodisperse size distribution. The average size of the droplets was 32.6 ± 3.2 μm, which was measured by a laser diffraction particle size analyzer (Beckman Coulter, Inc. LS 13 320). The stable crystalline phase of C_{16} is a triclinic structure where the axis of molecules is tilted off the layer normal by 19.4° [1]. In the reported transient-rotator phase of C_{16}, untilted molecules were suggested [2].

We performed SAXS-WAXS measurements to investigate the structural change of the sample near its freezing temperature at BL44XU, SPring-8 (Hyogo, Japan). The x-ray beam was monochromated at 0.9 Å, and the beam size at the sample position was 50 μm × 50 μm (FWHM). The samples were sealed in a 1.0 mm thick cell made of aluminum and set in a heating and cooling stage (LINKAM, THMS-600). The sample was heated to ~10 °C above the melting temperature and then cooled to −5 °C with a cooling rate of 1 °C/ min. Images of SAXS and WAXS were measured simultaneously with a DIP-6040 (Bruker-AXS), which has five imaging plates (IPs) [23]. Successive images were collected every minute by exchanging IPs, overcoming the 150 s readout time for one plate. The exposure time for one image was 30 s, and the interval between the exposures was around 25 s. The camera length was around 600 mm, which was calibrated with a silver behenate diffraction peak of 58.4 Å [24].

Figure 1(a) shows the SAXS-WAXS intensity profiles of C_{16} from 14.2 °C to 10.1 °C, which are obtained by azimuthally averaging the data. The scattering vector $q = \frac{4\pi}{\lambda}\sin\theta$ is given in the abscissa, where λ is the wavelength and 2θ is scattering angle. It is clearly shown that only the metastable peaks ($q = 0.281$ and 1.49 Å^{-1}) develop initially, and that the stable peaks which correspond to scattering from the triclinic phase appear later. The positions of the metastable peaks agree with those observed by Sirota and Herhold [2]. This shows that the structure at the initial stage of crystallization in emulsion droplets is the rotator phase of C_{16} observed in bulk samples. One of the SAXS-WAXS images taken by IP between 13.3 °C and 12.8 °C is shown in Fig. 1(b), corresponding to the second profile from the bottom in Fig. 1(a). The scattering from the rotator phase at $q = 1.63$ Å^{-1} was also observed, the position of which agrees with that of bulk sample observed by Sirota and Herhold. The expanded view of the central part of Fig. 1(b) shows that scattering from the rotator phase and the triclinic phase are both highly anisotropic

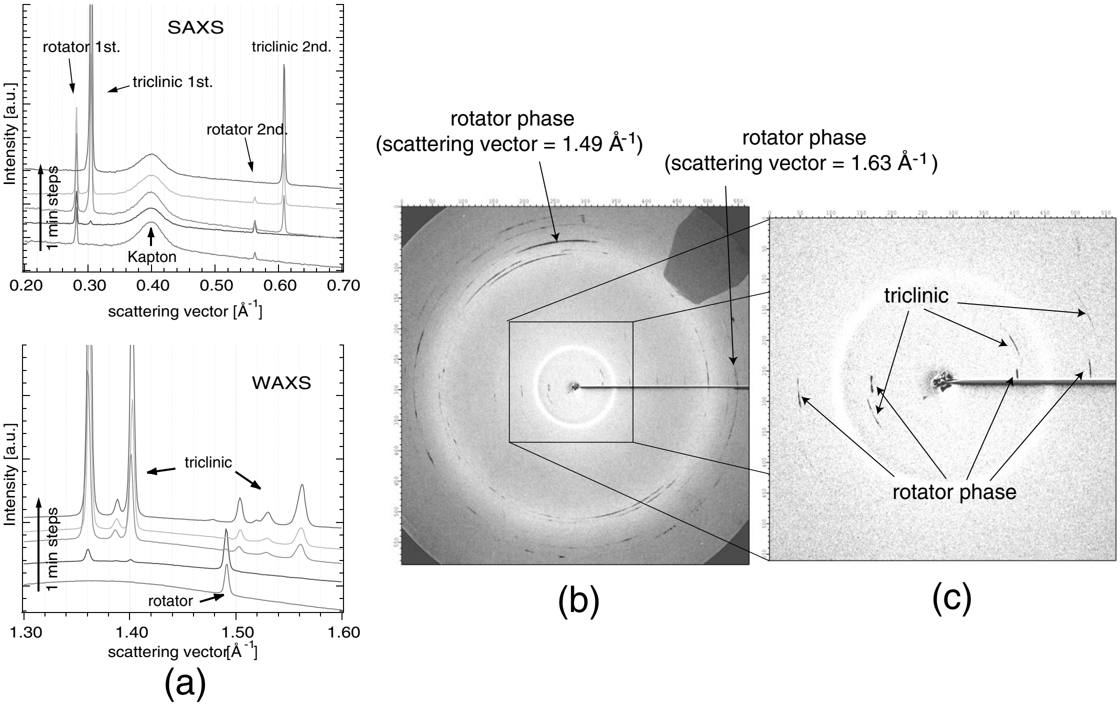

FIG. 1. (a) SAXS (upper) and WAXS (lower) intensity profiles which were obtained by azimuthally averaging the IP data during the cooling of C_{16} emulsion droplets from 14.2 °C to 10.1 °C. The cooling rate was 1 °C/ min. The exposure time was 30 s and the interval between the data acquisition was around 25 s (the profiles are offset vertically). (b) SAXS-WAXS image of C_{16} taken by an IP at 13.3 °C–12.8 °C. Two characteristic WAXS peaks, which are different from the triclinic structure peak, are clearly seen. A blowup of the central part of (b) is shown in (c), where the SAXS peaks from the rotator phase are clearly seen.

[Fig. 1(c)]. This indicates that a small number of domains of the rotator phase satisfies the Bragg condition.

To investigate the relationship between the structural change and the thermal behavior, time-resolved simultaneous SAXS-WAXS measurements together with differential scanning calorimetry, a modified Mettler model FP99A-V4.0 (SAXS-WAXS-DSC), were performed at BL-15A [25], Photon Factory (KEK, Tsukuba, Japan). The x-ray wavelength was 1.50 Å and the beam size was about 500 μm × 500 μm at the sample position. The images of SAXS and WAXS were measured with an x-ray CCD detector coupled with a 230 mm diameter x-ray image intensifier (XRII) [26], which was set at a distance of 160 mm from the sample. In addition to a usual beam stop, a small beam stop of 3 mm in diameter was set at a distance of 35 mm from the sample in order to reduce air scattering. The samples were sealed in the same cell as used in the previous experiments and were heated to 25 °C and cooled with a cooling rate of 2 °C/ min . SAXS-WAXS data were recorded at 3 s intervals and the exposure time was 1.5 s.

Figure 2 shows the relationship between the thermal data and the azimuthally averaged SAXS-WAXS intensity from the C_{16} emulsion during crystallization. It is shown that both SAXS and WAXS from the rotator phase appear at 14.0 °C and disappear at 10.8 °C. Three exothermic peaks appear on cooling of the sample. The first exothermic peak at 14.5 °C corresponds to the crystallization of C_{16} in bulk form or very large droplets made by coalescence. The corresponding scattering was not detected, because they existed at the upper part or the surface of the cell, which were not irradiated by x rays. The second weak peak of DSC at 10.8 °C corresponds to the sudden increase of scattering intensity from the triclinic phase and the disappearance of scattering from the rotator phase. Also a sudden decrease of halo scattering from the liquid oil is clearly seen at this temperature. These results suggest that at this cooling rate (2 °C/ min) the transition from the rotator phase to the triclinic phase occurs at this temperature (10.8 °C) and that at the same time the rapid crystallization from the liquid oil into the triclinic phase begins. The third broad DSC peak can be related to crystallization from the liquid to the triclinic structure. At this crystallization, no rotator phase was observed and C_{16} appears to crystallize directly into the triclinic crystal. This agrees with the previous results where no rotator phase was detected at low temperature [13]. In this regime, either the rotator phase no longer exists or the conversion from rotator to triclinic crystal occurs too rapidly to be observed at low temperature. The SAXS-WAXS-DSC experiments were repeated 7 times and SAXS-DSC experiments were repeated 10 times, and the same results were obtained in all experiments, though some fluctuation existed in temperature (± 0.5 °C).

From the above results, it is confirmed that the C_{16} in emulsion droplets crystallize into the triclinic structure through the rotator phase. This rotator phase corresponds to that observed by Sirota and Herhold in bulk samples. A significant difference between our results and the previous bulk studies is that we observed this rotator phase in every measurement, while Sirota and Herhold observed it only 9 times out of 85 measurements. The reason for the difference in observation probability can be understood simply. First, we used a 2D x-ray detector, whereas previous studies used 1D detectors. Since the scattering is anisotropic as shown in Fig. 1, it is reasonable that the use of 1D detectors often fails to detect the scattering which would be detected by a 2D detector. We note that the scattering from the rotator phase was not detected in our experiments using a 1D position-sensitive proportional counter detector. Second, rather than bulk oil, we used emulsion droplets as a sample. If C_{16} in each emulsion droplet nucleates individually, the use of emulsion droplets increases the number of crystallization events and hence the possibility of observing a transient scattering peak. In the case of the experiment at SPring-8, the beam size at the sample position was 50 μm × 50 μm, while the diameter of emulsion

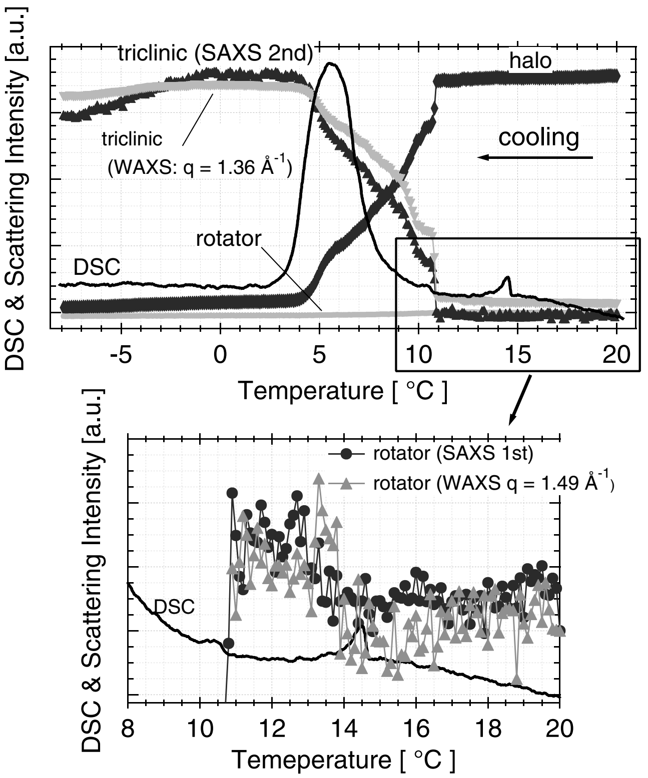

FIG. 2. (Upper) DSC and time evolution of scattering intensity profiles from the rotator phase (SAXS 1st), triclinic structure [SAXS 2nd, WAXS ($q = 1.36$ Å^{-1})], and halo during cooling of C_{16} emulsion droplets. The cooling rate was 2 °C/ min . Each scattering intensity is arbitrary in scale. (Lower) An expanded view of the marked portion of the upper figure. The scattering intensity profiles from the rotator phase [SAXS 1st and WAXS ($q = 1.49$ Å^{-1})] are shown.

droplets was around 30 μm. Therefore, the number of droplets which could satisfy the Bragg condition was of the order of 10^2, while it was of the order of 10^4 in the experiments at Photon Factory where the beam size was around 500 μm.

Now we further discuss the difference of crystallization mechanism between the bulk form and the emulsion droplets. As shown in Fig. 2, it seems that crystallization effectively began at 10.8 °C, because the scattering from the triclinic phase suddenly increased and the halo scattering from the liquid alkane suddenly decreased. The rotator phase was formed at 14.0 °C, which was about 3 °C above the effective crystallization temperature and disappeared at 10.8 °C when the cooling rate is 2 °C/ min. This situation is very similar to the surface freezing phenomenon for bulk samples where the surface monolayer is formed at temperatures above the bulk freezing temperature. These facts suggest that the rotator phase was formed at the oil-water interface of the emulsion droplets as a precursor for crystallization inside the droplets, although the reversibility observed for the equilibrium surface freezing phenomena has not been confirmed in this study. Previous studies demonstrate that the oil-water interface of emulsion droplets influences the behavior of C_{16} crystallization. Recently, Ueno *et al.* found that the even-numbered *n*-alkanes in O/W emulsion droplets with additives had a crystal structure different from that of the stable phase [20]. We have also found that the scattering intensity of the rotator phase greatly increased when additives (decaglycerine-decasterate: lypophilic surfactant) are included in the C_{16} droplets [27]. Furthermore, the behavior of DSC and the scattering intensity depends on droplet size and surfactant type [27]. These results imply that the rotator phase is affected by the interface of droplets at the initial stage of crystallization, and that the oil-water interface plays a vital role in forming the rotator phase as a precursor, which is also very similar to the surface freezing phenomenon.

In summary, we have performed time-resolved two-dimensional SAXS-WAXS experiments using IPs, and time-resolved two-dimensional SAXS-WAXS-*in situ* DSC experiments during crystallization of C_{16}. In these experiments, we have observed that C_{16} in O/W emulsion droplets nucleates via the rotator phase. We have obtained these results, which we would often fail to observe with a one-dimensional detector, in every experiment by using 2D detectors: IP and x-ray CCD detector coupled with XRII. This study conclusively demonstrated the existence of the transient-rotator phase in C_{16}, which plays a key role in the crystallization process. Also, the formation of the rotator phase at temperatures above the effective crystallization temperature has been discussed in relation to the surface freezing phenomenon and the crystallization at the oil-water interface. Further study is needed to reveal the origin and role of the rotator phase in emulsion droplets. Finally, our experiments have demonstrated that the two-dimensional SAXS-WAXS-*in situ* DSC technique is a very powerful tool for the study of transient phenomena.

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[1] D. M. Small, *The Physical Chemistry of Lipids* (Prenum, New York, 1986).
[2] E. B. Sirota and A. B. Herhold, Science **283**, 529 (1999).
[3] E. B. Sirota, H. E. King, Jr., D. M. Singer, and H. H. Shao, J. Chem. Phys. **98**, 5809 (1993).
[4] E. B. Sirota and D. M. Singer, J. Chem. Phys. **101**, 10 873 (1994).
[5] J. C. Earnshow and C. J. Hughes, Phys. Rev. A **46**, R4494 (1992).
[6] X. Z. Wu *et al.*, Phys. Rev. Lett. **70**, 958 (1993).
[7] B. M. Ocko *et al.*, Phys. Rev. E **55**, 3164 (1997).
[8] A. Müller, Proc. R. Soc. London A **138**, 514 (1932).
[9] A. Keller and Z. D. Cheng, Polymer **39**, 4461 (1998).
[10] G. Strobl, Eur. Phys. J. E **3**, 165 (2000).
[11] E. B. Sirota and A. B. Herhold, Polymer **41**, 8781 (2000).
[12] R. Montenegro and K. Landfester, Langmuir **19**, 5996 (2003).
[13] A. B. Herhold, D. Ertas, A. J. Levine, and H. E. King, Jr., Phys. Rev. E **59**, 6946 (1999).
[14] H. Kraack, E. B. Sirota, and M. Deutsch, J. Chem. Phys. **112**, 6873 (2000).
[15] A. B. Herhold, H. E. King, Jr., and E. B. Sirota, J. Chem. Phys. **116**, 9036 (2002).
[16] D. Turnbull and R. L. Cormia, J. Chem. Phys. **34**, 820 (1961).
[17] J. N. Coupland, Curr. Opin. Colloid Interface Sci. **7**, 445 (2002).
[18] W. Skoda and M. Van den Tempel, J. Colloid Sci. **18**, 568 (1963).
[19] D. J. McClements *et al.*, J. Food Sci. **58**, 1148 (1993).
[20] S. Ueno, Y. Hamada, and K. Sato, Cryst. Growth Design **3**, 935 (2003).
[21] S. M. Gruner, M. W. Tate, and E. F. Eikenberry, Rev. Sci. Instrum. **73**, 2815 (2002).
[22] T. Kawakatsu, U. Kikuchi, and M. Nakajima, J. Am. Oil Chem. Soc. **74**, 317 (1997).
[23] Y. Amemiya and J. Miyahara, Nature (London) **336**, 89 (1988).
[24] T. N. Blanton, C. L. Barnes, and M. Lelental, J. Appl. Crystallogr. **33**, 172 (2000).
[25] Y. Amemiya *et al.*, Nucl. Instrum. Methods **208**, 471 (1983).
[26] Y. Amemiya *et al.*, Rev. Sci. Instrum. **66**, 2290 (1995).
[27] Y. Shinohara, S. Ueno, and Y. Amemiya (to be published).