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Enclosure of Secondary Chirality Based on Highly-Oriented Lipid Aggregates into a Polymer Sheet by Photo-Induced Polymerization of Polymerizable Monomer Gels

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Summary: The L-glutamide derivative with double long-chain alkyl groups and a pyrenyl head group (Pyr-lipid) worked as organogelator in benzene and cyclohexane. Gelation was brought about through network formation with well-developed fibrous aggregates based on lipid membrane-like highly-oriented structures. Gelation was observed in polymerizable monomers such as methyl methacrylate (MMA) and styrene (St). Extremely intense CD spectra were observed at the absorption band of pyrenyl group in a monomer solution at gel-forming temperature, but the intensity decreased remarkably at the sol-forming temperature. The transparent solid sheet was obtained by photo-irradiation to the MMA gel in a quartz cell. More than 99% of MMA was polymerized even in the gel state and the weight-average molecular weight (M_w) of the resultant polymer was $7.2 \times 10^4\,g\,mol^{-1}$. CD spectra indicated that chirally-oriented structures in the gel state were maintained after polymerization of MMA and were stable at the temperature above the original gel-to-sol transition temperature. Fluorescence spectra of Pyr-lipid in the monomer solutions supported that the pyrene excimer was formed in the lipid aggregates and the stabilization of the excimer formation was achieved by polymerization of MMA in the gel state.

Keywords: L-glutamide-derived lipid; nanofibrillar aggregates; organogel; pyrene excimer; secondary circular dichroism; self-assembly

Introduction

Many research groups have been reporting possible techniques and methods to develop nano-scaled uniquely shaped materials by using supramolecular assemblies as templates.[1] The self-assembled organogels[2] are one of the promising candidates to create such nano-materials for bottom-up nanotechnology. Molecular orientation induced by intermolecular orientation is essential to form supramolecular assemblies and the highly-oriented structure has great impact on Materials Science and Technology. We have previously reported that the L-glutamide-derived lipids, shown in Figure 1, formed organogels in various organic solvents, in which the lipids formed highly-oriented aggregates with lipid membrane properties even in organic media. Scanning electron microscopy observations showed that the xerogel from the organogels was constructed by a 3D network structure through the formation of well-developed fibrous aggregates.[3] We have also clarified that the lipid aggregates showed phase separation behavior[4] and chirality induction for achiral dyes such as the cyanine dye,[5,6] which are usually observed in aqueous lipid membranes. In

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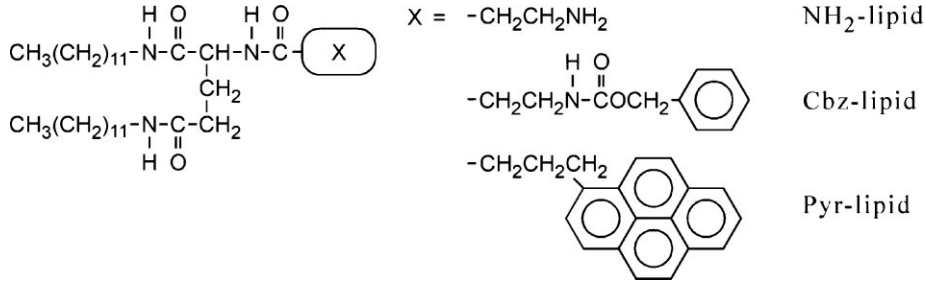

Figure 1.
Chemical structures of the L-glutamide-derived lipids.

our previous work,[7] we succeeded to stabilize the enhanced chirality from pyrene-containing L-glutamide lipid (Pyr-lipid) in methyl methacrylate (MMA) by photo-induced polymerization. In this paper, we describe the gelation properties, aggregation morphologies and chirally-oriented structures of the L-glutamide-derived lipid with pyrenyl head group in various polymerizable monomers and evaluation of the stability of the optical properties in oriented-structures of Pyr-lipid enclosed into a cross-linked polymer sheet by photo-induced polymerization of the bulk monomer.

Experimental Part

The pyrene-attached L-glutamide-derived lipid (Pyr-lipid) was synthesized according to our previous report.[8] The lipid was characterized by FT-IR and ^{1}H-NMR spectroscopic measurements, and elemental analysis. The Pyr-lipid was dissolved in polymerizable monomer at 70 °C and cooled down to 15 °C. The gelation properties of Pyr-lipid in each polymerizable monomer were estimated by the inversion fluid method using a ϕ 14 mm test tube at 15 °C. UV-vis and CD spectra were measured using a JASCO V-560 UV-vis spectrophotometer and a JASCO J-725 spectropolarimeter, respectively. Transmission electron micrographs (TEM) of the organogels were recorded using a JEOL 2000FX transmission electron microscope. The sample solutions were cast on copper grids and were stained with 2 wt% ammonium molybdate. The organogels were put into the glass or quartz cell and UV light was irradiated to polymerize the monomers. UV irradiation was shone from a high-pressure mercury lamp (USHIO UM-452) at a distance of 15 cm through an optical filter ($>$ 390 nm) at 10–15 °C. A radical photo-initiator, benzoin ethyl ether (1 wt%), was used for the photo-induced polymerization. After photo-irradiation, UV-vis and CD spectra were measured for evaluating the molecular orientation. Reversed phase HPLC and size exclusion chromatography (SEC) analyses were performed for the determination of the polymerization rate of the monomer and the polymerization degree of the obtained polymer. The solid sheet was taken out of the glass or the quartz cell and it was dissolved in THF. An octadecylated silica column (Grand pack 120-STC, 4.60 mm $\times$ 150 mm) was used with a multi-wavelength detector (JASCO MD-910). A TOSOH TSKgel Super HM-L column was used to determine the polymerization degree of the polymer. Scanning electron micrographs of the cross-section of polymer sheets were recorded using a JEOL JSM-5310LV scanning electron microscope.

Results and Discussion

Gelation of Polymerizable Monomers

We have reported that the Pyr-lipid forms fibrous aggregates in various organic solvents

such as benzene and toluene, and the fibrous aggregates form network structures which entrap organic solvents to form organogels.[8] The gelation properties of Pyr-lipid dissolved at different concentrations in various polymerizable monomers were investigated. Figure 2 shows a typical photograph of polymerizable solutions of Pyr-lipid in different monomers. The Pyr-lipid formed transparent gels in styrene (St) and divinylbenzene (DVB), and turbid gels in methyl methacrylate (MMA) and methyl acrylate (MA), whereas no gelation was observed in methacrylic acid (MAA) and acrylic acid at 5 mM. As shown in Table 1, the critical gelation concentrations (cgc) were different in each polymerizable monomer. Figure 3 shows TEM images of organogels in the various polymerizable monomers. The Pyr-lipid formed fibrous aggregates in St, DVB, MMA and MA, but their density of reticulation was very different. The cross-linking points would be formed by entanglement and/or fusion of several fibrous aggregates. The network structures in St and DVB are coarser than those in MMA and MA. On the other hand, fragmented fibrous aggregates were observed in MAA and MA. These results indicate that the organogels consist of three-dimensional network structures with well-developed fibrous aggregates and their turbidity is probably related to the reticulation density of the network structure. According to our previous report,[3] gelation properties and aggregation properties (critical aggregation concentration, cac) of glutamide-derived lipids are strongly affected by the solvent polarity, and oriented aggregates can be formed even at the sol-forming concentration. In MAA and AA solutions, gelatinization was not observed even at Pyr-lipid concentrations of 50 mM which is 100 times higher than the gelation concentration of Pyr-lipid in DVB.

Table 1.
Gelation ability of Pyr-lipid in polymerizable monomers (15 °C).

Concentration of **Pyr-lipid** (mM)	10	5	1	0.5	0.1
Styrene (**St**)	G	G	G	S	S
Divinylbenzene (**DVB**)	G	G	G	G	S
Methyl methacrylate (**MMA**)	G^t	G^t	S	S	S
Methyl acrylate (**MA**)	G^t	G^t	S	S	S
Methacrylic acid (**MAA**)	S	S	S	S	S
Acrylic acid (**AA**)	S	S	S	S	S
Benzene	G	G	G	S	S

G: gel, G^t: turbid gel, S: solution.

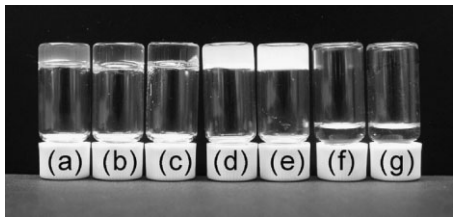

Figure 2.
Gelation properties of Pyr-lipid in various polymerizable monomers. (a) benzene, (b) styrene, (c) divinylbenzene, (d) methyl methacrylate, (e) methyl acrylate, (f) methacrylic acid, (g) acrylic acid. [Pyr-lipid] = 5 mM.

Secondary Chirality Based on Highly-Oriented Lipid Aggregates

Spectroscopic measurements are useful to detect the molecular orientation of the Pyr-lipid. Figure 4 shows CD and UV-vis spectra of Pyr-lipid in polymerizable monomers at 15 °C and 70 °C. Extremely intense positive CD signals were observed in the St solution at 354 nm (2.97×10^5 deg cm dmol^{-1}). Similar positive CD signals were observed in DVB (353 nm, 4.16×10^5 deg cm dmol^{-1}) and MMA (353 nm, 3.80×10^4 deg cm dmol^{-1}), but a negative CD signal was observed in MA (357 nm, -2.70×10^4 deg cm dmol^{-1}). Since these CD signals appeared at the absorption band of the pyrenyl group of the lipid, the intense CD spectra can be explained by an induction of chirality by the pyrenyl group through a chirally-oriented structure of the lipid. The CD intensities in MMA and MA were almost 10 times weaker than those in St and DVB. The induction of secondary chirality by the Pyr-lipid aggregates was accompanied by a red-shift in the absorption spectra of the pyrenyl group. These results indicate that the pyrenyl moieties form possibly J-aggregates with a chiral arrangement. In contrast, the CD signal was very weak in the AA solution

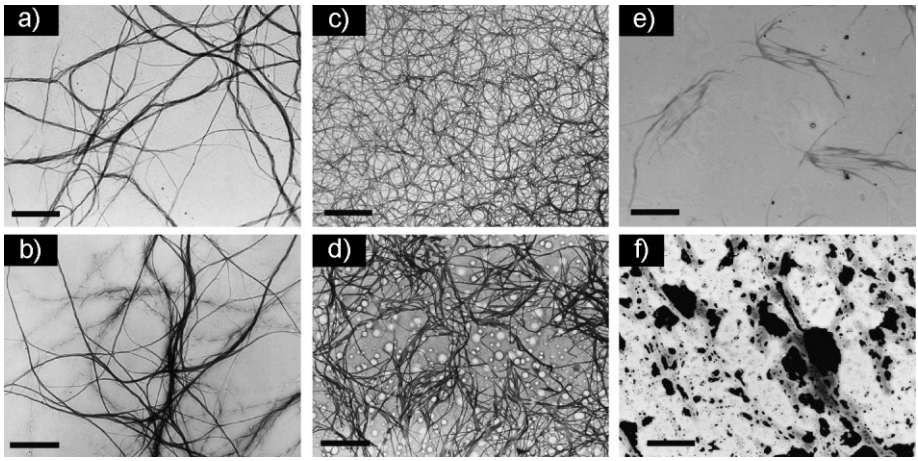

Figure 3.
Typical TEM images of Pyr-lipid aggregates in various polymerizable monomers. (a) styrene, (b) divinylbenzene, (c) methyl methacrylate, (d) methyl acrylate, (e) methacrylic acid, (f) acrylic acid. The lipid aggregates were stained with 2 wt% ammonium molybdate. The scale bars indicate 1 μm.

(353 nm, 8.9×10^3 deg cm dmol^{-1}), in which no gelation was observed as above-described. The characteristic pattern and the strength of the CD signals were affected by the chiral arrangement of the oriented-chromophore group.[9] The induced CD spectra disappeared at 70 °C which was above the gel-to-sol phase transition temperatures of the organogels. The secondary chirality based on the highly-oriented structures remarkably decreased around the gel-to-sol phase transition temperature and this change was thermally reversible. The instability of the secondary chirality can be useful for sensor applications but it has no obvious utility for optical devices.

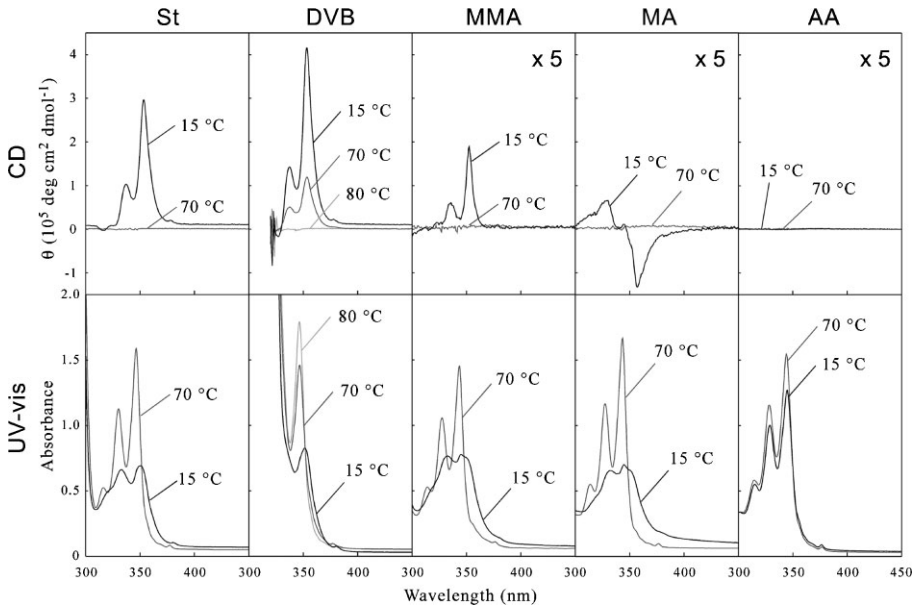

Figure 4.
CD and UV-vis spectra of Pyr-lipid in polymerizable monomers. [Pyr-lipid] = 5.0 mM.

Photo-Induced Polymerization

To increase the thermal and the mechanical stabilities, polymerizable monomer solutions of Pyr-lipid aggregates were polymerized by photo-induced polymerization. When 1 wt% of the benzoin ethyl ether photo-initiator was added to the polymerizable monomers and then UV-light was irradiated to the gel using the high-pressure mercury lamp for 10 hours at low temperature (10–15 °C), a solid sheet was obtained. The remaining monomer in the solid sheet was quantified by HPLC analysis. It was confirmed that the unreacted MMA monomer was less than 1% after photo-irradiation for 10 hours. The weight-average molecular weight (M_w) and polydispersity index (M_w/M_n) of the resultant polymer was evaluated by SEC measurements. The M_ws and the M_w/M_ns, with and without the Pyr-lipid, were 72,000 g mol^{-1} and 2.0, and 55,000 g mol^{-1} and 2.0, respectively. These results suggest that the polymerization reaction of the MMA monomer can occur even in gel state, and in the presence of lipid aggregates which do not seem to have any major effect on the polymerization reaction. Figure 5 shows the CD spectra of the organogels in MMA before and after the photo-polymerization. The CD intensity in the gel state was preserved in the solid sheet after the photo-polymerization and no decrease was observed at 70 °C. The light blue emission could be observed visually by UV-light (365 nm) irradiation to Pyr-lipid in the gel state whereas the color turned to purple in the solution state. According to the fluorescence spectra, it can be understood that the light blue and purple colors are caused by pyrene excimer formation and the monomeric pyrene respectively. The pyrene excimer formation was confirmed in the Pyr-lipid containing polyMMA sheet prepared by the polymerization of MMA in the gel state at 15 °C. But no excimer emission was observed in the polyMMA sheet prepared in the solution state at 70 °C. Figure 6 shows typical fluorescence spectra of polyMMA sheet and photographs of polyMMA sheet without and with UV light irradiation. These results indicate that highly-oriented structures of the Pyr-lipid in the gel state can be maintained after photo-induced polymerization of MMA and are stable even at temperatures above the original gel-to-sol phase transition.

Ethylene glycol dimethacrylate (EGDMA) was added to the MMA gel as a cross-linking reagent and a photo-induced polymerization of a 95: 5 MMA-EGDMA mixture was carried out by the same method. A similar solid sheet was obtained and the CD signal was preserved in its original pattern (as shown in Figure 8). To observe the aggregation structures of Pyr-lipid in the polymer sheet, the polymer sheet was frozen by liquid nitrogen and broken to make a cross section. Typical SEM images of the cross section of poly(MMA-EGDMA) with and without Pyr-lipid aggregates are shown in Figure 7. The fiber-like aggregates

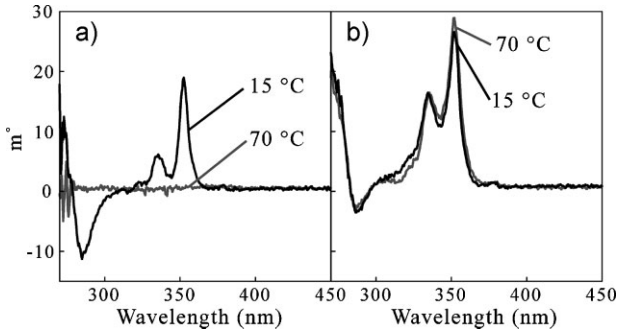

Figure 5.
CD spectra of Pyr-lipid in MMA monomer (a) before and (b) after photo-induced polymerization. [Pyr-lipid] = 5.0 mM.

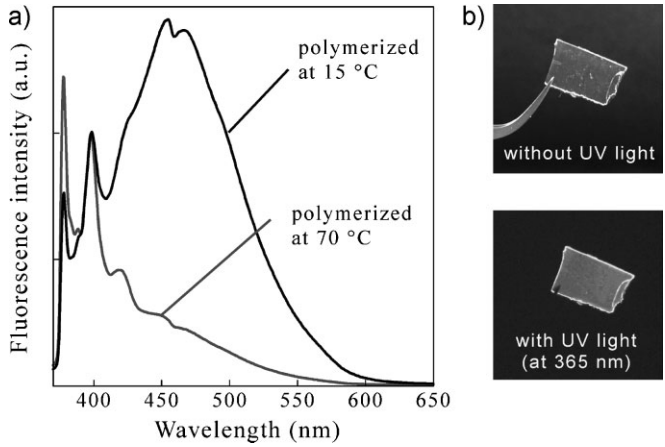

Figure 6.
(a) Fluorescence spectra of a polyMMA sheet prepared by photo-induced polymerization in the gel state (15 °C) and the solution state (70 °C) and (b) typical photographs of a polyMMA sheet containing Pyr-lipid prepared in gel state under the UV light irradiation and in its absence.

can be seen on the uneven cross section surface of the polymer sheet with Pyr-lipid, whereas a flat cross section surface appeared in the polymer sheet without Pyr-lipid. The polymer sheet was dipped in chloroform, which is a good solvent for Pyr-lipid, to extract the lipid aggregates from the polymer sheet. The uncross-linked polymer sheet (polyMMA) was dissolved completely in a few minutes, but the cross-linked polymer sheet (poly(MMA-EGDMA)) was not dissolved even after immersing more than 1 hour. The polymer sheet was dried *in vacuo* and evaluated by CD and UV-vis spectroscopic measurements. The absorption of the pyrenyl moiety decreased by about 90% and the CD strength almost disappeared. Thus, the weight of the polymer sheet after immersion is almost equal to that before immersion. These results indicate that the Pyr-lipids were mostly removed from the cross-linked polymer sheet by solvent extraction. Less than 10% of monomers remained in the polymer sheet, but their oriented-structures would be damaged by invaded solvent. Similar observations have been reported by Gu et al.[10] and Hafkamp et al.[11] According to optical and microscopic measurements, the fibrous pores were observed after solvent extraction of the lipid and their diameters were bigger than those of original fibrous aggregates before extraction. It was expected that the monomers near the surface of fibrous aggregates

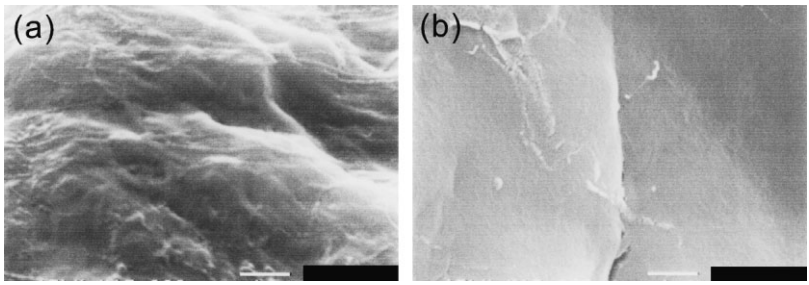

Figure 7.
Typical SEM images of the cross-section of poly(MMA-EGDMA) sheets (a) with and (b) without Pyr-lipid aggregates.

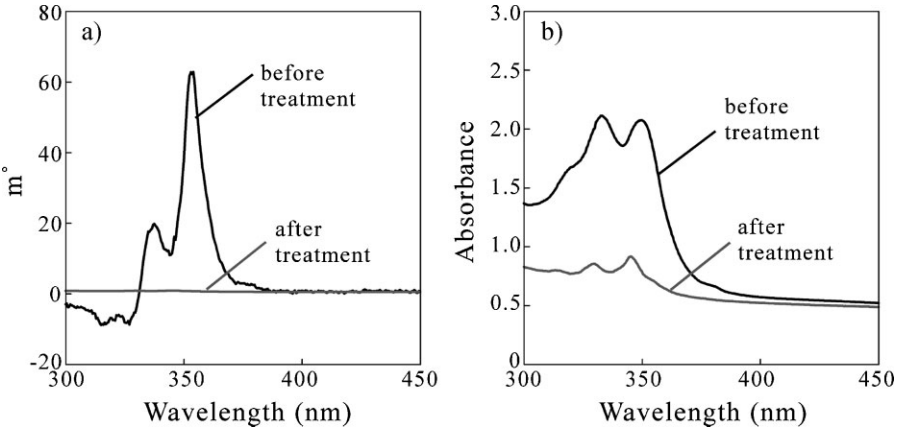

Figure 8.
CD and UV-vis spectra of cross-linked polymer (poly(MMA-EGDMA)) before and after immersion in chloroform for 1 h at room temperature.

could not react because their mobility was strongly restricted at the surface.

Conclusion

In this paper, we reported the aggregation morphologies and secondary chirality based on highly-oriented structures of Pyr-lipid in various polymerizable monomers and the enclosure of secondary chirality by the photo-induced polymerization of bulk monomer of organogels. The unique oriented-structures of Pyr-lipid aggregates in polymerizable monomers were maintained after polymerization and remained stable at a wide range of temperatures. The polymer sheet with specific chirality would give us potential applications for optical materials.

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