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Anisotropic Swelling Behavior Induced by Helix–Coil Transition in Liquid Crystalline Polypeptide Gels

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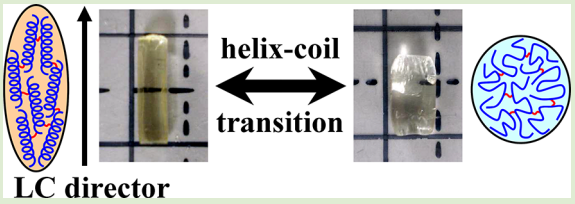
Supporting Information

ABSTRACT: Uniaxially oriented liquid crystalline poly(γ-benzyl L-glutamate) (PBLG) gel was prepared by cross-linking the lyotropic liquid crystalline PBLG solution in magnetic field, and PBLG was converted to poly[N^5-(2-hydroxyethyl) L-glutamine] (PHEG) by side-chain aminolysis reaction. The prepared PHEG gel retained the liquid crystallinity after the conversion from PBLG to PHEG when immersed in α-helix-forming solvent such as ethylene glycol. By the addition of water into the immersion solvent, conformational transition of PHEG from α-helix to random coil was occurred, and the optical anisotropy of the gel was disappeared. With this helix-to-coil transition of PHEG, the gel was swollen in the direction perpendicular to the orientational axis of PHEG, and was shrunk in the parallel direction. As the result, the gel in cylindrical shape in ethylene glycol, in which α-helical PHEG is oriented along the cylindrical axis, changed to more isotropic shape in water. This anisotropic shape change of the gel was described by the conformational transition of the cross-linked PHEG from the anisotropic rodlike α-helix to the isotropic random coil.



Polypeptides can change their overall molecular shape by a conformational change of repeating amino acid residues. Because of a regularly formed intramolecular hydrogen bond between oxygen atom at carbonyl group and hydrogen atom in amide group in the main chain, some polypeptides take stable α-helical conformation with rigid rodlike shape. If the formation of the intramolecular hydrogen bonds is disturbed by, for example, a change of solvent quality, the chain takes random coil state without regular structure. Poly[N^5-(2-hydroxyethyl) L-glutamine] (PHEG) is nonionic water-soluble polypeptide, and it takes α-helical or random coil conformation when dissolved in alcohol or water, respectively.[1−4] Therefore, PHEG chain can change its structure between rodlike α-helix and flexible random coil by changing solvent composition of water/alcohol mixed solvent.[5,6]

Because of the rigid rodlike structure of the α-helix, concentrated polypeptide solution is known to form lyotropic liquid crystalline solution in which the rodlike chains prefer to be aligned in parallel with each other in order to reduce an excluded volume of the rodlike molecule in the concentrated solution.[7,8] Liquid crystallinity of poly(γ-benzyl L-glutamate) (PBLG) in helix-forming solvent was widely investigated because of a good solubility and stability of α-helical PBLG.[9,10]

Preparation of liquid crystalline polypeptide gel and effect of the conformational change of the constituent polypeptide on swelling behavior of the gel was first reported by Kishi et al.[11−13] In their pioneering study,[12] liquid crystalline PBLG solution was uniaxially oriented by magnetic field and cross-linked, and the obtained PBLG gel was immersed in 1,4-dioxane (DOX) and dichloroacetic acid (DCA), in which PBLG takes α-helix and random coil, respectively. With the increase of DCA content in the mixed solvent, the gel was largely swollen in the perpendicular direction to the magnetic field, and swelling parallel to the magnetic field was negligible. In their subsequent report,[13] uniaxially oriented poly(L-glutamic acid) (PGA) hydrogels, which were prepared from the uniaxially oriented PBLG gel, were investigated. However, anisotropy of its swelling behavior was less significant than the uniaxial PBLG gel in the organic solvent.

In this study, liquid crystalline PHEG gel, in which PHEG chains dissolved in hydrophilic aqueous solvent were chemically cross-linked, was prepared. At first, a stably formed lyotropic liquid crystalline solution of PBLG was set in a magnetic field, and uniaxially oriented PBLG chains were cross-linked chemically by the cross-linker, pentaethylenehexamine (H_2N-$(CH_2CH_2NH)_5$-H, PEHA). Liquid crystalline PHEG gel was prepared by transformation of PBLG to PHEG by aminolysis reaction with 2-aminoethanol. As mentioned above, PHEG can change its conformation by changing the immersing solvent, that is, α-helix conformation having an anisotropic rodlike shape in alcohol, such as ethylene glycol (EG), and flexible random coil having isotropic dimension in water. Therefore, if the rodlike PHEG chains are preferably oriented uniaxially in the liquid crystalline state, the anisotropic conformational change of the constituent polymers is expected to influence an anisotropic shape change of the gel in macroscopic scale. This anisotropic feature is in contrast to a conventional stimuli-sensitive polymer gel in the disordered isotropic state and can be applicable as shape-memory or shape-changing hydrogels[14]

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based on polypeptide materials.[15,16] In this letter, uniaxially oriented PHEG gel was prepared and immersed in water/EG mixed solvent with various compositions. Relationships between the conformational change of the constituent PHEG, liquid crystallinity, and anisotropic swelling behavior of the PHEG gel were investigated.

Lyotropic liquid crystalline PBLG solution in *N*,*N*-dimethylacetamide (DMAc), with a polymer concentration of 25 wt %, was cross-linked by PEHA, and liquid crystalline PBLG gel (LC-PBLG gel) was prepared. Degree of swelling of the LC-PBLG gel in DMAc, which was defined as weight ratio of swollen gel to dried gel ($Q = W_{gel}/W_{dry}$), was $Q = 3.9$. After the conversion of LC-PBLG gel to PHEG gel and immersion in EG, the Q value increased to 6.0. This result should correspond to conformational flexibility of PHEG in EG, even though the fraction of α-helical conformation in EG was almost 100%, as mentioned in the previous report.[6] This PHEG gel revealed a bright image when observed under crossed polarizers, as shown in Figure 1a, suggesting it to be in the lyotropic liquid

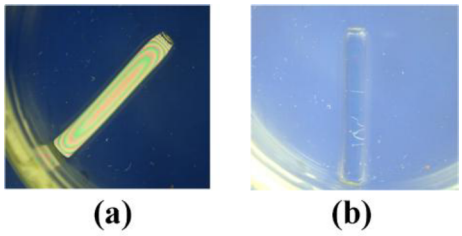

Figure 1. Photo images of PHEG gel immersed in DMAc. The sample was placed so that the cylindrical axis was (a) 45° and (b) parallel to the plane of polarized light. In (b), to show the image of the gel clearly, brightness of the image was more enhanced than (a).

crystalline state. The photo image became dark when the sample was rotated 45° (Figure 1b), so the α-helical PHEG chains were oriented uniaxially along the cylindrical axis of the PHEG gel. This result means that the uniaxial liquid crystalline order, which was formed in PBLG liquid crystalline solution in the magnetic field, was retained even after the cross-linking reaction and the side-chain exchanging reaction from PBLG to PHEG.

Optical anisotropy of the uniaxial PHEG gels immersed in deuterium oxide (D_2O)/EG mixed solvent with various EG weight fraction (W_{EG}) was observed by polarized optical microscopy (POM) and shown in Figure 2. With the decrease in W_{EG}, the optically anisotropic bright image became more dark, and the dark images of the solutions at $W_{EG} = 0.2$ and 0 suggests that the liquid crystalline order disappeared. After the gel was immersed in pure D_2O, W_{EG} of the solvent was increased from 0 to 1.0 stepwise. The result in Figure 2 suggests that the brightness of the image at the same W_{EG} in the

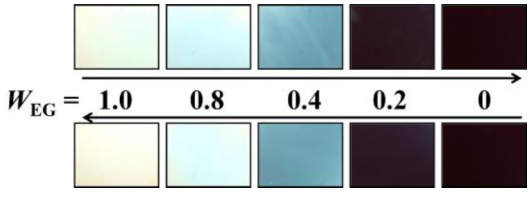

Figure 2. Variation of the POM image of the PHEG gel with the change of W_{EG} in EG → D_2O process (upper) and D_2O → EG process (lower).

increasing and decreasing process was almost the same, that is, the optical anisotropy of the PHEG gel was reversibly recovered.

α-Helix content (f^H) of the PHEG homopolymer in D_2O/EG mixed solvent, evaluated from molar ellipticity at 222 nm in circular dichroism (CD) spectrum, was increased with W_{EG}, as shown in Figure 3a. In Fourier transform infrared (FT-IR)

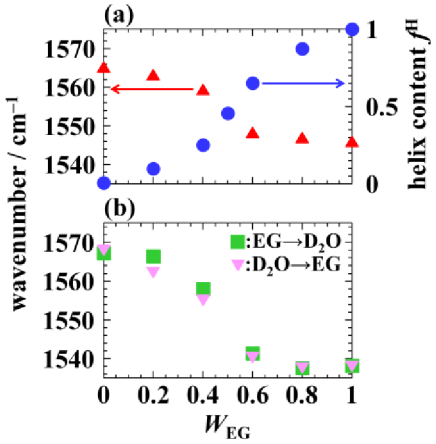

Figure 3. (a) Plots of the helix content f^H (right axis) and peak position of FT-IR amide II band (left axis) of PHEG homopolymer against W_{EG}. (b) Plots of the peak position of the amide II band for PHEG gel against W_{EG} in the EG → D_2O process (green square) and D_2O → EG process (pink inversed triangle).

spectrum for PHEG homopolymer/D_2O/EG solutions, the amide II band for PHEG was located at 1565 cm^{-1} in pure D_2O, shifted to a lower wavenumber region with the increase in W_{EG}, and was observed at 1546 cm^{-1} when $W_{EG} = 1.0$, as shown in Figure 3a. It was reported that the difference in the IR peak positions of the amide II band for random coil and α-helix conformations were small.[17] In Figure 3a, however, a clear peak shift with W_{EG} change can be recognized. In PHEG, there exists two kinds of amide groups, that is, one is in the main chain and the other is in the side chain. It may be possible that the conformational dependency of the peak position of amide II band is not same with ordinary polypeptides. From Figure 3a, we concluded that the peak shift of the IR amide II band was caused by the conformation change of PHEG with the solvent composition.

FT-IR spectra for PHEG gels immersed in D_2O/EG were also measured, and the peak position for the amide II band is plotted against W_{EG} for both processes of EG → D_2O and D_2O → EG, as shown in Figure 3b. The peak shift with W_{EG} was also observed in the PHEG gel, in the same manner with PHEG homopolymer in Figure 3a. Therefore, it can be concluded that the helix−coil transition of PHEG with the change of W_{EG} also occurred reversibly, even in the cross-linked PHEG gel.

The size of the uniaxial PHEG gel immersed in water/EG mixed solvent with various W_{EG} was measured. The axial length and diameter of the PHEG gel in cylinder shape were respectively defined as $d_{\parallel}$ and $d_{\perp}$, and the size of the dried gel was indicated by suffix 0, that is, $d_{\parallel,0}$ and $d_{\perp,0}$. In Figure 4, the values of $d_{\parallel}/d_{\parallel,0}$ and $d_{\perp}/d_{\perp,0}$ are plotted against W_{EG}. In EG, the length of the cylinder axis was almost identical with the dry gel, that is, $d_{\parallel}/d_{\parallel,0} = 1.06$, and the gel was more swollen in the diameter direction as found that $d_{\perp}/d_{\perp,0} = 2.15$. This result means that the absorbed solvent makes the interchain distance

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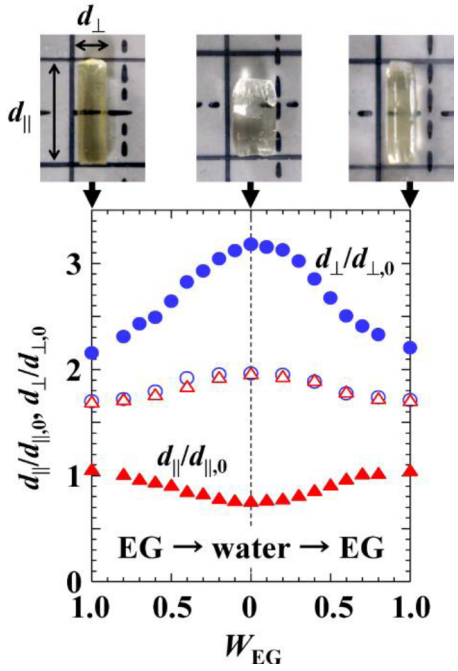

Figure 4. Relative size of uniaxial (filled symbols) and polydomain (open symbols) PHEG gel against the dried state for diameter ($d_\perp/d_{\perp,0}$, circle) and cylinder length ($d_\parallel/d_{\parallel,0}$, triangle) plotted against W_{EG}. Results for the processes of EG → water (left side of the graph) and water → EG (right side) are indicated. Photo images for the uniaxial PHEG gel at the corresponding W_{EG} are indicated at the top of the figure.

in the direction perpendicular to the orientational axis larger, and the molecular position along the orientational direction was almost identical in the dried and swollen sample.

With the decrease of W_{EG} from 1.0 to 0.0, the length of the cylinder was shrunk gradually, and $d_\parallel/d_{\parallel,0}$ decreased to 0.76 when immersed in water. On the other hand, the diameter of the cylinder was swollen and $d_\perp/d_{\perp,0}$ = 3.17 at W_{EG} = 0, that is, the diameter of the uniaxial PHEG gel in water was 1.5 times larger than that in EG. This result means that the uniaxial PHEG gel changed its shape to a more isotopic one with the conformational change of PHEG from anisotropic α-helix to isotropic random coil. This anisotropic swelling and shrinking behavior is clearly observed visually in the photo images in Figure 4.

For comparison, a polydomain PHEG gel was prepared by cross-linking of PBLG liquid crystalline solution without using uniaxial magnetic field. Because the liquid crystalline director of each domain is oriented randomly in the polydomain PHEG gel, the gel changed its size in an isotropic manner, as shown in Figure 4. Both $d_\parallel/d_{\parallel,0}$ and $d_\perp/d_{\perp,0}$ are 1.70 in EG and 1.96 in water. Therefore, in comparison with the dried state, the volume of the polydomain PHEG gel was swollen to be 1.70^3 = 4.91× in EG and 1.96^3 = 7.53× in water. These values were almost identical with the uniaxial PHEG gel, that is, 1.06 × 2.15 × 2.15 = 4.90 in EG and 0.76 × 3.17 × 3.17 = 7.64 in water. These results indicate that the overall swelling ratio is independent of the macroscopic orientation of the liquid crystalline director.

In Figure 5, plausible schematic molecular structure of uniaxially oriented PHEG gel is illustrated. PBLG molecules were cross-linked with each other when they were oriented uniaxially, and the orientational direction of the α-helical

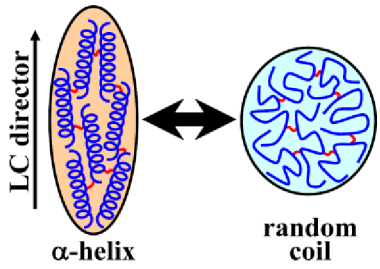

Figure 5. Schematic illustration of the helix−coil transition, anisotropic−isotropic transition, and shape change of the uniaxial PHEG gel.

polypeptide was retained even after the transformation to PHEG and immersed in EG. With the decrease in f^H, PHEG became more flexible and the anisotropy of the molecular shape was lowered, that is, the dimension of PHEG along the α-helix axis was decreased, at the same time, the dimension perpendicular to the α-helix axis was increased. This change of PHEG molecular shape from rodlike α-helix to isotropic random coil should be the reason of the anisotropic shape change of the uniaxial PHEG gel. Additionally, the increase in chain flexibility of PHEG would decrease the orientation order of PHEG in the liquid crystalline state, which also changed the macroscopic shape to be more isotropic. After the complete conformational change to the random coil, the liquid crystalline order could not be recognized as shown in the very weak birefringence in the POM image at W_{EG} = 0 in Figure 2.

In the increasing process of W_{EG} in Figure 4, the shape of the gel recovered to the original state reversibly. These results suggest that, even after the conformational change from α-helix to random coil, the cross-linking in the PHEG gel retained not only the position of the PHEG chain, but also the orientational characteristics of the rodlike PHEG. The original network structure could be recovered completely even after the destruction and reconstruction of the α-helix conformation of PHEG.

The volume of the PHEG gel in random coil conformation was about 1.5× larger than that in α-helix. Because water is a good solvent, the random coil PHEG could take more stretched conformation when immersed in water. On the other hand, conformational freedom of the α-helical PHEG in EG is very low, and PHEG cannot change its dimension. This should be the reason that the gel in water was more swollen than in EG. A much larger difference in the volume of the gel in random coil and the α-helix conformation was reported in PBLG gel in DOX/DCA[11,12] and in PGA hydrogel at various pH.[13] In these samples, good solubility of the randomly coiled polypeptide could cause very large swelling. Therefore, even though the conformational change from α-helix to random coil occurred, the decrease in the dimension along the α-helix axis was canceled out by the overall swelling. As a result, although the anisotropic swelling was observed, the macroscopic shape change with simultaneous swelling and shrinking was not reported. In our polypeptide gels with nonionic PHEG, the difference in the volume in water and in EG was relatively small, which might be the reason that the macroscopic shape change of the cylindrical gel with reflecting the molecular shape change in helix−coil transition was remarkably observed. In that sense, the present observation of the simultaneous swelling and shrinking in macroscopic scale, accompanied with the anisotropic conformational change of the polypeptide, is a

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novel phenomenon and has not been reported to our knowledge. These results could be achieved by appropriate molecular designs such as conformation transition condition, solubility, and cross-linking density of the constituent polymers.

Recently, liquid crystalline elastomers have been paid wide attention because of their unique hybrid characteristics of liquid crystals and elastomer solids.[18−20] In the uniaxial liquid crystalline gel in this study, some hierarchical structure changes are also coupled with each other, such as helix−coil transition, change of polymer rigidity, formation of liquid crystalline phase, deformation of the polymer network, and the macroscopic shape change of the gel. Detailed analyses of the relationships of the swelling and shrinking behavior in Figure 4 with the optical anisotropy (Figure 2) and the conformational change (Figure 3) are in progress.

In conclusion, uniaxially oriented PHEG hydrogels were prepared, and their anisotropic swelling and shrinking behavior in water/EG mixed solvent was investigated. The original cylindrical shape in liquid crystalline state in EG changed to the more isotropic shape in water as a result of the helix to coil transition of PHEG, that is, was swollen in the direction perpendicular to the orientational axis and was shrunk in the parallel direction. These observations should be responsible for the change of the molecular shape of the cross-linked PHEG from the anisotropic rodlike α-helix to the isotropic random coil. Because the gel was cross-linked when the original solution was in liquid crystalline state, not only the position but also the orientational order of the rodlike polypeptide chains was fixed. As the results, the anisotropic swelling and shrinking behavior was observed reversely with the change of W_{EG}. This unique behavior observed in polypeptide gels in water/EG systems has a potential applicability such as novel biomaterials because of the biocompatibilities of the used components.

EXPERIMENTAL METHODS

PBLG was dissolved in mixture of DMAc/PEHA/2-hydroxypyridine (2-HP), and lyotropic liquid crystalline solution with PBLG concentration of 25 wt % was prepared. The amount of the cross-linker (PEHA) against the PBLG benzyl group was 10 mol %. The mixture was stirred sufficiently, placed in a Teflon tube with a 2.0 mm inner diameter, and set in a NMR apparatus (Bruker Avance600) at room temperature for 4 days. The α-helical PBLG chains were aligned along the magnetic field direction, which is parallel to the cylindrical axis of the Teflon tube. Thus obtained LC-PBLG gel was converted to PHEG gel by immersing in DMAc solution of 2-aminoethanol and 2-HP at 37 °C for 48 h. Finally, the obtained PHEG gel was immersed in excess DMAc for three days to remove impurities. Details are described in Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Detailed explanation of the experimental procedure for the PBLG and PHEG gels and their swelling behavior. 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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