Macromolecules **2006,** *39,* 1313−1315

Spontaneous Organization of Helical Polypeptide Molecules into Polar Packing Structure

Chu-Chun Yen, Masatoshi Tokita, Byoungchoo Park,[†] Hideo Takezoe, and Junji Watanabe*

Department of Organic and Polymeric Materials,
Tokyo Institute of Technology, Ookayama,
Meguro-ku, Tokyo 152-8552, Japan

Received November 13, 2005
Revised Manuscript Received January 12, 2006

Rigidity and chirality are the topical characteristics of α-helical polypeptides.[1] These two characteristics are regarded as the cause of the natural tendency for polypeptide molecules to form a chiral nematic (or cholesteric) liquid crystal in concentrated solutions.[2,3] The transition behavior from isotropic to liquid crystal solution and the structural feature of the resulting superhelicoidal cholesterics have been significant research subjects for clarifying the self-organization of biological polymers in a living system.[4,5] In this Communication, we describe another important characteristic of biological polypeptide helices, that is, the polarity. Through the observation of the second harmonic generation (SHG) effect, we show here a polar packing structure can spontaneously form from the typical helical polypeptide, poly(γ-benzyl L-glutamate) (PBLG). This result will produce a new interesting insight into the dipole−dipole interaction of polar rodlike polymers, simultaneously indicating the potentiality of the self-organization of the biological helical proteins into the polar packing structure.

Since the helical conformation of polypeptides is built up by regular intramolecular hydrogen bonds between the N−H and C═O groups, there is a direct addition of residual dipole components along the polymer chain producing a huge dipole moment, μ (for example, μ = ca. 500 D for an α-helical PBLG with a molecular weight, M_w, of 30 000).[6] This huge dipole moment is attractive because of the possibility of forming a polar liquid crystal. However, up to now few researches have been done concerning studies on subjects related to the polar packing of the polar polypeptides.[7−9] The reason may be simple because researchers in this field consider that the helical polypeptides are packed with less regularity with respect to the "up" and "down" directionality. Until recent theoretical investigations[10−12] and computer simulations,[13] they suggested that rigid-rod polymers with a huge dipole moment along their long axis like PBLG can form a polar packing structure in their liquid crystals.

With respect to the polarity, we recall the interesting fact that there are two crystal modifications, forms B and C, in PBLG films.[14] These forms are prepared by casting from different kinds of solvents. From the polar solvents such as *N*,*N*-dimethylformamide (DMF: dielectric constant $\epsilon = 36.7$), form B is obtained, while form C is formed from solvents like chloroform ($\epsilon = 4.7$) and ethylene dichloride (EDC: $\epsilon = 10.7$). Form B has a well-defined crystalline structure with a three-dimensional monoclinic unit cell,[14,15] while form C shows a poor X-ray pattern with continuous scattering on the layer lines and diffuse reflections on the equatorial line. Poor periodicity is thus likely to arise for this form C, with its chains packed laterally into a rather irregular two-dimensional hexagonal lattice and displaced randomly along their chain axes.[15] Of interest is the fact that the packing feature of these two forms is not altered at all by annealing at high temperatures above 150 °C, where the side chains are freely rotated. This means that the difference in the packing structure cannot be attributed to a difference in side-chain conformation that might have been induced by some specific interaction between the side chains and solvents. Thus, we consider another structural factor, the "up" and "down" arrangement of polar molecules; it may be regular in form B but irregular in form C.[15] Unfortunately, X-ray analyses have hardly allowed the clarification of this difference because there are so many structural factors included for examination. SHG measurement will likely be the simplest method to clarify whether the polar packing exists or not.[16]

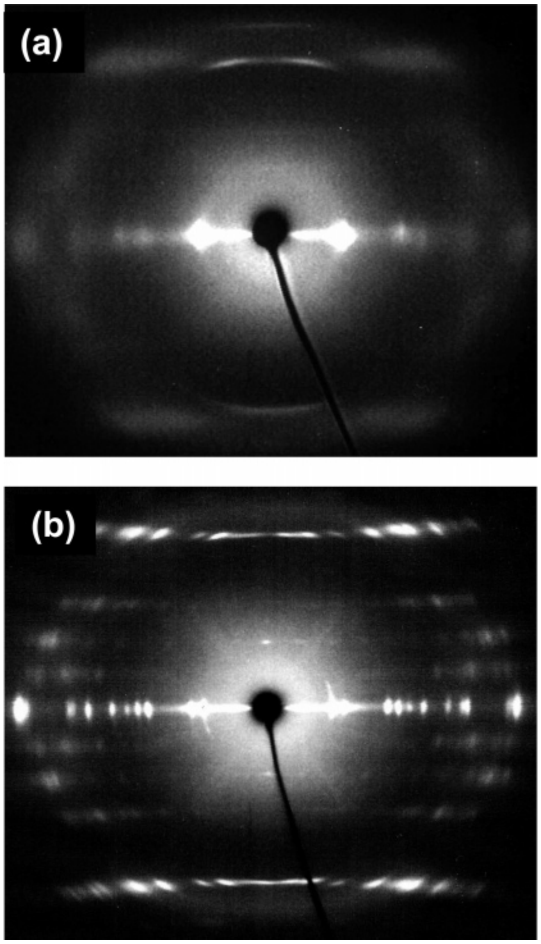

Figure 1. X-ray diffraction photographs of oriented PBLG (M_w = 27 000) films prepared from lyotropic solutions in EDC (15 wt %). The highly oriented samples were obtained under (a) magnetic (5 T) and (b) electric (0.6 V/μm) fields. The molecular long axis corresponding to the field direction is set along the vertical direction.

We first prepared the oriented films of PBLG (M_w = 27 000) for the analysis of SHG. High orientation was achieved by applying an external electric (0.6 V/μm) or magnetic field (5 T) to lyotropic solutions (initial concentration: 15 wt %) in EDC (form C solvent). The X-ray patterns (Ni-filtered Cu Kα radiation by a Rigaku-Denki X-ray RU200BH generator) are shown for the magnetically and electrically oriented films in

---

* To whom correspondence should be addressed: e-mail jwatanab@polymer.titech.ac.jp.

[†] Present address: Department of Electrophysics, Kwangwoon University, Seoul 137-701, Korea.

10.1021/ma052424p CCC: $33.50

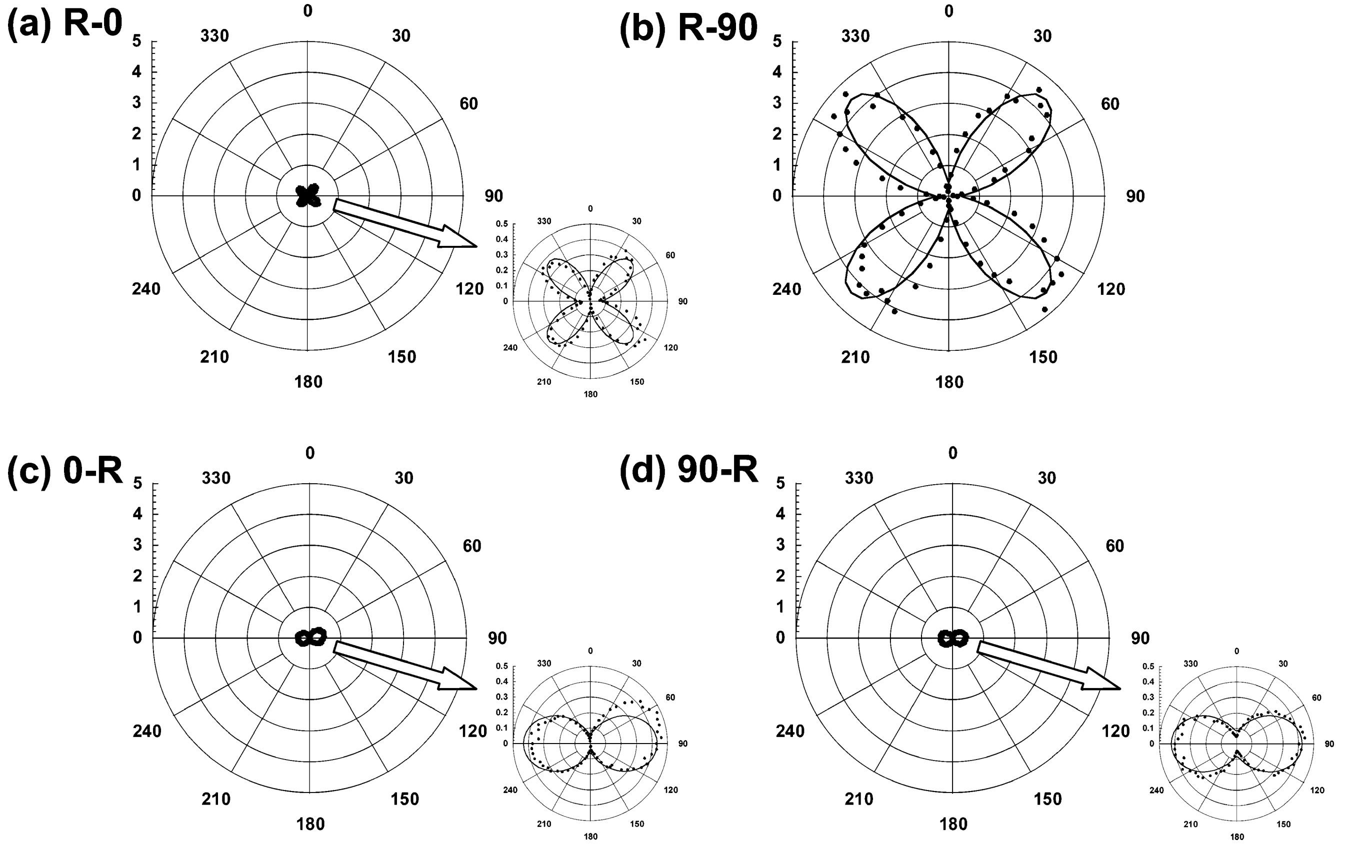

Figure 2. Intensity profiles of SHG light observed for the electrically aligned PBLG film from EDC solution, with various angular combinations of φ_p and φ_a. Here, the Nd:YAG laser (1064 nm) was irradiated perpendicular to the film surface, and then the SH light (532 nm) generated was detected by a Hamamatsu model-R955 photomultiplier tube in the transmitted direction after passing through an IR cut filter, an interference filter and an analyzer, as a function of polarizer rotation angle (φ_p) for the fundamental beam and the analyzer rotation angle (φ_a) for the SHG light. The clockwise rotation is defined as the view along the beam, and the molecular polar axis is set to be 0°. Intensities are polar-plotted with four configurations ((a) R-0, (b) R-90, (c) 0-R, and (d) 90-R). Combinations of R-0 and R-90 indicate that the polarization of fundamental light is rotated about the beam direction (i.e., R = φ_p varied from 0° to 360°), while φ_a is fixed at 0° and 90°, respectively. The combinations of 0-R and 90-R indicate that the analyzer direction (R = φ_a) is rotated with φ_p fixed at 0° and 90°, respectively. The solid curves show the validity of our theoretical calculations based on C_s symmetry, where the angle between the experimental and the sample coordinates has been averaged. The reduced second-order nonlinear optical susceptibility (χ) parameters are as follows: $\chi_{11} + \chi_{12} = 1.7$, $\chi_{13} = 3.7$, $\chi_{26} = 2.8$, $\chi_{15} + \chi_{24} = 16.0$, $\chi_{31} + \chi_{32} = 2.3$, $\chi_{33} = 1.0$, and $\chi_{35} = 3.1$.

parts a and b of Figure 1, respectively. The magnetically aligned film (Figure 1a) shows the hexagonal pattern characteristic of form C with broad reflections on the equatorial line and diffuse streaks on the layer lines. The equatorial reflections with spacings of 12.8, 7.28, and 6.39 Å indicate the molecular packing with the pseudohexagonal lattice of $a = b = 14.8$ Å. The turn layer line near 5.3 Å and the 1.5 Å meridional reflection accounted for the α-helical conformation with 18 residues in five turns. An SHG signal is not detected at all from this oriented form C film (nor from the cast film), which ensures that the α-helices in the form C film are placed randomly with respect to their "up" and "down" directionality.

In contrast to the magnetically aligned film, the electrically aligned one shows the well-defined crystalline X-ray pattern in Figure 1b irrespective of the use of the same form C solvent. It includes many sharp reflections, which can be indexed into a three-dimensional monoclinic lattice containing two chains with lattice parameters of $a = 29.0$ Å, $b = 13.4$ Å, $c = 27.0$ Å, $\alpha = \gamma = 90°$, and $\beta = 96°$. This result is remarkable since the crystal structure agrees exactly with that of form B, which can be formed from the DMF solvent.[15] Figure 2 shows the polar SHG intensity profiles obtained with the various angular combinations of the polarizer and analyzer. The relatively strong SHG signals indicate that the electrical poling by the electric field of 0.6 V/μm is successful in inducing the noncentrosymmetry of molecular packing,[17] that is, polar packing with the same "up" or "down" directionality. As given by the solid curves in Figure 2, the intensity profiles are well fitted by the C_s symmetry attributable to the polar monoclinic lattice. We thus reach the important conclusion that the polar packing of molecules forced by electric poling results in form B crystal even when the form C solvent is used.

The magnetically oriented film was prepared from the DMF form B solvent at 50 °C. The orientation was not completed in this case because the twisting power of cholesteric liquid crystal in the DMF solvent is much stronger than that of the form C solvent.[18] Irrespective of this ambiguity, the SHG signal is clearly detected for this form B film with a similar intensity as that in the electrically poled film prepared from form C solvent (Figure S1). The simple DMF-cast film also shows the clear SHG signal while the EDC-cast one does not. These results lead to another interesting conclusion: in form B from DMF solution, polypeptide molecules have been self-organized into the polar packing without electric poling.

Note that if the PBLG with the relatively lower $M_w = 27\,000$ is used, the form B type of packing in the DMF-cast film is gradually altered to the form C type upon increasing the casting temperature from 45 to 100 °C (Figure S2). According to the above results, this change in the packing structure suggests the transformation of the polar structure to a nonpolar structure. Figure 3 shows the dependence of SHG intensity on casting temperature. The SHG intensity is highest by casting at 45 °C,

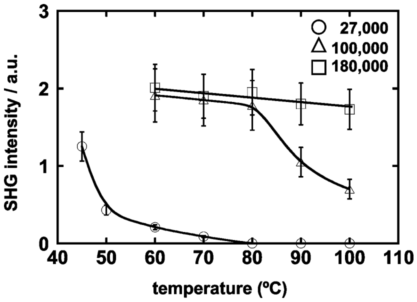

Figure 3. Dependence of SHG intensity on the casting temperature in the PBLG films cast from the liquid crystal DMF solutions. Three kinds of PBLG with different molecular weights of 27 000, 100 000, and 180 000 were examined, and the casting temperatures were varied from 45 to 100 °C. In these cast films (a thickness of ca. 100 μm), the PBLG molecules assume the uniplanar orientation, with their helical axes lying parallel to the film plane and being randomly oriented as an average in this plane. The laser light was irradiated perpendicular to the film surface and the SH light intensity without the analyzer was simply obtained as an average value from 10 different positions of the film.

decreases steadily with the increase in casting temperature, and then falls down to 0 at temperatures higher than 80 °C. Again, we recognize the close relationship between the crystal form and SHG activity response. The loss of the SHG activity at the elevated casting temperatures indicates the polar to nonpolar transformation at some temperature in the liquid crystal phase of DMF solution.

The theory based on the dipole–dipole interactions and the hard-core repulsions predicts the occurrence of the transformation in nematic polymers with directional polar segments similar to that in the present polypeptide.[10,12] The polarization instability takes place at some temperature T_c that is proportional to $\rho\mu^2$, where ρ is the number density of the molecule. Hence, the increase in M_w in the present system may increase the instability temperature. From this expectation, we performed the same experiment for two other PBLG samples with higher M_w = 100 000 and 180 000. As can be seen in Figure 3, the SHG intensity is sustained for PBLG with highest M_w = 180 000 over the temperature region from 60 to 100 °C, while it decreases significantly at around 80 °C for PBLG with M_w = 100 000. When the casting temperature dependence of SHG is compared between three polymers with different M_w, we find the qualitative but clear tendency that the instability temperature increases with the increase of μ, as predicted theoretically.

In conclusion, polar helical PBLG molecules can spontaneously form a polar packing structure in their lyotropic solution. Form B solvents such as DMF are responsible for the polar ordering (Figure S3), suggesting that the polar alignment may occur in solvents with a high dielectric constant.[19] The occurrence of polar structure and its temperature instability is consistent with the theoretical prediction. This finding of the polar packing structure in the liquid-crystal phase of rigid-rod polypeptides is important since a similar polarity packing may possibly arise in the self-organized structure of biological helical proteins and its electrical properties such as ferroelectricity and piezoelectricity may serve in living systems.

Acknowledgment. The research is financially supported by a Graint-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, Sports, and Technology.

Supporting Information Available: Figures S1–S3 showing more detailed experimental results. This material is available free of charge via the Internet at http:// pubs.acs.org.

References and Notes

(1) Pauling L.; Corey, R. B. *J. Am. Chem. Soc.* **1950**, *72*, 5349.
(2) Flory, P. J. *Proc. R. Soc. London* **1956**, *A234*, 60.
(3) Robinson, C. *Trans. Faraday Soc.* **1956**, *52*, 571.
(4) Kelker, H.; Hatz, R. *Handbook of Liquid Crystals*; Verlag Chemie GmbH: Weinheim, 1980; pp 553–592.
(5) Bouligand, Y. *Liquid Crystalline Order in Polymers*; Blumstein, A., Ed.; Academic Press: New York, 1978; pp 262–298.
(6) Wada, A. *Adv. Biophys.* **1976**, *9*, 1.
(7) Park, B.; Kinoshita, Y.; Takezoe, H.; Watanabe, J. *Jpn. J. Appl. Phys.* **1998**, *37*, L136.
(8) Watanabe, J.; Hirose, Y.; Tokita, M.; Watanabe, T.; Miyata, S. *Macromolecules* **1998**, *31*, 5937.
(9) Watanabe, T.; Miyata, S.; Furukawa, T.; Takezoe, H.; Nishi, T.; Sone, M.; Migita, A.; Watanabe, J. *Jpn. J. Appl. Phys.* **1996**, *35*, l505.
(10) Terentjev, E. M.; Osipov, M. A.; Sluckin, T. J. *J. Phys. A* **1994**, *27*, 7047.
(11) Groh, B.; Dietrich, S. *Phys. Rev. E* **1997**, *55*, 2892.
(12) Yu, C.-J.; Yu, M.; Lee, S.-D. *Jpn. J. Appl. Phys.* **2002**, *41*, L102.
(13) Biscarini, F.; Zannoni, C.; Chiccoli, C.; Pasini, P. *Mol. Phys.* **1991**, *73*, 439.
(14) (a) McKinnon, A. J.; Tobolsky, A. V. *J. Phys. Chem.* **1966**, *70*, 1453. (b) Samulski, E. T.; Tobolsky, A. V. *Nature (London)* **1967**, *216*, 997. (c) Mckinnon, A. J.; Tobolsky, A. V. *J. Phys. Chem.* **1968**, *72*, 1157.
(15) Watanabe, J.; Imai, Z,; Gehani, R.; Uematsu, I. *J. Polym. Sci., Polym. Phys. Ed.* **1981**, *19*, 653.
(16) Prasad, P. N.; Williams, D. J. *Introduction to Nonlinear Optical Effects in Molecules & Polymers*; John Wiley & Sons: New York, 1991.
(17) Levine, B. F.; Bethea, C. G. *J. Chem. Phys.* **1976**, *65*, 1989.
(18) Uematsu, I.; Uematsu, Y. *Adv. Polym. Sci.* **1984**, *59*, 37.
(19) This solvent effect should be the opposite, since all electrostatics such as dipole–dipole interactions decrease in a strongly polarizable media, and then the polar ordering is not promoted. In this case, however, the solvents with high dielectric constant seem to interact with the side chains of PBLG. As a result some short-range interactions may occur, leading to the complex situation on formation of polar packing structure of PBLG molecules.

MA052424P