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Polymer 40 (1999) 6769–6775

polymer

Conformational transition of nanoparticles composed of poly(γ-benzyl L-glutamate) as the core and poly(ethylene oxide) as the shell

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Received 9 June 1998; received in revised form 7 December 1998; accepted 18 December 1998

Abstract

Diblock copolymers composed of poly(γ-benzyl L-glutamate) (PBLG) as the hydrophobic component and poly(ethylene oxide) (PEO) as the hydrophilic component were obtained by the polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride, initiated by the primary amino end group of the α-methoxy-ω-amino PEO. Nanoparticles are formed from an organic solution of the block copolymers by the diafiltration method. From the NMR measurement of the nanoparticle in water, the PEO segments in the copolymer extend out as the shell from the nanoparticle core into the aqueous environment. From circular dichroism measurements in ethylene dichloride solution, it was found that the polypeptide block exists in the α-helical conformation and adopts right-handed helix sense, as in PBLG homopolymer. However, the helix sense of PBLG in the copolymer nanoparticles prepared from organic solvent depends on the PEO content in the copolymer and the nature of solvent.

Keywords: Nanoparticle; Conformational transition; Diafiltration method

1. Introduction

Nanoparticles are colloidal particles of a size below 1 μm. Nanotechnology are currently developing as advanced conceptual approaches in many areas of modern science and engineering [1]. In particular, they are widely employed in the various fields of life sciences such as separation technologies, histological studies, clinical diagnostic assays, and drug delivery systems. Of particular interest is the application of nanoparticles in pharmaceutical technology because they have some advantages such as easy purification and sterilization, drug targeting possibilities, and sustained release action [2]. Recently, the diafiltration method was developed for the preparation of nanoparticles based on the polymeric micelles because of the following advantages: simple, small particle size, high yield, no-aggregation, microreservoir in the hydrophobic core and high structural stability [3,4]. The nanoparticles based polymeric micelles are stable in aqueous medium and can solubilize hydrophobic drugs in their inner core [5]. Kwon et al. also reported that poly(β-benzyl L-aspartate)(PBLA)/PEO micelles obtained by the dialysis against water of an organic solvent of the block copolymers, are composed of an outer shell of PEO and an inner core of PBLA [6].

Nishikawa et al. reported that self-assembled nanoparticle formed by the hydrophobized pullulan binds various proteins and the thermal stability of the proteins drastically increases upon complexation [7]. However, few studies were carried out to characterize the conformation of the inner core in organic solvents [8]. In particular, the effect of the PEO block on the conformation of the inner core in the nanoparticles obtained by dialysis was not reported elsewhere.

In this study, we wish to report conformational transitions of nanoparticles composed of PBLG as the core and poly(ethylene oxide) as the shell. We are particularly interested in the effects of the soft segment-induced conformational transition in the block copolymer on the chiroptical properties of the nanoparticles in water. Chiroptical properties are mainly dependent on the effect of external factors such as solvent, temperature, added salt, and ionization [9]. It was

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0032-3861/99/$ - see front matter
PII: S0032-3861(99)00007-5

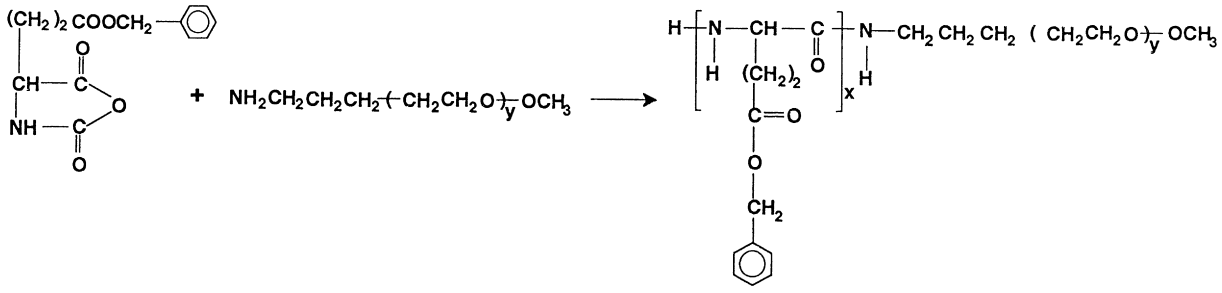

Fig. 1. Synthesis scheme of PBLG/PEO diblock copolymer.

reported that PBLG formed an α-helix in the right-handed (RH) screw sense whereas poly(L-aspartic acid) (PLA) esters formed an α-helix in either the RH or left-handed (LH) one depending on the solvent, temperature or chemical structure of the ester group [10–13]. The knowledge on the conformation of the inner core in the block copolymer nanoparticles appears to be of importance as some properties such as stability and drug loading content of the carriers might depend on the conformation of the inner core of the nanoparticles.

2. Experimental part

2.1. Materials

Amine-terminated PEO: The amine-terminated α-methoxy-ω-amino PEO (M_W = 12 000) was supplied from Nippon Oil and Fats Co., Ltd., Japan. γ-benzyl L-glutamate *N*-carboxyanhydride (γ-BLG-NCA) was synthesized from γ-benzyl L-glutamate using triphosgene [14]. The PBLG (M_W = 60 000) was purchased from Sigma and used without further purification. Hexane, tetrahydrofuran (THF), dimethylformamide (DMF), dimethylsulfoxide (DMSO), dimethylacetamide (DMAc) and 1,4-dioxane were purchased from Aldrich and dried with molecular sieve.

2.2. Synthesis of PBLG/PEO (abbreviated as GE) diblock copolymer

GE copolymer was synthesized according to a similar method previously reported [14–16]. Briefly, GE block copolymers were obtained by the ring-opening polymerization of γ-BLG-NCA initiated by the α-methoxy-ω-amino PEO as a polymeric initiator in CH_2Cl_2 at room temperature for 72 h. After the characteristic absorption of BLG-NCA (1785, 1860 cm^{-1}) had disappeared in the IR spectrum (72 h), the reaction mixture was poured into a large excess of diethyl ether. The precipitated copolymer was dried in vacuum. The reaction scheme is shown in Fig. 1.

2.3. Preparation of nanoparticles

The nanoparticles were prepared by the diafiltration method according to a similar method reported previously in Ref. [14]. Briefly, the block copolymer (20 mg) solution dissolved in a mixture of 10 ml of THF/DMF (7/3 : V/V) was dialyzed against distilled water using cellulose membrane tubing (12 000 molecular weight cutoff) to remove the organic solvent at room temperature.

2.4. ^1H NMR measurement

^1H NMR spectra of the copolymers were measured in solvent $CDCl_3$ to estimate the copolymer compositions and the molecular weight of PBLG blocks, using a JEOL FX 90Q NMR spectrometer. As the number-average molecular weight (M_n = 12 000) of PEO is known, one can estimate the number-average molecular weights of the PBLG and of the block copolymer from the block copolymer composition calculated from the peak intensities in the spectrum assigned to both polymers.

2.5. Dynamic light scattering measurements

Dynamic light scattering (DLS) was measured using a S 4700 (Malvern Instrument, England) with an argon laser beam at a wavelength of 488 nm at 25°C and the value is expressed in number-averaged scales as unimode. A scattering angle of 90° was used. The average particle size based

Table 1
Particle size distribution of nanoparticles[a] against PBLG chain length

Sample	PBLG content (mol%)	Particle size (nm)	Polydispersity
PBLG	100	405.4 ± 59.7	0.44
GE-1	60.5	541.0 ± 111.3	0.64
GE-2	40.0	242.8 ± 47.4	0.18
GE-3	12.4	182.7 ± 25.2	0.24

[a] The nanoparticles were prepared by the mixture of THF/DMF(7/3 : V/V).

Table 2
Characterization of PBLG and PBLG/PEO diblock copolymers (●M_W of PEO: 12 000)

Sample	Contents of monomeric units in mol %[a]		$\overline{M_n}$
	PBLG	PEO	
PBLG	100	0	60 000
GE-1	60.5	39.5	103 700
GE-2	40.0	60.0	51 800
GE-3	12.4	87.6	20 400

[a] Contents were obtained by NMR measurement.

on the number distribution for the nanoparticles prepared from the dialysis of the mixture of THF/DMF (7/3 : V/V) solution against water is shown in Table. 1. The concentration of the sample was 0.1 g/100 ml in deionized water and the sample was filtered using Millipore filters of pore size 0.45 μm.

2.6. Circular dichroism measurement

The circular dichroism (CD) spectra were measured at room temperature on a JASCO J-500A spectropolarimeter equipped with a quartz cell having a path length of 1 mm.

3. Results and discussion

The block copolymer was prepared by polymerization of BLG-NCA initiated by the amine-terminated PEO in CH_2Cl_2 as shown in Fig. 1. In Table 2 are listed the contents of PBLG and the molecular weights of the copolymers estimated ^{1}H NMR spectra. The block copolymer composition was estimated from the peak intensities of the signal of methylene protons (5.0 ppm) of the PBLG block and of the signal of the ethylene protons (3.7 ppm) of the PEO block in the spectrum. Assuming that all the amino groups of PEO participate in the polymerization, the M_n of the block and the PBLG block can be calculated from the block copolymer composition and the molecular weight of PEO chains.

In Table 1 (see experimental part), are listed the size distribution of the PBLG homopolymer and block copolymers prepared by the diafiltration method. It was found that the particle sizes of the nanoparticles at room temperature were 405.4 ± 59.7, 541.0 ± 111.3, 242.8 ± 47.4 and 182.7 ± 25.2 for PBLG, GE-1, GE-2 and GE-3, respectively. The sizes of the nanoparticles were dependent on the chain length of the PBLG in the block copolymer. The nanoparticle sizes became larger as the content of PBLG increased, as expected. Also, the sizes of the nanoparticles were dependent on the organic solvents and composition of the mixed solvents used. On an average, the nanoparticle sizes of the GE-3 for the THF/DMF system among the used solvents and 7/3 : (V/V) composition in the mixture were the smallest.

Usually, an amphiphilic block copolymer containing hydrophobic and hydrophilic components, exhibits surfactant behavior [14] and may form micelles. In an aqueous media, these materials contain structures comprising hydrophobic cores surrounded by water-soluble polar groups, which extend into the medium. As a matter of fact, in Fig. 2(a), the spectrum of GE-3 in $CDCl_3$ is shown. The ethylene protons of the PEO block and, phenyl protons and methylene protons adjacent to the benzyl group of the PBLG blocks are visible. In Fig. 2(b), the spectrum of a suspension of GE-3 nanoparticles in D_2O is presented. In the spectrum the peaks of phenyl protons (7.2 ppm) and methylene protons (5.1 ppm) adjacent to the benzyl group of the PBLG blocks are not visible whereas the ethylene protons (3.7 ppm) of the PEO block are visible. The spectrum of GE-3 nanoparticles redissolved in $CDCl_3$ can be compared to that of a suspension of nanoparticles. Therefore, it can be said that the copolymers assemble as nanoparticles with PBLG cores and PEO shells from the NMR results. However, the assembled core-shell type nanoparticle size distribution obtained by the diafiltration method was extremely large. At this moment, the nature of the larger particle size is still unclear. We consider several possibilities namely: (1) the individual micelles are further associated by the hydrophobic–hydrophobic interactions between exposed cores [17]; (2) multilayer structure with alternating concentric layers of solvated and undissolved blocks [18]; (3) secondary aggregates with time because of the weak steric stabilization of PEO chains [16]; (4) a mixture of micelles and a considerable amount of secondary aggregates [19].

Fig. 3 shows the CD spectra of the copolymers and PBLG dissolved in dichloroethane, which all having troughs [Θ] at ca. 222 nm, indicating the existence of RH α-helical conformations with different contents, as expected. Assuming of $[\Theta]_{222}$ of 36 000 for PBLG as a reference for 100% helicity, the helical content of the copolymers was estimated from the ratio of $[\Theta]_{222}$ of the copolymer to that of PBLG. The helical content thus estimated was 60.0, 38.2 and 22.9% for GE-1, GE-2 and GE-3, respectively. These helical contents of the GE-1 and GE-2 are consistent with the content (60.5 and 40.0 mol%, respectively, see Table 2) of PBLG in the copolymer chain, as evaluated from ^{1}H NMR measurements. However, the helical content of the GE-3 is larger than that evaluated from NMR measurement (12.4 mol%). It is thought that longer PEO segments in the block copolymer induce more α-helix conformation of the PBLG segment. Cammas et al. reported that PEO segment in the block copolymer influenced to take the α-helical structure of the poly(β-benzyl L-aspartate) [8].

Fig. 4 shows the CD spectra of the nanoparticles of PBLG homopolymer and block copolymers in water, prepared by dialysis against water after dissolving them in a mixture of THF/DMF (7/3 : V/V). These results indicated that the

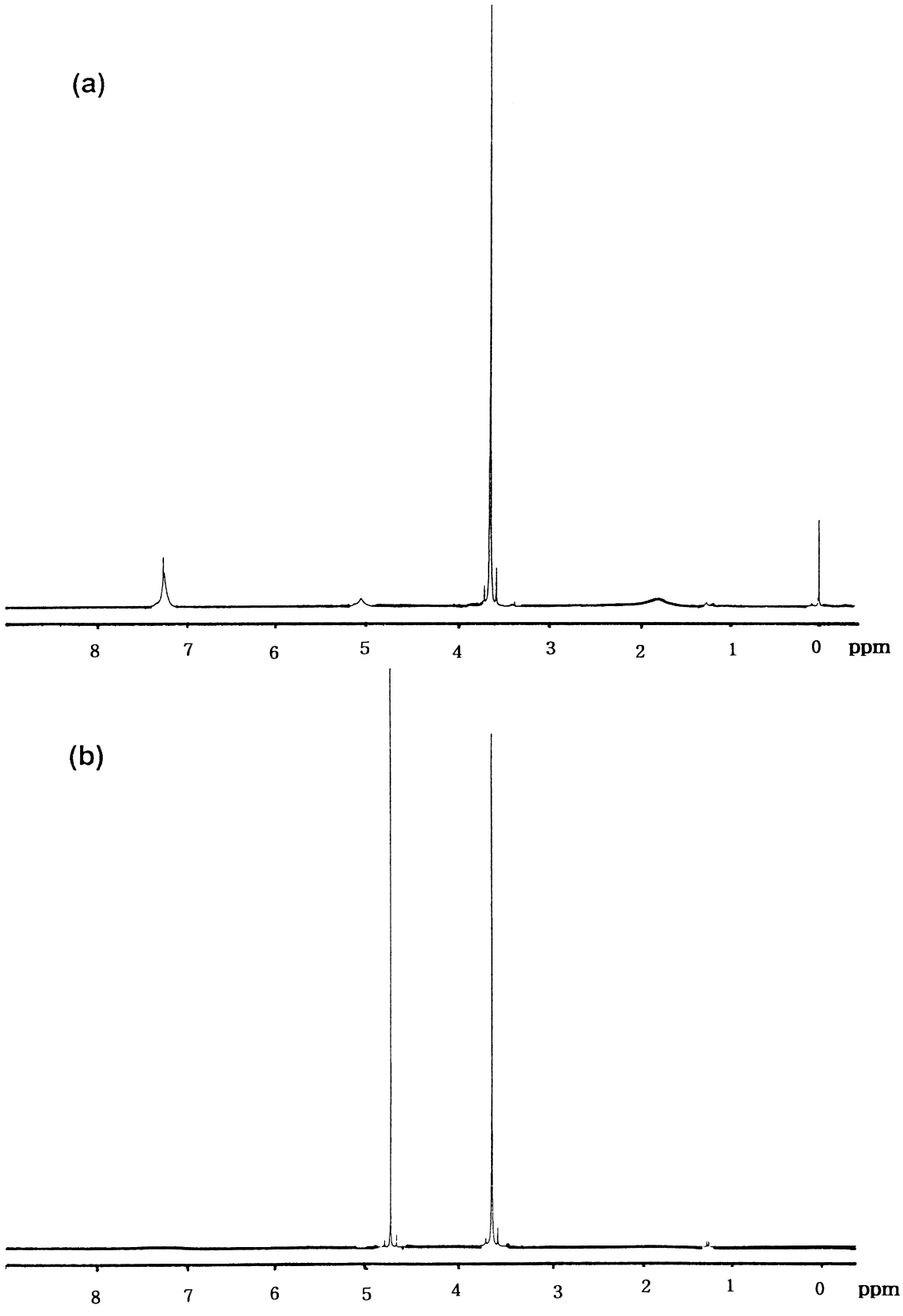

Fig. 2. ^{1}H NMR spectra of GE-3 nanoparticles: (a) redissolved in $CDCl_3$; (b) as a suspension in D_2O.

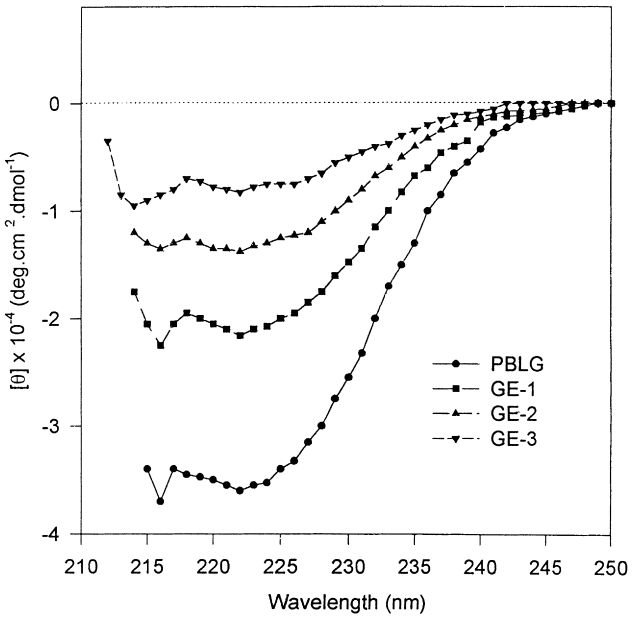

Fig. 3. CD spectra of PBLG and PBLG/PEO diblock copolymers in 1,2-dichloroethane solution at 25°C.

spectra of PBLG homopolymer and GE-1 copolymer nanoparticles in water showed negative Cotton effects characteristic of an α-helical conformation, with a band at ca 222 nm assigned to the n-π^* transition, whereas those of GE-2 and GE-3 copolymers nanoparticles showed positive ones. However, the second peak, due to the $\pi-\pi^*$ transition, appearing at 208 nm [20] for the PBLG and copolymers nanoparticles appeared as the shoulder instead of a strong band. It is probable that α-helices in the nanoparticles are either aggregated artifacts on strong optical interaction between nanoparticles or the α-helices in the nanoparticles are distorted [21]. Plots of [Θ] at maximum wavelength (λ_{max}) against PEO content in the copolymer nanoparticles are shown in Fig. 5. It was found that the sign of the CD band changed from negative to positive when PEO content reached 60 mol% in the block copolymer. This CD data supports the fact that the helix sense of PBLG in the copolymer inverts from the RH to the LH with an incorporation of PEO segment in the block copolymer. It is well known that PLA esters form an α-helix in either the RH or LH screw sense depending on the solvent or temperature, whereas PBLG forms only in the RH helix. It is thought that the RH α-helical form is more stable than the LH α-helical form in the formation of nanoparticles for the longer PBLG chain length similar to the tendency of the PBLG homopolymer. On the contrary, for the shorter PBLG chain length, the difference in stability between the two helical forms of GE-2 (PEO content: 60.0 mol%) or GE-3 (PEO content: 87.6 mol%) copolymer nanoparticles is considerably small. Thus, the RH α → LH α transition can be induced easily because the PEO component as a

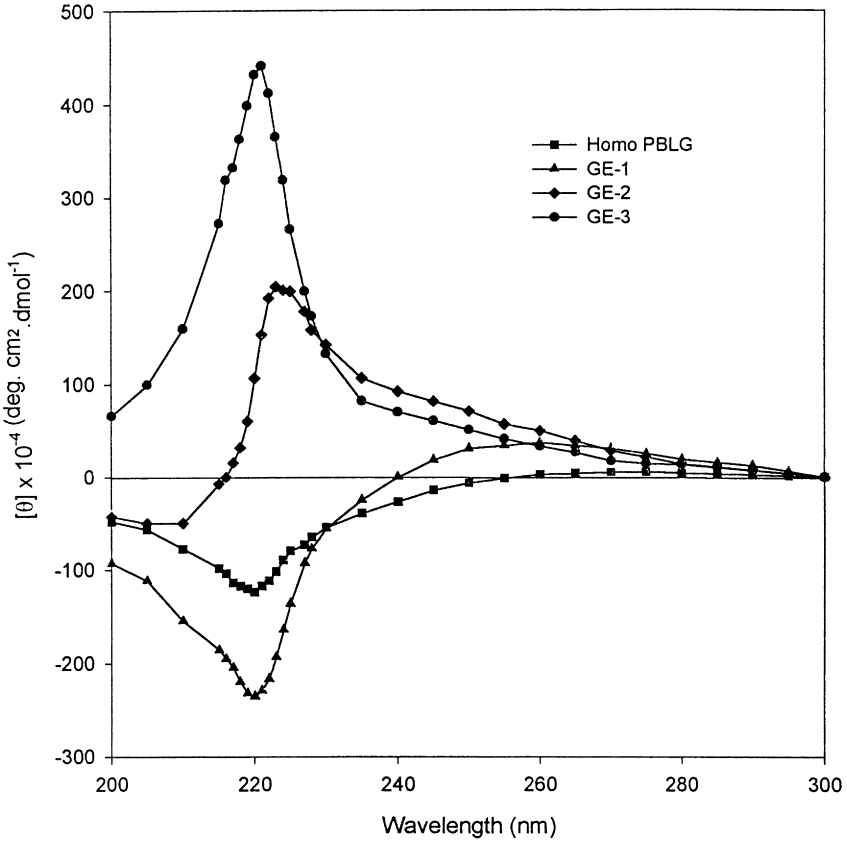

Fig. 4. CD spectra of PBLG and PBLG/PEO copolymers nanoparticles in distilled water at 25°C.

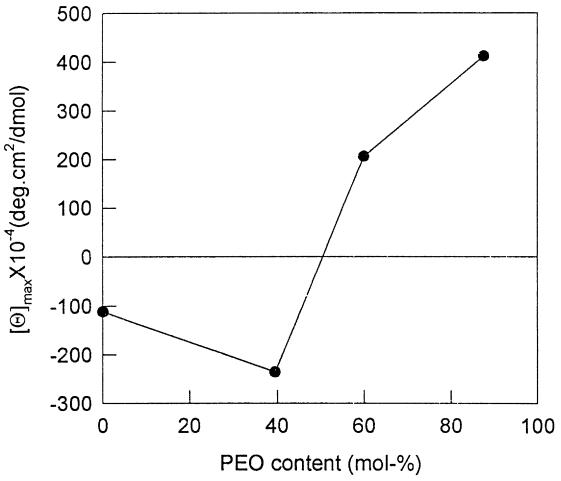

Fig. 5. Plots of $[\Theta]\lambda_{max}$ against PEO content in the copolymer nanoparticles.

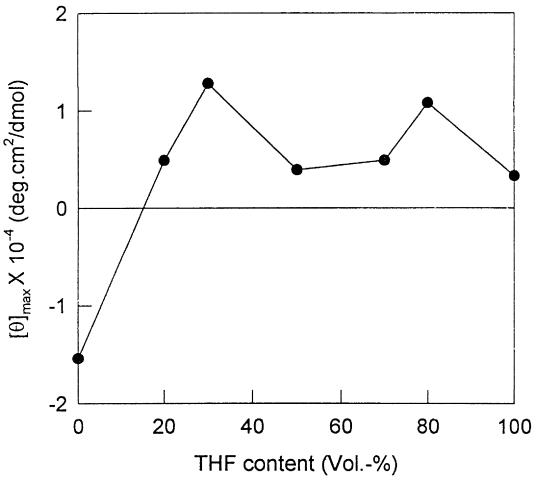

Fig. 6. Plots of $[\Theta]\lambda_{max}$ for GE-3 nanoparticles obtained against THF content in the mixture of THF/dioxane.

soft segment is in a random, liquid-like conformation and has a plasticizing effect on the block copolymer main chains [22]. Toriumi et al. reported that the regularity of the side chain conformation of the PLA affected the stability of the RH α form [12]. Therefore, it may be regarded that the regularity of main chain conformation has a close relationship with the stability of the α form. The molar ellipticity of [Θ] at a maximum wavelength for the PBLG, GE-1, GE-2 and GE-3 are 1 116 000, 2 166 000, 1 920 000 and 4 120 000, respectively. Assuming that PBLG has 100% helical content in the solution state, the value of ellipticity for the PBLG is about 36 000 as shown in Fig. 3. The values of the molar ellipticity for the GE-1, GE-2 and GE-3 in the dichloroethane solution are 21 600, 13 750, and 8250, respectively. Also, the ratio of molar ellipticity of nanoparticles in water to the molar ellipticity at 222 nm in dichloroethane solution against PEO content is shown in Table 3. The ratios for the PBLG, GE-1, GE-2 and GE-3 are 31, 100, 140 and 499, respectively. Although the ratios for the nanoparticles indicated that the PBLG chains aggregated during nanoparticle formation, it is not clear that the ratios mean the association number of the nanoparticles because the particle size is comparable to the wavelength of the light and the internal structure of the nanoparticles is not optically homogeneous. Generally, amphiphilic block copolymers in various selective solvent can self-assemble to form aggregates on micellar structure with a solvophobic innercore and a solvophilic outershell [23]. This phenomenon occurs above a critical concentration of the block copolymer, termed as the critical micelle concentration (CMC). Therefore, it can be said that PBLG chains as the innercore and PEO chains as the outershell are assembled to form aggregates.

Plots of $[\Theta]\lambda_{max}$ for GE-3 nanoparticles obtained against THF content in a mixture of THF/dioxane are shown in Fig. 6. These results indicate that GE-3 nanoparticles form an α-helix in the RH screw sense on the dioxane itself. However, the helix sense of PBLG in the GE-3 nanoparticles inverts from the RH to the LH with the mixing of 20 vol% THF on the dioxane. Also, the effect of various solvents on the α-helix sense of the GE-3 nanoparticles prepared by the diafiltration method is shown in Table 4. It was found that the conformation of the GE-3 nanoparticles adopted by the PBLG depended on the solvent. The helix sense of PBLG in the GE-3 nanoparticles showed RH α-helix on the dioxane or THF/DMSO (7/3 : V/V) whereas PBLG showed LH one on the THF, THF/dioxane (7/3 : V/V), THF/DMF (7/3 : V/V) or THF/DMAc (7/3 : V/V). In future, it should be checked to see whether the nanoparticles seem to behave optically like small liquid crystals.

Table 3
The molar ellipticity of PBLG and PBLG/PEO copolymers nanoparticles at maximum wavelength

Sample	PEO content (mol%)	$[\Theta]_N$[a]	$[\Theta]_S$[b]	$[\Theta]_N/[\Theta]_S$
PBLG	0	−1 116 000	−36 000	31
GE-1	39.5	−2 166 000	−21 600	100
GE-2	60.0	1 920 000	−13 750	140
GE-3	87.6	4 120 000	−8250	499

[a] Molar ellipticity of nanoparticles in distilled water.
[b] Molar ellipticity of PBLG and copolymers in dichloroethane.

4. Conclusion

Conformational studies of the nanoparticles for the PBLG homopolymer and GE copolymers in water, prepared by the diafiltration method was checked by NMR and CD. The results described in the previous sections confirm that the nanoparticles adopt α-helical conformation and the helix sense of PBLG inverts from the RH to the LH with an incorporation of PEO in the copolymer. The helix sense of PBLG in the GE-3 nanoparticles depends on the solvent. At

Table 4
Effect of various solvents on the α helix sense of the GE-3 nanoparticles prepared by the diafiltration method

Solvent	Dioxane	THF	THF/Dioxane (7/3 : V/V)	THF/DMF (7/3 : V/V)	THF/DDMAc (7/3 : V/V)	THF/DMSO (7/3 : V/V)
α-helix sence	L[a]	D[b]	D	D	D	L

[a] Right-handed α-helix.
[b] Left-handed α-helix.

present, the mechanism of the conformation of PBLG in the nanoparticles is under investigation.

Acknowledgements

This work was supported from the 1997 Korean Ministry of Education Research Funds for Advanced Materials.

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