Synthesis and Conformational Properties of Poly(N^4-1-phenylethyl-L-asparagines)

YUN CHEN,* SHIN-SHING JUNG, and JENN HWA WANG

Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan, 70101 Republic of China

SYNOPSIS

Poly(N^4-1-phenylethyl-L-asparagines) (**1a, 1b,** and **1c**) containing DL-, (*R*)-, and (*S*)-side group chirality were prepared by aminolysis of poly(β-methyl-L-aspartate) (PMLA) with DL-, (*R*)-(+)-, and (*S*)-(−)-1-phenylethylamine, respectively. The PMLA was synthesized by ring-opening polymerization of β-methyl-L-aspartate-*N*-carboxy anhydride (NCA) using triethylamine as initiator. The conformations of these polypeptides in the film state were investigated by IR and circular dichroic spectra. The PMLA, **1a,** and **1b** exist mainly in β-sheet conformation, while **1c** forms α-helix that is induced by the (*S*)-chirality of the side groups. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Higher-order structure of proteins plays an important role in determining their catalytic and structural functions in biochemical processes. To mimic the conformation, structure, and function of proteins, a lot of synthetic polypeptides containing different side groups have been prepared and their conformations or structures have been studied.[1] The main conformations of polypeptides are α-helix, β-sheet, and random coil, depending on the molecular weight, concentration, temperature, and solvent.[1-3] Methods for the characterization of polypeptide conformation are infrared spectra,[4,5] nuclear magnetic resonance,[6-9] wide-angle X-ray scattering,[10] and circular dichroic spectra.[10-13] The conformation and structure of poly(γ-methyl-L-glutamate) and poly(γ-benzyl-L-glutamate) are among the most extensively studied topics.[14-17] However, the influence of side group chirality on the conformation of poly(N^4-alkyl-L-asparagines) has not been reported so far. The objective of this work is to report the synthesis and conformational properties of poly(N^4-1-phenylethyl-L-asparagines) (**1a, 1b,** and **1c**) in the film state. These polypeptides were obtained by aminolysis of PMLA with DL-, (*R*)-(+)-, and (*S*)-(−)-1-phenylethylamine, respectively.

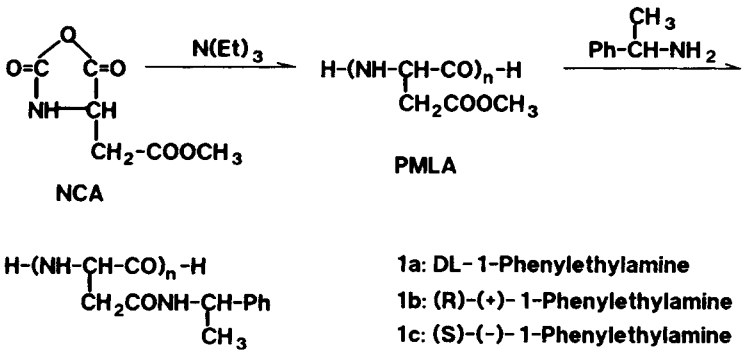
EXPERIMENTAL

Chemicals

β-Methyl-L-aspartate-*N*-carboxy anhydride (NCA) was prepared from L-aspartic acid (Merck) by the modified methods reported by Berger et al.[24] in the synthesis of β-benzyl-L-aspartate-*N*-carboxy anhydride.[18,19] The DL-, (R)-(+)-, and (S)-(−)-1-phenylethylamine and triethylamine (initiator) were pure reagents (Merck) and used as received. The solvents for polymerization and aminolysis such as tetrahydrofuran (THF), dioxane, pyridine, chloroform, toluene, *N,N*-dimethylformamide (DMF), and *N,N*-dimethylacetamide (DMAc) were dried and purified by usual methods. Dichloroacetic acid (DCA) was purchased from Merck and used without further purification.

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 47, 1303–1308 (1993)
 CCC 0021-8995/93/071303-06



Scheme 1

Synthesis of Poly(β-methyl-L-aspartate) (PMLA) (Scheme 1)

The ring-opening polymerization of the NCA was initiated by triethylamine ([NCA]/[I] = 200, 50, 40, 33, 20) in different solvents. For example, into 10 mL of dried THF were added 2.0 g of the NCA (11.6 mmol) and 0.030 g of triethylamine (0.29 mmol). The mixtures were allowed to react at room temperature for 6 days and then precipitated in 160 mL of ethyl ether. The precipitates were collected by filtration and vacuum-dried to obtain 1.28 g of PMLA. Yield 86%, $[\alpha]_D^{26} = -34.1°$ (c 1.6, DCA), $[\eta]$ = 0.17 dL/g. IR(KBr): ν 3300 cm^{-1} (amide A), 1740 cm^{-1} (— $COOCH_3$), 1636 cm^{-1} (amide I), 1534 cm^{-1} (amide II), 1180–1300 cm^{-1} (— CO — O — C). ^{1}H-NMR (CF_3COOD) 3.21 (d, 2H, — CH_2 —), 3.88 (s, 3H, — CH_3), 5.14 (t, 1H, — CH —).

ANAL: calcd for $(C_5H_7NO_3)_n$: C, 46.51%; H, 5.46%; N, 10.85%.
Found: C, 46.78%; H, 5.32%; N, 10.61%.

Synthesis of 1a, 1b, and 1c by Aminolysis of PMLA (Scheme 1)

Poly(N^4-1-phenylethyl-L-asparagines) (**1a, 1b,** and **1c**) were prepared by the modified aminolysis method reported by Tompa.[20] For example, into a mixture of toluene and pyridine (10 mL, v/v = 80/20) were added 0.90 g of PMLA (6.97 repeating-unit-mmol) that was obtained in THF at [NCA]/[I] = 40, 0.01 g of ethylene glycol as catalyst, and 3.53 g of DL-1-phenylethylamine (34.8 mmol). The mixtures were allowed to react at 65°C for 4 days, then precipitated in 160 mL ethyl ether. The solids were collected by filtration and dried to obtain 1.35 g of **1a** (6.20 repeating-unit-mmol). Yield 89%, $[\alpha]_D^{26} = -5.4°$ (c 1.6, DCA), $[\eta]$ = 0.13 dL/g. IR(KBr): ν 3200–3300 cm^{-1} (amide A), 1640 cm^{-1} (amide I), 1540 cm^{-1} (amide II), 700–800 cm^{-1} (phenyl). ^{1}H-NMR(CF_3COOD) 1.72 (b, 3H, — CH_3), 1.97 [d, 1H, — CH(CH_3) —], 3.2 (b, 2H, — CH_2 —), 5.15 (b, 1H, — NH — CH — CO —), 7.2–7.8 (m, 5H, phenyl).

ANAL: Calcd for $(C_{12}H_{14}N_2O_2)_n$: C, 66.04%; H, 6.47%; N, 12.84%.
Found: C, 65.61%; H, 6.30%; N, 12.51%.

MEASUREMENTS

Infrared spectra (IR) were recorded on an infrared spectrophotometer, model IR-810, from the Japan Spectroscopic Co, at a resolution of 4 cm^{-1}. The ^{1}H-NMR spectra were recorded using a Bruker 100 MHz spectrometer. The optical rotations were measured at 26°C using a Jasco DIP-360 digital polarimeter. The concentration was 1.6 g/dL in dichloroacetic acid (DCA). The viscosities of the polypeptides were measured in DCA at 30°C using an Ubbelohde viscometer. The circular dichroic (CD) spectra of the polypeptides in film state were measured at room temperature on a Jasco J-720 spectropolarimeter. The thin films for CD measurement were prepared by casting polypeptide solutions (0.1 g/mL in DMF) on the outside surface of a quartz cell. The coated films were dried at ambient air and then *in vacuo* for 24 h at room temperature. Each measurement were repeated four times by rotating the sample cell by 60°, 120°, and 180° from the first position around the axis of the incident light beam to ascertain the absence of birefringence (linear dichroism).

RESULTS AND DISCUSSION

Synthesis of Poly(β-methyl-L-aspartate) (PMLA)

The results of ring-opening polymerization of the NCA under different conditions are summarized in

Table I Synthesis of PMLA by Ring-opening Polymerization of NCA[a]

	Polymerization Solvent						
	THF			Dioxane		Pyridine	DMF
[NCA]/[I]	50	40	20	33	200	—[b]	200
Yield (%)	81	86	87	84	—	87	—
$[\eta]$ (dL/g)[c]	0.13	0.17	0.14	0.14	—	0.14	—
M_w, ($\times 10^3$)[d]	11	15	12.4	12.4	—	12.4	—
$[\alpha]^{26}D$[e]	−45.4°	−34.1°	−28.6°	−42.3°	—	−57.6°	—

[a] 2.0 g of the NCA in 10 mL solvent; triethylamine as initiator; reacted at room temperature for 6 days.
[b] Pyridine as solvent and initiator.
[c] Measured in DCA at 30°C using an Ubbelohde viscometer.
[d] Calculated from $[\eta] = 1.39 \times 10^{-4} M_w^{0.737}$ for poly(β-benzyl-L-aspartate).[21]
[e] Measured in DCA at a concentration of 1.6 g/dL.

Table I. The yields are higher than 80% except in dioxane and DMF with [NCA]/[I] = 200. The intrinsic viscosity $[\eta]$ and weight-average molecular weight (M_w) are at 0.13–0.17 dL/g and 11,000–15,000, respectively. Increasing the [NCA]/[I] ratio to 200 to obtain high molecular weight PMLA was not successful and resulted in the ring-opening of the NCA. In THF and dioxane, the PMLA precipitated out during the polymerization due its insolubility. This limits the formation of high molecular weight PMLA. Thus, obtained PMLA readily dissolves in high polar solvent such as dichloroacetic acid (DCA), trifluoroacetic acid, and DMF, but it cannot be dissolved in common solvents such as dioxane, THF, and chloroform.

Aminolysis of PMLA

The aminolysis of PMLA with DL-1-phenylethylamine was first studied to investigate the proper reaction conditions. The results under different conditions are summarized in Table II. From ^{1}H-NMR, the aminolysis in toluene/pyridine and in DL-1-phenylethylamine (bulk) results in 96% and 100% substitution, respectively. For other conditions, the substitution ratio is low or even results in the cleavage of the main chain (in DMAc/LiCl). Accordingly, the aminolysis of PMLA with (*R*)-(+)- and (*S*)-(−)-1-phenylethylamine were all conducted in toluene/pyridine at 65°C. The results are shown in Table III with that of PMLA. The IR and ^{1}H-NMR spectra of PMLA and **1a** are shown in Figures 1 and 2, respectively. The $[\eta]$'s of the poly(N^4-1-phenylethyl-L-asparagines) (**1a, 1b,** and **1c**) are between 0.10 and 0.13 dL/g, which are smaller than that of the starting PMLA (0.17 dL/g), indicating that slight chain scission of PMLA has occurred during the aminolysis. The substitution ratios are between 90% and 96% determined from ^{1}H-NMR.

Conformational Properties of PMLA, 1a, 1b, and 1c

IR and CD spectra have been proved to be effective methods for conformational study of polypep-

Table II Aminolysis of PMLA with DL-1-Phenylethylamine under Different Conditions[a]

	$CHCl_3$	DMAc/LiCl	CH_3OH	Dioxane	.TL/Pyridine	Bulk[b]
Temp (°C)	50	70	50	50	65	65
Solution	Het.[c]	Hom.[c]	Hom.	Hom.	Hom.	Hom.
IR[d]	—	Decomposed	—	—	—	—
Substitution ratio (%)[e]	34	—	41	32	96	100

[a] The PMLA with M_w = 15,000 was used.
[b] DL-1-Phenylethylamine as reacting solvent.
[c] Het.: heterogeneous; Hom.: homogeneous.
[d] Determined from 1740 cm^{-1} (ester), 1600–1700 cm^{-1} (amide I), and 1500–1600 cm^{-1} (amide II).
[e] Substitution ratio was determined from ^{1}H-NMR spectra.

Table III The Poly(N^4-1-phenylethyl-L-asparagines) Obtained from the Aminolysis of PMLA with 1-Phenylethylamines

	PMLA	1a	1b	1c
Yield (%)	86	89	87	82
$[\eta]$ (dL/g)[a]	0.17	0.13	0.11	0.10
$[\alpha]^{26}D$[b]	−34.1[c]	−5.4°	+58.1°	−64.5°
Substitution ratio (%)[c]		96	90	93

[a] Measured in DCA with an Ubbelohde viscometer at 30°C.
[b] Measured in DCA at 1.6 g/dL.
[c] Calculated from ^{1}H-NMR spectra.

tides.[10-13] The characteristic IR absorptions of PMLA forming the α-helix are at 1664, 1553, and 784 cm^{-1}, whereas those of the β-sheet are at 1636, 1534, and 778 cm^{-1}.[4,5] The PMLAs obtained under different conditions show similar IR spectra (Fig. 1) and exhibit characteristic absorptions at 1636 and 1534 cm^{-1}, suggesting that the PMLA exist mainly in the β-sheet conformation. Similarly, both **1a** and **1b** show characteristic absorptions at 1640 and 1536 cm^{-1}. However, in **1c**, the absorptions appear at 1662 and 1550 cm^{-1}, which is due to the formation of the α-helix conformation.

The polypeptides forming the right-handed α-helix conformation possess characteristic CD bands of amide groups at 190 nm (peak), 210 nm (trough), and 222 nm (trough), whereas those forming the β-sheet conformation show a peak at 195 nm and a trough at 216 nm.[13] The random coil has two bands centered at 197 nm (trough) and 215 nm (peak).[13] However, depending on measurement conditions and the side-chain groups, the amplitude and wavelength of the CD bands may shift slightly. As shown in Figure 3, the CD spectra of PMLA in the film state shows a trough at 230 nm, which can be assigned to components of the split $n-\pi^*$ transition due to an exciton coupling of the amide chromophores in the β-sheet conformation. These results also support the formation of the β-sheet as stated in IR analysis.

It has been reported that chirality of side-chain groups may induce the conformation of polymer main chain.[22,23] Aminolysis of PMLA with DL-, (*R*)-(+)-, and (*S*)-(−)-1-phenylethylamine introduce DL-, (*R*)-, and (*S*)-chiral centers in the side chain, respectively. The CD spectrum of **1a** with racemic side groups shows one positive peak at 190 nm and one trough at 224 nm (Fig. 3), which is similar to the CD spectra of polypeptides forming the β-sheet conformation. Accordingly, similar to PMLA, **1a** exists mainly in the β-sheet conformation, in which

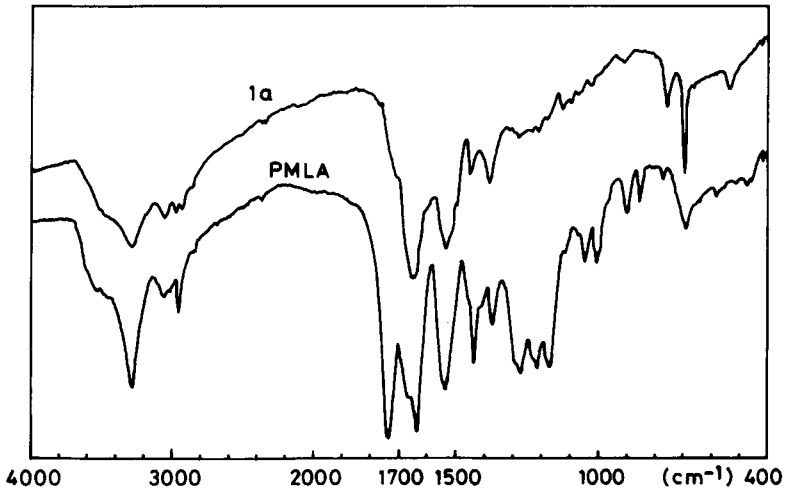


Figure 1 Infrared spectra (KBr pellet) of PMLA and **1a**.

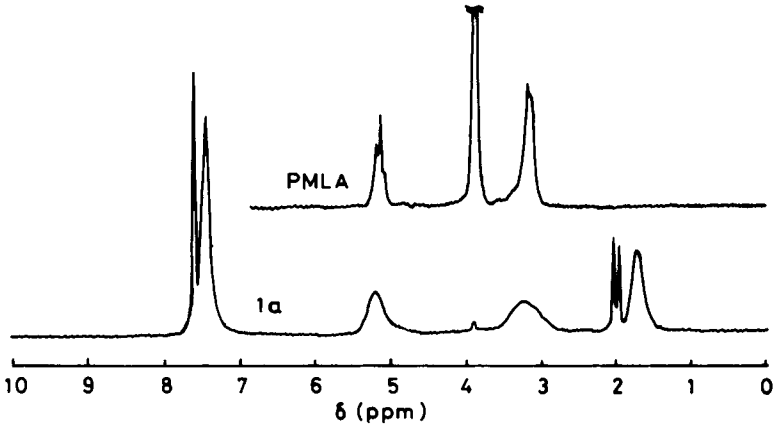


Figure 2 ^{1}H-NMR spectra (100 MHz) of PMLA and **1a**.

the side groups exhibit little influence on its conformation.

The CD spectra of **1b** and **1c** that contain (*R*)- and (*S*)-chiral centers, respectively, are shown in Figure 4. For **1b**, the CD bands of main-chain amides are located at ca. 190 nm (positive) and 236 nm (negative), whereas that of the side-chain aromatic amide is located at 305 nm (negative) with a small shoulder. From the bands below 250 nm, clearly, **1b** forms the β-sheet conformation in the film state. For **1c**, the CD bands of side groups show a peak at 300 nm with a shoulder at 270 nm, which is just opposite to those of **1b** due to its (*S*)-chirality. However, below 250 nm, **1c** shows three characteristic CD bands of the α-helix, i.e., one peak at 190 nm and two troughs centered at 202 and 220 nm. These results indicate clearly that the (*S*)-chiral centers of the side groups in **1c** strongly induce the formation of the α-helix. This offers an effective method for the control of the conformation in polypeptides.

CONCLUSION

We have successfully synthesized poly(N^4-1-phenylethyl-L-asparagines) (**1a, 1b,** and **1c**) by aminolysis of PMLA ([η] = 0.17 dL/g) with corresponding DL-, (*R*)-(+)-, and (*S*)-(−)-1-phenylethylamine, respectively. The intrinsic viscosity is between 0.10 and 0.13 dL/g. The results of IR and circular dichroic (CD) studies show that PMLA, **1a,** and **1b** exist mainly in the β-sheet conformation in the film state. However, **1c** forms the α-helical conformation that is induced by the (*S*)-chirality of side groups.

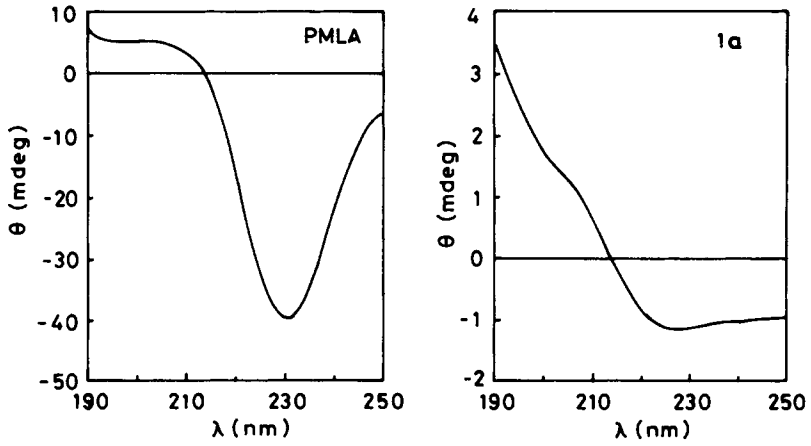


Figure 3 The CD spectra of PMLA and **1a** in the film state.

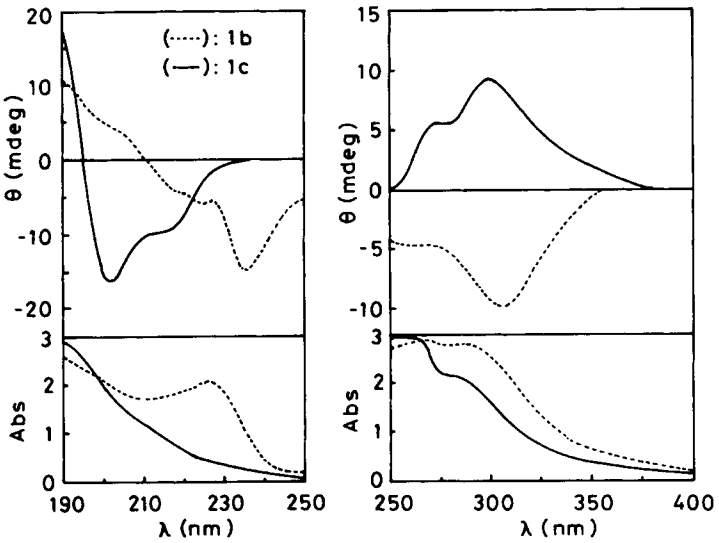


Figure 4 The CD spectra of **1b** and **1c** in the film state.

REFERENCES

1. A. G. Waton, *Polypeptides and Protein Structures,* Elsevier, New York, 1981.
2. Y. Mohadger and G. Wilkes, *J. Polym. Sci. Polym. Phys. Ed.,* **14,** 963 (1976).
3. F. A. Bovey and F. H. Winslow, Ed., *Macromolecules: An Introduction to Polymer Science,* Academic Press, New York, 1979, Chap. 8.
4. T. Miyazama, Y. Masuda, and K. Fukushima, *J. Polym. Sci.,* **62,** 174 (1962).
5. F. Suzuki, K. Onozato, and K. Ebe, *Polym. J.,* **12**(10), 759 (1980).
6. J. A. Ferrettic, *Chem. Commun.,* 1030 (1967).
7. H. Pivcova, V. Saudek, P. Schmidt, D. Hlavata, and J. Plestil, *Polymer,* **28,** 991 (1987).
8. A. Shoji and T. Ozaki, *Macromolecules,* **20,** 2442 (1987).
9. W. Moffitt, D. D. Fitts, and J. G. Kirwood, *Proc. Natl. Acad. Sci. U.S.A.,* **43,** 723 (1957).
10. D. M. Byler and H. Susi, *Biopolymers,* **25,** 469 (1986).
11. N. Greenfield and G. D. Fasman, *Biochemistry,* **8,** 4108 (1969).
12. R. W. Woody, *J. Polym. Sci. Macromol. Rev.,* **12,** 181 (1977).
13. M. Goodman, E. Peggion, and F. Naider, *Encyclopedia of Chemical Technology,* 3rd Ed., Vol. 18, Wiley, New York, 1982, p. 888.
14. C. Robinson, *Tetrahedron,* **13,** 219 (1961).
15. D. L. Patel and D. B. Dupre, *J. Polym. Sci. Polym. Phys. Ed.,* **18,** 1599 (1980).
16. K. Ito, T. Kajiyama, and M. Takayanagi, *Polym. J.,* **12**(5), 305 (1980).
17. D. L. Patel and D. B. Dupre, *J. Chem. Phys.,* **72,** 2515 (1980).
18. K. Y. Kim, T. Komoto, and T. Kawai, *Makromol. Chem.,* **180,** 465 (1979).
19. R. D. Lundberg and P. Doty, *J. Am. Chem. Soc.,* **79,** 3961 (1957).
20. H. Tompa, *Monatsh. Chem.,* **86,** 542 (1955).
21. Y. Hayashi, A. Teramoto, K. Kawahara, and H. Fujita, *Biopolymers,* **8,** 403 (1969).
22. F. Ciardelli, P. Salvadori, C. Carlini, and R. Menicagli, *Makromol. Chem.,* **61,** 207 (1963).
23. R. Nishizima, Y. Tsujita, and A. Takizana, *Polymer,* **26,** 379 (1985).
24. A. Berger and E. Katchalski, *J. Am. Chem. Soc.,* **73,** 4084 (1951).

Received November 26, 1991
Accepted March 25, 1992