

Preparation and Conformation of Hexarmed Star Poly(β -benzyl-L-aspartates) Utilizing Hexakis(4-aminophenoxy)cyclotriphosphazene

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Received April 25, 1994

Considerable attention has been paid to the synthesis of structurally unique polymers, such as dendrimers and arborols,^{1–9} with well-defined molecular composition and constitution. These polymers have been prepared by convergent and divergent growth approaches, and new physical properties and functionalities are expected to result from their globular structures and large number of terminal groups. On the other hand, the polymerization of *N*-carboxy amino acid anhydride (NCA) with a primary amine is well-established to proceed by a mechanism involving the attack of the amino group at the C₃ carbonyl group of the NCA, giving rise to well-ordered polymer chains. Some synthetic poly(amino acid) derivatives or block and graft copolymers containing these polymer units have also been of interest, as their properties and functionalities make them useful as selective membrane and biomedical materials.¹⁰ Although many interesting studies concerning star-shaped polymers have been reported, there have been few reports on the preparation of star-shaped polymers with defined tertiary structures. Recently, Sasaki and Kaiser^{11,12} have reported the synthesis of an attractive model enzyme, namely, helichrome, which has four polypeptide arms on the porphyrin ring. One of the most distinguishing characteristics of this polymer is that the arms in the helichrome take mainly α -helical structure, although the single polypeptide alone has a disordered conformation. In this communication, we report the preparation of a novel star-shaped polymer, poly(β -benzyl-L-aspartate), using hexakis(4-aminophenoxy)cyclotriphosphazene (AOP) as a core,¹³ and the conformation of poly(β -benzyl-L-aspartate) chains.

The *N*-carboxy anhydride of β -benzyl-L-aspartate (BLA-NCA)¹⁴ and AOP¹⁵ as an initiator were prepared according to procedures reported in the literatures. In principle, the six amino groups in AOP would be expected to have the same ability to initiate the polymerization of BLA-NCA. The polymerization of BLA-NCA was carried out at different molar ratios of [BLA-

Table 1. Polymerization of BLA-NCA Initiated by AOP in Dioxane at Room Temperature

run	[BLA-NCA] ^a [AOP]	time (h)	conversion (%)	$M_{\text{calcd.}}^b$ $\times 10^{-3}$	$M_{n,\text{VPO}}$ $\times 10^{-3}$	n^c
1	31	6	83	6.1	5.9	2.9
2	58	3	61	8.0	8.4	2.6
3	60	24	89 ^d	11.7	10.4	1.7
4	100	1	25	5.8	5.8	2.0
5	100	12	79	16.8	16.8	0
6	500	0.17	9 ^d	10.0	11.3	0
7	500	12	57 ^d	59.4	53.5	0

^a Run 1, [BLA-NCA] = 0.18 mol/L; runs 2–7, [BLA-NCA] = 0.19 mol/L; [AOP] = 5.9–0.38 mmol/L. ^b Calculated from the equation, $M_{\text{calcd.}} = [\text{BLA-NCA}] \times \text{conversion} \times M_{\text{BLA}}/[\text{AOP}] + M_i$, where M_{BLA} and M_i represent the molecular weights of monomer units in PBLA and AOP, respectively. ^c Number of residual amino group in AOP. ^d At 50 °C.

NCA]/[AOP] in dioxane at room temperature and 50 °C under an atmosphere of dry nitrogen.¹⁶ The results are summarized in Table 1.

The molecular weights of poly(β -benzyl-L-aspartate) (PBLA) calculated from the feed ratio of [BLA-NCA]/[AOP] and the conversion were essentially consistent with those determined by vapor pressure osmometry, suggesting that only AOP acts as initiator for the polymerization of BLA-NCA. For the gel permeation chromatography (GPC) measurement of PBLA, the polymers with $M_{n,\text{VPO}} = 11\,300$ and $M_{n,\text{VPO}} = 5900$ (determined by VPO) showed the number average molecular weights of 9600 ($M_w/M_n = 1.3$) and 5800 ($M_w/M_n = 1.2$), respectively. However, the PBLA with $M_{n,\text{VPO}} = 10\,400$ exhibited two peaks in the GPC trace.¹⁷

¹³C NMR spectra of PBLA are very useful for detection of any residual amino groups in AOP, since C₃ of the 4-aminophenoxy moiety in AOP was observed at 114.1 ppm (from TMS, in DMSO-*d*₆) as a strong signal, while C₃ of hexakis(4-acetylaminophenoxy)cyclotriphosphazene, the model compound of the initial product for the reaction of AOP with BLA-NCA, shifted to 120.1 ppm. As shown in Figure 1, the PBLA with $M_{n,\text{VPO}} = 4500$ obtained at [BLA-NCA]/[AOP] = 500 and 4.8% conversion showed the peak at 114.1 ppm, indicating that some amino groups do not participate in the polymerization of BLA-NCA. However, for the PBLA with $M_{n,\text{VPO}} = 11\,300$ obtained at the same ratio but slightly higher conversion (9%), the peak of C₃ of AOP was not observed. This disappearance was not due to the lowering of the relative concentration of aminophenoxy group in AOP caused by high molecular weight of PBLA, since the PBLA with $M_{n,\text{VPO}} = 10\,400$ obtained from [BLA-NCA]/[AOP] = 60 showed the signal at 114.1 ppm. Further evidence supporting the involvement of all aromatic amino groups in the initiation was obtained from the reaction of the polymer with 5-(4-(dimethylamino)phenyl)-2,4-pentadienal (DMAPP), which is known to react selectively with aromatic primary amines to give the protonated Schiff bases in trichlo-

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(16) A solution of AOP in dry dioxane was added to a stirred solution of NCA-BLA in dry dioxane under N₂ atmosphere. After being stirred for a definite time, the mixture was poured into 100 mL of ether. The polymer was purified by reprecipitations from a chloroform solution into ether.

(17) The GPC measurement was performed on a Shimadzu LC-6A instrument, equipped with TSK_{gel}G4000HR, using 10 mM LiBr-DMF as eluent (polystyrene standards). When DMF was used as eluent, all polymers exhibited two peaks in GPC traces; one gave a reasonable molecular weight, but the other showed a molecular weight above the exclusion limit of the column ($M_n > 200\,000$). The latter peak disappears or shifts to a lower molecular weight region in the presence of LiBr. These results suggest that the aggregation of polymers, which might be affected by the number and length of the PBLA chain, is responsible for the two peaks in GPC traces.

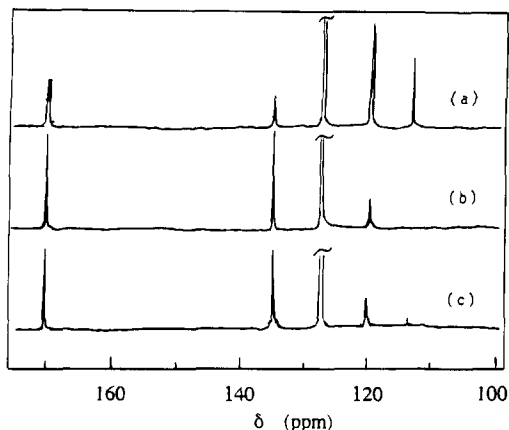
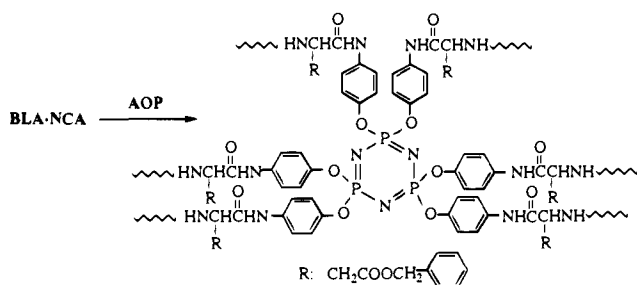


Figure 1. ^{13}C NMR spectra of PBLA in $\text{DMSO-}d_6$. (a) PBLA with $M_n = 4500$ obtained at $[\text{BLA-NCA}]/[\text{AOP}] = 500$ and 4.8% conversion. (b) PBLA with $M_n = 11\,300$ obtained at $[\text{BLA-NCA}]/[\text{AOP}] = 500$ and 9% conversion. (c) PBLA with $M_n = 10\,400$ obtained at $[\text{BLA-NCA}]/[\text{AOP}] = 60$ and 89% conversion.

Scheme 1



roacetic acid-methanol.¹⁸ As a control experiment, the UV spectrum of the reaction mixture of AOP and DMAPP was measured.¹⁹ The absorbance due to the protonated Schiff base prepared from the mixture of 6:1 molar ratio of DMAPP to AOP was observed at $\lambda_{\text{max}} = 560$ nm with $\epsilon = 19\,600$. This value was 6 times larger than that of the mixture of 1:1 molar ratio of DMAPP to AOP, suggesting that all amino groups react quantitatively with DMAPP without suffering steric hindrance under the conditions. When a slight excess of DMAPP was added to the PBLA ($M_{n,\text{VPO}} = 11\,300$), a very weak absorption around 560 nm was observed. However, the PBLA prepared by aniline initiator showed the same UV spectrum, suggesting that the weak absorption was not due to the protonated Schiff base originated from the reaction of aromatic amine with DMAPP. Thus, the lack of any significant absorption around 560 nm for the PBLA ($M_{n,\text{VPO}} = 11\,300$)-DMAPP system suggests that all amino groups in AOP participate in the polymerization of BLA-NCA (Scheme 1), in agreement with the result of ^{13}C NMR spectra, and that the hexaarmed star PBLA diverging from cyclotriphosphazene core is formed. Similarly, the PBLA prepared at $[\text{BLA-NCA}]/[\text{AOP}] = 100$ and 79% conversion has a hexaarmed structure, as evidenced by the UV spectrum (Table 1). However, for the PBLA with relatively low molecular weight ($M_{n,\text{VPO}} = 5800$) prepared under the same feed ratio but at low conversion (25%), the formation of the protonated Schiff base was observed, and the number of unreacted aromatic amino groups in AOP was calculated to be 2.0. Such an existence of AOP primary amine was observed for the polymers prepared at a low ratio of $[\text{BLA-NCA}]/[\text{AOP}]$.

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(19) To a sample solution (0.05–0.07 mg/1 mL) were added 0.1% (w/v) DMAPP-MeOH solution (1 mL) and 50% (w/v) trichloroacetic acid-MeOH (1.0 mL), and then the whole was diluted to 10 mL with MeOH. The solution was allowed to stand for 10 min, and then the absorbance was measured with a Shimadzu UV 260 spectrometer.

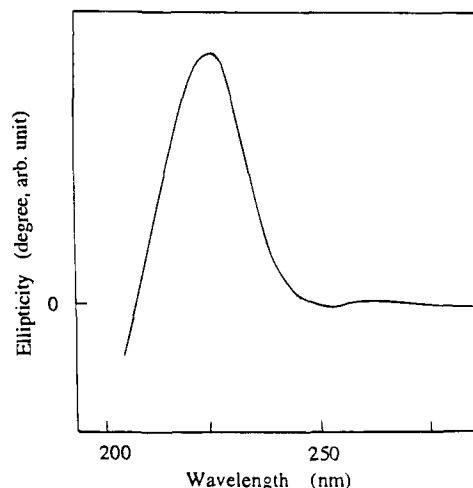


Figure 2. CD spectrum of hexaarmed PBLA.

Thus, the formation of hexaarmed star PBLA could be achieved at a high ratio of $[\text{BLA-NCA}]/[\text{AOP}]$ and high conversion.

These results also indicate that some of aromatic amino groups initiate the polymerization after delay. Furthermore, it appears that, for the propagation process, the same situation is also operative, i.e., the chain lengths of the PBLAs might be different. These ideas imply that the molecular weight distribution of the hexaarmed PBLA should be broad. However, as described earlier, the values of polydispersity (M_w/M_n) are rather small. At the present time it is not clear whether the low degree of polydispersity is due to the formation of PBLA chains with narrow molecular weight distributions, differing from the prediction of a spherical structure for the star-shaped PBLA. Further information about the mechanism of polymerization and characteristics of the hexaarmed PBLA is needed to solve the question.

It is highly of interest to examine whether the PBLA chains diverging from the phosphazene core take an α -helical structure. FT-IR spectra of hexaarmed PBLA films with high molecular weights of $M_{n,\text{VPO}} = 53\,500$ cast from chloroform solution at room temperature showed an amide I band at 1669 cm^{-1} and an amide II band at 1555 cm^{-1} , which are characteristic of an α -helical polypeptide.²⁰

Furthermore, the circular dichroism (CD) spectrum of the hexaarmed PBLA film on a quartz cell exhibited a positive curve at 226 nm, as shown in Figure 2. These results suggest that most of the PBLA chains diverging from AOP take mainly a left-handed α -helical structure, in a manner similar to that of common PBLA in the solid state.²⁰ Contrary to this, IR spectra of the PBLA chains with low molecular weights ($M_n < 10\,000$) showed the absorptions at 1672 , 1659 , and 1646 cm^{-1} in the amide I band and at 1557 , 1544 , and 1529 cm^{-1} in the amide II band, and CD spectra exhibited a negative curve, indicating the involvement of disordered conformations.

In conclusion, the polymerization of BLA-NCA with AOP was found to give hexaarmed star polypeptides that take the α -helix mainly when their molecular weights are high. Such synthetic polypeptides would be expected to possess such functionalities as the channel for transporting a specific substrate and the active sites of enzymes. Further studies of the polymerization of NCA and the structural characterizations and properties of hexaarmed poly(amino acid) derivatives containing PBLA are now in progress.

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