Enantioselective Permeation of Amino Acids across Membranes Prepared from 3α-Helix Bundle Polyglutamates with Oxyethylene Chains

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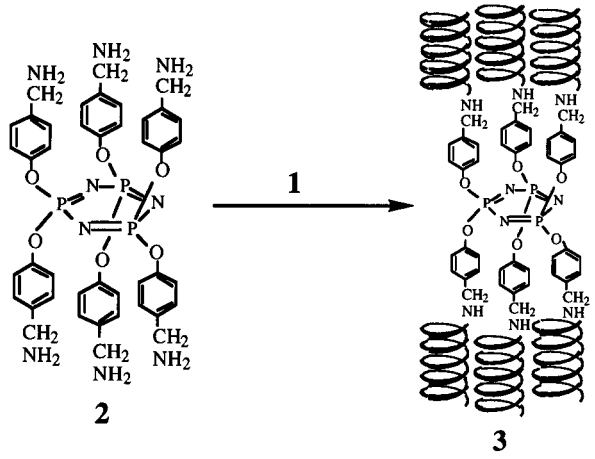
Synthetic poly(amino acid) derivatives with well-ordered polymer chains or block and graft copolymers containing these polymer units are interesting in their properties and functionalities such as selective membrane and biomedical materials.[1−4] Recently, Ogata et al.[2] reported that a polyglutamate with amphiphilic side chains consisting of oligo(oxyethylene) and long alkyl chains could act as an effective membrane for resolving optical isomers and demonstrated the importance of the formation of an ordered α-helical structure for such a resolution. On the other hand, polymers with well-defined molecular composition and constitution such as dendrimers and hyperbranched polymers have been prepared, and applications of these polymers have been explored in various fields such as drug delivery system or liquid crystals.[5−12] More recently, we reported the preparation of a novel star-shaped poly(*β*-benzyl-L-aspartate) using hexakis(4-aminophenoxy)cyclotriphosphazene as a core, which takes an α-helical structure.[13] A well-defined three-dimensional structure is the fundamental prerequisite for proteins to manifest their biological functions. If the hexaarmed star poly(amino acid) derivatives form a 3α-helix bundle structure on both sides of a nearly planar phosphazene ring, new developments or improvements of functionalities based on α-helical structure would be expected, especially in the fields of the ion channels and optical resolutions. In this paper, we report the preparation of hexaarmed poly(L-glutamates) with di- (**4**) and triethylene glycol monomethyl ether units (**5**) and their functionalities as enantioselective membranes of tryptophan (Trp), phenylalanine (Phe), and tyrosine (Tyr).

A hexaarmed poly(*γ*-benzyl-L-glutamate) with a phosphazene core (**3**) was prepared by ring-opening polymerization of the *N*-carboxyanhydride of *γ*-benzyl-L-glutamate (**1**)[14] with a new initiator, hexakis(4-(aminomethyl)phenoxy)cyclotriphosphazene

Scheme 1. Preparation of **3**



(**2**).[15] In principle, the amino group in **2** has the same ability to initiate the polymerization of **1**. ^{1}H NMR spectra of the mixture of [**1**]/[**2**] = 10 in $CDCl_3$ showed that the peak at 3.8 ppm assignable to benzyl protons disappeared immediately, together with the conversion of **1** of ca. 57%. The molecular weight of **3** ($M_w = 15.3 \times 10^4$, $M_n = 11.4 \times 10^4$) obtained from [**1**]/[**2**] = 600 (conversion, 81%), determined by the combination of gel permeation chromatography (GPC) and light scattering method, was in agreement with the calculated value ($M_{n,cal} = 10.7 \times 10^4$),[16] and the molecular weight distribution was remarkably narrow. Furthermore, IR spectra of **3** with M_n = 5400, for which the degree of polymerization of each chain would be expected to be DP = 3∼4 if the simultaneous propagation occurs at six reactive sites, exhibited peaks at 1660 and 1631 (amide **I**) and 1538 and 1526 (amide II) cm^{-1}, suggesting that the chains take *β*-form and random coil but not α-helical structure.[17] From these results, it appears that all of amino groups in **2** initiate the polymerization of **1** without delay and that the lengths of polymer chains diverging from the phosphazene core are well controlled. As expected, IR and CD spectra of **3** with $M_n = 11.4 \times 10^4$ cast from $CHCl_3$ showed peaks at 1653 and 1550 cm^{-1} and a negative peak at 226 nm, respectively, indicating that the hexaarmed polyglutamates take a right-handed α-helical structure, in a manner similar to that of conventional glutamates.[2,15]

The displacement of benzyl groups with di- (DEG) and triethylene glycol monomethyl ethers (TEG) was carried out in the presence of *p*-toluenesulfonic acid. The degree of displacement determined from ^{1}H NMR spectra was in the range 52−63%, in spite of showing that the value of the conventional linear polyglutamate was 85% under the same conditions. This suggests the existence of sterically hindered sites in **3**. The helix contents of **4** and **5** were almost the same as the value for the parent **3**.

Since the polymers obtained did not offer a self-standing membrane, Teflon was used as a supporting material for the permeation experiments. The permeation behaviors of Trp, Tyr, and Phe were examined using two-chamber cells at room temperature.[18] As shown in Figure 1, the membrane **5** permeates only the D-isomer for Trp. The rate of permeation of D-Trp

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(16) The molecular weights were calculated from the combination of a refractive index detector and laser light scattering in the GPC line (eluent, 10 mM LiBr−DMF). The differential refractive index increment required for the calculation of molecular weights of the polymers was computed from the value of the detector calibration constant and the amount of the polymer injected into the GPC line.[15] The $M_{n,cal}$ was determined from $M_{n,cal} = 219.3 \times$ [**1**]/[**2**] × conversion + M_{core}, where M_{core} denotes the molecular weight of phosphazene moiety.

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Table 1. Preparation of Hexaarmed Star Poly(L-glutamates) with Oxyethylene Chains[a]

3						poly(L-glutamate) with oxyethylene chains		
α-HC (%)[b]	10^{-4} M_w	M_w/M_n	$H(OCH_2CH_2)_nOCH_3$	temp (°C)	time (h)	DS (%)[c]	α-HC (%)[b]	
85	15.3	1.3	$n = 2$	80	3	57	82	**4**
			$n = 3$	70	9	52	83	**5**
55	5.8	1.1	$n = 3$	80	6	63	58	
65[d]	7.0	1.1	$n = 3$	80	6	85	54	

[a] [**3**] = 2.3 mmol. $[H(OCH_2CH_2)_nOCH_3]$ = 0.047 mol. [*p*-TsOH] = 2.3 mmol. [b] α-Helix content in DCE. [c] Degree of substitution. [d] Poly(γ-benzyl-L-glutamate) prepared from *n*-propylamine.

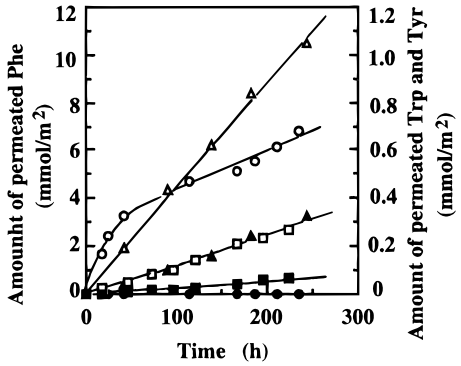
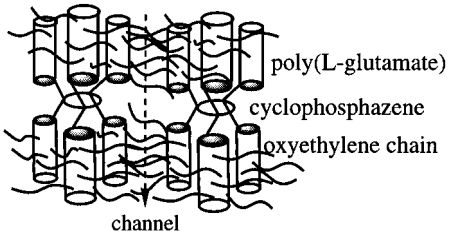


Figure 1. Plots of permeated D- (○, △, □) and L- (●, ▲, ■) isomers from racemates of Trp (○, ●), Phe (△, ▲), and Tyr (□, ■). The membrane used was as follows: Phe, **4**; Trp and Tyr, **5**.

decreased in the time course of permeation, but a complete optical resolution held for over 200 h.[19] A similar result has been reported for the resolution of Trp with polyglutamates carrying amphiphilic side chains.[2] The addition of antibacterial reagent NaN_3 did not show any significant influence on the amount of permeate or the resolution. When the conventional polyglutamate with TEG units (degree of displacement = 85%; α-helix content = 54%), which was prepared from the polymerization of **1** with *n*-propylamine, was used as control experiment, the resolution of racemate of Trp was not observed. For the permeation of Tyr through membrane **5**, the D isomer permeated predominantly, and the enantioselective permeation of 60% ee was achieved. A similar enantioselective permeation was observed in Phe, but the enantioselectivity of D isomer was somewhat low (30% ee).[20] Interestingly, the optical resolution of Phe was improved by changing the length of side oxyethylene chains, i.e., the enantioselectivity by **4** as high as 64% ee was attained. The improvement of the optical resolution by changing the membrane, however, was not observed for Tyr. Thus, these results indicate that hexaarmed polyglutamates with short oxyethylene chains function as excellent membranes of the optical resolution of amino acids.

In the helical structure, the intramolecular hydrogen bonds confer a rigidity to the polyglutamate so that the chain behaves like a rigid rod. Recent X-ray analysis of phenoxycyclotriphosphazenes showed that the organic groups are aligned approximately perpendicular to the almost planar cyclotriphosphazene.[21] If this geometrical arrangement holds for **4** and **5**, it seems reasonable to assume that the rigid polyglutamate

Scheme 2. Schematic Representation of Assembly of a 3α-Helix Bundle Polyglutamate with Oligo(oxyethylene) Chains



chains form a 3α-helix bundle structure on both sides of the phosphazene ring and the oxyethylene phase is formed along the polymer chains. The fact that the degree of displacement of benzyl group in **3** with TEG and DEG is relatively low seems to support the formation of bundle structure, i.e., it is difficult for the benzyl group located at the inner site of the bundle structure to be substituted by an oxyethylene group due to steric hindrance. It is safe to say that the α-helical structure is essential for the chiral recognition of amino acids and oxyethylene chains are concerned mainly in adsorption and transportation of the substrate.[2] No functionality of conventional polyglutamate with TEG units indicates that the additional factor, the well-ordered assembly of the recognition sites, is required to attain a high enantioselectivity. For membranes **4** and **5**, it appears that the short oxyethylene chains around hexaarmed polyglutamate could assemble side to side to form a relatively narrow channel along the α-helical structure, which makes possible effective molecular recognition of racemates in the vicinity of the α-helical polyglutamate backbone. The increase in the enantioselectivity of Phe for **4** seems to be related to the size of the channel, i.e., in such a narrow channel, Phe with relatively small molecular size could interact efficiently with the recognition site of the α-helical polymer backbone. This is consistent with the observation that the enantioselective permeation of Phe could not be attained for the membrane prepared from poly(L-glutamate) with relatively long oxyethylene and alkyl chains.[2] The reason why D isomers permeate predominantly through the membrane is unclear, although D-Trp is known to be adsorbed much more than L-Trp in poly(L-glutamates).[2]

In conclusion, our preliminary work has shown that the hexaarmed star polyglutamates carrying short oxyethylene chains function as excellent enantioselective membranes for Trp, Phe, and Tyr. It is worth noting that the structural change from linear to star-shaped polymers improves the functionality of molecular recognition. Detailed studies concerning optical resolutions of racemates of amino acids by the membrane of hexaarmed poly(amino acid) derivatives and further structural characterizations of these polymers containing **4** and **5** are now in progress.

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(18) Teflon (diameter, 15 mm; porosity, 0.1 μm) was placed in a solution of the polyglutamate (0.5−1 wt %) in $CHCl_3$ and dried. This coating process was repeated several times, and then the membrane was placed in deionized water for 1 day. The chamber volumes of the donor and acceptor were 50 and 20 cm^3, respectively. The concentrations of Trp, Phe, and Tyr in the donor chamber were 4, 2, and 1.8 mmol/L, respectively. The amounts of amino acids were determined by a UV spectrometer, and the compositions of D and L isomers permeated through the membrane were determined by HPLC equipped with an enantioselective column (Tosoh, TSK gel Enantio L1; eluent, 1 or 5 mM $CuSO_4-H_2O$) and a UV detector.

(19) The reason for the change of permeation rate remains unclear. One plausible explanation might be the conformational change of the membrane due to the interaction between poly(L-glutamate) and Trp.

(20) No enantioselective permeation of Try and Phe was observed for the membrane of linear polyglutamate with TEG units.

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