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Structure and Biomedical Properties of Block Copolymer Membranes Containing Poly-α-Aminoacids

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Poly-α-aminoacids have been investigated for possible use in various biomedical applications[1~6] ranging from dialysis membranes to artificial skin substitutes. On one hand, some kinds of block copolymers such as BIOMER and AVCOTHANE are known to have excellent anti-thrombogenic properties[7,8]. Morphlogically, block copolymer membranes exhibit micro-heterophase structures[9~14] as a result of micro-phase separation from solution. Such micro-heterophase structures are also observed in biomembranes which include hydrophilic and hydrophobic domains. Block copolymers so far investigated are mainly A-B or A-B-A type polymers in which both block component chains are such kind as exists in random coil conformation in solution.

For reason that poly-α-aminoacids can reveal α-helical conformation in solution and in the bulk, block copolymers containing poly-α-aminoacid as one component should be an interesting subject as a novel class of biomaterials from morphological and functional points of view. However, little is reported about such class of block copolymers except papers of Gallot et al[15,16]. On an A-B di-block copolymer.

The aim of this research is to extend fundamental investigations on the formation and structure of micro-heterophase and to elucidate the functional properties of the materials for A-B-A tri-block copolymers in which A is a poly-α-aminoacid and B is polybutadiene (PB). Three series of block copolymers are investigated: Poly-γ-benzyl-L-glutamate (PBLG), Poly-γ-methyl-L-glutamate (PMLG), and poly-N^{ϵ}-carbobenzoxy-L-lysine (PCBL) are used as the A component.

Experimental

Synthesis and Characterization of Tri-block Copolymers

A cyclo-aliphatic secondary amine-terminated polybutadiene[17] having a number-average molecular weight of 3,600 was used as the middle block. The molecular weight distribution of this middle block is very sharp. The A-B-A tri-block copolymers were prepared by reacting the middle block with N-carboxy anhydride of γ-benzyl-L-glutamate, γ-methyl L-glutamate, or N^{ϵ}-carbobenzoxy-L-lysine. The polymerization was carried out in the absence of moisture at room temperature in dioxane-methylene dichloride mixture at 3% total concentration of aminoacid-NCA and the middle block. The polymerization was followed by infrared spectroscopy. After the polymerization was terminated, the copolymer was precipitated in methanol for purification and then dried in vacuo. The A-B-A tri-block copolymers obtained are in the form of

$H-(NH-CHR-CO)_n-X-NH-CO-(CH_2-CH=CH-CH_2)_m-CO-NH-X-(CO-CHR-NH)_n-H$

where R indicates

R: $-(CH_2)_2-COOCH_2-$⟨benzene⟩ (BLG)

$-(CH_2)_2-COOCH_3$ (MLG)

$-(CH_2)_4-NH-CO-CH_2-$⟨benzene⟩ (CBL)

and X represents ⟨piperazine⟩$-CH_2-CH_2$

The copolymer composition was determined by ultraviolet spectra and elemental analysis. Since the molecular weight of the middle block is known, the degree of polymerization of the polypeptide block (A-block). P_A was estimated from the copolymer composition. The chain conformation of A block in solution and in bulk, as represented by the helical content X_H, was evaluated from the circular dichroism and infrared spectra.

Electron Microscopy, Mechanical Property, Permeability: and Tissue Compatibility

Copolymer samples were dissolved in solvent from which they are cast. A drop of the solution was introduced onto the sheet mesh for electron microscope and allowed to form thin membrane. The membrane was then treated with osmium tetraoxide vapor to stain the PB portions.

The dynamic mechanical relaxation behaviors were studied with rheovibron at 110 Hz and in a range of -20 to 100°C.

Water permeability of the membranes was measured with a ultrafiltration cell at 25-60°C under pressure of 1 to 5 atmospheric pressure.

Tissue compatibility of the block copolymers was examined with in vivo implantation tests by using rabbits. For this test, the copolymers were coated onto DACRON fabric from solution.

Results and Discussion

Molecular Conformation of Block Copolymers

Table I. Molecular Characterization of Block Copolymer A-B-A Having $P_B=64$

Code	A-Mol%	P_A	X_H
	(1) PBLG-PB-PBLG		
GBG-1	67.5	53	0.681
GBG-2	71.6	78	0.719
GBG-4	81.3	188	0.812
GBG-6	89.5	275	0.900
PBLG	100	588	1
	(2) PMLG-PB-PMLG		
MBM-1	84.4	176	0.851
MBM-2	89.2	266	0.891
MBM-3	91.5	346	0.915
PMLG	100	215	1
	(3) PCBL-PB-PCBL		
LBL-1	47.7	28	
LBL-3	76.3	98	
LBL-4	83.8	158	
LBL-5	88.1	226	0.880
PCBL	100	227	1

As is obvious from Table I, the A-mol% is in good agreement with X_H. This means that the A-block are perfectly in α-helical conformation in the solution. The same aspect was also confirmed for solid membranes cast from solution. The polybutadiene block chain should be in random coil conformation, thus we have Fig. 1 as a model. in which $\langle r^2_{B/2}\rangle$ and $\langle s^2_{B/2}\rangle$ are, respectively, the mean square end-to-end distance and mean square radius of gyration of the chain having $P_{B/2}$ chain length. An A-B-A

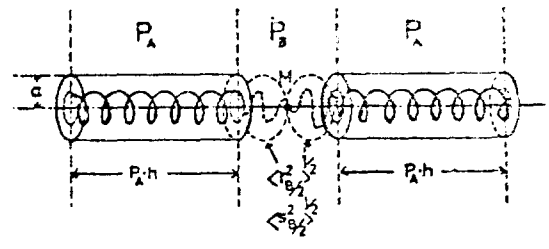

Figure 1.

chain of this type is regarded as a chain com-

pose of two A-B di-block chains connected at M, each consisting of an A-block of P_A length and a B-block of $P_{B/2}$ length.

Formation of Micro-heterophase Structure

At critical micelle concentration, each of the A and B blocks undergoes micro-phase separation and aggregates into characteristic micelles such as spherical, cylindrical, and lamella-like micelles, as illustrated in Fig. 2, in accordance with the copolymer composition, dimensions of blocks in the copolymer, and environmental conditions.

The Gibbs free energy ΔG per unit volume for the micelle formation is represented by

$$\Delta G = \Gamma \Delta W - T\Delta S \qquad (1)$$

where Γ is the area of A/B interface per unit volume of micelle. ΔW is the interfacial free energy per unit area of A/B interface, and Δs is the entropy change accompanied with the micelle formation. Now we assume that all the junction points of A-block and B-block locate on spherical, cylindrical, and planar interface, respectively, for spherical, cylindrical, and lamella-like micelle, and furthermore the midpoint M of the B-block chain locates at the center of micelle as illustrated in Fig. 2.

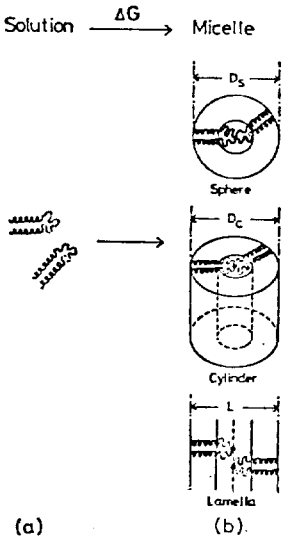

Figure 2.

The volume fraction φ_B of the domain occupied by B blocks relative to the total volume occupied by the copolymer in solution was given by

$$\varphi_B = [(4/3)\pi\langle s^2_{B/2}\rangle^{3/2}] / [(4/3)\pi\langle s^2_{B/2}\rangle^{3/2} + \pi a^2 P_A h] \qquad (2)$$

The number of junction point between A and B-block per unit volume of micelle is denoted by N.

$$N = 1/[(4/3)\pi\langle s^2_{B/2}\rangle^{3/2} + \pi a^2 P_A h] \qquad (3)$$

The A-block was assumed not to affect on ΔS, i.e., ΔS for micelle formation was regarded to be attributed only to the B-block chain. As mentioned above, a B-block chain is assumed to be composed of two Gaussian chains connected at M, neglecting the effect of the junction. Thus, ΔS is given by

$$\Delta S = N\Delta S_{B/2} = -\frac{3}{2}kN[(R^2_{B/2}/\langle r^2_{B/2}\rangle) - 1] \qquad (4)$$

where, $R_{B/2}$ is the end-to-end distance of B/2 chain in the micelle, and $r_{B/2}$ is that in solution at the critical micelle concentration.

Details of calculation will appear elsewhere[18]. The final results on the equilibrium micelle dimensions with respect to D_S, D_C, and L are as follows:

$$D_{s,eq} = [8\Delta W\langle r^2_{B/2}\rangle / kTN]^{1/3} \qquad (5)$$

$$D_{c,eq} = [16\Delta W\langle r^2_{B/2}\rangle / 3\varphi_B^{1/2} kTN]^{1/3} \qquad (6)$$

$$L_{eq} = [8\Delta W\langle r^2_{B/2}\rangle / 3\varphi_B kTN]^{1/3} \qquad (7)$$

If we have numerical data on $\langle r^2_{B/2}\rangle$, $\langle s^2_{B/2}\rangle$, and ΔW, then we can estimate the micelle dimensions from eqs (5)-(7). The polybutadiene used is mainly composed of trans-1, 4-butadiene, so we treat the B-block as poly(trans-1, 4-butadiene). The chain dimensions of B/2-chain in chloroform are estimated as[19,20] $\langle r^2_{B/2}\rangle^{1/2} = 41.34$Å and $\langle s^2_{B/2}\rangle^{1/2} = 16.88$Å. The interfacial free energy ΔW (erg/cm^2), on one hand, is equal to the interfacial tension γ_{AB} (dyne/cm),

and is related[21,22] to the surface tensions, γ_A and γ_B, of A-and B-component by the equations:

$$\gamma_{AB}=\gamma_A+\gamma_B-2\sqrt{\gamma_A^a\gamma_B^a}-2\sqrt{\gamma_A^b\gamma_B^b}-2\sqrt{\gamma_A^c\gamma_B^c}$$
$$=(\sqrt{\gamma_A^a}-\sqrt{\gamma_B^a})^2+(\sqrt{\gamma_A^b}-\sqrt{\gamma_B^b})^2+(\sqrt{\gamma_A^c}-\sqrt{\gamma_B^c})^2 \quad (8)$$

Where the surface tension is assumed to be the sum of three contributions, γ^a, γ^b, and γ^c, respectively, from dispersion force, polar force, and hydrogenbonding force. However, data are not available to use eq(8) directly for the present systems. An alternative method to obtain the quantities as γ_P^a, γ_P^b, and γ_P^c (P represent A or B) is to measure contact angle θ of various liquid L, of known values of γ_L, γ_L^a, γ_L^b, and γ_L^c, on component P. The work of adhesion W_a of liquid L on liquid P is represented by Dupre's equation[23]:

$$W_a=\gamma_L+\gamma_P-\gamma_{LP}$$
$$=2\sqrt{\gamma_L^a\gamma_P^a}+2\sqrt{\gamma_L^b\gamma_P^b}+2\sqrt{\gamma_L^c\gamma_P^c} \quad (9)$$

and is related to θ by

$$W_a=\gamma_L(1+\cos\theta) \quad (10)$$

So, we can obtain W_a from eq(10), and γ_P^a, γ_P^b, and γ_P^c are estimated from eq(9). Finally, we have γ_{AB} from eq(8). Though details are not shown here, ΔW's(erg/Å^2) obtained are 28.9×10^{-16}, 25.7×10^{-16}, and 21.1×10^{-16} for PBLG-PB, PMLG-PB, PCBL-PB, respectively.

Comparison of Micelle Dimensions Calculated with Electronmicrograph Data

Table Ⅱ. Dimension of Micelle

Code	φ_B	$D_{s,eq}$(Å)	$D_{c,eq}$(Å)	L_{eq}(Å)	D_{EM}(Å)
(1) PBLG-PB-PBLG ($\Delta W=28.9\times10^{-16}$ erg/Å^2, a=7.5Å)					
GBG-1-2	0.603			309	300 (lamella)
GBG-1-4	0.355		394		400 (cylinder)
GBG-4	0.288		437		450 (cylinder)
GBG-6	0.216	447			430 (sphere)
(2) PMLG-PB-PMLG ($\Delta W=25.7\times10^{-16}$ erg/Å^2, $a=6.0$Å)					
MBM-1-2	0.646			277	300 (lamella)
MBM-1-3	0.489		322		370 (cylinder)
MBM-1-4	0.280		426		450 (cylinder)
(3) PCBL-PB-PCBL ($\Delta W=21.1\times10^{-16}$ erg/Å^2, $a=9.0$Å)					
LBL-1	0.653			257	350 (lamella)
LBL-3	0.350		357		400 (cylinder)

GBG-series and LBL-series were cast from chloroform, but MBM-series was cast from chloroform containing 10% trifluoro ethanol. These solvents are helicogenic solvents for A-block chains and good solvents for B-block chains. The micelle dimension D_{EM} estimated from electron micrographs are compared with dimensions, $D_{s,eq}$, $D_{c,eq}$, and L_{eq}, calculated from eqs (5)-(7). Agreement between both values seems satisfactory.

Dynamic Mechanical Spectroscopy on Membranes

Dynamic mechanical spectroscopy is used for the characterization of multicomponent polymer systems such as micro-heterophase formed by block copolymer chains. Takayanagi's equivalent mechanical model[24] comprising of elements connecting partly in series and partly in parallel as shown in Fig. 3 was applied to the present systems.

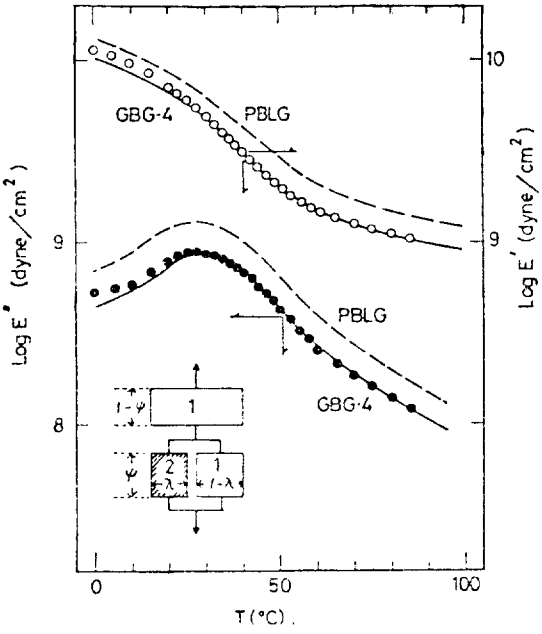

Figure 3.

The relative magnitude of λ to ϕ can be interpreted and representing the extent of parallel ans series character of the observed behavior, and $\lambda\phi$ is equal to the volume fraction φ_B of the inclusion phase (in this case, B-domain); i.e., $\varphi_B=\lambda\phi$. The dynamic Young's modulus E* for such model is given by

$$\frac{1}{E^*}=\frac{\phi}{E_B^*+(1-\lambda)E_A^*}+\frac{1-\phi}{E_A^*} \qquad (11)$$

The expression for E* can be separated into real and imaginary parts to give explicit expressions for the dynamic elastic modulus E′ and the loss modulus E''.

$$E'=|E^*|\cos\delta,\quad E''=|E^*|\sin\delta \qquad (12)$$

Fig. 3 shows the comparison of experimental result with theoretical curve for GBG-4 as an example. The numerical value of E′ for polybutadiene at the temperature range investigated here was cited from paper of Keskkula as $E'=3.45\times10^7$ dyne/cm². To estimate φ_B, it was assumed that polybutadien chains exist in unperturbed state in the membrane. The solid curves in Fig. 3 for GBG-4 were obtained with $\varphi_B=0.19$, $\lambda=0.56$, $\phi=0.34$.

Permeability of Water through Membrane

The hydraulic permeability of water K is defined by

$$J_f=K(\Delta P/\Delta X) \qquad (13)$$

where J_f is the flux of water per unit area of membrane of thickness ΔX. The most remarkable characteristic of the block copolymer membranes compared to homopolymer membrane is that the values of K for the former are dramatically higher than that of the latter. Such drastic increases in the K values for the A-B-A tri-block copolymer membranes should be attributed to the specific feature of the interfacial zone between A and B domains in the A-B-A block copolymer membranes. As mentioned above, interfacial zone is made up of coiled peptide residues near the end of the polypeptide chain and terminal residues of the amine-terminated polybutadiene. The NH and CO residues in this region do not be incorporated in the intramolecular hydrogen bondings of α-helix of polypeptide backbone, but can bind water through hydrogen bond. Thus, the amount of the bonded water molecules should closely relate to the volume of the interfacial zone. Furthermore, such bonded water may contribute to reduce the size of clusters of water. These factors may result in large K values for block copolymer membranes.

Tissue Compatibility of Block Copolymers

Dacron fabric coated with the copolymers was implanted[25] in the paravertebrate muscle of rabbits for 4 weeks, and then remove with the surrounding tissues. In Table Ⅲ, some preliminary results on the extents of foreign-body reaction observed under the microscope were

Table Ⅲ. Implantation tests

Sample	Butadiene Content (Mol%)	Extent of Foreign-body Reaction
GBG-1-1	50.8	(++)
MBM-1-2	43.3	(±)
LBL-1-2	42.3	(+)

shown. The order of increasing foreign-body reaction is MBM-1-2(±)<LBL-2(+)<GBG-1-1(++). MBM-1-2 seems to have fairly good issue compatibility, and LBL-2 is also not so bad.

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References and Notes

(1) E. C. Martin, P. D. May and W. A. McMahon, *J. Biomed. Mat. Res.*, **5**, 53 (1971).

(2) E. Klein, P. D. May, J. K. Smith and N. Leger, *Biopolymes.* **10**, 647 (1971).

(3) J. M. Anderson, A. Hiltner et al., *J. Biomed. Mat. Res. Symp.*, **3**, 25 (1972).

(4) C. H. Dohlman, D. Pavan-Langston and J. Rose, *J. Am. Ophtalol.* **4**, 823 (1972).

(5) J. M. Anderson et al., *J. Biomed. Mat. Res. Symp.*, **5**, 197 (1974).

(6) W. L. Sederel "The potential of copoly (α-aminoacids) base on L-aspartic acid and its esters as biomaterials", Ph. D. Thesis, Case Western, Res. U.

(7) E. Nyilas, *J. Biomed. Mat. Res. Symp.*, **3**, 97 (1972).

(8) D. J. Lyman et al., *Trans. Am. Soc. Artif. Intern. Organs*, **21**, 49 (1975).

(9) C. Sadron, *Pure & Appl. Chem.*, **4**, 347 (1962); *Angew. Chem.*, **75**, 472 (1963).

(10) T. Inoue, T. Soen, T. Hashimoto and H. Kawai, J. P. S., A-2, **7**, 1283 (1969).

(11) T. Uchida, T. Soen, T. Inoue and H. Kawai, *ibid.*, **10**, 101 (1972).

(12) D. J. Meier, J. P. S., C **26**, 81 (1969).

(13) S. Krause, *Macromolecules*, **3**, 84 (1970).

(14) A. Nakajima and T. Korenaga, *Bull. Inst. Chem. Res.* Kyoto Univ., **52**, 295 (1974).

(15) B. Perly, A. Douy and B. Gallot, *Makro. Chem.*, **177**, 2569 (1976).

(16) A. Douy and B. Gallot, *Polym. Eng. Sci.*, **17**, 523 (1977).

(17) Kindly supplied by Drs. K. Rieu and R. Drake of B. F. Goodrich Chem. Co., Cleveland, Ohio.

(18) A. Nakajima, K. Kugo, and T. Hayashi, *Macromolecules in press* (1979).

(19) F. T. Wall, *J. Chem. Phys.*, **11**, 67 (1943).

(20) M. Kurata, Y. Uchiyama, T. Koyama, and H. Fujita, Report at 12th Polymer Symposium of Soc. Polymer Science Japan (Nov. 5, 1963).

(21) Y. Kitazaki and T. Hata, *J. Adhesion Soc.* Japan **8**, 131 (1972).

(22) F. M. Fowkes, *Ind. Eng. Chem.*, **56**, 40 (1964).

(23) W. A. Zisman and F. M. Fowkes, "Contact Angle, Wettability and Adhesion", ACS Advances in Chemistry Series No. 43, 4, 60, 103 (1964).

(24) M. Takayanagi, *Mem. Fac.* Engineering Kyushu Univ., **23**, 41 (1963).

(25) Dr. Yasuharu Noishiki kindly carried out the implantation tests.