PREPARATION AND CHARACTERIZATION OF LIPOSOMES INCORPORATING HYDROPHOBIC POLY(AMINO ACID)S WITH DIFFERENT SECONDARY STRUCTURE

Akira SHIBATA,* Kazumi NAKAJIMA, Satoru UENO, and Takuya YAMASHITA
Faculty of Pharmaceutical Sciences, University of Tokushima, 1 Shomachi, Tokushima 770, Japan

Incorporation of hydrophobic poly(amino acid) into liposomal membranes was achieved by the polymerization of N-carboxy anhydrides of amino acids in the egg yolk phosphatidylcholine (EyPC) bilayer under reduced pressure at 30 °C. The trapped volumes for the liposomes with and without polypeptides were 8.1 - 10.3 $l\,mol^{-1}$ and 11.4 $l\,mol^{-1}$, respectively. The permeability barrier abilities of liposomal membranes were lowered by the incorporation of polypeptides into the membrane bilayers. Water permeation across the membrane bilayer was more effective for the liposome with the α-helical polypeptide than for that with the β-sheeted one.

KEYWORDS liposome; polypeptide; trapped volume; water permeability; secondary structure

The behavior of liposomes in vivo is strongly dependent upon lipid composition together with vesicle size and lipid dose [1]. Actually, there is a good correlation between the impact of the lipid composition on residence time in blood and uptake by tumors and various normal tissues [1]. From a wide variety of effects of lipid composition, one could expect that liposome-incorporating proteins also function characteristically in vivo because of their possible extension to the molecular self-organization, membrane permeability, and recognition [2]. Therefore, our attention was focused on the incorporation of a hydrophobic polypeptide in various conformations as a protein model into the liposomal membranes and on the evaluation of their liposome characteristics [3].

In the present study, the incorporation of hydrophobic polypeptides into EyPC liposomal membranes was examined by polymerization of N-carboxy anhydrides (NCA) of L-valine, L-leucine, and γ-dodecyl L-glutamate in the membranes. The difficulty of incorporation of poly(L-valine) (PLVal) or poly(L-leucine) (PLLeu) into the membrane bilayer was overcome by the use of the NCA of amino acids as a starting material for the incorporation under water vacuum at 30 °C. Characterization of the prepared liposome was performed by determination of the trapped volume, the barrier ability of the membrane bilayer to water permeability, and conformation of the polypeptide incorporated in the membranes.

Large unilamellar liposomes were prepared according to a modification of the ether injection method [4]. Three components of EyPC (86 mg), phosphatidic acid (PA) and the NCA of an amino acid (L-Val-NCA, L-Leu-NCA, and DoLG-NCA) were dissolved in a molar ratio of 1.0: 0.1:0.7 in a mixture (5.0 ml) of diethyl ether and dichloromethane (3:2 v/v). Polymerization of the amino acid was carried out by gradual injection of 4 ml of this solution with the aid of a mechanical drive through a needle into the pure water phase (6.0 ml) located within the internal chamber of the modified Liebig condenser. The apparatus was then placed under water vacuum (30 °C and 460 mmHg (1 mmHg=133.322 Pa)). Gel filtration (Cellulofine-CG-700) was used to remove any residual amino acid and organic solvent mixing with the liposome suspension. The size of the liposomes determined with a particle sizer (Nicomp model 370) was a heterogeneous mixture of about 70 nm and about 250 nm. To isolate the polypeptide from the liposomes incorporating the polypeptide, lyophilized liposomes were added to methanol. The precipitated polypeptide was filtered off, washed

© 1994 Pharmaceutical Society of Japan

NII-Electronic Library Service

with methanol, and dried in vacuo: yields were 15% for PLVal; 17% for PLLeu; 24% for PDoLG. Surface viscosities, η_s, as an indication of molecular weight were meausred by the monolayer technique [5]: η_s (surface poise, sp) at a fixed surface pressure of 1.0 mN m^{-1} was 0.009 for PLVal; 0.014 for PLLeu; 0.021 for PDoLG. The η_s value of 0.021 for PDoLG polymerized in the membrane bilayer is compared with the value of η_s=0.231 for bulk polymerized PDoLG with molecular weight of 32,000.

A. Barrier Ability of Liposomal Membrane to Water Permeation For an osmometer with a membrane completely impermeable to the solute, a proportionality exists between the volume change and the difference in bsmotic pressure of the outer and inner volumes. Since the initial shrinking rate, v_o, for the liposome is directly proportional to the initial rate of its volume change, dV/dt, the following relationship holds [6]:

$$v_o = (d(1/A)/dt)_{t=o}/(1/A_{t=o}) = k(dV/dt)_{t=o} \qquad (1)$$

where $A_{t=o}$ is the absorbance extrapolated to the time of glucose injection (t=0). Water permeability of the liposomal membrane is a sensitive function based on the extent of interaction between constituents in the membrane, and the rate of water permeation is closely correlated to the initial shrinking rate in the liposomes.

Figure 1 shows the time course of absorbance change of a liposome with PLLeu polymerized in the EyPC/PA bilayer membranes together with that of the polypeptide-free EyPC/PA liposome at 20 °C. The osmotic shrinking of liposomes was followed by the time course of turbidity at 450 nm using a rapid mixing device (within less than 0.5 s) with 50 mM hypertonic glucose solution. The initial shrinking rates (initial rate of the absorbance change) for the various liposomes are given in Table I. There are explicit differences in the initial shrinking rates of the liposomes with different polypeptides, and the shrinking rate is in the order of the liposome with PLLeu > the liposome with PDoLG > the liposome with PLVal > the polypeptide-free liposome.

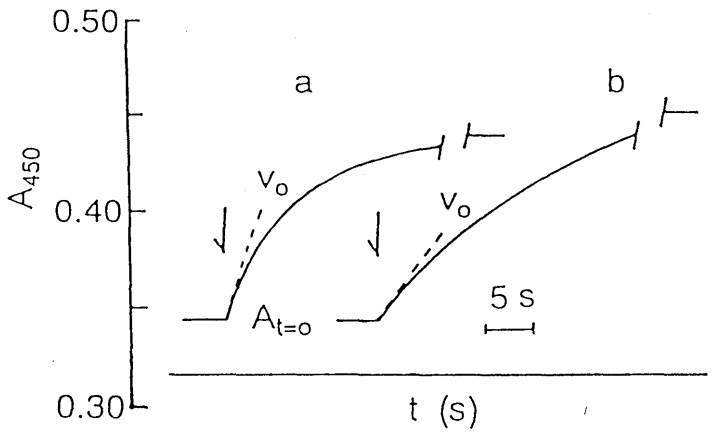

Fig. 1.
Time Courses of Absorbance Change at 450 nm Due to Osmotic Shrinking for Liposomes of EyPC/PA/PLLeu (a) and EyPC/PA (c) Caused by Addition of Hypertonic Glucose Solution
v_o: initial shrinking rate.
At the moment ($A_{t=o}$) indicated by the first arrow, the liposome suspension was mixed with hypertonic glucose solution.

B. Trapped Volume

The trapped volume, estimated by the solute diffusion method [7], of the liposomes with and without polypeptides is also given in Table I. The trapped volume tends to be decreased by the incorporation of polypeptides into the EyPC membrane bilayers.

Table I. Initial Shrinking Rate (v_o) and Trapped Volume (V_t) of EyPC Liposomes with and without Polypeptides

Liposome	v_o (x10^{-2} s^{-1})	V_t (lmol^{-1})
PLLeu-EyPC	1.56	8.8
PDoLG-EyPC	1.21	8.1
PLVal-EyPC	0.95	10.3
EyPC	0.70	11.4

NII-Electronic Library Service

C. Secondary Structure of Polypeptide Figure 2 shows the infrared spectra for PLLeu, PLVal, and PDoLG isolated from liposomes with polypeptides prepared under a reduced pressure of 450 mmHg at 30 °C, together with PDoLG polymerized in 1,4-dioxane. The polypeptide having an α-helix shows major peaks at about 1650 cm^{-1} (amide I band) and at about 1550 cm^{-1} (amide II band). The β-sheet generally gives rise to bands at lower frequency (at about 1630 cm^{-1} for amide I band and at about 1525 cm^{-1} for amide II band) than the α-helix. IR spectra indicated that these polypeptides were incorporated into the liposomal membranes with the mixed conformation of the α-helix and β-sheet for PLLeu and PDoLG, and with the single conformation β-sheet for PLVal. The difference in water permeability through the bilayer membranes between the liposomes with PLLeu and PLVal may be attributed to the secondary structure of the polypeptides in the membranes (Table I). The PDoLG polymerized in 1,4-dioxane takes the α-helical conformation (Fig. 2-d). However, the conformations of PLLeu, PLVal, and PDoLG polymerized in the membrane-free aqueous phase were enhanced in the β-sheet (data not shown). Taking into account the fact that the content of the secondary structure for PLLeu and PDoLG polymerized in the liposomal membranes is larger for the α-helix than for the β-sheet, the liposomal environments may promote the formation of the α-helix in polymerization of the amino acid-NCA.

The method of liposome preparation used here can be widely applied to incorporation of polypeptides with various conformations into the liposomal membranes.

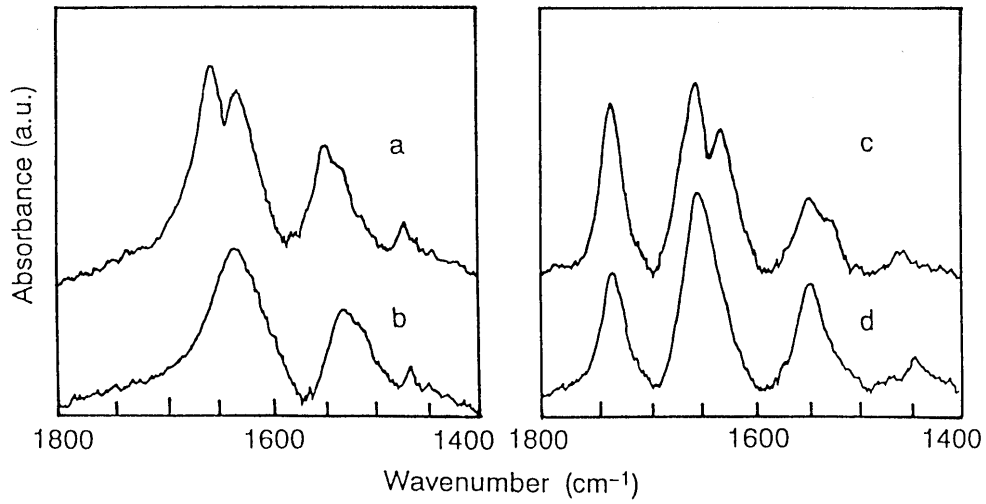

Fig. 2. Infrared Spectra in the Amide I and II Bands Region for PLLeu (a), PLVal (b) and PDoLG (c) Isolated from Mixed EyPC/PA/Polypeptide Liposomes Prepared under Reduced Pressure at 30 °C and for PDoLG (d) Polymerized in 1,4-Dioxane

REFERENCES

1) A. Gabizon, D. Papahadjopoulos, Proc. Natl. Acad. Sci. USA, 85, 6949 (1988).
2) R.L. Juliano, "Liposomes," ed. by M.J. Ostro, Marcel Dekker Inc., New York, 1983, pp. 53-86.
3) A. Shibata, S. Yamashita, Y. Itoh, T. Yamashita, Biochim. Biophys. Acta, 854, 147 (1986).
4) D.W. Deamer, A.D. Bangham, Biochim. Biophys. Acta, 443, 629 (1976).
5) L.G. Fourt, W.D. Harkins, J. Phys. Chem., 42, 897 (1938).
6) T. Inoue, H. Kamaya, I. Ueda, Biochim. Biophys. Acta, 812, 393 (1985).
7) D.W. Deamer, Ann. N. Y. Acad. Sci., 308, 250 (1978).

(Received February 16, 1994; accepted March 24, 1994)

NII-Electronic Library Service