pH-Responsive Liposomes Which Contain Amphiphiles Prepared by Using Lipophilic Radical Initiator†

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ABSTRACT: A lipophilic azo radical initiator having two *N,N*-dioctadecylamide groups was prepared, and oligomerization of acrylic acid was carried out by using the initiator. The amphiphiles obtained formed a stable liposome with dipalmitoylphosphatidylcholine (DPPC), and the liposome showed a pH-responsive release of fluorophore, Eosin Y, from an inner water pool of the liposome to the bulk phase. Effects of the degree of oligomerization, content of the liposome, salt concentration, and temperature on the pH sensitivity were examined. In conclusion, the amphiphiles can be used as a component of a pH-responsive device.

Polymer systems that are highly sensitive to the external stimuli, such as light, electric field, pH, heat, and so on, have been studied during these 10 years in various research fields, such as medical engineering, microelectronics, information technology, and bioengineering. In this report we examined the pH-responsive release of the fluorophore from the inner water pool of liposomes, which are composed of acrylic acid oligomer-carrying lipid and phosphatidylcholine, to the bulk phase.

To reduce the side effects of antitumor drugs for patients, selective targeting of the drugs to tumor cells has been extensively studied.[1] Since targeting using specific homing devices (monoclonal antibodies, for example) is still difficult to carry out, we have to seek a nonspecific targeting technique that utilizes differences in physical and chemical properties between malignant tumor cells and normal cells.

The microenvironment of tumor cells is more acidic than that of normal cells due to a larger amount of neuramic acid on the surface of the tumor cell.[2] In addition, tumor cells are very active in their metabolic functions, and they vigorously produce acidic compounds. When glucose is added to the medium, for example, tumor cells produce a large amount of lactic acid, which results in a lower pH around the tumor cells than around normal cells.[3] By taking this difference into consideration, several groups prepared a pH-sensitive drug delivery system for the chemotherapy of tumors.[1] Previously, we reported a pH-responsive release of Eosin Y from the *N*-methacryloylhomocysteine-containing polymerized liposomes.[4] Tirrell and his co-workers have been studying the pH-responsive interaction between poly(acrylic acid) derivatives and phosphatidylcholine liposomes.[5] They reported the pH sensitivity of phosphatidylcholine liposomes covered with poly(2-ethylacrylic acid) carrying anchor groups too.[6]

In this regard, therefore, we prepared amphiphilic compounds by the oligomerization of a hydrophilic monomer, acrylic acid, using a lipophilic initiator. By dispersing the amphiphilic compounds obtained and phospholipid into water, we obtained stable liposomes. We examined the usability of the amphiphiles as a component of pH-responsive devices.

Experimental Section

Materials. Disuccinimidyl 4,4′-azobis(4-cyanovalerate) (abbreviated as A-501 hereafter) was prepared by mixing 4,4′-azobis(cyanovaleric acid) (V-501; donated from Wako Pure Chemicals, Osaka, Japan) with 1.25 equiv of *N*-hydroxysuccinimide in the presence of 1.5 equiv of 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride in dry THF/CH_3CN at room temperature overnight. After evaporation A-501 was purified by washing with cold water and dried in vacuo. Yield: 25%. Anal. Calcd for $C_{20}H_{22}O_8N_6$: C, 50.63; H, 4.68; O, 27.00; N, 17.71. Found: C, 51.20; H, 4.71; O, 27.05; N, 17.04. IR: 2970 (ν_{as} of CH_2), 2890 (ν_s of CH_2), 2290 (C≡N), 1740 (C=O of ester), 1635 cm^{-1} (C=O of succinimide).

A lipophilic radical initiator, DODA-501 (Figure 1a) was prepared by the reaction of dioctadecylamine (Fluka, Switzerland; recrystallized twice from acetone before use; abbreviated as DODA hereafter) with 0.8 equiv of A-501 in dry THF/$CHCl_3$ (2:1). The amphiphile was purified by silica gel chromatography (mobile phase, hexane:ethyl acetate = 3:1). The compound purified was finally precipitated in acetonitrile. Yield: 26.4%. Anal. Calcd for $C_{84}H_{162}N_6O_2$: C, 78.32; H, 12.67; N, 6.52. Found: C, 78.23; H, 12.88; N, 6.42. Mp: 49.5 °C. IR: 2910 (ν_{as} of CH_2), 2830 (ν_s of CH_2), 2300 (C≡N), 1630 cm^{-1} (C=O of tertiary amide). ^1H NMR ($CDCl_3$): δ 0.9 (12 H, t, $-CH_3$), 1.1-1.6 (132 H, m, $-CH_2$), 1.7 (6 H, t, $-C(CH_3)-$), 2.5 (4 H, t, $-CH_2C(=O)-$), 3.1-3.5 (8 H, m, $-NCH_2-$).

Succinic *N,N*-dioctadecylamide (DODA/SUC) was prepared by coupling dioctadecylamine (6.8 mmol) with succinic anhydride (11.3 mmol) in CH_2Cl_2/DMF (1:1) at room temperature overnight. After evaporation the oily mixture was dissolved in $CHCl_3$ and washed with 5 w/v % aqueous citric acid solution and water. After drying with anhydrous Na_2SO_4, the solvent was evaporated, and the white solid obtained was precipitated in acetonitrile. Yield: 79%. Anal. Calcd for $C_{40}N_{79}NO_3$: C, 77.23; H, 12.80; N, 2.25. Found: C, 77.41; H, 13.07; N, 2.35.

L-α-Dipalmitoylphosphatidylcholine (DPPC) of the highest grade (98.2% by HPLC) was kindly donated from Wako Pure Chemicals, Osaka, Japan. Acrylic acid was purified by the distillation in vacuo. Polyacrylic acid (HPAA; M = 90 000; Aldrich, Milwaukee, WI) was purified by an ultrafiltration technique (Amicon 202 type cell, membrane XM-50 (exclusion limit 50 000)), and after filtration through an Acrodisc membrane filter (pore size 0.45 μm; Gelman Science Japan, Tokyo, Japan), HPAA was lyophilized. For preparation of aqueous sample solutions, deionized water was distilled just prior to use.

Preparation of Amphiphiles. Polymerization of acrylic acid using the radical initiator in homogeneous phase was carried out in dry THF (deoxygenated beforehand) in a tightly sealed test tube at 70 °C for 20 h. Molar ratios of acrylic acid and the initiator were 5:1, 15:1, 20:1, 30:1, 50:1, and 100:1. After evaporation, the unreacted lipophilic initiator and monomer were removed by washing with hexane using a centrifuge at 3000 g and 5 °C for 30 min. The precipitates were further washed with a 0.2 M aqueous NaCl solution. Polyacrylic acid formed by chain transfer via the solvent could be removed by washing with the salt solution and subsequent centrifugation (spontaneous polymerization was confirmed to be absent). The precipitates were dispersed in water by using a sonicator (Astrason W-385,

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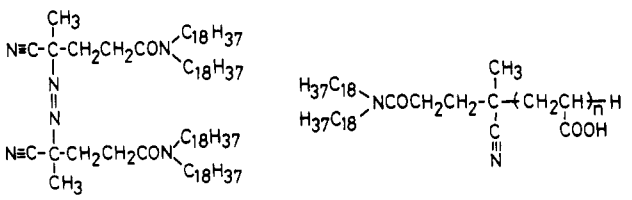

(a) DODA-501 (b) DODA/PAA

Figure 1. Chemical structures of DODA-501 (a) and DODA/PAA (b).

Heat Systems-Ultrasonics Inc., Farmingdale, NY), and after removal of NaCl by gel permeation chromatography (Sephadex G-10, i.d. 2 × 15 cm), the suspension was lyophilized to give the amphiphilic compound DODA/PAA (Figure 1b).

Characterization of Liposomes. Diameters of liposomes were estimated by using a dynamic light-scattering (DLS) apparatus (BI 2230, Brookhaven, NY, light source; He–Ne laser, 6328 Å, NEO-15 MS, Japan Science Engineering, Osaka, Japan).[7] Phase transition phenomena of the liposomes were followed by differential scanning calorimetry (SSC 580, Daini-Seikosha, Tokyo, Japan). The rate of temperature rise was 2 °C min^{-1}.

Characterization of Amphiphiles. The degree of polymerization ($\overline{DP}$) of the amphiphiles was estimated from the number of protons (-CH_2CH(COOH)- (δ 1.5–2.2), -CH(COOH)- (δ 2.2–2.7), and -(CH_2)- (δ 1.2–1.5)) (in $CDCl_3$–CD_3OD (1:1)) as evaluated by using a 270-MHz ^{1}H NMR apparatus (JSX 270, JEOL, Tokyo, Japan).

pH-Responsive Release of Eosin Y. The amphiphiles obtained and DPPC were dissolved into a THF/chloroform mixture in a small round-bottomed flask. After evaporation of the solvent using a vacuum pump, the lipids, which formed a thin film on the inner wall of the flask, were dispersed into *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (5 mM, pH 8.0) containing 0.1 M Eosin Y (a fluorophore that is known to show self-quenching of fluorescence intensity)[4] using a vortex mixer and the ultrasonicator at 50 °C for 15 min. The Eosin Y entrapped in liposomes was separated from free Eosin Y by gel permeation chromatography (Sephacryl S-1000 Superfine, Pharmacia, i.d. 2 × 20 cm).

The liposome suspension was quickly mixed with an equal volume of 5 mM HEPES at various pH values (adjusted by 0.1 N HCl), and the release of Eosin Y from the liposome was followed by using a fluorescence stopped-flow spectrophotometer (RA-401, Otsuka Electronics, Hirakata, Osaka, Japan) (light source D_2 lamp, Model L1314, Hamamatsu Photonics, Hamamatsu, Japan; excitation 305 nm, emission >460 nm (cut filter, Hoya Y 46)) at 35 °C. The dead time of the apparatus was claimed to be about 1 ms by the manufacturer. The pressure of nitrogen gas to drive the mixing equipment was 4 atm. The observation cell was thermostated at 35 ± 0.05 °C by using a Neslab RTE-8 waterbath.

Association Processes of Polyacrylic Acid with DPPC Liposome. By mixing with a HPAA solution, the DPPC liposome suspension became more turbid due to an association of the polymer onto the liposome surface.[8] To avoid coagulation of the liposomes via HPAA molecules, a molecular concentration (not equivalent concentration) of HPAA was set to be in excess of the particle concentration of the liposomes ($\simeq 10^4$ times). The average diameter of the DPPC liposome and the occupied area per one lipid molecule in the gel state were 1700 Å and 48 Å^2,[9] respectively. The association process could be followed by the increase in turbidity at 300 nm using the RA-401 stopped-flow apparatus. The apparent first-order rate constant, k_{obs}, was estimated from the initial exponential curve of the increase in turbidity.

The turbidity change of the DODA/PAA–DPPC liposome suspension induced by the pH change was followed by the same method.

Recovery of Lipids. Amounts of the lipids (DODA/PAA and DPPC) contained in the liposomes were determined before and after passing through the GPC column by conductometric titration (DODA/PAA) or the ammonium molybdate method (DPPC),[10] respectively.

Table I
Oligomerization of Acrylic Acid (AA) by Using DODA-501 as Initiator[a]

DODA-501:AA	yield,[b] %	$\overline{DP}$
1:5	36(50)	7
1:15	59(83)	21
1:20	29(54)	37
1:20	73(45)	10[c]
1:30	89(83)	28
1:50	56(85)	76
1:50	50(92)	91[c]
1:100	54(84)	156

[a] In THF (10 mL) at 70 °C for 20 h; DODA-501, 64.3 mg (0.05 mmol). [b] Ratios of the amounts of the DODA group supplied and recovered. In the parentheses, those of the amounts of acrylic acid. [c] In the presence of diethyl 2-allylmalonate (0.05 mmol).

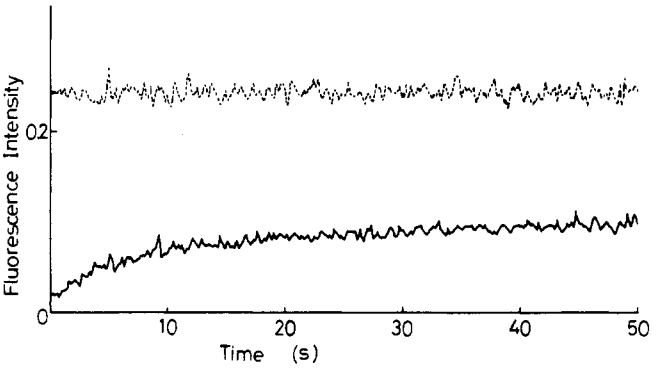

Figure 2. Typical profile of the release of Eosin Y from the DODA/PAA–DPPC liposome at 35 °C. $\overline{DP}$ of DODA/PAA, 76; DODA/PAA:DPPC = 1:1; initial pH, 8.0 (5 mM HEPES); final pH, 5.5. (- - -) After sonication (100% release).

Results and Discussion

A. pH-Responsive Release of Eosin Y. Table I shows the degree of polymerization and yields of amphiphiles prepared at various molar ratios of the initiator and acrylic acid. The diameters of the mixed liposomes of DODA/PAA and DPPC were about 1700–1800 Å. By mixing the suspension of the mixed liposome with an acidic HEPES solution, we could observe a rapid release of Eosin Y from the inner waterpool of the liposome as exemplified in Figure 2.

The percent release of Eosin Y through the liposome membrane in 50 s evaluated from Figure 2 was strongly influenced by the pH value after mixing as exemplified in Figure 3. When a suspension of DPPC liposomes was mixed with an acidic HEPES solution, on the contrary, we did not observe a distinct pH-responsive release of Eosin Y. Such a pH-responsive release from the mixed liposomes is due to the hydrogen bonding of carboxyl groups of the acrylic acid residues of the DODA/PAA with the phosphate group of DPPC.[5] This will be discussed in a later part (part B).

Figure 4 shows the effect of the content of the liposome on the pH sensitivity. By the increase in the weight content of DPPC in the DODA/PAA ($\overline{DP}$ = 76)–DPPC mixed liposome, the pH sensitivity around pH 6 became larger (DODA/PAA–DPPC 2:1 → 1:1 → 1:2), and after passing the maximum (at 1:4), the sensitivity became smaller (at 1:10 and 0:1). Furthermore, the physical stability of the liposomes that contained a large amount of DODA/PAA was not so high (we could confirm the presence of liposomal structures in the suspension of

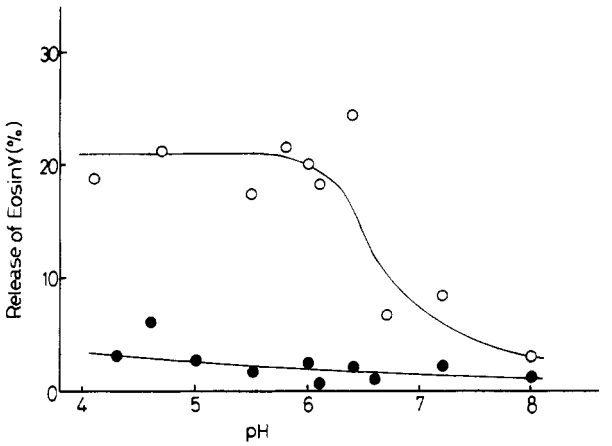

Figure 3. Effect of pH on the percent release of Eosin Y from the liposomes in 50 s at 35 °C. Initial pH, 8.0; $\overline{DP}$ = 76. (○) DODA/PAA-DPPC (1:1); (●) DPPC.

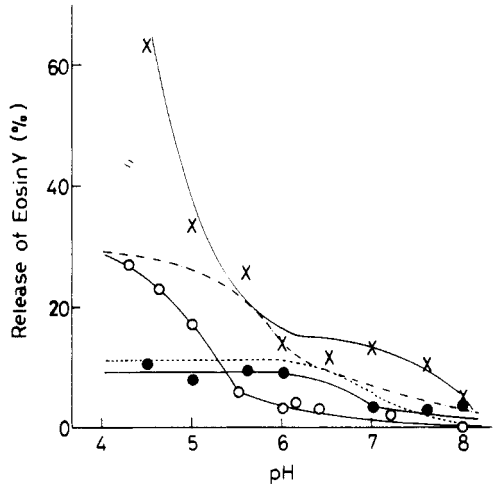

Figure 4. Effect of the content of the liposome on the release of Eosin Y from liposomes in 10 s at 35 °C. $\overline{DP}$ = 76. (●) DODA/PAA:DPPC = 1:10; (×) 1:4; (– · –) 1:2; (· · ·) 1:1; (○) 2:1.

DODA/PAA ($\overline{DP}$ = 76) by the capability to encapsulate Eosin Y. However, the percent recovery of the DODA/PAA liposomes from the GPC column was low due to the release of DODA/PAA molecules themselves from the liposomes). These results show that the coexistence of DPPC and DODA/PAA molecules is essential to get pH-sensitive liposomes.

Figure 5a shows the effect of pH on the release of Eosin Y in 50 s from the DODA/PAA-DPPC liposomes with various $\overline{DP}$ values, and Figure 5b shows the effect of the $\overline{DP}$ value on the percent of release at pH 5.5 (DODA/SUC is considered as DODA/PAA with $\overline{DP}$ = 1). By the increase in the degree of oligomerization ($\overline{DP}$) the pH sensitivity increased and, after passing a maximum at $\overline{DP}$ = 37, decreased.

We checked the percent of recovery of DODA/PAA and DPPC in the mixed liposome suspensions from the GPC column. The recovery of DODA/PAA monotonously decreased with $\overline{DP}$ (83% ($\overline{DP}$ = 7), 65% ($\overline{DP}$ = 21), 58% ($\overline{DP}$ = 28), 50% ($\overline{DP}$ = 37), 30% ($\overline{DP}$ = 76), and 27% ($\overline{DP}$ = 91)) whereas that of DPPC was quantitative. The percents of release of Eosin Y per 1 mg of DODA/PAA in 50 s, therefore, were estimated to be 0.5% ($\overline{DP}$ = 7), 7% ($\overline{DP}$ = 21), 15% ($\overline{DP}$ = 28), 27% ($\overline{DP}$ = 37), 14% ($\overline{DP}$ = 76), and 18% ($\overline{DP}$ = 91), respectively. These results

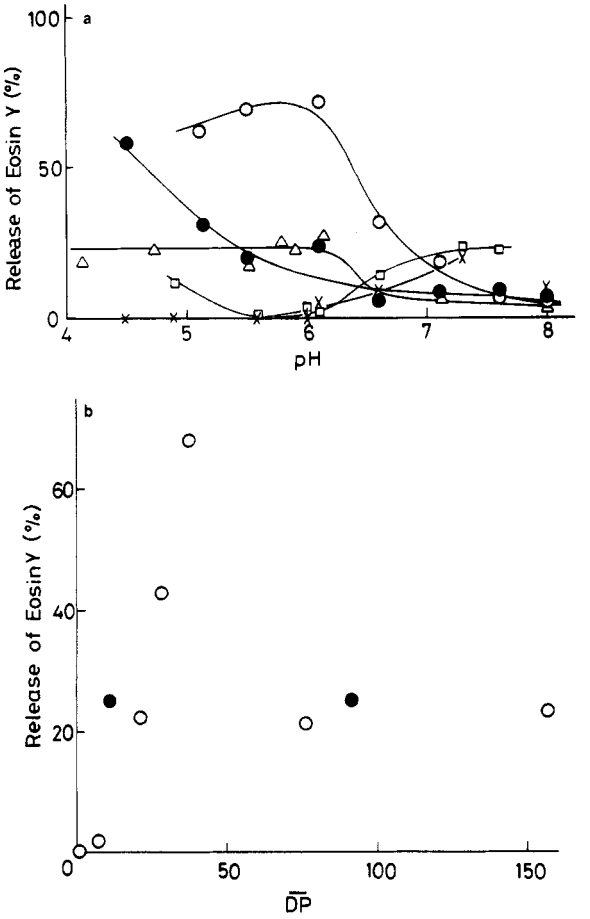

Figure 5. (a) Effect of $\overline{DP}$ on the release of Eosin Y from the DPPC-DODA/PAA liposome in 50 s at 35 °C. DODA/PAA:DPPC = 1:1: $\overline{DP}$ (□) 7, (○) 37, (△) 76, (●) 156. DODA/SUC: DPPC = 1:1 (×). (b) Effect of $\overline{DP}$ on the percent of release in 50 s at pH 5.5 and 35 °C. Initial pH, 8.0; DODA/PAA:DPPC = 1:1. (○) Prepared without diethyl 2-allylmalonate ($\overline{DP}$ = 1 corresponds to DODA/SUC). (●) Prepared with diethyl 2-allylmalonate.

suggest that an optimum degree of oligomerization might be required to realize an effective release at weakly acidic regions. For realization of distinct pH sensitivity some acrylic acid residues per one DODA group might have to associate with the polar head groups of DPPC molecules. There is a possibility that a contribution of acrylic acid residues on the pH sensitivity decreases with distance from the DODA group (in other words, distance from the liposome surface), though we do not have any direct evidences.

The mixed liposome of DPPC with DODA/SUC or DODA/PAA ($\overline{DP}$ = 7) showed no large release at acidic regions, and moderate release at basic conditions, though we cannot explain this phenomenon at the present time.

The amphiphile may have two hydrophobic DODA groups at its ends due to the termination reaction between propagating oligomer chains. By polymerization in the presence of a degradative chain-transfer agent, diethyl 2-allylmalonate,[11] we could obtain the amphiphile with only one hydrophobic DODA group. There was, however, no distinct difference between the pH sensitivity of amphiphiles whether they were prepared in the presence of diethyl 2-allylmalonate or not. Therefore, we cannot definitely say whether the substitution of two DODA groups into the oligoacrylic acid did occur or not in the absence of diethyl 2-allylmalonate.

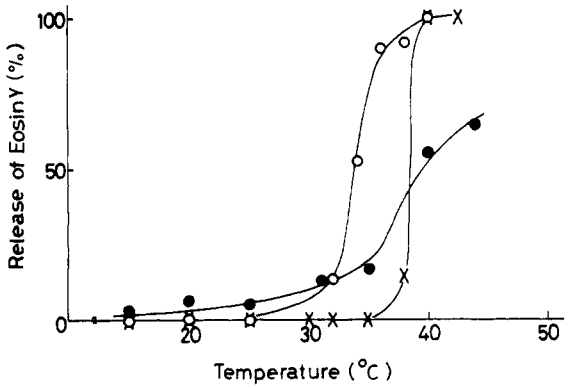

Figure 6. Effect of temperature on the release of Eosin Y from the liposomes in 50 s: initial pH, 8.0; final pH, 5.0; $\overline{DP}$ = 76. (×) DPPC; (●) DODA/PAA-DPPC (1:1); (○) DODA/PAA-DPPC (1:4 in 10 s).

Table II
Temperature of the Midpoint of Phase Transition (T_m)

DODA/PAA:DPPC[a]	T_m, °C pH 6	pH 8
1:1	40.7	42
1:4	40.7	39.5
1:0	34[b]	51[b]
0:1[c]	40.5	41

[a] Weight ratio. $\overline{DP}$ of DODA/PAA = 76. [b] Very broad peak. [c] Lit. 41 °C.[12]

Figure 6 shows the effect of temperature on the release of Eosin Y from the DODA/PAA-DPPC liposome at pH 5.0. In the range between 10 and 25 °C there was no high pH sensitivity. When the temperature was raised to 30–40°C the pH sensitivity was highly increased. By DSC measurement, temperatures of the midpoint of the phase transition (T_m) of the DODA/PAA ($\overline{DP}$ = 76)-DPPC liposome (1:1) at pH 8 and 6 were 42 and 40.7 °C, respectively (Table II). This means that the perturbation of the packing of lipid molecules by the association with acrylic acid residues, which causes a rapid release of Eosin Y, did not affect the T_m value of the liposomes. A similar tendency was previously reported concerning the effect of poly(acrylic acid) derivatives on the DSC behavior of phospholipid liposomes.[5]

The prompt perturbation of the liposomal structure by the reduction of pH might cause the rapid release of the inner content even in the temperature regions lower than T_m. However, the structure of the liposome would be gradually recovered afterward, although we have no direct evidence for such relaxation except for the DSC data, which reflect the packing of lipid molecules long (>20 min) after the pH reduction.

It should be mentioned here that at the phase transition region release of Eosin Y from all the liposomes examined was very high (Figure 6). In physiological temperature ranges around 35 °C, however, the DPPC liposome showed a much smaller pH sensitivity than the DODA/PAA-DPPC liposome.

We also examined the salt effect on the pH sensitivity. Figure 7 shows that, by the increase in salt concentration, the pH profile shifted to more acidic regions probably because of the changes in the ionization of PAA.

B. Motive Force of the pH Response. As mentioned above the oligoacrylic acid (DODA/PAA)-carrying liposomes showed a distinct pH sensitivity. To clarify the reason for this phenomenon, we investigated the association process of polyacrylic acid (HPAA) with DPPC liposomes. Under acidic conditions, HPAA quickly associated with the DPPC liposome as detected by an increase in turbidity at 300 nm (Figure 8). Under weakly alkaline conditions (pH 8.0), however, polyacrylate anion did not associate with the DPPC liposome at all. The pK value of the phosphate group of lecithin was reported to be 2–3,[13] and the DPPC molecule is electrically neutral at the pH regions examined in this work, which excludes the probability that the binding of HPAA to the liposome surface is due to the electrostatic interaction between a carboxyl group of acrylate residues and the quaternary ammonium group of the DPPC molecule.

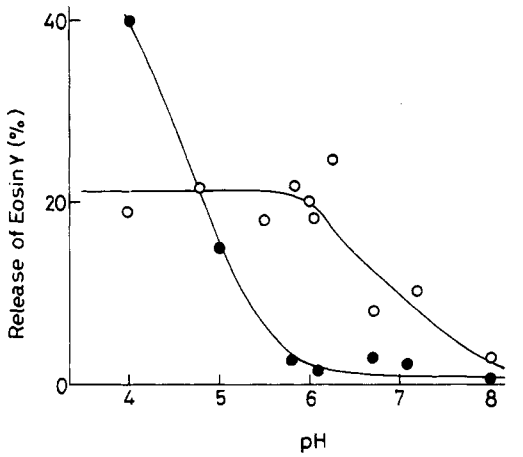

Figure 7. Effect of salt concentration on the pH-responsive release of Eosin Y in 50 s from the liposome at 35 °C: initial pH, 8.0. (○) [NaCl] = 0 M, (●) 0.15 M. DODA/PAA-DPPC (1:1) $\overline{DP}$ = 76.

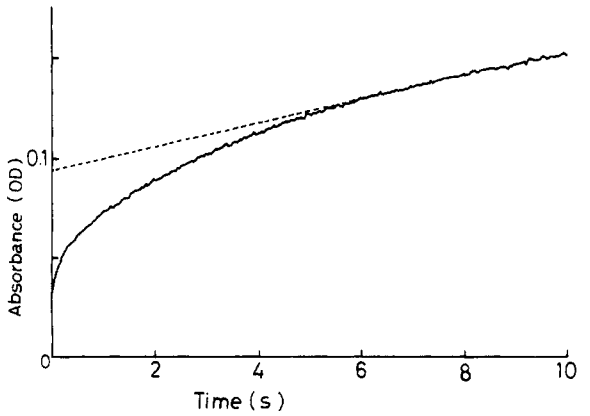

Figure 8. Typical profile of the turbidity change after mixing the DPPC suspension with HPAA solution at pH 5.0 and 25 °C. [DPPC] = 0.68 mequiv·L^{-1}, [HPAA] = 25 mequiv·L^{-1} = 20 μM (molecular concn). M_w of HPAA was 90 000.

By the addition of urea, which is known to destroy hydrogen bonding,[14] the association of HPAA with the DPPC liposome at pH 5.0 was highly decelerated (Figure 9), though the size of the liposome was not affected by the presence of urea as confirmed by the DLS technique ([urea] = 0 M, average diameter, 1750 Å; 0.5 M, 1770 Å; 1.0 M, 1770 Å). A more detailed result concerning the association processes between HPAA and phosphatidylcholine liposome will be given elsewhere.[8] Furthermore, by the addition of urea into the acidic HEPES solution, the release of Eosin Y from the DODA/PAA-containing liposome was largely reduced, and the pH-dependent release was diminished.

These results suggest that the motive force of the association of poly- or oligoacrylic acid with phosphatidylcholine molecules is hydrogen bonding between a pro-

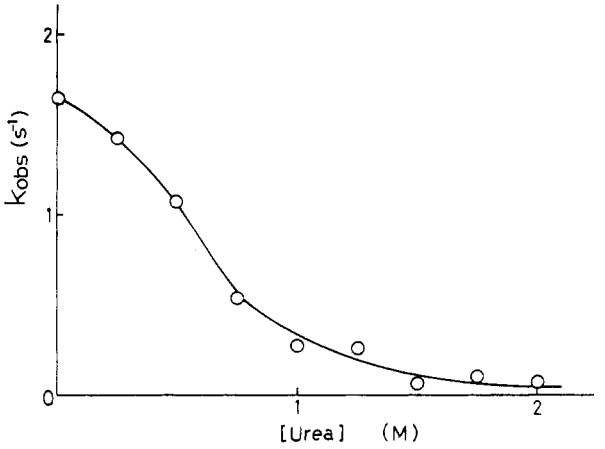

Figure 9. Effect of the concentration of urea on the apparent reaction rate constant, k_{obs}, at pH 5.0 and 25 °C. [DPPC] = 0.68 mequiv·L^{-1}, [HPAA] = 25 mequiv·L^{-1} = 20 μM (molecular concn). M_w of HPAA was 90 000.

tonated carboxyl group and a phosphate group.[5] As for liposomes that contain both phosphatidylcholine molecules and DODA/PAA molecules, hydrogen bonding largely perturbs the packing of lipid molecules (a phosphate group exists several Å from the polar end of the DPPC molecule), which results in a rapid release of Eosin Y from the inner waterpool. The increase in hydrophobicity of the DODA/PAA by the reduction of pH would be less important because the hydrophobicity of polyacrylic acid at low pH is not so high.[15] There was a report on the pH sensitivity of phosphatidylcholine liposomes covered with poly(2-ethylacrylic acid) carrying anchor groups.[6]

There is a possibility that the pH-responsive release is due to the coagulation and subsequent destruction of liposomes induced by the protonation of PAA chains on the liposome surface. We checked the time dependence of the diameter of the liposomes (DODA/PAA:DPPC = 1:1, $\overline{DP}$ = 76) after the change in pH from 10.5 to 3.5 by using the DLS technique. The diameter was constant within experimental uncertainties for several minutes and, after that, increased very slowly.

These bimodal processes were accompanied by the bimodal increases in turbidity, that is, a slight but rapid increase and a subsequent slow but large increase. The reciprocal of the relaxation time of the first slight increase was independent of the concentration of the liposomes, and the slope of the following larger increase was highly dependent on the liposome concentration.[8] These results suggest that the first step corresponds to the change in the liposomal structure and the second one to the coagulation of the liposomes, which excludes the possibility that the coagulation of liposomes induces the pH-responsive release in 50 s after the pH change.

In conclusion, liposomes composed of phosphatidylcholine and the amphiphilic compounds prepared by the oligomerization of acrylic acid using the lipophilic initiator showed a pH sensitivity. The amphiphilic compound prepared here is quite simple and can be easily prepared, and, furthermore, it can be quite easily incorporated into any liposomes. Therefore, it would be quite useful as a component of pH-responsive devices.

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References and Notes

(1) Tietze, L.-F. *Nachr. Chem., Tech. Lab.* **1988**, *36*, 728–737.
(2) Yamada, T. *Sci. Biol.* **1970**, *8*, 372.
(3) (a) Gullino, P. M.; Grantham, F. H.; Smith, S. H.; Haggerty, A. C. *J. Natl. Cancer Inst.* **1965**, *34*, 857. (b) Kahler, H.; Robertson, W. V. B. *Ibid.* **1943**, *3*, 495.
(4) Kitano, H.; Wolf, H.; Ise, N. *Macromolecules* **1990**, *23*, 1958–1961.
(5) Seki, K.; Tirrell, D. A. *Macromolecules* **1984**, *17*, 1692–1698.
(6) Maeda, M.; Kumano, A.; Tirrell, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 7455–7459.
(7) Ito, K.; Okumura, H.; Yoshida, H.; Ueno, Y.; Ise, N. *Phys. Rev. B* **1988**, *38*, 10852–10859.
(8) Kitano, H.; Akatsuka, Y.; Ise, N., to be submitted for publication.
(9) Levin, I. W. *Proceedings of the International Conference on Raman Spectroscopy*, 7th; North-Holland: Amsterdam, The Netherlands, 1980; p 528.
(10) Allen, R. J. L. *Biochem. J.* **1940**, *34*, 858.
(11) Tanaka, M.; Miyagama, T.; Shiraki, K. *Nippon Kokai* 63-54413.
(12) Chapman, D.; Williams, R. M.; Ladbrooke, B. D. *Chem. Phys. Lipids* **1967**, *1*, 445.
(13) Boggs, J. M. *Can. J. Biochem.* **1980**, *58*, 755.
(14) *Water—A Comprehensive Treatise*; Franks, F., Ed.; Plenum Press: New York, 1975; Vol 4.
(15) Ben-Naim, A. *Hydrophobic Interactions*; Plenum Press: New York, 1980; p 180.

Registry No. A-501, 130468-55-2; V-501, 2638-94-0; DODA-501, 130468-56-3; DODA/SUC, 130468-57-4; DPPC, 63-89-8; H_2C=$CHCO_2H$ (homopolymer), 9003-01-4; succinic anhydride, 108-30-5; dioctadecylamine, 112-99-2; *N*-hydroxysuccinimide, 6066-82-6; Eosin Y, 17372-87-1.