evolution of hydrogen gas ceased. The slurry was diluted with 10 mL of THF, and 2.75 g (9.0 mmol) of **8** was added. The heterogeneous light yellow mixture changed to a dark yellow viscous solution. The mixture was stirred at ambient temperature for 3 h, and the solvent was evaporated under reduced pressure to yield 4.18 g of crude **9** (68% yield). This material was about 80% pure by ^{1}H NMR. Purification by silica gel chromatography caused decomposition, so the product was used without further purification: mp 204-210 °C dec; TLC R_f = 0.4, 6% methanol-CH_2Cl_2; ^{1}H NMR δ 2.45 (s, 4 H, $COCH_2$), 2.68 (t, 4 H, J = 6.0, CH_2S), 3.58 (dd, 4 H, J_1 = 6.0, J_2 = 6.0, CH_2N), 4.52 (s, 4 H, $ArCH_2$), 6.50 (br s, NH), 7.56 (dd, 2 H, $J_{1,2}$ = 9.0, $J_{2,3}$ = 7.0, H-2), 7.62 (dd, 2 H, $J_{6,7}$ = 7.0, $J_{7,8}$ = 9.0, H-7), 7.77 (m, 4 H, H-3, H-6), 8.23 (d, 2 H, $J_{5,6}$ = 9.0, H-5), 8.35 (dd, 2 H, $J_{1,2}$ = 9.0, $J_{1,3}$ = 1.0, H-1), 8.41 (d, 2 H, $J_{7,8}$ = 9.0, H-8); MS (FD, 22 mA) m/z (relative intensity) 686 (M^+, 100).

***N,N'*-Bis[2-[[(9-phenoxyacridin-4-yl)methyl]thio]ethyl]butane-1,4-diamide (10).** A mixture of 3.0 g (4.36 mmol) of crude **9** and 56 g (596 mmol) of phenol was immersed in a 95 °C oil bath until the phenol melted. One 980-mg (17.4-mmol) portion was added of powdered KOH. The solution was stirred at 100 °C for 1 h and cooled slightly and the viscous solution poured into 650 mL of 1 M NaOH. This solution was washed four times with 50 mL of 10% 2-propanol-chloroform, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The solid residue was purified by flash chromatography (10% CH_2Cl_2-ethyl acetate) and then recrystallized from acetonitrile to yield 1.1 g (31%) of **10** as a light yellow powder: mp 167-169 °C; TLC R_f = 0.3, 10% CH_2Cl_2-ethyl acetate; IR (KBr) 3300, 1634 cm^{-1}; ^{1}H NMR δ 2.50 (s, 4 H, $COCH_2$), 2.74 (t, 4 H, J = 6.0, CH_2S), 3.65 (dd, 4 H, J_1 = 6.0, J_2 = 6.0, CH_2N), 4.56 (s, 4 H, Ar-CH_2), 6.73 (t, 2 H, J = 6.0, NH), 6.83 (d, 4 H, $J_{10,11}$ = 8.0, H-10), 7.03 (t, 2 H, $J_{11,12}$ = 7.0, H-12), 7.26 (m, 4 H, H-11), 7.41 (m, 4 H, H-2, H-7), 7.75 (m, 4 H, H-3, H-6), 8.02 (dd, 2 H, $J_{5,6}$ = 8.8, $J_{5,7}$ = 1.0, H-5), 8.05 (d, 2 H, $J_{1,2}$ = 8.5, H-1), 8.26 (d, 2 H, $J_{7,8}$ = 8.8, H-8); ^{13}C NMR (500 MHz) δ 31.20, 31.73, 32.06, 38.67, 115.53, 120.25, 120.25, 122.19, 122.54, 122.63, 125.52, 126.04, 129.92, 130.25, 130.56, 137.23, 148.65, 149.83, 155.36, 159.54, 172.16; MS (FD, 18 mA) m/z (relative intensity) 802 (M^+, 100), 401 (M^{2+}, 10); Anal. Calcd for $C_{48}H_{42}N_4S_2O_4$: C, 71.80; H, 5.27; N, 6.98; S, 7.99. Found: C, 71.71; H, 5.30; N, 7.10; S, 7.93.

1,5,10,14,33,38-Hexaaza-30,41-dithia[14.14](4,9)acridinophane-34,37-dione (4). To a solution of 365 mg (1.05 mmol) of spermine tetrahydrochloride in 1 L of dry methanol was added 825 mg (1.03 mmol) of **10** followed by 30 mL of methanol (1 mM in bisacridine). The reaction was stirred at reflux for 24 h and then concentrated at reduced pressure to yield 1.07 g of crude product. The solid was slurried in ethanol, absorbed onto silica gel, and flash chromatographed, eluting with 100% ethanol until the first yellow band came off. The column was eluted with 4% diethylamine-ethanol to produce 360 mg (42%) of **4** as a dark yellow oily solid. The tetrahydrochloride of **4** was formed by dissolving the free base in methanol and adding methanol saturated with gaseous HCl at 0 °C. The methanol was removed under reduced pressure to yield a light yellow powder. The solid was repeatedly dissolved in water and lyophilized to yield a fluffy canary yellow solid: mp 189-191 °C dec; TLC R_f = 0.30, 4% diethylamine-ethanol; IR (KBr) 1625 cm^{-1}; ^{1}H NMR (500 MHz, D_2O, 16 mM, 20 °C) δ 1.91 (br s, 4 H, H-7), 2.32 (br s, 4 H, H-35), 2.38 (t, 4 H, $J_{2,3}$ = 6.8, $J_{3,4}$ = 6.8, H-3), 2.54 (t, 4 H, $J_{31,32}$ = 5.7, H-31), 3.21 (br s, 4 H, H-6), 3.29 (br s, 8 H, H-4, H-32), 3.93 (br s, 4 H, H-29), 4.02 (t, 4 H, $J_{2,3}$ = 6.8, H-2), 6.80 (br s, 2 H, H-26), 7.32 (m, 4 H, H-18, H-25), 7.52 (d, 2 H, $J_{18,19}$ = 6.2, H-19), 7.59 (d, 2 H, $J_{24,25}$ = 8.3, H-24), 7.77 (d, 2 H, $J_{26,27}$ = 7.8, H-27), 8.00 (d, 2 H, $J_{17,18}$ = 8.6, H-17); ^{13}C NMR (500 MHz, D_2O) δ 24.92, 28.59, 33.06, 33.78, 34.03, 40.85, 47.07, 48.53, 48.87, 120.73, 126.07, 126.43, 126.47, 128.00, 137.82, 139.01, 160.55, 177.05 (missing resonances due to low S/N); MS (FAB) m/z (relative intensity) 817 (M^+ + 1, 100 for peaks m/z > 650). Anal. Calcd for $C_{46}H_{56}N_8S_2O_2 \cdot 4HCl \cdot 6H_2O$: C, 51.57; H, 6.78; N, 10.47; S, 5.97; Cl, 13.25. Found: C, 51.29; H, 6.19; N, 10.07; S, 5.64; Cl, 13.48.

***N,N'*-Bis[3-(9-acridinylamino)propyl]butane-1,4-diamine (Spermine Bisacridine) (5).** By the method outlined for **4** (1 mM in 9-phenoxyacridine), 356 mg (56%) of **5** was obtained as a yellow solid: mp >235 °C; TLC R_f = 0.3, 6% diethylamine-ethanol; ^{1}H NMR (D_2O) δ 1.87 (br s, 4 H, H-5'), 2.40 (m, 4 H, H-2'), 3.18 (br s, 4 H, H-4'), 3.29 (t, 4 H, $J_{2',3'}$ = 7.8, H-3'), 4.20 (t, 4 H, $J_{1',2'}$ = 7.2, H-1'), 7.55 (dd, 4 H, $J_{1,2}$ = 8.7, $J_{2,3}$ = 7.7, H-2), 7.66 (d, 4 H, $J_{3,4}$ = 8.5, H-4), 7.94 (dd, 4 H, $J_{3,4}$ = 8.5, $J_{2,3}$ = 7.7, H-3), 8.25 (d, 4 H, J = 8.7, H-1); MS (FD, 8 mA) m/z (relative intensity) 556 (M^+, 100). Anal. Calcd for $C_{36}H_{40}N_6 \cdot 4HCl \cdot 1.5H_2O$: C, 59.26; H, 6.49; N, 11.52. Found: C, 59.40, H, 6.59; N, 11.33.

Acknowledgment. We thank Heidi Guzalo for preliminary synthetic efforts, Jack Starmer and Lee Chenier for assistance with the DNA binding studies, and Dr. Lynn Elwell for assistance in preparing the plasmid used in these experiments. Funding from the donors of the Petroleum Research Fund, administered by the American Chemical Society, the American Cancer Society (Junior Faculty Award to S.C.Z.), and the National Science Foundation PYI Program (Grant CHE-8858202) is gratefully acknowledged.

Mutual Recognition between Polymerized Liposomes: Macrophage Model System by Polymerized Liposomes†

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Received June 30, 1988

Abstract: A macrophage model system using polymerized liposomes was examined. Recognition and subsequent attack of an avidin-carrying *partly* polymerized liposome by a biotin-carrying and phospholipase A_2 (PLA_2) carrying polymerized liposome were studied by the turbidimetry and fluorescence methods. While the hydrolysis of dimyristoylphosphatidylcholine (DMPC) molecules was lowered, the introduction of avidin and biotin onto the surface of liposomes promoted the concentrated attack of PLA_2 to DMPC molecules in a narrow region on the avidin-carrying liposome; an effective "uncorking" of the avidin-carrying liposome is thus realized. Effects of the surface morphology and the surface density of the complementary ligands on the recognition phenomena were examined.

Recognition of invading cells and subsequent attack on cells by other cells having immunological or phagocytic roles are essential processes in the protective system of living bodies. For example, activated macrophage cells are reported to attack tumor cells and make a lysis of the wall of the tumor cells.[1] To mimic such phenomena, we examined here the recognition and subsequent attack (lysis) of a model cell by another model cell using a polymerized liposome system.

† Presented at the 37th Annual Meeting of the Society of Polymer Science, Japan, at Nagoya, May 1988.

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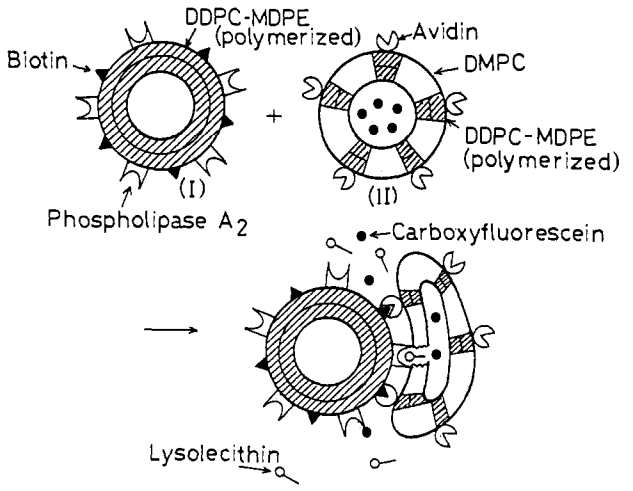

Figure 1. Schematic picture of the recognition and subsequent lysis of partly polymerized liposome II by polymerized liposome I.

Polymerized liposomes, which are lipid bilayers stabilized by polymer chains, were used here because they are quite suitable analogues of biomembranes which are physically stabilized by polypeptides and polysaccharides.[2-6] Previously, we examined a mutual recognition between polymerized liposomes modified with complementary ligands (trypsin and soybean trypsin inhibitor) as analogues of ligand and receptor on cell surfaces.[7] In this paper we have constructed a system that has a higher functionality (recognition, association, and lysis of the target cell) than the previous one (recognition, association, and inhibition of enzymatic activity).[7]

As analogues of ligand and receptor on cell membranes, we chose biotin and avidin here, because they have a strong affinity with each other ($K = 10^{15}$ M^{-1}).[8] We used a polymerized liposome which has phospholipase A_2 (PLA_2) and biotin on its surface (I) and an avidin-carrying *partly* polymerized liposome (II) as model cells (Figure 1). It should be noted here that we ignored the biological meaning (raison d'être) of biotin and avidin in nature. We only need a pair of compounds, which have a strong affinity with each other, to attain higher sensitivities and exclude complexities which might be caused by the dissociation of the associated products.

Experimental Section

Materials. Polymerizable lipids such as mono- and didienoylphosphatidylcholine (MDPC and DDPC, Figure 2) and mono-dienoylphosphatidylethanolamine (MDPE) were kindly donated by Dr. Yoshihiko Nagata, Tosoh Corp., Tokyo. L-α-Dimyristoylphosphatidylcholine (DMPC), avidin, phospholipase A_2 (PLA_2, EC 3.1.1.4, porcine pancreas, 725 units/mg), 1,6-disuccinimidylsuberate (DSS), and biotin hydroxysuccinimide ester were from Sigma, St. Louis, MO. Biotin and *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) were from Nacalai Tesque, Kyoto, Japan. Sulfosuccinimidyl 6-(biotinamido)hexanoate (NHS-LC-biotin) was from Pierce Chemical Co., Rockford, IL. Milli-Q grade water was used for a preparation of sample solutions.

Polymerized Liposomes. To prepare the liposome (I) in Figure 1, a mixed liposome made of didienoylphosphatidylcholine (DDPC) and monodienoylphosphatidylethanolamine (MDPE) (DDPC:MDPE = 9:1)

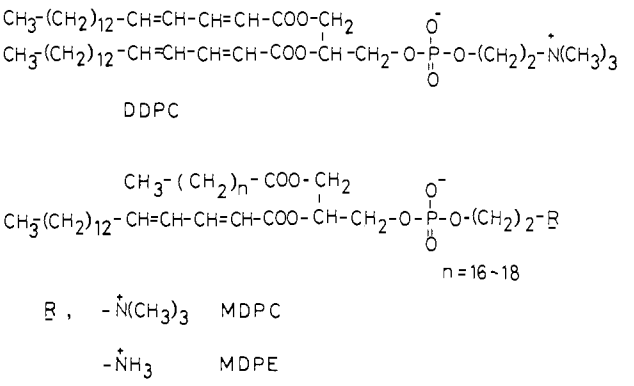

Figure 2. Chemical structures of polymerizable lipids used.

was prepared according to a conventional thin-layer formation–vortexing–ultrasonication method using a Bransonic 42 ultrasonifier (Branson, CT). The liposome suspension obtained (10 mg of lipid/2 mL of H_2O) was UV irradiated for 10 h with a mercury lamp (UI-501C, 250 W, Ushio Electric Co., Tokyo, Japan). The polymerization process of these lipids was followed by the decrease in absorbance of dienoyl groups at 258 nm.

To prepare the partly polymerized liposome (II), a suspension of mixed liposome composed of DDPC, MDPE, and DMPC (DDPC:MDPE:DMPC = 4:1:5) was UV irradiated. 6-Carboxyfluorescein (6-CF) was included in the liposome by dissolving 6-CF in the suspension prior to sonication and subsequent gel permeation chromatography (GPC, Sepharose 4B column, i.d. 2 × 14 cm) processes.

Modification of Liposomes. Phospholipase A_2 (0.30 mg) was immobilized onto the liposome with a bifunctional reagent (1,6-disuccinimidylsuberate, DSS).[7] An excess amount of DSS (5 equiv to the amino group of MDPE) was coupled with the MDPE-carrying polymerized liposome at pH 8.5 for 15 min to avoid a cross-linking of liposomes via the DSS molecule. After a prompt gel permeation chromatography [Sepharose 4B column, i.d. 2 × 10 cm; a fraction collector (KM-100, Pharmacia) was connected to a mini-UV detector (type II, Atto, Tokyo)], the activated liposome obtained was coupled with proteins at pH 8.0 and room temperature for 1 h and, afterward, at 10 °C overnight.

After purification of the protein-carrying liposome with GPC (Sepharose 4B column, i.d. 2 × 25 cm), biotin hydroxysuccinimide ester[9] (0.45 mg) was mixed with the liposome suspension (5 mg of lipid/mL of H_2O) to react with amino groups on the liposome surface at pH 8.5 and room temperature for 1 h and, afterward, at 10 °C overnight. The polymerized liposome modified with both biotin and PLA_2 was finally purified by GPC (liposome I). The amount of biotin bound to the liposome was estimated from the amount of unbound biotin in the eluate by HPLC [Model 440, Waters, Milford, MA; column, Cosmosil $5C_{18}$ (Nacalai Tesque, Kyoto, Japan); mobile phase, H_2O].

Similarly, the partly polymerized liposome (DDPC:MDPE:DMPC = 4:1:5) was coupled with avidin by use of DSS. The avidin-carrying partly polymerized liposome (liposome II) was finally purified by GPC. We also prepared a mixed liposome with another monomer ratio (DDPC:MDPE:DMPC = 1.5:1:7.5). The physical stability of the liposome was, however, very poor during the modification processes with avidin.

Spectrophotometric Measurements. Amounts of proteins, avidin ($A_{280}^{1\%} = 15.4$)[8b] and phospholipase A_2 ($A_{280}^{1\%} \simeq 12.4$),[10] bound to the liposomes were estimated from the absorbance of the eluate from the GPC column with a high-sensitivity spectrophotometer (SM-401, Union Giken, Hirakata, Japan). An association process of liposomes due to the mutual recognition was followed from the increase in turbidity at 300 nm by the same equipment. The observation cell was thermostated at 25 ± 0.05 °C by a Lauda K-2R water bath.

Hydrolytic Reaction. The hydrolysis of DMPC catalyzed by various kinds of phospholipase A_2 was followed with a pH-stat titrator (RTS-622, Radiometer, Copenhagen). The reaction vessel (5 mL) was thermostated at 25 ± 0.05 °C by a Neslab RTE-8 water bath. Nitrogen gas (CO_2 free) was passed through the suspension gradually before and during the reaction. The initial slopes of five experiments were averaged to estimate the initial reaction rate of the catalysis. The uncertainties of the reaction rates were within 10%.

Fluorescence Measurements. Releasing processes of 6-carboxyfluorescein (6-CF) from the liposomes were followed at 520 nm (exci-

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tation 310 nm) with a fluorescence spectrophotometer (FS-401, Union Giken). 6-Carboxyfluorescein is well-known to show a self-quenching phenomenon at high concentrations, and by dilution, it shows an intensive fluorescence.[11]

Dynamic Light Scattering Measurements. Diameters of the polymerized liposomes were estimated with a dynamic light scattering apparatus (BI 2230, Bookhaven, New York) with a He–Ne laser (6328 Å, NEO-15MS, Japan Science Engineering, Osaka, Japan).[12]

Differential Scanning Calorimetry (DSC). The phase-transition point of the liposomes was determined by differential scanning calorimetry on a SSC 580 (Daini-Seikosha, Tokyo, Japan). The concentration of lipid suspension was 25 mg·mL^{-1}, and the sample volume was 50 μL. The temperature raising rate was 0.5 °C·min^{-1}.

Microscopic Observation. To confirm the absence of aggregates of liposomes during the modification processes, an ultramicroscope (AXIOMAT, Carl-Zeiss) was used.

Fluorescence Anisotropy Depolarization. The lipid structural order of the polymerized liposome was measured with a fluorescence anisotropy depolarization method. The details of the apparatus were described elsewhere.[7] As a fluorophore, we used 1,6-diphenylhexatriene (DPH). The concentration of the liposome was 0.75 mg·mL^{-1}, and the molar ratio of DPH and lipids in the liposomes was 1:1000. The time dependences of the emission anisotropy, $r(t)$, were analyzed by setting

$$r(t) = (r_0 - r_\infty) \exp(-t/\phi) + r_\infty \quad (1)$$

where r_0 and r_∞ are the degree of anisotropy of DPH extrapolated to time zero and that in equilibrium distribution and ϕ is the relaxation time to approach the anisotropic equilibrium distribution of excited DPH.

Results and Discussion

DDPC Liposome System. By use of a dynamic light scattering technique, the average diameters of the liposomes before and after polymerization were estimated to be 1500 and 1400 Å (DDPC–MDPE liposome) and 1700 and 1700 Å (DDPC–MDPE–DMPC liposome), respectively.

The phase transition temperature (T_c, the point of departure of the thermogram from the base line) and the temperature of the midpoint of phase transition (T_m) of the DDPC–MDPE–DMPC liposome after polymerization were 25 and 27 °C, respectively. Since the T_m of the polymerized DDPC region could not be detected by DSC,[7] we concluded that DMPC molecules, which show a phase transition at about 24 °C in a homogeneous liposome,[13] still have similar flexibility and mobility in the partly polymerized liposome to be hydrolyzed by phospholipase A_2 (most active in the phase transition regions).[14]

By the fluorescence anisotropy depolarization technique a similar tendency was observed: The value of r_∞,[15] which reflects the orientational freedom of chains in the DMPC region of the partly polymerized DDPC–MDPE–DMPC (4:1:5) liposome, was roughly estimated to be 0.32 by assuming that partition coefficients and fluorescence life times of DPH in DMPC and in polymerized lipid regions were the same. This value was not so largely different from that of a DMPC homogeneous liposome at 25 °C (r_∞ = 0.29).

Strictly speaking, however, we could not definitely talk about the lipid structural order in the *partly* polymerized liposome system using the anisotropy depolarization technique, because of the probability of the localized distribution of fluorescent probe, DPH, in the clefts formed between blocks of polymerized lipids on the liposome surface, for example.[16]

The amount of biotin, phospholipase A_2, and avidin bound to the liposomes was 0.066, 0.3, and 0.4 mg/10 mg of lipid, respectively. Phospholipase A_2 bound to liposome I fully retained its catalytic activity after storage for 100 days in H_2O at 10 °C.

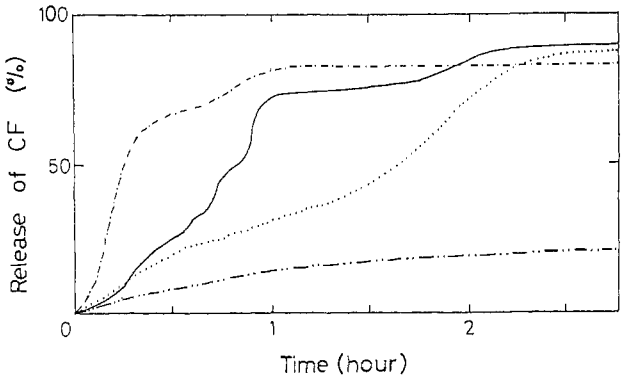

Figure 3. Release of 6-CF from liposome II catalyzed by various phospholipases A_2 at 25 °C. [Enzyme] = 6.9 μg/4 mL; [$CaCl_2$] = 2.5 mM; [HEPES] = 5 mM; pH 8.1. 100% release was attained by the addition of Triton X-100 [final concentration 0.15% (v/v)] to the suspension. (a) (–·–) Release by free phospholipase A_2. (b) (–··–) Release in the presence of biotin-carrying DDPC–MDPE liposome (without PLA_2's). (c) (···) A partly polymerized liposome (DDPC:MDPE:DMPC = 4:1:5) (without avidins) was incubated with liposome I. (d) (—) Release in the presence of liposome I.

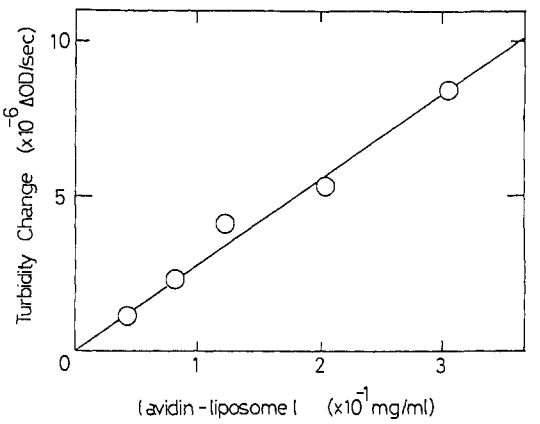

Figure 4. Turbidity measurements of the avidin liposome–biotin liposome system at 25 °C: [avidin liposome (DDPC:MDPE:DMPC = 4:1:5)] and [biotin liposome (DDPC:MDPE = 9:1)] = 0.08 mg/mL.

(A) Release of 6-CF at 25 °C. By the addition of free phospholipase A_2 to the liposome II suspension at 25 °C (near the T_m of the DMPC region), the fluorescence intensity of the suspension at 520 nm was increased (curve a in Figure 3). This is due to the hydrolysis of DMPC on the liposome surface to produce water-soluble lysolecithin, which induces the formation of pores of DMPC region (uncorking)[2] on the liposome surface to release the fluorescent 6-CF gradually. Note here that phospholipase A_2 did not hydrolyze the polymerized lipids at all.[2b,17] The enzyme catalysis on DMPC was not inhibited by the polymerized lipids, either.

By the addition of the liposome which has biotin molecules on its surface (without PLA_2's), a very slow release of 6-CF from liposome II was observed (curve b in Figure 3), probably because of a *destabilization* of liposome II due to the tight association with the biotin-carrying liposome.

When a suspension of liposome I was added to a suspension of a partly polymerized liposome (DDPC:MDPE:DMPC = 4:1:5) without avidins, a similar but slightly faster release than that in the curve b was observed (curve c in Figure 3). The rate of the increase in fluorescence intensity was, however, still much smaller than that of curve a.

By the introduction of avidin molecules onto the surface of the partly polymerized liposome, however, the situation largely

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Table I. Hydrolysis of DMPC on the Partly Polymerized Liposome Catalyzed by Phospholipase A_2 (PLA_2) Carrying Liposomes[a]

substrate liposome	enzyme liposome	reaction rate[b]
DDPC–DMPC	free PLA_2	7.6
DDPC–DMPC	DDPC–PLA_2	3.3
DDPC–DMPC	DDPC–PLA_2–biotin[c]	1.5
DDPC–DMPC–avidin[d]	DDPC–PLA_2	1.5
DDPC–DMPC–avidin[d]	DDPC–PLA_2–biotin[c]	1.9

[a] [Enzyme] = 6.9 μg/5 mL; [$CaCl_2$] = 2.5 mM; pH 8.0; 25 °C. [b] 10^{-5} M^{-1}·min^{-1}. [c] Liposome I. [d] Liposome II.

changed. As shown by curve d in Figure 3, the fluorescence intensity increased rapidly, which means that the recognition of biotin on liposome I by avidin on liposome II promotes the tight association of these liposomes to realize an "uncorking".

The association of these liposomes was confirmed by microscopic observation and also by the increase in turbidity of the suspension at 300 nm (Figure 4). The rate of turbidity change depended on the surface density of the complementary ligands on the liposome surface. However, there was no strict proportionality between the amount of ligands on the liposomes and the rate of turbidity change; when the amount of biotin on the liposome surface was reduced to 25% of that of liposome I, the rate of turbidity change was 87% of that of the liposome I–liposome II system, and when the amount of avidin on the liposome surface was 17% of that of liposome II, the rate of turbidity change was 76% of that of the liposome I–liposome II system.

This is probably because several avidin–biotin pairs are enough for a liposomal association. A similar tendency was observed in association processes of latex particles modified with antigens and antibodies.[18] Wolff et al. reported that 1.3 molecules of immunoglobulin G per vesicle are enough for interaction of the vesicle with antigen-carrying cells.[19]

The rate of the increase in fluorescence intensity in the liposome I–liposome II system (curve d in Figure 3) was still smaller than that of the liposome II–free PLA_2 system (curve a in Figure 3). This is due to the slower enzymatic reaction than that in the free enzyme system, partly because of the steric hindrance of carrier liposomes (catalytic activities of immobilized enzymes are very often lower than those of free enzymes due to the steric hindrance)[7,20] and partly because of the liposomal association being too tight for catalysis.

Actually, the reaction rate of hydrolysis of DMPC on liposome II by phospholipase A_2 immobilized on the polymerized liposome was slowed by the introduction of biotin and avidin (Table I). In spite of such a disadvantageous effect, the tight liposomal association enhances the concentrated attack of phospholipase A_2 to the DMPC molecules in a narrow region on another liposome surface to induce an effective "uncorking".

(B) Release of 6-CF at 37 °C. We also examined the same liposomal systems at 37 °C (Figure 5). With increase in temperature the DMPC-carrying partly polymerized liposomes were destabilized, and the release of CF from the liposome was highly accelerated in the liposome II–biotin liposome (without PLA_2) system and slightly accelerated in the liposome I–partly polymerized liposome (without avidin) system as compared to those at 25 °C. In other liposome systems, however, the releasing rate was reduced due to the slower catalytic acitivity of PLA_2 at higher temperature in comparison with the activity around the phase-transition point of DMPC region (T_m, 27 °C).[13] Especially in the free PLA_2 system, the releasing rate was only slightly larger than that of the liposome I–liposome II system in the initial stage. In spite of such a destabilization factor, the releasing rate of 6-CF from the partly polymerized liposome was still in an order similar to that at 25 °C: that is, free enzyme–liposome II ≳ liposome I–liposome II > liposome I–DDPC–MDPE–DMPC liposome

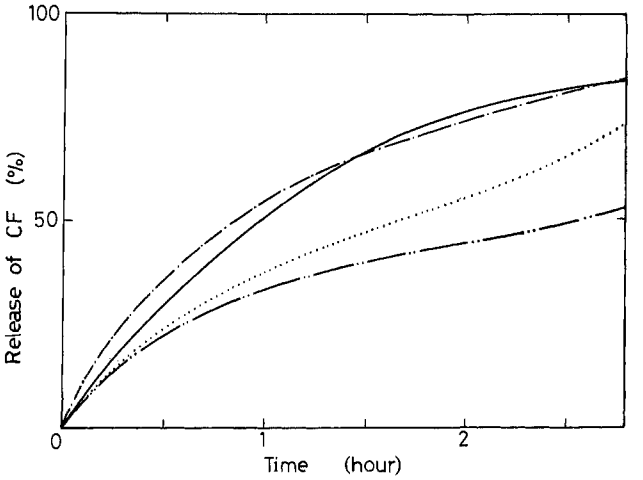

Figure 5. Release of 6-CF from the avidin-carrying partly polymerized liposome (DDPC:MDPE:DMPC = 4:1:5) at 37 °C. The conditions (except experimental temperature) and symbols are the same as those in Figure 3.

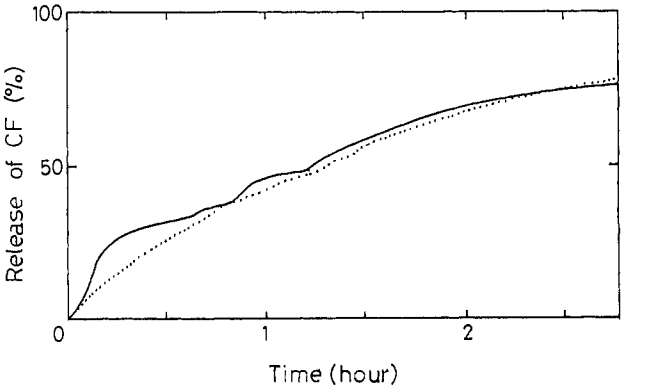

Figure 6. Release of 6-CF from the avidin-carrying partly polymerized liposome (MDPC:MDPE:DMPC = 4:1:5) at 25 °C. [Enzyme] = 6.9 μg/4 mL; [$CaCl_2$] = 2.5 mM; [HEPES] = 5 mM. (a) (···) A partly polymerized liposome (MDPC:MDPE:DMPC = 4:1:5) (without avidins) was incubated with liposome I. (b) (—) Release in the presence of liposome I.

(without avidin) > biotin liposome (without PLA_2)–liposome II. These results suggest that the main factor to induce the uncorking at 37 °C is also hydrolysis of DMPC molecules by PLA_2.

(C) Effects of Spacer Group. We also used a liposome I on which biotin was bound via a long spacer group (modified by using NHS-LC-biotin, 0.07 mg of biotin/10 mg of lipid). The releasing rate of 6-CF from liposome II, however, was not different from that of the system in which liposome I without a spacer was used. We had expected effective recognition–lysis processes. The too-tight association of liposomes through many biotin–avidin pairs might not be so highly effective for an uncorking.

MDPC Liposome System. We also examined recognition and subsequent lysis of an avidin-carrying polymerized liposome composed of monodienoylphosphatidylcholine (MDPC), MDPE, and DMPC (MDPC:MDPE:DMPC = 4:1:5) by liposome I. The diameters of the MDPC–MDPE–DMPC liposomes were 2800 and 2600 Å before and after polymerization, respectively. The T_c and T_m of the DMPC region in the partly polymerized liposome were 24 and 26.5 °C, respectively. The amount of avidin bound to the liposome was 0.4 mg/10 mg of lipid.

The release of 6-CF from the avidin-carrying MDPC–MDPE–DMPC liposome was *not* largely faster than that from the liposome without avidin (Figure 6). We had expected that the MDPC liposomes with a larger deformability[7] might be more suitable to realize a macrophage model system than the DDPC liposomes. The unexpected results obtained here are probably because, in the MDPC system, the hydrolysis of DMPC does not induce a pore (which has to be large enough for 6-CF to be

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released to the bulk solution) on the liposome surface more easily than that in the DDPC system.

The blocks of polymerized MDPC lipids (tetramer, estimated by GPC) on the liposome surface are more mobile than those of DDPC (two dimensionally cross-linked).[7] On a fluid surface like the mixed liposome of DMPC and oligomerized MDPC, pores induced by the release of lysolecithin might be quickly closed by the neighboring lipids.

Such a system would be quite useful to promote understanding of the sophisticated and complicated processes in living bodies.

Acknowledgment. We thank Dr. Yoshihiko Nagata, Tosoh Corp., for a kind donation of the polymerizable lipids. We also thank Prof. Masahide Yamamoto and Dr. Shinzaburo Ito of this department for generously allowing us to use the fluorescence anisotropy depolarization equipment.

Direct Cleavage versus Transpeptidation in the Autodecomposition of Peptides Containing 2,4-Diaminobutanoic Acid (DABA) and 2,3-Diaminopropanoic Acid (DAPA) Residues. Specific Cleavage of DAPA-Containing Peptides[1,2]

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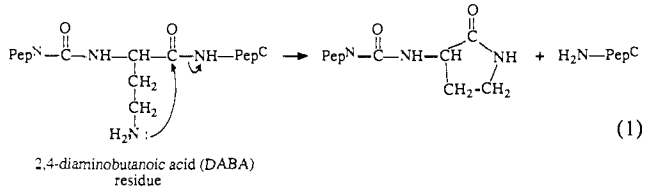
Abstract: Peptides containing 2,4-diaminobutanoic acid and 2,3-diaminopropanoic acid residues undergo transpeptidation by attack of their side-chain amino groups on the *N*-carbonyl (eq 2). Little or no direct cleavage by attack on the *C*-carbonyl (eq 1) is observed. The transpeptidation reactions of peptides containing 2,4-diaminobutanoic acid (DABA) or 2,3-diaminopropanoic acid (DAPA) residues reach an equilibrium in which the various peptides studied are about 70–80% transpeptidized; this extent of transpeptidation is in agreement with the equilibrium constants for other transamination reactions. The transpeptidation reaction is strongly catalyzed by phosphate and bicarbonate buffers, and the pH dependence of the reaction suggests that an unprotonated side-chain amino group is required for significant reactivity. The rate of the transpeptidation reaction is retarded by bulky substituents at the α-carbon of the residue at the amino-terminal side of the DAPA or DABA residue. The preference for transpeptidation over direct cleavage in the case of DABA residues can be explained by one or more of the following factors: (1) a preference for (*Z*)-amide (transpeptidation) over (*E*)-amide (direct cleavage); (2) greater ring strain in the tetrahedral intermediate for direct cleavage; (3) a steric effect resulting from unfavorable interactions in the possible transition states for direct cleavage (Scheme III). A stereoelectronic explanation is considered and rejected. Peptides containing *transpeptidized* DABA and DAPA residues (isoDABA and isoDAPA residues, respectively) undergo cleavage at the carboxy-terminal side of these residues on treatment with the Edman reagent followed by treatment with trifluoroacetic acid. Peptides can be induced to undergo direct cleavage at the carboxy-terminal side of *untranspeptidized* DAPA residues by treatment with the Edman reagent followed by heptafluorobutyric acid. The chemical and biological significance of these observations is discussed.

Although the 2,4-diaminobutanoic acid (DABA) residue does not occur naturally in most peptides, a number of investigators have been intrigued by the possibility that such a residue, synthetically produced from one of the standard amino acids by peptide modification, might serve as a site for specific cleavage of proteins, as shown in eq 1. In this paper, we shall refer to this

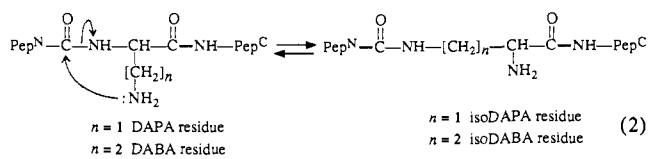


(1)

2,4-diaminobutanoic acid (DABA) residue

type of process as *direct cleavage*. For example, LeQuesne and Young[4] wrote, "It is tempting to suggest that under suitable conditions, α,γ-diaminobutyric acid [DABA] might serve as a source of instability in peptide chains". Similarly, Rudinger[5] suggested, "Selective peptide-bond fission based on the presence of amino groups in the γ-position of amino-acid chains might well find application in [structural] studies". Indeed, several groups have demonstrated that peptide-bond fission like that shown in eq 1 does occur in favorable cases.[6]

The reaction in eq 1, however, is not the only process by which nucleophilic side chains may become covalently involved with the peptide backbone. A side-chain amino group can in principle also attack the carbonyl group of the preceding residue (eq 2). In



n = 1 DAPA residue
n = 2 DABA residue

n = 1 isoDAPA residue
n = 2 isoDABA residue

(2)

(1) This paper is dedicated to Professor Donald S. Noyce following his retirement from the Department of Chemistry of the University of California, Berkeley.

(2) A preliminary account of this work has appeared: Blodgett, J. K.; Loudon, G. M. In *Peptides, Structure and Function: Proceedings of the Ninth American Peptide Symposium*; Deber, C. M.; Hruby, V. J.; Kopple, K. D., Eds.; Pierce: Rockford, IL, 1985; pp 371–374.

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