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Membrane-damaging action of *Clostridium perfringens* alpha-toxin on phospholipid liposomes

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Abstract

The effect of *Clostridium perfringens* alpha-toxin on multilamellar liposomes prepared from various phospholipids and cholesterol was investigated. The toxin induced carboxyfluorescein leakage from liposomes composed of the choline-containing phospholipids such as egg-yolk phosphatidylcholine and bovine brain sphingomyelin in a dose-dependent manner, but did not induce leakage from those liposomes composed of bovine brain phosphatidylethanolamine, egg-yolk phosphatidylserine or phosphatidylglycerol. The toxin-induced carboxyfluorescein leakage from egg-yolk phosphatidylcholine liposomes was increased by addition of divalent cations. The toxin induced carboxyfluorescein release from liposomes composed of phosphatidylcholine containing unsaturated fatty acyl residues or shorter chain length saturated fatty acyl residues (12 or 14 carbon atoms), but did not induce such release from liposomes composed of phosphatidylcholine containing saturated fatty acyl residues of between 16 and 20 carbon atoms. Furthermore, the toxin-induced carboxyfluorescein release decreased with increasing chain length of the acyl residues of phosphatidylcholine used. The toxin bound to liposomes composed of phospholipids which are hydrolyzed by the toxin, but did not bind to those composed of phospholipids which are not attacked by the toxin. The toxin-induced carboxyfluorescein release from liposomes composed of dipalmitoleoyl-L-α-phosphatidylcholine and cholesterol and the toxin binding to the liposomes decreased with decreasing cholesterol contents. These observations suggest that the specific binding site formed by the choline-containing phospholipids and cholesterol, and membrane fluidity in liposomes are essential for the membrane-damaging activity of alpha-toxin.

Keywords: Alpha-toxin; Toxin; Liposome; Phospholipase C; Phospholipid; Fluidity; (*C. perfringens*)

1. Introduction

C. perfringens produces alpha-toxin, which is thought to be an important virulence factor in gas gangrene of the organism, and is a single polypeptide with a molecular weight of about 43 000 [1,2]. The toxin is hemolytic, dermonecrotic and lethal. Furthermore, it also has phospholipase C and sphingomyelinase activities as enzyme activity. The toxin has been shown to cause membrane damage to a variety of erythrocytes and cultured mammalian cells [1,2]. Consequently, it is suggested that the toxin-induced membrane damage is related to the enzymatic activities of the toxin. Extensive studies on the mechanism of membrane damage by the toxin have been performed with erythrocytes. We have reported that the toxin activates endogenous phospholipase C and phospholipase D in rabbit erythrocyte membranes so that phosphatidic acid is produced by phosphorylation of diacylglycerol (DG) formed from phosphatidylinositol 4,5-bisphosphate by endogenous phospholipase C and DG via phosphatidic acid produced by endogenous phospholipase D, and that the toxin-induced phosphatidic acid formation is closely related to hemolysis induced by the toxin [3,4]. Furthermore, we have reported that the toxin activates endogenous phospholipase C through GTP-binding protein, suggesting that the toxin may interact with receptors on erythrocyte membranes and activate endogenous phospholipase C [4]. Cai et al. reported that treatment of biological membranes with *Bacillus cereus* phospholipase C results in activation of GTP-binding protein [5]. The findings support the concept that the enzymatic activity of the toxin may activate GTP-binding protein in membranes. Recently, when we determined biological activities of variant alpha-toxins at the histidine residue which were replaced with neutral amino acids by site directed mutagenesis, the hemolytic

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activity of the toxin was unable to be separated from the phospholipase C activity of the toxin [6]. It is therefore possible that the initial step of hemolysis induced by the toxin is hydrolysis of phospholipids by the phospholipase C activity of the toxin in membranes. However, little is known about the interaction between the toxin and phospholipids in biological membranes, although the toxin is known to hydrolyze phosphatidylcholine and sphingomyelin. Biological membranes being very complicated, one approach seems to be to study much more simple model systems such as liposomes composed of phospholipids and cholesterol. Several studies have been reported dealing with proteins which display electrostatic interactions with the polar head groups of phospholipids and hydrophobic interactions with aliphatic chains of phospholipids [7–9], and dealing with proteins which penetrate into the lipid bilayer [10–13].

In the present paper, we investigated the influence of polar head groups of phospholipids and membrane fluidity on the membrane-damaging action and the binding of alpha-toxin by using multilamellar liposomes composed of phosphatidylcholine (PC) with different acyl chain composition or various contents of cholesterol as model membranes.

2. Materials and methods

2.1. Materials

Dilauroyl-L-α-PC (DLPC), dimyristoyl-L-α-PC (DMPC), dipalmitoyl-L-α-PC (DPPC), dipalmitoleoyl-L-α-PC (DPOPC), distearoyl-L-α-PC (DSPC), dioleoyl-L-α-PC (DOPC), diarachidoyl-L-α-PC (DAPC), sphingomyelin (SM) from bovine brain, L-α-phosphatidylethanolamine (PE) from egg yolk, L-α-phosphatidyl-DL-glycerol (PG) from egg yolk, L-α-phosphatidyl-L-serine (PS) from bovine brain and 5(6)-carboxyfluorescein diacetate (CF) were purchased from Sigma (St. Louis, MO). PC from egg yolk and cholesterol were purchased from Nacalai Tesque (Kyoto). Dieicosenoyl-L-α-PC (DEPC) was obtained from Avanti Polar Lipids (Alabaster, AL).

2.2. Purification of alpha-toxin

Alpha-toxin was purified from the culture supernatant of *C. perfringens* type A (NCTC 8237) as described by Fujii et al [14].

2.3. Preparation of liposomes

Multilamellar liposomes composed of phospholipid and cholesterol were prepared according to the method of Tomita et al. [8,9]. A mixture of phospholipid (0.5 μmol) and cholesterol (0.5 μmol) in chloroform was evaporated under reduced pressure to form a lipid film on the wall of a conical-bottomed flask. After drying under reduced pressure for 1 h, the lipid film was hydrated by vortexing at 45–50 C or above the phase transition temperature (T_m) of the PC used in 100 μl of 20 mM Tris-HCl buffer (pH 7.5) containing 0.1 M CF and 0.9% NaCl. The liposome suspensions were centrifuged at 22000 × *g* for 15 min at 4°C to remove the nonencapsulated marker, and washed three times by centrifugation in 20 mM Tris-HCl buffer (pH 7.5) containing 0.9% NaCl (Tris-buffered saline). The resulting liposomes were suspended in 200 μl of Tris-buffered saline.

2.4. Assay of marker release from liposomes

In a 96-well U-bottomed microplate (Nunc, Roskilde, Denmark), alpha-toxin and 5 μl of CF-liposomes were mixed with the Tris-buffered saline to give a total volume of 300 μl. After the mixture had been incubated at 37°C or 4°C for 30 min, fluorescence intensity was measured with microplate fluorometer MTP-32 (Corona Electric Co., Katsuda, Japan) at an excitation wavelength of 490 nm and an emission wavelength of 530 nm [7]. The toxin-induced CF release was maximal after 30 min of incubation. 100% CF release was defined as the fluorescence intensity that was gained upon exposure of liposomes to 1% Triton X-100 at 37°C for 30 min.

2.5. Binding of alpha-toxin to liposomes

Mixture of alpha-toxin (1 μg) and liposomes (0.02 μmol of lipids) in 500 μl of Tris-buffered saline in the presence or absence of 1 mM $CaCl_2$ were incubated at 4°C for 30 min. After the incubation, to remove unbound toxin, the mixture was centrifuged at 22000 × *g* at 4°C for 20 min. The precipitated liposomes were washed three times by centrifugation with 1 ml of Tris-buffered saline. The washed liposomes were dissolved in 20 μl of 20 mM Tris-HCl buffer (pH 7.5) containing 1% Triton X-100. SDS-polyacrylamide gel electrophoresis (PAGE) was performed on 12.5% gels, and the protein bands were then transferred to a poly(vinylidene fluoride) membrane (Immobilon P; Millipore). The detection of alpha-toxin by Western blotting was performed as described previously [5].

2.6. Determination of phosphorylcholine and protein

Release of phosphorylcholine from phospholipids by alpha-toxin was determined by the method described by Ikezawa et al. [15]. Protein determination was carried out according to the method of Lowry et al. [16] using bovine serum albumin as a standard.

3. Results

3.1. The sensitivity of liposomes composed of phospholipid and cholesterol to alpha-toxin

Clostridium perfringens alpha-toxin has been reported to hydrolyze PC and SM [2]. To investigate the sensitivity of artificial membranes composed of phospholipids to alpha-toxin, CF-loaded liposomes composed of phospholipids and cholesterol in a molar ratio of 1:1 were incubated with alpha-toxin in the presence or absence of 1 mM $CaCl_2$ at 37°C for 30 min. The toxin in the range over 1.5 and 15 ng/ml induced CF leakage from liposomes composed of egg-yolk PC and bovine brain SM, respectively, in the presence of $CaCl_2$ at 37°C (Fig. 1), but the toxin at a concentration of 1000 ng/ml did not from both liposomes in the absence of $CaCl_2$ (data not shown). On the other hand, the toxin caused no effect on liposomes composed of PE, PS or PG, which are known to be rarely hydrolyzed by alpha-toxin, at the concentration of 10 μg /ml in the presence of 1 mM $CaCl_2$ (Fig. 1). It has been reported that divalent cations such as Zn^{2+}, Co^{2+} and Mn^{2+} are required for enzymatic activity of the toxin treated with chelating agents [17]. The effect of divalent cations on the toxin-induced CF release from liposomes composed of egg-yolk PC (Fig. 2) or bovine brain SM was investigated (data not shown). Zn^{2+}, Co^{2+} and Mn^{2+} stimulated the EDTA-treated toxin-induced CF release from both liposomes, but Mg^{2+}, Ba^{2+} and Cu^{2+}, which do not stimulate enzymatic activity of the EDTA-treated toxin, did not. Therefore, it appears that the toxin-induced CF release from liposomes is related to enzymatic activity of the toxin.

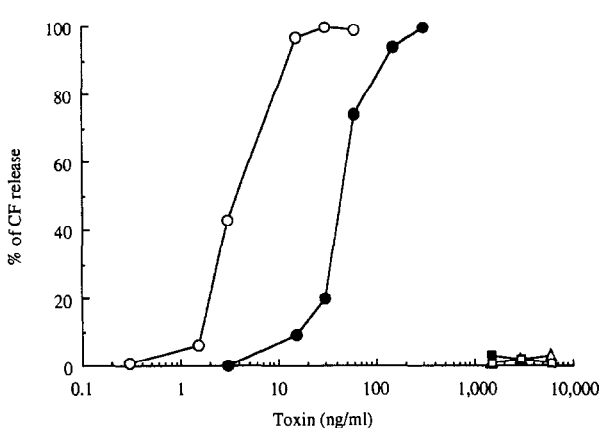

Fig. 1. Alpha-toxin-induced CF release from various phospholipid-cholesterol liposomes. CF release from various liposomes upon exposure to alpha-toxin was assayed as described in Section 2. The liposomes used in the experiments were composed of egg-yolk PC (○), bovine brain SM (●), egg-yolk PE (□), bovine brain PS (■) or egg-yolk PG (△) plus cholesterol in a molar ratio of 1:1. Mean values obtained from three independent experiments were plotted for all illustrations.

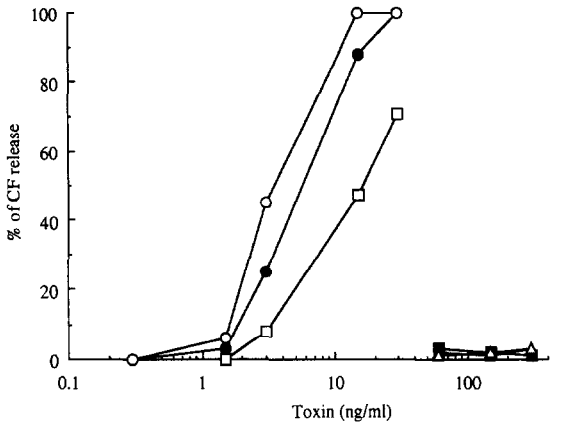

Fig. 2. Effect of divalent cations on alpha-toxin-induced CF release from egg-yolk PC-cholesterol liposomes. CF-loaded liposomes composed of phospholipid and cholesterol in a molar ratio of 1:1 were exposed in the presence of 1 mM divalent cations at 37°C for 30 min to alpha-toxin pretreated with 0.5 mM EDTA. CF release was measured as described in Section 2. Mean values obtained from three independent experiments were plotted for all illustrations. Symbols: Zn^{2+} (□), Co^{2+} (●), Mn^{2+} (○), Mg^{2+} (▲), Ba^{2+} (△), Cu^{2+} (■).

3.2. The sensitivity of liposomes composed of synthetic PC and cholesterol to alpha-toxin

As shown in Fig. 3, the toxin in the range from 1.5 ng/ml to 15 μg/ml dose-dependently induced CF release from liposomes composed of unsaturated fatty acyl residue-carrying PC such as DPOPC ($T_m = -36$°C) [18], DOPC ($T_m = -22$°C) [8] and DEPC ($T_m = 9.5$°C) [18], and saturated fatty acyl residue-carrying PC such as DLPC ($T_m = 0$°C) and DMPC ($T_m = 23$°C) [8] upon incubation at 37°C. In addition, the data indicated that the toxin-induced

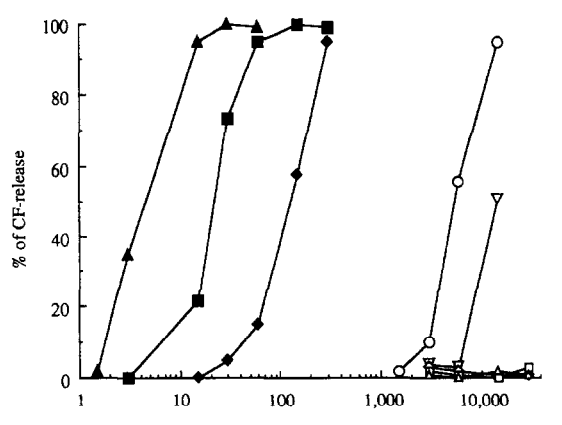

Fig. 3. Effect of various PC on the sensitivity of PC-cholesterol liposomes to alpha-toxin. CF-loaded liposomes each composed of DPOPC (▲), DOPC (■), DEPC (◆), DPPC (△), DSPC (□), DAPC (◇), DMPC (▽) or DLPC (○) and cholesterol in a molar ratio of 1:1 were exposed to various doses of alpha-toxin in the presence of 1 mM $CaCl_2$ at 37°C for 30 min. Leakage of CF from the liposomes was measure as described in Section 2. Means values obtained from three independent experiments were plotted for all illustrations.

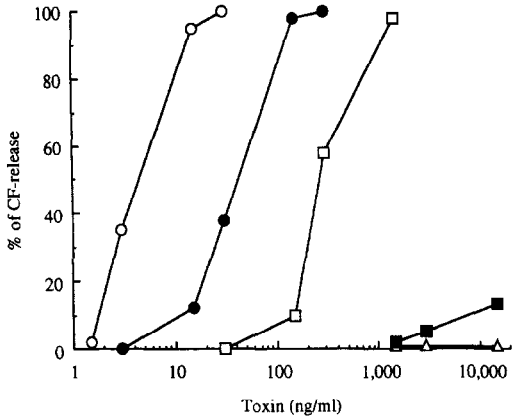

Fig. 4. Alpha-toxin-induced CF release in solid and fluid PC-cholesterol membranes. DPOPC was mixed with DPPC as follows. The molar ratio of DPOPC to DPPC was 100 (○), 75 (●), 50 (□), 25 (■) and 0 (△). Liposomal membranes composed of the mixture and cholesterol of a molar ratio 1:1 were incubated with the toxin in the presence of 1 mM $CaCl_2$ at 37°C for 30 min. Marker release from liposomes upon exposure to alpha-toxin was assayed as described in Section 2. Mean values obtained from three independent experiments were plotted for all illustration.

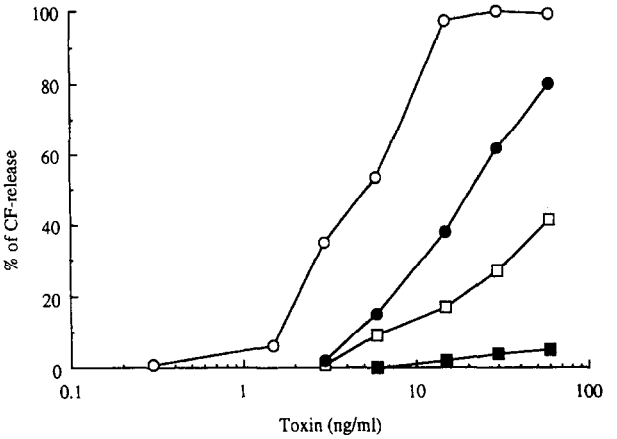

Fig. 5. Effect of cholesterol contents on alpha-toxin-induced CF release from PC-cholesterol liposomes. Alpha-toxin was incubated with liposomes composed of DPOPC and cholesterol in various molar ratios in the presence of 1 mM $CaCl_2$. Marker release from liposomes upon exposure to alpha-toxin was assayed as described in Section 2. Mean values obtained from three independent experiments were plotted for all illustrations. The molar ratio of cholesterol to DPOPC (mol%) was 50 (○), 25 (●), 12.5 (□) and 0 (■).

CF release from liposomes composed of PC containing unsaturated fatty acyl residues between 16 and 20 carbon atoms or containing saturated fatty acyl residues between 12 and 14 carbon atoms was decreased with increasing chain length of the fatty acyl residue. On the other hand, the toxin did not induce CF release from liposomes composed of PC with saturated fatty acid such as DPPC (T_m = 41°C), DSPC (T_m = 55°C) and DAPC (T_m = 75°C) [8] at the concentration of 30 μg/ml of the toxin upon incubation at 37°C. From these results, the T_m of PC used appears to affect the activity of the toxin. To confirm the relationship between the toxin-induced CF release and T_m of PC in liposomes, the effect of DPPC contents on the toxin-induced CF release from liposomes composed of DPPC, DPOPC and cholesterol was investigated by the incubation of the toxin with liposomes at 37°C for 30 min. The toxin-induced CF leakage decreased with the increase of DPPC content in liposomes (Fig. 4). Furthermore, the toxin-induced CF release from liposomes composed of DSPC, DOPC and cholesterol also decreased with increasing DSPC content (data not shown). From these data, the toxin-induced CF release was found to be related to the T_m of PC in liposomes.

To clarify the cholesterol effect on the toxin-induced CF release from liposomes composed of PC and cholesterol, we prepared DPOPC liposomes with various cholesterol contents between 0 and 50 mol%. The toxin-induced CF release was measured after the toxin had been incubated with liposomes at 37°C for 30 min. Fig. 5 shows that the toxin-induced CF release decreased with decreasing of cholesterol contents and that no CF release induced by the toxin was observed from liposomes without cholesterol. On the other hand, when the toxin (165 ng/ml) was incubated with the egg-yolk suspension in the presence of 2.5 mM cholesterol solubilized in 0.2% sodium deoxycholate (300 μl) and 3 mM Ca^{2+} at 37°C for 30 min, cholesterol caused no effect on phospholipase C activity of the toxin (data not shown), suggesting that cholesterol itself does not interact with the toxin.

3.3. Release of phosphorylcholine from liposomes composed of PC or SM by alpha-toxin

To determine whether or not the toxin hydrolyzes phospholipids in liposomes, liposomes with egg-yolk PC,

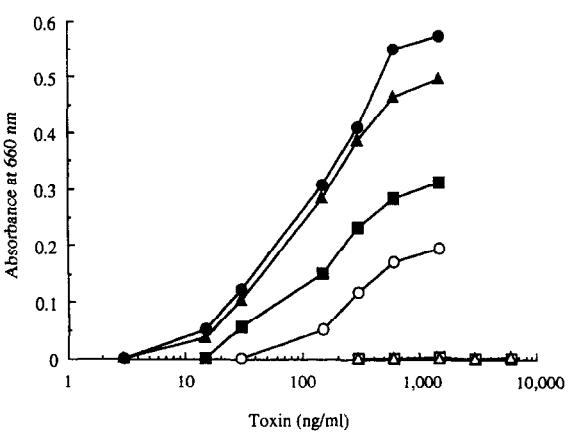

Fig. 6. Hydrolysis of PC in phospholipid-cholesterol liposomes by alpha-toxin. Alpha-toxin was incubated with liposomes prepared from various liposomes at 37°C for 30 min in the presence of 1 mM $CaCl_2$. Phosphorylcholine release was assayed as described in Section 2. The liposomes composed of egg-yolk PC (●), bovine brain SM (○), DPOPC (▲), DOPC (■), DPPC (△) or DSPC (□) and cholesterol in a molar ratio of 1:1 were used. Mean values obtained from three independent experiments were plotted for all illustrations.

bovine brain SM, DPOPC, DOPC, DPPC or DSPC (phospholipid and cholesterol in a molar ratio of 1:1) were incubated with various concentrations of the toxin at 37°C for 30 min and phosphorylcholine release was measured. The toxin induced phosphorylcholine release from liposomes in the following order: egg-yolk PC = DPOPC > DOPC > bovine brain SM (Fig. 6). However, the toxin induced no release of phosphorylcholine from liposomes composed of DPPC and DSPC. The results were coincident with those of the toxin-induced CF release from liposomes composed of phospholipids tested.

3.4. Binding of alpha-toxin to liposomes

When the toxin was incubated with liposomes composed of egg-yolk PC and cholesterol (PC/cholesterol, 1:1) in the presence of 1 mM $CaCl_2$ at 37°C for 30 min, oil layer was observed on the surface of the reaction solution, but the precipitated liposomes were not collected by centrifugation at 22 000 × *g* for 20 min. Accordingly, it appears that liposomes were completely destroyed by the toxin under the condition. On the other hand, when the toxin at the concentrations of 100 and 1000 ng/ml was incubated with liposomes composed of egg-yolk PC and bovine brain SM, respectively, at 4°C for 30 min in the presence or absence of 1 mM $CaCl_2$, no CF release from liposomes and no degradation of liposomes occurred. When the resulted liposomes were washed by centrifugation (22 000 × *g*, 20 min) and then incubated at 37°C for 30 min, CF release was observed from liposomes incubated in the presence of $CaCl_2$, but did not from those incubated in the absence of $CaCl_2$ (data not shown). Accordingly, it is likely that the toxin binds to liposomes at 4°C in the presence of $CaCl_2$. We have reported that the toxin binds

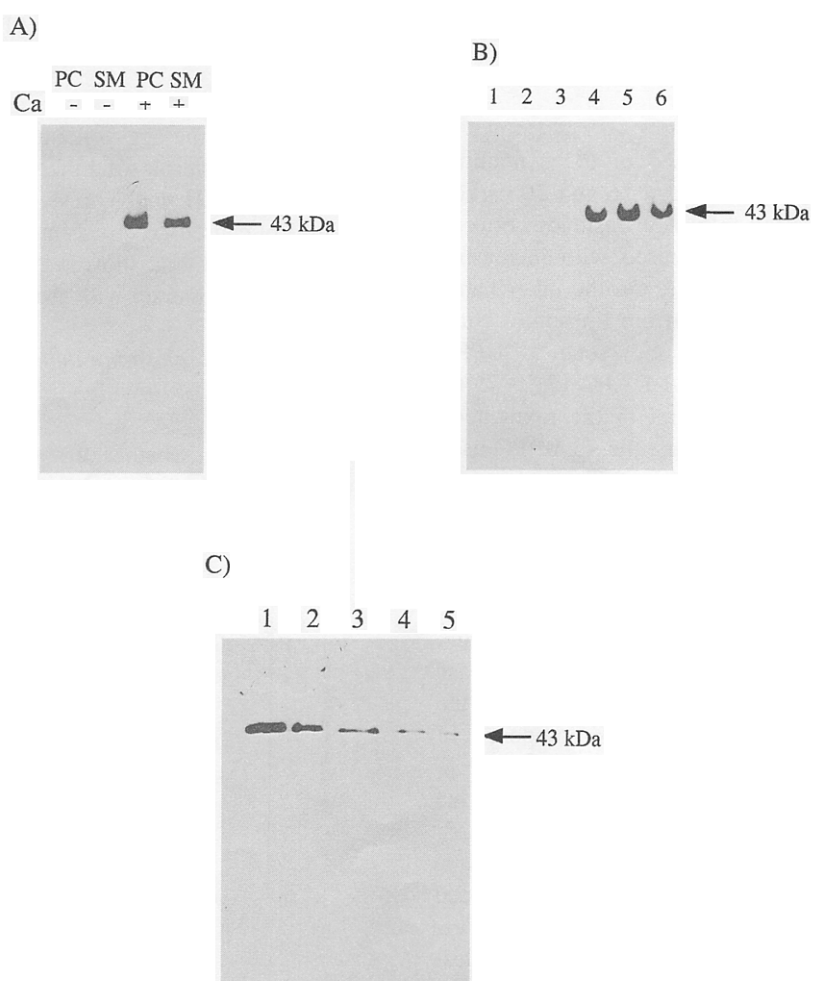

Fig. 7. Binding of alpha-toxin to various phospholipid-cholesterol liposomes. (A) Alpha-toxin was incubated with liposomes prepared from egg-yolk PC (PC) or bovine brain SM (SM) and cholesterol in a molar ratio of 1:1 in the presence (+) or absence (−) of 1 mM $CaCl_2$. Liposome-bound toxin was determined upon incubation at 4°C for 30 min as described in Section 2. (B) Alpha-toxin was incubated with liposomes composed of various PC (DPPC, DSPC, DAPC, DPOPC, DOPC, and DEPC) and cholesterol in a molar ratio of 1:1 in the presence of 1 mM $CaCl_2$ at 4°C for 30 min. Liposome-bound toxin was determined as described in Section 2. Lanes; 1, DPPC; 2, DSPC; 3, DAPC; 4, DPOPC; 5, DOPC; 6, DEPC. (C) Alpha-toxin was incubated with liposomes compared of DPOPC and cholesterol in various molar ratios (50, 37.5, 25, 12.5 and 0 mol%) as described in Fig. 7B. Lanes (mol%); 1, 50; 2, 37.5; 3, 25; 4, 12.5; 5, 0.

to rabbit erythrocytes in the presence of $CaCl_2$, but does not in the absence of $CaCl_2$ [6], indicating that the effect of Ca^{2+} on the toxin binding to liposomes is in coincidence with that on the toxin binding to erythrocytes. Binding of the toxin to liposomes composed of egg-yolk PC or bovine brain SM was investigated. After the incubation of the toxin with liposomes in the presence or absence of $CaCl_2$ at 4°C for 30 min, liposomes were collected by centrifugation and then assayed on SDS-PAGE after solubilizing liposomes in 1% Triton X-100. As shown in Fig. 7A, the toxin alone was recovered as a monomer of about 43 kDa from liposomes incubated in the presence of $CaCl_2$, suggesting that no oligomerization of the toxin occurred under the condition. However, the band of the toxin was not observed on SDS-PAGE from liposomes incubated in the absence of $CaCl_2$. To determine the effect of PC in liposomes on the toxin binding to liposomes, the toxin was incubated with liposomes composed of various PC in the presence of 1 mM $CaCl_2$ at 4°C for 30 min (Fig. 7B). The toxin bound to liposomes composed of DPOPC, DOPC and DEPC ($10°C > T_m$), but not to those prepared from DPPC, DSPC and DAPC ($40°C < T_m$), suggesting that the toxin binding to liposomes may be related to the T_m of the PC used. Furthermore, the effect of cholesterol contents on the toxin binding to liposomes was investigated on SDS-PAGE. The toxin was incubated with DPOPC liposomes with various cholesterol contents between 0 and 50 mol% at 4°C for 30 min. Fig. 7C shows that the toxin binding decreased with decreasing cholesterol contents under the conditions, indicating that the binding of the toxin and the toxin-induced CF release are dependent on cholesterol content in liposomes.

4. Discussion

In the work presented here, it was shown that alpha-toxin-induced CF release from liposomes composed of phospholipids which are hydrolyzed by the toxin, was dependent upon the incubation temperature and divalent metal cations. It has been reported that these conditions affect the enzymatic activity of the toxin [17]. The observations suggest that the toxin-induced CF release is due to degradation of the choline-containing phospholipids in liposomes by the enzymatic activity of the toxin.

After the incubation of the toxin with egg yolk PC-cholesterol liposomes in the presence of $CaCl_2$ at 4°C, the monomeric toxin was only detected from the liposomes (Fig. 7A), suggesting that the toxin binds to liposomes under the condition, but does not form oligomer on liposomes. The experimental method employed does not completely exclude the possibility of formation of unstable oligomeric states, which was dissociated to monomer during the solubilization or electrophoresis of liposome-bound toxin. It has been reported that the precipitated liposomes are able to be collected by centrifugation after the incubation with Triton X-100 or *Staphylococcus aureus* alpha-toxin, which form transmembrane channel (oligomeric state), but does not degrade phospholipids, with liposomes [9,11]. *C. perfringens* alpha-toxin caused hydrolysis of phospholipids in membranes at 37°C and appearance of oil layer on the reaction solution. From these findings, the toxin seems not to form a transmembrane channel of oligomeric toxin. It is likely that the membrane-bound active state of the toxin corresponds to the monomeric toxin. This is furthermore supported by the finding that the toxin-induced CF release is related to enzymatic activities of the toxin.

The toxin induced leakage of internal CF from various PC liposomes at 37°C in the following order: DPOPC > DOPC > DEPC > DLPC > DMPC. The toxin induced no leakage of CF from DPPC-, DSPC- or DAPC-cholesterol liposomes at 37°C. As judged from the relationship between the incubation temperature and T_m of the PC used, the toxin-induced CF release correlates well with the T_m of the PC used. It has been reported that PC liposome membranes are fluidized over or at the T_m of the PC used [9]. Moreover, the toxin-induced CF release decreased with an increase of chain length of unsaturated fatty acyl residues or saturated fatty acyl residues in PC. Membrane fluidity also is thought to decrease with increasing chain length of fatty acyl residues in PC [8]. Therefore, the activity of the toxin is suggested to be closely related to membrane fluidity in liposomes.

It was found that the toxin bound to DPOPC-, DOPC- and DEPC-cholesterol liposomes, but not to DPPC-, DSPC- and DAPC-cholesterol liposomes, suggesting that membrane fluidity may affect the toxin binding of phospholipids in liposomes. However, the toxin-induced CF leakage and the toxin binding decreased with a decrease in cholesterol contents in liposomes, indicating that cholesterol contents in liposomes are important for the toxin activity. Demel and De Kruijff have reported that cholesterol causes a strong reduction in permeability of liposome systems [19], suggesting that cholesterol will reduce membrane fluidity in liposome. It therefore appears that membrane fluidity plays an important role in binding and/or action of the toxin, but is not absolutely decisive. Slavik suggested, from studies of 1-anilinonaphthalene 8-sulfonate (ANS) binding to liposomes, that ANS molecules are bound in both pockets formed by four phosphatidylcholines and also by phosphatidylcholine and cholesterol [20]. It is likely that another type of ANS binding site appears in liposomes when cholesterol is added. Accordingly the toxin may be specifically bound in the pocket formed by phosphatidylcholine and cholesterol.

Bhamidipati and Hamilton have reported that the phospholipase C of *B. cereus*, which does not possess hemolytic activity, hydrolyzes PC in liposomes, but does not alter bilayer structure [21]. Furthermore, they showed that the cleavage of phosphorylcholine group from PC by the *B. cereus* phospholipase C does not alter the molecular con-

formation of interfacial region and that DG produced does not induce any gross changes in the structure organization of bilayer. It therefore is deemed that the toxin-induced CF leakage from liposomes is unable to be only explained by cleavage of phosphorylcholine from PC. It has been reported that the deduced amino acid sequences of the *B. cereus* phospholipase C and the toxin have significant homology up to approximately 250 residues and that the active site and binding site of the proteins are in the region [2,6]. Moreover, the toxin has been reported to have an additional C-terminal domain (approximately 150 residues), which is important for the biological activities of the toxin [22,23]. Therefore, the C-terminal domain of the toxin may induce changes in the structural organization of membranes after hydrolysis of phospholipids in membranes by the toxin.

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