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Effect of Aluminum Ion on Fe^{2+}-Induced Lipid Peroxidation in Phospholipid Liposomes under Acidic Conditions[1]

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The effects of Al^{3+} on Fe^{2+}-induced lipid peroxidation in phospholipid liposomes consisting of phosphatidylcholine (PC) and phosphatidylserine (PS) were examined under acidic conditions. The stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation in the liposomes showed a biphasic response against pH variation, and the maximum stimulation was observed around pH 6.0. In addition, it was found that the stimulatory effect of Al^{3+} on the lipid peroxidation was dependent on the proportion of PS in the liposomes. On the other hand, the lipid peroxidation in PC liposomes was not stimulated by the addition of Al^{3+}. From these findings, it is suggested that the Al^{3+} effect on Fe^{2+}-induced lipid peroxidation under acidic conditions is largely dependent on the phospholipid composition. Trivalent cations such as Tb^{3+} and Ga^{3+} also stimulated Fe^{2+}-induced lipid peroxidation in PC/PS liposomes under acidic conditions, but divalent cations (Zn^{2+} and Mn^{2+}) showed no stimulatory effect. The extents of Fe^{2+} disappearance and Fe^{3+} formation during the reaction were enhanced by the addition of Al^{3+} or Ga^{2+}, but Tb^{3+} had no effect on Fe^{2+} disappearance. The results with 1,6-diphenyl-1,3,5-hexatriene (DPH) showed that the fluorescence anisotropy of DPH-labeled PC/PS liposomes under acidic conditions was increased by the addition of Al^{3+}. Furthermore, there is a relation between the extents of the fluorescence anisotropy of the complex and TBARS production. In contrast, the fluorescence anisotropy of DPH molecules embedded in PC liposomes was not changed by the addition of Al^{3+}. Based on these results, a possible mechanism of the stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation under acidic conditions is discussed.

Key words: acidosis, Al^{3+} effect, lipid peroxidation, phospholipid liposome.

It has been reported that elevated levels of aluminum in the brain may be related to several neurological disorders such as Alzheimer's disease and Parkinsonian dementia (*1-4*). In addition, it has been suggested that neurological disorders are accompanied by an increased rate of lipid peroxidation in the brain, resulting in accumulation of large amounts of "age pigments" such as lipofuscin and ceroid (*5*). There is evidence that production of thiobarbituric acid-reactive substances (TBARS) in rat (*6*) and mouse (*7*) brains is accelerated by the administration of aluminum salts.

It has recently been reported that aluminum ion stimulates lipid peroxidation in phospholipid liposomes (*8-10*) and membranes such as rat liver microsomes (*11*), human erythrocytes (*8*), and mouse brain membranes (*12*). These findings suggest that the stimulatory effect of Al^{3+} on lipid peroxidation plays an important role in various neurological disorders associated with increased levels of aluminum ion. On the other hand, Halliwell and Gutteridge have also reported (*13*) that the end-products of lipid peroxidation may produce secondary damage in a number of neurological disorders.

It has been widely accepted (*14-16*), that extracellular and intracellular acidosis leads to ischemic brain damage. In addition, Siesjo *et al.* have reported (*17*) that the intracellular acidosis results in an enhancement of lipid peroxidation in brain tissue. In the previous paper, we also reported (*9*) that Fe^{2+}-induced lipid peroxidation in PC liposomes is markedly stimulated by lowering the pH of the reaction mixture. These findings suggest that acidification of the cellular fluid plays an important role in the enhancement in Fe^{2+}-induced lipid peroxidation of cellular membranes *in vivo*.

To investigate this possibility, we examined the effects of Al^{3+} on Fe^{2+}-induced lipid peroxidation in phospholipid liposomes under acidic conditions and chose pH 5.0 as a model pH of acidosis, because aluminum ions form numerous types of the hydrated aluminum complexes above pH 5.0 (*18*).

The results suggested that a marked stimulatory effect of Al^{3+} on the lipid peroxidation under acidic conditions is related to phospholipid composition and the physical

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Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; DPH, 1,6-diphenyl-1,3,5-hexatriene; TBARS, thiobarbituric acid-reactive substances; BHA, 3(2)-*tert*-butyl-4-hydroxyanisole; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonate; MES, 2-(*N*-morpholino)ethanesulfonic acid monohydrate; BPT, basophenanthroline disulfonic acid disodium salt.

properties of the membrane lipids.

MATERIALS AND METHODS

Materials—Phosphatidylcholine (egg yolk, PC) and phosphatidylserine (bovine brain, PS) were purchased from Sigma (St. Louis, MO). 2-Thiobarbituric acid, 3(2)-*tert*-butyl-4-hydroxyanisole (BHA), *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), 2-(*N*-morpholino)ethanesulfonic acid monohydrate (MES), 1,6-diphenyl-1,3,5-hexatriene (DPH), and basophenanthroline disulfonic acid disodium salt (BPT) were obtained from Wako Pure Chemical (Osaka). The amounts of lipid hydroperoxides present in phospholipids were measured by iodometric method (*19*), and estimated to be 0.90 and 0.013 nmol/mg phospholipid (as cumene hydroperoxide) for PC and PS, respectively. Other chemicals used were of the purest grade commercially obtainable.

Preparation of Phospholipid Liposomes—Chloroform solutions of PC (30 mg/ml) and PS (5 mg/ml) were mixed, unless otherwise specified, and then evaporated to dryness with a stream of nitrogen gas. The residual solvent was completely removed under vacuum, the appropriate amount of 10 mM Tris-HCl buffer (pH 7.4) was added, and the mixture was sonicated with an Ultra Sonic Disrupter UR-200 P (Tomy Seiko, Tokyo) until the dispersion became clear. This clear solution was then centrifuged at $25,000 \times g$ for 20 min, and the supernatant was used in the present study. PC liposomes were prepared similarly, but without PS.

Lipid Peroxidation—The liposomal suspension (0.1 mg/ml) was incubated with 0.1 mM $FeSO_4$ in 50 mM acetate buffer (pH 5.0) or 50 mM TES/NaOH buffer (pH 7.4) for 30 min at 37°C in the presence and absence of $AlCl_3$, unless otherwise specified. The reaction was terminated by the addition of 5 mM BHA (as a final concentration). TBARS production was measured by the procedure reported previously (*20*), and represented as the absorbance at 530 nm. On the other hand, the amount of conjugated diene was calculated using the molar extinction coefficient of 25,200 at 233 nm (*19*).

Labeling of the Liposomes with DPH—The PC/PS or PC liposomes (1 mg/ml) were incubated with 1 μM DPH (dissolved in tetrahydrofuran) in 10 mM Tris-HCl buffer (pH 7.4) for 5 min at 37°C. The final concentration of tetrahydrofuran in the reaction mixture was 0.33%.

Fluorescence Measurements—The fluorescence measurements were performed by using a Hitachi 850 spectrofluorometer equipped with a rhodamine B quantum counter at 25°C. The excitation and emission wavelengths used for the DPH fluorescence measurement were 340 and 430 nm, respectively. To prevent error due to light scattering of the sample emission, a 390-nm cut off filter was used. The steady-state fluorescence polarization was expressed as the fluorescence anisotropy, γ, using the following equation: $\gamma = (I_V - I_H)/(I_V + 2I_H)$, where I_V and I_H represent the fluorescence intensities of the vertically and horizontally polarized light of the emission with vertically polarized excitation, respectively.

Measurement of Turbidity—The turbidity of the liposomal suspension was measured by monitoring the absorbance at 450 nm after the addition of $AlCl_3$ to the suspension (0.1 mg/ml liposomes in 50 mM acetate buffer, pH 5.0) as described in our previous paper (*9*).

Assay of Ferrous Ion Oxidation—The concentration of Fe^{2+} was colorimetrically measured by the use of BPT (*21*). The reaction was started by the addition of 0.1 mM $FeSO_4$ (as a final concentration) to the reaction mixture containing 0.1 mg/ml liposomes and 50 mM acetate buffer (pH 5.0) in the presence and absence of 0.5 mM $AlCl_3$ at 37°C. The total volume of the reaction mixture was 1 ml. The reaction was periodically terminated by the addition of 0.025% BPT containing 1.5 M sodium acetate (1.5 ml). Then 0.5 ml of 1 N HCl was added and the mixture was left for 10 min at room temperature. The amount of BPT-ferrous complex was calculated using the molar extinction coefficient of 22,350 at 535 nm. On the other hand, Fe^{3+} formation was determined by recording the increase in the absorbance at 310 nm (*22*) after the addition of 0.1 mM $FeSO_4$ to the liposomal suspension (0.1 mg/ml) with and without 0.5 mM $AlCl_3$ in 50 mM acetate buffer (pH 5.0).

RESULTS

Effects of pH Variation—The effects of $AlCl_3$ on Fe^{2+}-induced lipid peroxidation in PC/PS liposomes were examined in the pH range of 5.0-7.4.

As shown in Fig. 1, the stimulatory effect of Al^{3+} on Fe^{2+}-induced TBARS production in the liposomes showed a biphasic response to the pH variation. The maximum stimulation was observed at pH 6.0. In the absence of Fe^{2+}, $AlCl_3$ itself did not induce TBARS production in the pH range tested (data not shown). On the other hand, an appreciable stimulation of Fe^{2+}-induced TBARS production in PC liposomes by Al^{3+} was not observed below pH 6.0. ($A_{530} = 0.374 \pm 0.012$ and 0.400 ± 0.012 for the systems without and with 0.5 mM $AlCl_3$ at pH 5.0, respectively).

The difference in the Al^{3+} effect on the lipid peroxidation between PC/PS and PC liposomes under acidic conditions was also confirmed in terms of conjugated diene formation (Fig. 2).

The effects of the content of PS in PC/PS liposomes upon

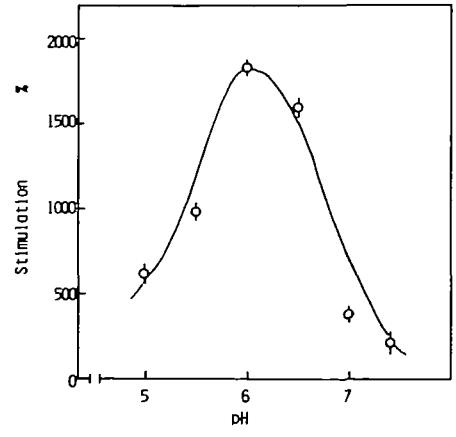

Fig. 1. **pH dependence of stimulatory effect of $AlCl_3$ on Fe^{2+}-induced lipid peroxidation in PC/PS liposomes.** The liposomes (0.1 mM) were incubated with 0.1 mM $FeSO_4$ in the presence and absence of 0.5 mM $AlCl_3$ in 50 mM acetate buffer (pH 5.0), 50 mM MES/NaOH buffer (pH 5.5-7.0) or 50 mM TES/NaOH buffer (pH 7.4) for 30 min at 37°C. Values are expressed as relative to those in the absence of Fe^{2+} at each pH, and represented as means±SD of three independent determinations.

the stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation at pH 5.0 are presented in Fig. 3.

As shown in the figure, the Al^{3+} effect increased with increasing proportion of PS in PC liposomes. On the other hand, the extent of Fe^{2+}-induced TBARS production in PC liposomes in the absence of Al^{3+} was not stimulated by increasing concentrations of PS (data not shown), indicating that enhancement of the Al^{3+} effect associated with increasing concentrations of PS is not due to the increase of the available substrate for peroxidation, and that the stimulatory effect of Al^{3+} on the lipid peroxidation under acidic conditions is largely dependent on the phospholipid composition.

Figure 4 shows the concentration dependence of $AlCl_3$ on Fe^{2+}-induced lipid peroxidation in PC/PS liposomes at pH 5.0. As can be seen in the figure, stimulation of Fe^{2+}-induced TBARS production by Al^{3+} was dependent on the concentration of $AlCl_3$, and the concentration of $AlCl_3$ required to induce the half-maximum effect was estimated to be approximately 80 μM.

Effect of Other Metal Ions on TBARS Production—In order to investigate Al^{3+} specificity for the stimulation of Fe^{2+}-induced lipid peroxidation in PC/PS liposomes under acidic conditions, other metal ions such as Tb^{3+}, Ga^{3+}, Zn^{2+}, and Mn^{2+} were used in place of Al^{3+}.

As shown in Table I, TBARS production in PC/PS liposomes was also markedly stimulated by the addition of

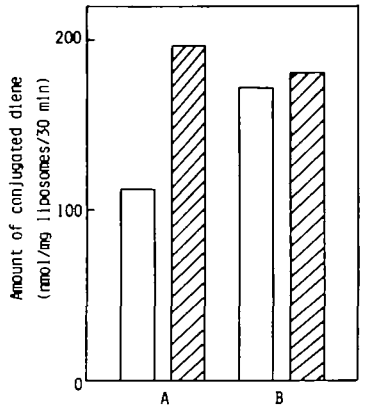

Fig. 2. **Effect of $AlCl_3$ on conjugated diene formation in PC/PS and PC liposomes at pH 5.0.** The liposomes were incubated with 0.1 mM $FeSO_4$ for 30 min at 37°C in the presence and absence of 0.5 mM $AlCl_3$. A and B represent PC/PS and PC liposomes, respectively. Symbols: □, in the absence of $AlCl_3$; ▨, in the presence of $AlCl_3$. Values are expressed as means of three independent determinations.

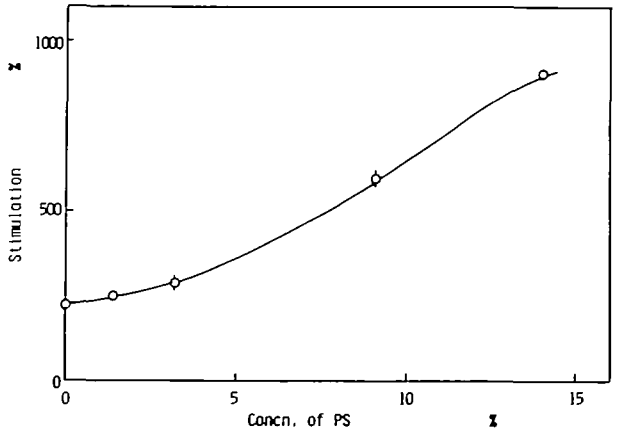

Fig. 3. **Effect of $AlCl_3$ on TBARS formation in PC liposomes with variable proportions of PS at pH 5.0.** The concentration of $AlCl_3$ was 0.5 mM. Values are expressed relative to that without Al^{3+} in each system, and expressed as means±SD of six independent determinations.

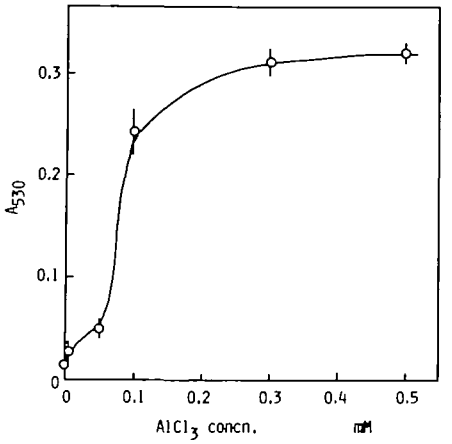

Fig. 4. **The $AlCl_3$ concentration dependence of Fe^{2+}-induced lipid peroxidation in PC/PS liposomes at pH 5.0.** The $AlCl_3$ concentration was varied from 0.01 to 0.5 mM. Values are expressed as mean±SD of six independent determinations.

TABLE I. **Effects of divalent and trivalent cations on Fe^{2+}-induced TBARS production in PC/PS liposomes at pH 5.0.** The concentrations of divalent and trivalent cations were 0.5 mM, except for Fe^{2+}. Values are expressed as means±SD of three independent determinations.

System	A_{530}
Fe^{2+} alone	0.059±0.003
Fe^{2+}/Zn^{2+}	0.067±0.007
Fe^{2+}/Mn^{2+}	0.013±0.003
Fe^{2+}/Tb^{3+}	0.327±0.027
Fe^{2+}/Ga^{3+}	0.283±0.004

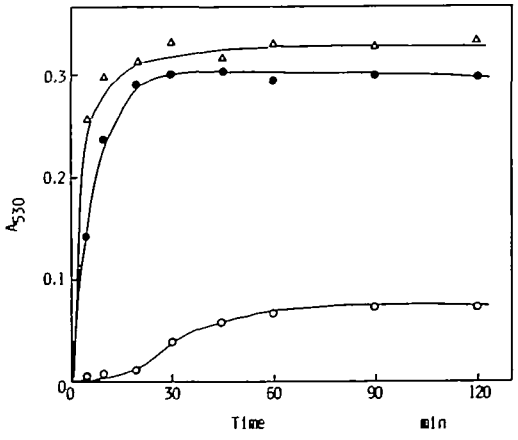

Fig. 5. **Time course of TBARS production in PC/PS liposomes at pH 5.0.** The concentrations of trivalent cations were 0.5 mM. Symbols: ○, control (Fe^{2+} alone); ●, $AlCl_3$; △, $GaCl_3$. Values are expressed as mean of three independent determinations.

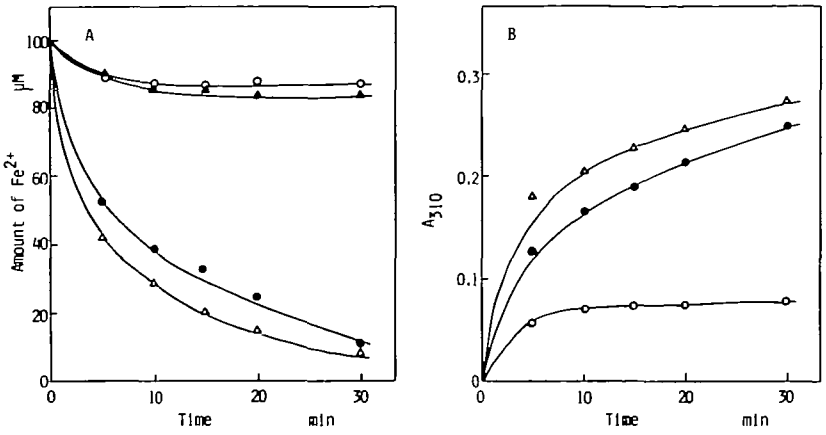

Fig. 6. **Time courses of ferrous iron oxidation in PC/PS liposomes at pH 5.0.** After the addition of 0.1 mM $FeSO_4$ to the liposomal suspensions, Fe^{2+} disappearance (A) and Fe^{3+} formation (B) were measured. The concentrations of trivalent cations were 0.5 mM. Symbols: ○, control (no Me^{3+}); ●, plus Al^{3+}; △, plus Ga^{3+}; ▲, plus Tb^{3+}. Values are expressed as means of three independent determinations.

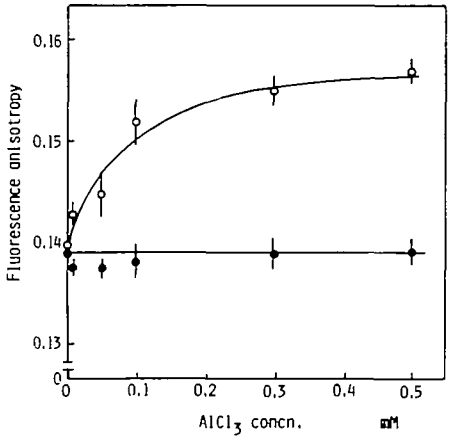

Fig. 7. **The $AlCl_3$ concentration dependence on the fluorescence anisotropy of DPH-labeled liposomes at 25°C.** The concentrations of DPH-labeled PC/PS and PC liposomes were 0.1 mg/ml. The concentration of $AlCl_3$ was varied from 0.01 to 0.5 mM. Symbols: ○, PC/PS liposomes; ●, PC liposomes. Values are expressed as means ± SD of three independent determinations.

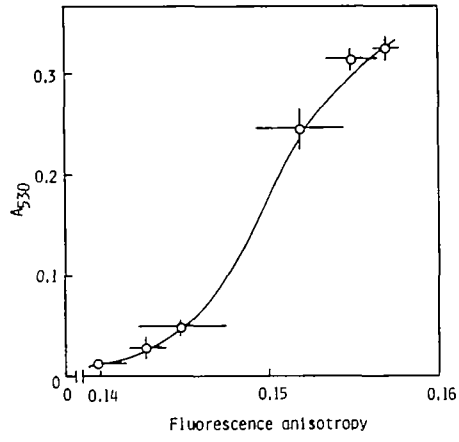

Fig. 8. **Relationship between the TBARS values and the fluorescence anisotropy of DPH-labeled PC/PS liposomes.** The data of TBARS production and the fluorescence anisotropy were obtained from Figs. 4 and 7, respectively.

$TbCl_3$ or $GaCl_3$. On the other hand, divalent cations showed no stimulatory effect on Fe^{2+}-induced TBARS production. The addition of $MnCl_2$ to the system rather effectively suppressed Fe^{2+}-induced TBARS production.

Next, the time courses of Fe^{2+}-induced TBARS production in PC/PS liposomes in the absence and presence of $AlCl_3$ or $GaCl_3$ were examined.

As can be seen in Fig. 5, TBARS production in PC/PS liposomes in the absence of these trivalent cations was initiated after a lag period of about 30 min. On the other hand, the addition of $AlCl_3$ or $GaCl_3$ to the reaction mixture virtually abolished the lag period and a constant level was reached after 30 min of incubation. In addition, it was found that the TBARS values in the systems with Al^{3+} and Ga^{3+} after 10-min incubation were almost the same.

Ferrous Ion Oxidation under Acidic Conditions—Figure 6 (A and B) shows Fe^{2+} disappearance and Fe^{3+} formation in PC/PS liposomes at pH 5.0 in the presence and absence of trivalent cations.

As can be seen in the figure, the addition of $AlCl_3$ or $GaCl_3$ to the liposomes resulted in a marked acceleration of Fe^{2+} disappearance and Fe^{3+} formation, suggesting that these trivalent cations stimulate Fe^{2+} oxidation. In contrast, Tb^{3+} had no effect on Fe^{2+} disappearance.

Change in Lipid Fluidity—The effects of Al^{3+} on the lipid organization in PC/PS and PC liposomes at pH 5.0 were examined by measuring the fluorescence anisotropy of DPH-labeled liposomes.

As can be seen in Fig. 7, the fluorescence anisotropy of DPH-labeled PC/PS liposomes increased depending on the $AlCl_3$ concentration, and the concentration of $AlCl_3$ required to induce half-maximal change in the anisotropy of the complex was estimated to be approximately 80 μM. Furthermore, it was found that there is a relationship between the extents of the fluorescence anisotropy of DPH-labeled PC/PS liposomes and the TBARS value under the same conditions (Fig. 8). On the other hand, the fluorescence anisotropy of DPH-labeled PC liposomes at pH 5.0 did not change in the concentration range of $AlCl_3$ tested.

Under these conditions, the turbidity of the PC/PS liposomal suspension (A_{450} = 0.020) did not change upon the addition of $AlCl_3$ in the concentration range tested (data not shown), indicating that the increased fluorescence anisotropy of PC/PS liposomes induced by the addition of $AlCl_3$ is not due to the formation of liposomal aggregates (*9*).

DISCUSSION

The present study suggests that the stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation under acidic conditions is dependent on the lipid composition and lipid organization.

As shown in Fig. 1, the pH profile of the stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation showed a biphasic response with a maximum effect at around pH 6.0. This finding is different from that reported by Oteiza (*10*), who found that the stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation in phospholipid liposomes decreased with increase of pH of the reaction mixture. The reason for the discrepancy is unclear at present. As is well known, Al^{3+} is readily hydrated above pH 5.0 and forms numerous types of hydrated products. Turner has reported (*18*) that aluminum mainly exist as the free ion (Al^{3+}) with a small amount (about 1%) of aluminum hydrated dimer, $Al_2(OH)_2^{4+}$ at pH 4.65. From this finding, it is suggested that the proportion of the hydrated products of Al^{3+} is closely related to the effect of Al^{3+} under acidic conditions. In the present study, we chose pH 5.0 as the experimental condition to examine the effects of interaction of Al^{3+} ions with the liposomal membranes on Fe^{2+}-induced lipid peroxidation, in order to avoid such complexity in the interpretation of the results.

As shown in Fig. 4, Fe^{2+}-induced lipid peroxidation in PC/PS liposomes at pH 5.0 was stimulated by Al^{3+} in a dose-dependent manner, although the lipid peroxidation in PC liposomes under the same conditions was almost unaffected by the addition of $AlCl_3$. This result suggests that the Al^{3+} effect on Fe^{2+}-induced lipid peroxidation under acidic conditions is dependent on the surface charge density of the liposomal membranes. In fact, an increase in the proportion of PS molecules in PC liposomes resulted in an enhancement of the Al^{3+} effect (Fig. 3). It has been reported (*18, 23*) that the concentration of free Al^{3+} is increased by lowering of the pH. Therefore, it seems that an increased Al^{3+} effect observed in PC/PS liposomes at pH 5.0 may be due to a facilitated interaction of Al^{3+} with the liposomal membranes, because Al^{3+} has a great affinity for PS (*24*). In a preliminary experiment, we found that the amount of Al^{3+} bound to the PC/PS liposomes at pH 5.0 was 75.0 ± 3.2 nmol/mg liposomes after incubation of the liposomes with 0.5 mM $AlCl_3$ for 4 h at room temperature, and that the amount of bound Al^{3+} decreased with increasing concentrations of KCl in the reaction mixture (64.5 ± 0.7 and 55.6 ± 4.5 nmol/mg liposomes for the systems with 10 and 50 mM KCl, respectively). On the other hand, the amount of Al^{3+} bound to PC liposomes was very small under the same conditions (0.4 nmol/mg liposomes). From these results, it seems that binding of Al^{3+} on the liposomal surface is an important factor for the Al^{3+} effect.

It is of interest that Ga^{3+} and Tb^{3+} also showed a stimulatory effect on Fe^{2+}-induced TBARS production in PC/PS liposomes, like Al^{3+} (Table I). Furthermore, the addition of Ga^{3+} to PC/PS liposomes at pH 5.0 resulted in disappearance of the lag period in TBARS production (Fig. 5) and enhancement of Fe^{2+} disappearance and Fe^{3+} formation (Fig. 6, A and B). From these results, it is suggested that the stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation under acidic conditions may be directly related to Fe^{2+} oxidation and that Ga^{3+} is able to replace Al^{3+} as a stimulator of the lipid peroxidation of liposomes in the presence of Fe^{2+}. As is known, Ga^{3+} belongs to the IIIa group of chemical elements, like Al^{3+}, and has a similar ionic radius (0.62 Å) and charge density to those of Al^{3+} (0.51 Å) (*25*). The similarity of chemical properties between Al^{3+} and Ga^{3+} strongly suggests that these cations would have similar binding capacities for ligands, *e.g.*, phosphate and carboxy groups. On the other hand, Tb^{3+} had no significant effect on Fe^{2+} disappearance (Fig. 6A), despite stimulation of TBARS production (Table I), suggesting that mechanism of the stimulation of lipid peroxidation associated with Tb^{3+} is different from that with Al^{3+} or Ga^{3+}. In addition, these results suggest that an Fe^{2+}-O_2-Fe^{3+} complex is not necessary as a specific initiator in lipid peroxidation induced by Fe^{2+}, although Minotti and Aust have reported (*26*) that an Fe^{2+}-O_2-Fe^{3+} complex or a 1:1 ratio of Fe^{2+} to Fe^{3+} plays an important role in the initiation of Fe^{2+}-induced lipid peroxidation. Our interpretation is consistent with the conclusion of Aruoma *et al.* (*27*) that Fe^{2+}-O_2-Fe^{3+} complex formation is not be required in the initiation process of Fe^{2+}-induced lipid peroxidation.

The fluorescence anisotropy of DPH-labeled PC/PS liposomes at pH 5.0 increased with increasing concentrations of $AlCl_3$ (Fig. 7). In contrast, the addition of $AlCl_3$ to PC liposomes under the same conditions did not induce an appreciable stimulation of Fe^{2+}-induced TBARS production or change in the fluorescence anisotropy of the dye molecules embedded in the liposomes. These results suggest that changes in the lipid dynamics in the liposomal membranes play an important role in the occurrence of the stimulatory effect of Al^{3+} on Fe^{2+}-induced lipid peroxidation in PC/PS liposomes under acidic conditions. In fact, it was found that there is a relationship between the extents of the fluorescence anisotropy and TBARS production of the liposomes in the presence of various concentrations of $AlCl_3$ (Fig. 8). In addition, it seems that the agreement of the $AlCl_3$ concentrations required to induce half-maximal production of TBARS and changes in the fluorescence anisotropy of DPH-labeled PC/PS liposomes (approximately 80 μM; Figs. 4 and 7) also supports this interpretation. Recently, McLean and Hagaman have reported (*28*) that an increase of the membrane fluidity decreases lipid peroxidation rates. Based on these results, we speculate that changes in the lipid organization induced by interaction of negatively charged liposomal constituents with Al^{3+} may be a mechanism for the stimulating effect of Al^{3+} under acidic conditions, and that an increased packing of acyl chains in the lipid layer may facilitate the initiation and/or propagation of the radical-mediated chain reactions in lipid peroxidation.

It is of interest that the $AlCl_3$ concentrations described above are close to those reported for inhibition of choline transport in erythrocytes and brain synaptosomes (*29, 30*). Although the concentration of Al^{3+} used in the present study may be much higher than that detected in patients suffering from Al^{3+} poisoning, this agreement of the Al^{3+} concentrations also suggested that changes in the ordering in the fatty acid chains in membrane lipids are closely related to the development of Al^{3+} toxicity *in vivo*. In addition, the present study raises the possibility that Al^{3+} toxicity may be enhanced under acidic conditions, *i.e.* acidosis. Further studies including *in vivo* experiments will

be necessary to clarify the relationship between acidosis and lipid peroxidation in the context of Al^{3+} toxicity.

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