Biochimica et Biophysica Acta 854 (1986) 93–101
Elsevier

BBA 72866

Cytolytic activity of liposomes containing stearylamine

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(Received June 3rd, 1985)

Key words: Liposome-cell interaction; Stearylamine; Hemolysis; Cytolytic activity

In order to develop the cytotoxic liposome, the cytolytic effect of polycationic liposome was examined. Upon incubation of the stearylamine-containing liposome (stearylamine-liposome) with rabbit erythrocyte, a significant extent of hemolysis was observed. Hemolytic activity of the liposome depends on the amount of stearylamine in the liposome membrane. The plots of the initial rate of hemolysis versus the concentration of stearylamine-liposome showed a sigmoidal curve, suggesting that stearylamine-liposomes act cooperatively on the erythrocyte membrane. Hemolytic activity of stearylamine-liposome was markedly influenced by the composition of hydrocarbon chains of the phospholipids in the liposome membrane, suggesting that the membrane fluidity of stearylamine-liposome is important to evoke the hemolysis. Since the liposomes containing acidic phospholipids inhibited markedly the stearylamine-liposome-caused hemolysis, it is likely that the primary target of stearylamine-liposome is the negatively charged component(s) such as acidic phospholipids on the erythrocyte membrane. Furthermore, stearylamine-liposome induced the release of the intravesicular contents from the liposome made of acidic phospholipids but not from the liposome made of phosphatidylcholine only. These results suggest that stearylamine-liposome interacted with the negative charges of the erythrocyte membrane and eventually damaged the cell. Erythrocytes from rabbit, horse and guinea pig are highly susceptible to stearylamine-liposome but those from man, sheep, cow and chicken are less so.

Introduction

Interactions of liposome membranes with intact cells or with other liposomes have been extensively studied as models for cell-cell interactions and physiological processes such as membrane fusions and adhesions (see Ref. 1 for a review). These studies are extended to the cell technology such as the transfer of genes, proteins or lipids to the specific cells [2–7]. Another interesting application of such studies is to develop the drug-carrier liposomes to deliver antitumor drugs, enzymes, etc., into the target cells [8–12]. Examples are the transfer of DNA from Simian virus 40 to the African green monkey kidney cells [3,4] and the delivery of methotrexate, an antitumor drug into the target cells by using the liposome bearing monoclonal antibody [10]. We are interested in developing the cytotoxic liposome in which the liposome itself has an ability to damage the target membrane rather than the cytotoxic agent into the vesicles.

It is well recognized that polycationic amphipathic antibiotics such as polymyxin, etc., are toxic to prokaryotic and eukaryotic cells [13,14]. Recently it was shown that polymyxin B as well as other polycations such as polylysine, polyhistidine, etc., caused the aggregation of acidic liposomes

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPOPC, dipalmitoleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PC, phosphatidylcholine.

and consequently induced membrane fusion [15–17]. On one hand, an early study showed that the liposome containing phosphatidylcholine, lysophosphatidylcholine and stearylamine had an ability to lyse red blood cells [18]. They interpreted the result as that the lysophosphatidylcholine in the vesicle membrane plays an essential role for the fusion of the membranes and for a subsequent cell lysis [19,20]. Therefore, we thought that the study on the interactions between the polycationic liposome and cell would be meaningful in understanding the mechanism of the cytotoxic effect of such liposome.

In the present report we have studied the cytotoxic effect of the polycationic liposome containing stearylamine against the erythrocyte and found that stearylamine-liposome caused the hemolysis of rabbit erythrocyte.

Materials and Methods

Materials. Heparinized animal erythrocytes (Nippon Bio-Test Laboratories, Tokyo, Japan) and human erythrocytes (volunteers) were used within 2 weeks. Erythrocytes were washed twice with 10-fold ice-cold buffer A (see below) by centrifugation at $700 \times g$ for 10 min and were resuspended in the same buffer. Egg yolk phosphatidylcholine and bovine brain sphingomyelin were purchased from Sigma, St. Louis, MO. Dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, dipalmitoylphosphatidic acid, bovine brain phosphatidylserine, egg phosphatidylcholine phosphatidylglycerol, pig liver phosphatidylinositol, bovine heart cardiolipin and pig liver phosphatidylethanolamine were from Serdary Research Laboratories, London, Canada, and dipalmitoleoylphosphatidylcholine was from Avanti Biochemical Co., Birmingham, AL. Stearylamine and hexadecylamine were obtained from Wako Pure Chemical Industries, Osaka, Japan. Neuraminidase from *Clostridium perfringens* and trypsin were purchased from Sigma and Worthington Biochemical Co., Freefold, NJ, respectively. 5,6-Carboxyfluorescein was from Eastman Kodak Co., Rochester, NY. Sepharose CL-4B was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were of reagent grade or the highest purity available.

Buffer. Buffer A (147 mM NaCl/6 mM glucose/20 mM Tris-HCl (pH 7.2)), buffer B (294 mM sucrose/6 mM glucose/20 mM Tris-HCl (pH 7.2)) and the mixture of buffers A and B were used throughout.

Preparation of liposomes. The essential technique to prepare the liposomes was described earlier [21,22]. The mixture of phospholipids and stearylamine in chloroform was dried in a test-tube under a nitrogen gas stream and kept in an evacuated desiccator for at least 2 h. The dried lipid film was suspended in buffer A and this was subjected to sonic oscillation for 5 min at around 50°C by using a Branson Sonifier 200 equipped with a microtip (Branson Sonic Power Co., Banbury, CT).

Treatment of rabbit erythrocytes with neuraminidase or proteinase. The washed rabbit erythrocytes (final hematocrit of 2%) were incubated with various concentrations of enzyme in buffer A at 37°C for 60 min. Then, the enzyme-treated erythrocytes were washed three times with ice-cold buffer A by centrifugation at $700 \times g$ for 10 min. No hemolysis was visible by these treatments.

Measurement of hemolysis. Hemolysis was quantified by determining the absorbance at 540 nm. In a typical experiment, washed erythrocytes and stearylamine-liposomes were mixed in 600 μl of a solution comprising 74 mM NaCl/147 mM sucrose/6 mM glucose/20 mM Tris-HCl (pH 7.2) and the mixture was incubated at 37°C for desired times with stirring by using a gyrorotatory waterbath (New Brunswick Scientific Co., New Brunswick, NJ). An aliquot of the mixture was centrifuged and $A_{540\,nm}$ of the supernatant was determined. The complete hemolysis was attained by mixing the erythrocytes with 0.2% of Triton X-100.

Measurement of the leakage of intravesicular contents. Dried phospholipids were suspended in buffer A containing 100 mM carboxyfluorescein and sonicated as above. The liposomes were passed through a Sepharose CL-4B column (1×45 cm) equilibrated with buffer A to separate the liposomes from free carboxyfluorescein. The liposomes encapsulating carboxyfluorescein were mixed with stearylamine-liposome and they were incubated at 37°C for 5 min. Fluorescence intensity was monitored by using a Hitachi 650-10S

fluorescence spectrophotometer at 510 nm with an excitation wavelength at 320 nm [15]. The total amount of carboxyfluorescein in the liposome was determined by mixing the liposome with 0.2% of Triton X-100.

Results

Hemolysis of rabbit erythrocyte by the stearylamine-containing liposome

Rabbit erythrocytes were mixed with the liposomes composed of 10% (w/w) of stearylamine and 90% (w/w) of egg-yolk PC (10%-stearylamine-liposome) and they were incubated at 37°C. Hemolysis was quantified at various times. The results depicted in Fig. 1 showed the following. (i) Stearylamine-liposome caused the hemolysis of rabbit erythrocytes with a lag time. The lag time was inversely proportional to the concentration of stearylamine-liposome and the minimum lag time was calculated to be about 3 min (Fig. 1c). (ii) The plots of the initial rate of hemolysis versus the concentration of stearylamine-liposome showed a sigmoidal curve, suggesting a cooperative action of stearylamine-liposomes on the erythrocyte membrane (Fig. 1b). These results were interpreted as that stearylamine-liposomes attacked the erythrocyte membrane at multiple sites in a cooperative manner and the minimum time required to begin the hemolysis was about 3 min.

In order to examine the effect of the stearylamine density in the liposome membrane, the lipo-

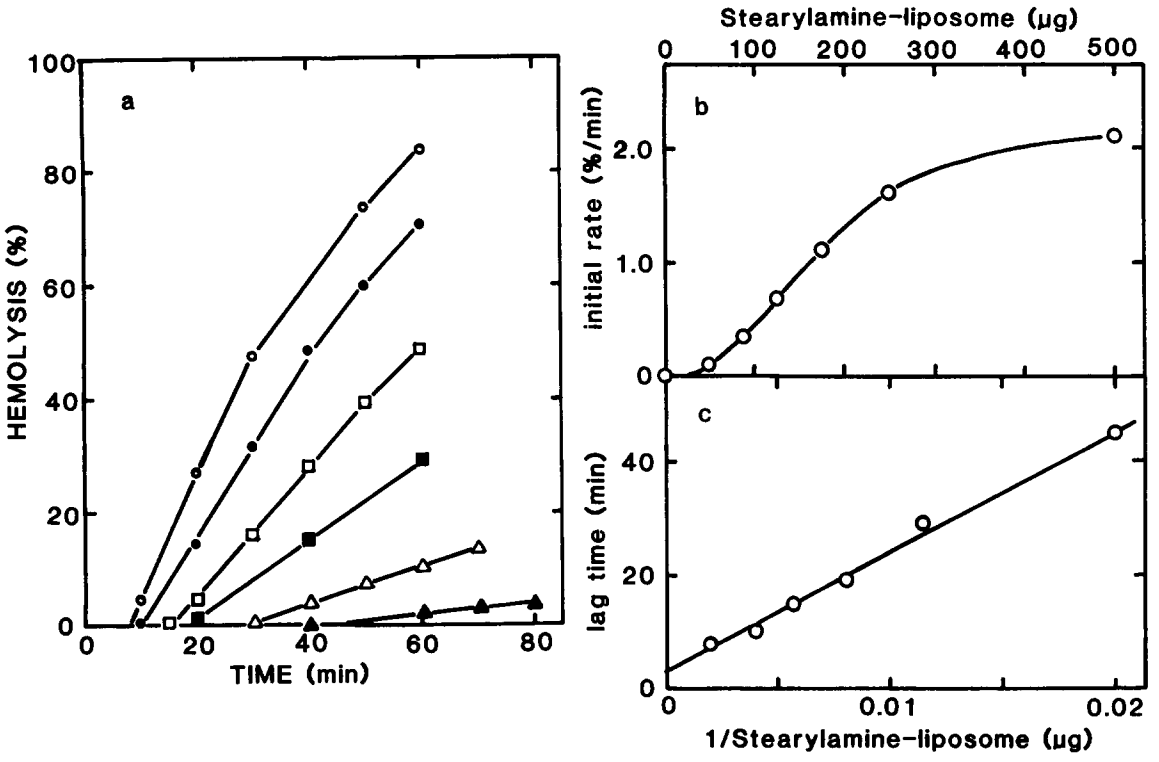

Fig. 1. Time-course of the hemolysis of rabbit erythrocytes by stearylamine-liposome. The liposomes were prepared from 10% (w/w) of stearylamine and 90% (w/w) of egg-yolk PC as described in Materials and Methods. Rabbit erythrocytes (final hematocrit 3%) were mixed with stearylamine-liposomes at 50 μg (▲), 87.5 μg (△), 125 μg (■), 175 μg (□), 250 μg (●) and 500 μg (○) in 600 μl of the buffer (74 mM NaCl/147 mM sucrose/6 mM glucose/20 mM Tris-HCl (pH 7.2)). The mixture was incubated at 37°C for the desired time with shaking (120 rpm on a gyrorotatory shaker). An aliquot of the mixture was centrifuged by using an Eppendorf centrifuge and A_{540nm} of the supernatant was determined. (a) The hemolysis was plotted against incubation time. (b) The initial rate of hemolysis in (a) was replotted against the amount of stearylamine-liposome. (c) The lag time of hemolysis in (a) was replotted against 1/stearylamine-liposome.

somes composed of various ratios of stearylamine/egg-yolk PC were prepared and they were tested for the hemolytic activity. The result depicted in Fig. 2 indicates that the liposomes containing less than 5% (w/w) of stearylamine lacked the hemolytic activity, and the liposomes containing 7.5–12.5% of stearylamine showed an abrupt increase of the hemolytic activity. Further increase of stearylamine in the liposome membrane resulted in the decrease of the hemolytic activity. The result suggests that only those liposomes with the critical density of stearylamine had the ability to cause the hemolysis.

Since the stearylamine molecule is positively charged at neutral pH, the experiment to confirm the involvement of the positive charge in the hemolytic activity was carried out. The liposomes containing phosphatidylserine, stearylamine and egg-yolk PC were prepared and they were tested for hemolytic activity (Fig. 3). The result shows that the hemolytic activity of such liposomes was inversely related to the amount of phosphatidylserine in the liposome membrane. This was interpreted as that the positive charge of stearylamine was neutralized by the negative charge of phosphatidylserine, which caused a reduced hemolytic activity. These results suggest that the polycationic nature of the liposome gained an ability to cause the hemolysis. Although the importance of the positive charge in hemolytic activity was confirmed, the question remained as to whether the hemolytic activity of stearylamine-liposome was related to the structure of the hydrophobic domain of stearylamine. In order to answer this, the hemolytic activity of the liposome containing hexadecylamine (C_{16}) was compared with that of stearylamine-liposome. The liposome containing 38 mol% of hexadecylamine had 76% hemolytic activity compared with that containing the same mol% of stearylamine (data not shown). The result suggests, thus, that the hydrophobic portion

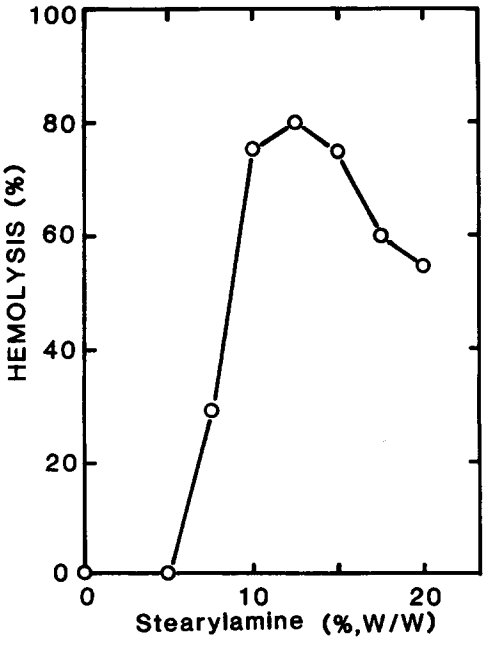

Fig. 2. Effect of the amount of stearylamine in the liposome membrane on hemolysis. Liposomes composed of various ratios of stearylamine/egg-yolk PC were prepared. Rabbit erythrocytes (final hematocrit 3%) were mixed with 250 μg of the liposomes in 600 μl of the buffer and the mixture was incubated at 37°C for 60 min with shaking. The extent of hemolysis was quantified as described in the legend to Fig. 1 and expressed as percent against that of Triton X-100-treated cells.

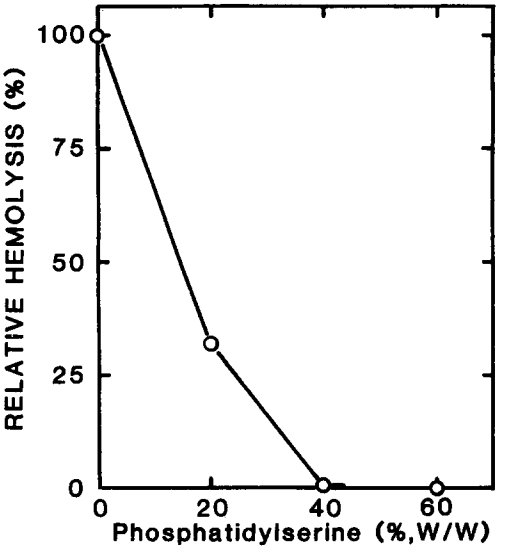

Fig. 3. Effect of phosphatidylserine on the hemolysis by stearylamine-liposome. The liposomes were prepared from 10% (w/w) of stearylamine and 90% (w/w) of various ratios of phosphatidylserine/egg-yolk PC. Rabbit erythrocytes (final hematocrit 3%) and the liposomes (250 μg) were mixed in 600 μl of the buffer and the mixture was incubated at 37°C for 60 min with shaking. The extent of hemolysis was plotted as percent against the hemolysis by stearylamine-liposome without phosphatidylserine.

of alkylamine is also involved in the hemolytic activity.

Effect of fatty acid composition of phosphatidylcholine in the stearylamine-liposome membrane on the hemolysis

The results described above indicate that the hydrocarbon chain of alkylamine had a slight effect on the hemolytic activity. A question which still remained was whether the fatty acid composition of PC in the liposome membrane is involved in the expression of the hemolytic activity. An experiment to answer this question was performed by examining hemolytic activities of the liposomes containing 90% (w/w) of either egg-yolk PC, DMPC (C_{14}), DPPC (C_{16}) or DPOPC (C_{16}, *cis*-9,10 diunsaturate) and 10% (w/w) of stearylamine. The result shows that all the liposomes showed a similar hemolytic activity, except that the liposomes containing DPPC had a significantly lower hemolytic activity (the liposomes containing DPPC had about 15% hemolytic activity compared with that of the liposomes containing egg-yolk PC, data not shown). Since the phase-transition temperatures of DPOPC, egg-yolk PC, DMPC and DPPC are shown to be $-38°C$, -15 to $-7°C$, 23°C and 41°C, respectively [21,23], the above result can be best interpreted as that the fluidity of the liposome membrane directly affects on the action of stearylamine-liposome.

Search for the target(s) of the stearylamine-liposome

As it was shown that the positive charge of stearylamine on the liposome is important to evoke the hemolysis, it is conceivable that the primary target(s) of stearylamine-liposome could be the negatively charged membrane component(s) of the erythrocyte. To test this, we measured the stearylamine-liposome-mediated hemolysis of the erythrocytes pretreated with neuraminidase (up to 5 μg/ml) or trypsin (up to 1.5 mg/ml). Hemolysis of the neuraminidase- or trypsin-treated erythrocytes appeared to be comparable with that of untreated cells (data not shown), suggesting that neither sialic acid nor the trypsin-sensitive membrane protein(s) is the target of stearylamine-liposome. Since the remaining candidate for the target of stearylamine-liposome could be acidic phospholipids, we have tested the effects of various phospholipids on the stearylamine-liposome-mediated hemolysis. The liposomes consisting of various phospholipids were mixed with stearylamine-liposome and erythrocyte, and the hemolysis was measured. The results are shown in Fig. 4. On the basis of the hemolysis in the presence of these liposomes, the phospholipids could be divided into two groups. One group included the liposomes made of zwitterionic phospholipids, in which the extents of the inhibition of the hemolysis by these phospholipids were less significant. More than 200 μM of phospholipids were required to obtain 50% inhibition of the hemolysis. Another class included the liposomes made of acidic phospholipids. The concentrations of phospholipids required to inhibit 50% of the hemolysis appeared to be 20, 60, 60, 80 and 100 μM for phosphatidic acid, phosphatidylserine, cardiolipin, phosphatidylglycerol and phosphatidylinositol, respectively. Among them, phosphatidic acid was the most efficient and followed by phosphatidylserine and cardiolipin. The result shows that stearylamine-liposome has a high affinity towards acidic phospholipids, although one cannot state firmly that these phospholipids are the targets of stearylamine-liposome attack.

Leakage of the vesicle contents by stearylamine-liposome

In order to know whether the binding of stearylamine-liposome to acidic group(s) in the membrane causes the damage of the membrane, the liposome encapsulating the fluorescence dye was used as the target of stearylamine-liposome. Liposomes were formed from various acidic phospholipids and/or egg-yolk PC entrapping carboxyfluorescein in the intravesicular space and they were treated with stearylamine-liposomes. As the release of the dye from the liposome causes the dequenching of the fluorescence, the membrane damage can be monitored by the fluorescence at 510 nm. The leakage of carboxyfluorescein from the liposome made of phosphatidylserine only increased linearly as the amount of stearylamine-liposome was increased to about one-third the weight of the target liposome and reached a plateau thereafter (Fig. 5). Stearylamine-liposome also caused the dye leakage from the liposomes composed of phosphatidylserine/PC or phosphatidic

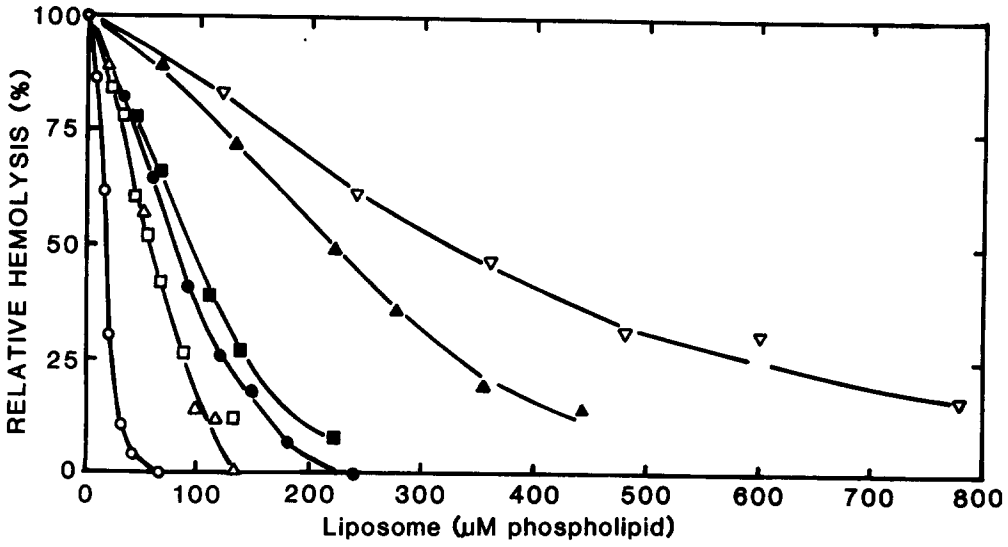

Fig. 4. Effect of various phospholipids on the hemolysis by stearylamine-liposome. The liposomes containing 20% (w/w) of egg-yolk PC and 80% (w/w) of either phosphatidic acid (○), phosphatidylserine (□), cardiolipin (△), phosphatidylglycerol (●), phosphatidylinositol (■), phosphatidylethanolamine (▲) or egg-yolk PC (▽) were prepared. The various concentrations of these liposomes were mixed with 3% of rabbit erythrocytes and 250 μg of the 10%-stearylamine-liposome in 600 μl of the buffer and the mixture was incubated at 37°C for 60 min with shaking. The extent of hemolysis in the presence of these liposomes was expressed as percent of that of stearylamine-liposome without these liposomes.

acid/PC, but not at all from the liposome made of PC only (data not shown). We confirmed that the liposome without stearylamine caused no measurable leakage of the dye from the acidic liposome (data not shown). These results clearly indicate that stearylamine-liposome acted on the acidic phospholipid and caused the membrane damage.

Susceptibility of erythrocytes from different animal sources to stearylamine-liposome

From the results of the above experiment, it became clear that the negative charge at the target membrane plays an important role on the accessibility and susceptibility towards stearylamine-liposome. However, it is not clear if the accessibility of

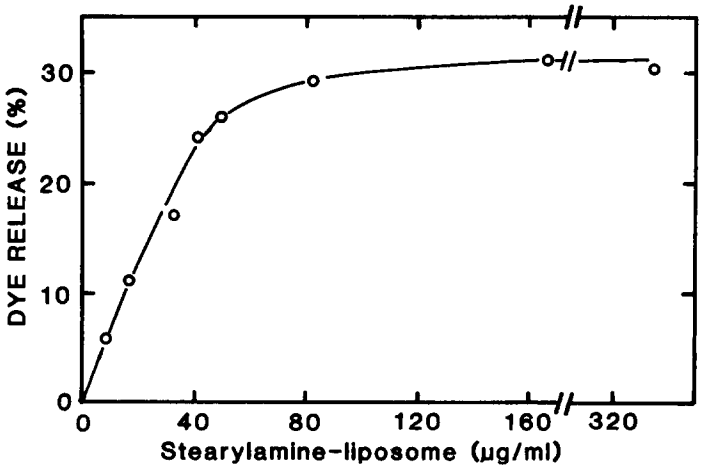

Fig. 5. Leakage of the intravesicular contents from the acidic liposome by stearylamine-liposome. The liposomes were prepared from phosphatidylserine only in the presence of 100 mM carboxyfluorescein as described in Materials and Methods and 100 μg of these liposomes were mixed with various amounts of 10%-stearylamine-liposomes in 600 μl of the buffer. The mixture was incubated at 37°C for 5 min with shaking. The extent of carboxyfluorescein release was determined by monitoring the increase of fluorescence intensity at 510 nm excited at 320 nm.

the liposome alone determines the susceptibility of the erythrocytes to stearylamine-liposome. To test this, the susceptibility of erythrocytes from various animals was examined. The result shows that the erythrocytes from horse, guinea pig and rabbit were highly susceptible to stearylamine-liposome, whereas the erythrocytes from man, sheep, chicken and cow were less so (Table I). Although the reason for this difference is not clear at present, we suspect that the topological distribution of the membrane component(s) accepting the stearylamine-liposome as well as unknown factors are involved in the susceptibility of the erythrocytes. In fact, it was reported that chicken erythrocyte membrane, being resistant to stearylamine-liposome, lacks both phosphatidic acid and phosphatidylserine [24]. Another suggestive datum to explain the different suceptibility among erythrocytes of these animals is the relative amounts of sphingomyelin/PC in the erythrocyte membranes [25]. The ratio of these lipids was found to be small in the stearylamine-liposome-susceptible membranes, whereas the ratio was found to be high in the membranes resistant to the stearylamine-liposome treatment. This therefore suggests the possibility that sphingomyelin somehow blocks the action of stearylamine-liposome. Since the sphingomyelin content in erythrocyte membrane cannot be modulated, rabbit erythrocytes were treated with the liposomes made of sphingomyelin

TABLE I

SUSCEPTIBILITY OF ERYTHROCYTES FROM VARIOUS ANIMAL SOURCES TO STEARYLAMINE-LIPOSOME

Erythrocytes (final hematocrit of 3%) were mixed with 250 μg of 10%-stearylamine-liposome in 600 μl of the buffer and they were incubated at 37°C for 60 min with shaking. The extent of hemolysis was determined as described in the legend to Fig. 1. The hemolysis of rabbit erythrocytes was taken as 100%.

Animal source	Relative hemolysis (%)
Horse	136
Guinea pig	133
Rabbit	100
Man	8
Sheep	5
Chicken	1
Cow	<1

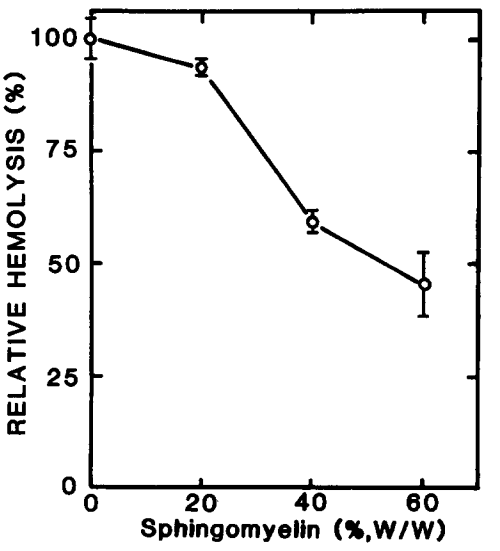

Fig. 6. Effect of sphingomyelin on hemolysis by stearylamine-liposome. The liposomes were prepared from 10% (w/w) of stearylamine and 90% (w/w) of various ratios of sphingomyelin/egg-yolk PC. The liposomes (250 μg) were mixed with 3% of rabbit erythrocytes in 600 μl of the buffer and they were incubated at 37°C for 60 min with shaking. The extent of hemolysis was plotted as percent of the hemolysis by the stearylamine-liposome without sphingomyelin.

(0–60%, w/w), egg-yolk PC and stearylamine. The result depicted in Fig. 6 shows that the hemolysis by the liposome containing 60% of sphingomyelin appeared to be 50% of that by stearylamine-liposome without sphingomyelin. This therefore suggests that the sphingomyelin content can be a factor that alters the susceptibility of erythrocyte to stearylamine-liposome. The result does not exclude other possibilities.

Discussion

On the study of the cytolytic property of the polycationic liposome, we have described the stearylamine-liposome-mediated hemolysis of rabbit erythrocytes. The experimental results revealed the following: (i) Stearylamine-liposome causes the hemolysis of rabbit erythrocyte acting on the cell membrane cooperatively. (ii) Both positive charge and the fluidity are important to potentiate the hemolytic activity of the liposome. (iii) The primary target of stearylamine-liposome seems to be the acidic group(s) such as acidic phospholipids of the

erythrocyte membrane. (iv) Erythrocytes from various animals show different susceptibility to stearylamine-liposome. This is the first case, to our knowledge, in which the hemolytic activity of polycationic liposome has been demonstrated.

The hemolytic activity of stearylamine-liposome depends on the liposome concentration. The amount of 10%-stearylamine-liposome required to yield the half-maximum rate of hemolysis appeared to be about 175 μg per 0.6 ml of the buffer (Fig. 1b). Stearylamine concentration for the half-maximum rate of hemolysis is about 0.1 mM or 26 μg/ml. As the plots of initial rate of hemolysis versus the concentration of stearylamine-liposome showed a sigmoidal curve and the hemolytic reaction showed a lag time, it is suggested that the hits by stearylamine-liposomes on the erythrocyte membrane make the cell more susceptible to the subsequent hits by these liposomes, and multiple hits on the erythrocyte cause the hemolysis.

We have investigated what physical conditions of the liposome membrane influence the hemolytic activity. It was indicated that both the positively charged amino groups and the membrane fluidity are important for the hemolytic activity of stearylamine-liposome. Since the polycationic nature of the liposome is essential for the hemolytic activity, it is suggested that multiple ionic interactions closely appose the liposome membrane to the erythrocyte membrane and such apposition induces the subsequent membrane damage. As the stearylamine-liposome containing DPPC showed a markedly lower hemolytic activity than that containing egg-yolk PC, DMPC or DPOPC and the phase-transition temperature of DPPC is only above the incubation temperature, it is suggested that the membrane fluidity of the liposome influenced the hemolytic activity of stearylamine-liposome. The effect of the membrane fluidity of the liposome on the hemolytic activity may be accounted for by the membrane fusion, because fusion efficiency is known to depend on the membrane fluidity [26]. However, we cannot exclude other possibilities.

In search of the receptor molecule(s) on the erythrocyte, it was suggested that sialic acid or membrane protein is not a likely candidate. We examined the effect of acidic phospholipids on the hemolytic activity of stearylamine-liposome, since the specific enzyme which cleaves acidic phospholipids is not available. As the acidic phospholipids inhibited the stearylamine-liposome-mediated hemolysis effectively (Fig. 4) and stearylamine-liposome damaged the liposome membrane made of acidic phospholipids as shown by the release of vesicle contents (Fig .5), it is possible that the primary target of stearylamine-liposome could be acidic phospholipid(s) of the erythrocyte membrane. Schlegel et al. [27] also used a similar technique to determine phosphatidylserine as the binding site for vesicular stomatitis virus, showing that phosphatidylserine totally inhibited the binding of the virus on Vero cell. Localization of acidic phospholipids such as phosphatidylserine at the outer leaflet of the plasma membranes is known [27,28].

Summing all these results together, we would like to propose a possible mechanism of stearylamine-liposome-mediated hemolysis as follows. Stearylamine-liposome binds to the acidic group(s) such as acidic phospholipid(s) of erythrocyte membrane at multiple sites by ionic interactions and the liposome and erythrocyte membranes come in close contact. After this, the damage of the target membrane is somehow induced by the multiple attacks of stearylamine-liposomes and eventually causes the release of intracellular contents. Since a polycation such as polylysine is known to induce membrane fusion [15–17] and membrane fluidity is reported to play a role in membrane fusion [26], and the above-described results fulfill these conditions, the membrane damage may be induced by the fusion between liposome and erythrocyte membranes. The precise mechanism of the membrane damage must still be worked out.

It has been reported that polycations such as polylysine, polyhistidine, polymyxin B, etc., are the substances that act on the membranes causing the aggregation, fusion and the lysis of the membranes [15–17]. These membrane-disordering agents are not useful drugs, since these agents attack cells randomly at relatively high concentrations. If a liposome membrane bound with the cationic component could be prepared, the liposome itself would act as the membrane-damaging agent. In such a case, the amount of free cationic component of the aqueous solution could be minimized and therefore the adverse effects of such

drugs could be significantly lowered. Therefore, such a liposome could be used as the cytolytic bullet attacking the specific cells upon targeting. The targeting facility could be introduced onto the liposome membrane by chemical cross-linking of monoclonal antibody against the target cells [29–33]. In this respect, this work may provide a new direction for the use of the liposome as the membrane-damaging agent. We believe that the liposome containing stearylamine or other cationic components and bearing the monoclonal antibody to the target cell kills the cell specifically. Such study is under progress in our laboratory.

Acknowledgements

This study was supported by grants from the Ministry of Education, Science and Culture of Japan and by the Organization of General Research of Tokai University.

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