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acid, *N*-(3-carboxy-1-oxopropyl)-, 1,5-dihexadecyl ester (SA), has been used as an anionic component. Vesicles containing SA have been demonstrated to efficiently encapsulated hemoglobin, and given their stability, can be infused in considerably large doses as a red blood cell substitute [15,16]. In addition, administration of this formulation is not associated with considerable rejection by the blood immune system and complement in animals [17,18]. Recent collaboration involving our group found that vesicles containing SA are selectively captured by bone marrow macrophages at small injection doses in rabbits [19]. This targeting of bone marrow has not yet been reported for conventional anionic vesicles. These findings suggest that the surface characteristics of the anionic vesicles have a marked effect on their associated biological events and that this is highly dependent upon the nature of the acidic groups.

Based on the hypothesis that the negative electrostatic charge of acidic phospholipids on the surface of vesicles is one of the factors responsible for inducing complement activation, the electrostatics of vesicles containing SA may differ from those of vesicles containing acidic phospholipids. Conversely, when the electrostatic interactivity of these acidic lipids is equal, biological events such as complement activation are likely to be mediated by the molecular specificity of acidic lipids. To elucidate what aspects are critical to the biocompatibility and performance of anionic vesicles, a comparative analysis of the electrostatic interfacial properties of vesicles containing either SA or acidic phospholipids is essential. In this study, we elucidated the electrostatic interfacial properties of vesicles containing SA or an acidic phospholipid by electrophoretic mobility measurement and observed complement activation after the infusion of these vesicles in animals. The results clearly demonstrated that the negatively charged group on the surface of anionic vesicles is not critical to the activation of complement.

## 2. Materials and methods

### 2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (PC), cholesterol (CH), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (PG), and L-glutamic acid, *N*-(3-carboxy-1-oxopropyl)-, 1,5-dihexadecyl ester (SA), were purchased from Nippon Fine Chemical Co. Ltd. (Osaka, Japan). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[monomethoxy poly(ethylene glycol) (5000)] (PEG-DSPE) was purchased from NOF Co. (Tokyo, Japan). Pentalysine and poly-L-lysine (Mw. 15–30 kDa) were purchased from SIGMA (St. Louis, MO).

### 2.2. Preparation of vesicles

Vesicle samples were produced using PC and CH (1/1, m/m, PC-vesicles), and varying amounts of SA (SA-vesicles) or PG (PG-vesicles). The mixed lipids were added to 10 mM phosphate buffer (NaCl; 20 mM, pH 7.4) and the dispersion was introduced into an extruder (Lipex Biomembrane, Canada) and extruded through the membrane filters (final pore size: 0.2 µm, Isopore®, Millipore, Tokyo, Japan) under pressure using nitrogen gas. For animal experiments, vesicles were composed of PC, CH, and SA or PG (1:1:0.2 molar ratio), with 0.3 mol% of PEG-DSPE incorporated to prevent the aggregation of vesicles [20]. Samples for animal experiments were prepared under sterile conditions.

### 2.3. Characterization of vesicles

The diameters of the resulting vesicles were determined with a COULTER submicron particle analyzer (N4SD, Coulter, Hialeah, FL), and the average diameter ± standard deviation (SD) was calculated. The phospholipid concentration was determined using a cholineoxidase method (Phospholipid C Test Wako; Wako Pure Chem., Tokyo). Endotoxin contamination in the samples prepared for the animal experiment was determined to be less than 0.1 EU/mL by the Limulus assay test [21].

### 2.4. Determination of zeta potential

A 10 µL aliquot of vesicles (lipid concentration: 2 g/dL) was diluted in 2 mL of 10 mM phosphate buffer (pH 3–9, 37 °C) containing 20 mM NaCl and incubated for 1 h at 37 °C. The pH was determined using a pH meter with a pH electrode (F-52, HORIBA, Kyoto, Japan) at 37 °C and the electrophoretic mobility of vesicles (lipid concentration: 0.01 g/dL) were determined by Laser Doppler Velocimetry (Zeta-Sizer Nano ZS, Malvern Instruments, Malvern, Worcestershire, UK). Measurement conditions and parameters were as follows: 37 °C, dielectric constant 74.4 (for dilute water solution), viscosity 0.6864 cP (for dilute water solution), and the applied voltage was 20 V/cm. The zeta potential was determined by measuring the electrophoretic mobility ($U_E$), and the zeta potential ($\zeta$) and applied to the Henry equation:

$$U_E = \frac{2\varepsilon\zeta f(\kappa R)}{3\eta} \quad (1)$$

where $\varepsilon$ is the dielectric constant, $\eta$ is the viscosity of the solvent, $f(\kappa R)$ is the Henry function, $\kappa$ is the Debye–Hückel parameter and the $R$ is the radius of the particle. The Smoluchowski equation was used with $f(\kappa R) = 1.5$ employed in the zeta potential calculations. Measurements were performed four times for each sample and statistical analysis was conducted using the average ± SD of the four measurements.

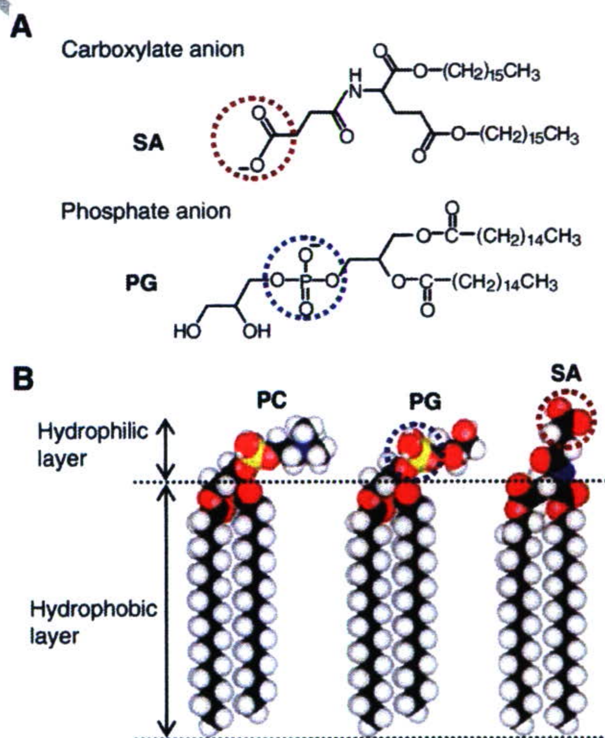


Fig. 1. Structure of anionic compounds for surface modification of vesicles. (A) Chemical structure of L-glutamic acid, *N*-(3-carboxy-1-oxopropyl)-, 1,5-dihexadecyl ester (SA) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (PG). The focus of this study is the different acidic groups shown in the dotted circle. (B) The CPK model of SA and PG with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (PC) to estimate the distance of acidic groups from the membrane surface of PC. CPK models show the structure with minimized energy in molecular mechanistic calculation.

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133 2.5. Electrostatic interactivity

134 The electrostatic interactivity of the anionic vesicles was evaluated using the change in the zeta potential in presence of $Ca^{2+}$, pentalysine, and poly-L-lysine (Mw. 15–30 kDa) as an index. A 10 μL aliquot of vesicles (lipid concentration: 2 g/dL) was diluted in 2 mL of 10 mM 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) buffer (pH 7.4, 37 °C) containing 0–3 mM $CaCl_2$ and 17–20 mM NaCl (total 20 mM), or containing varying amounts of pentalysine or poly-L-lysine (Mw. 15–30 kDa) with 20 mM NaCl. The dispersions were incubated for 1 h at 37 °C before mobility measurement of the vesicles was performed by electrophoresis as described in Section 2.4.

143 2.6. Animal experiments

144 Animal experiments were conducted under the guidelines recommended by the National Institutes of Health, Animal Use and Care and the protocol was approved by the Steering Committee for Animal Experimentation at Waseda University. Male Wistar rats (250±20 g) were anesthetized with ether. The vesicular dispersion (5 g/dL) was introduced into rats through the tail veins at 1 mL/min ($n$=5 for each sample). Each rat received 5.6 mL/kg of body weight of vesicle dispersion (lipids: 280 mg/kg of body weight). At 1 or 24 h after injection, the blood was collected and centrifuged to separate the serum ($1\times10^3$ g, 10 min). The collected serum was further ultracentrifuged to remove the vesicles ($3\times10^5$ g, 30 min). The 50% hemolytic unit of complement serum (CH50) was determined in accordance with general procedures for clinical laboratory tests by a commercial company (BML, Japan).

156 2.7. Statistical methods

157 The data from the animal experiments are reported as means±standard error of the mean. Statistical analysis was performed using Microsoft Excel for Windows and CH50 values were compared using Student's unpaired $t$ test.

160 **3. Results and discussion**

161 3.1. Samples

162 Two characteristic acidic lipids used in this study are shown in Fig. 1. SA and PG each have carboxylic acid and phosphoric acid as ionized groups, respectively. Both lipids have a strongly hydrophobic dialkyl structure to fix the ionized groups on vesicle surface. The molecular length indicated by the CPK model showed that the carboxylate anion of SA and the phosphate anion of PG would be located at the surface of PC-vesicles (Fig. 1B). Various amounts of SA and PG were incorporated into the PC/CH membrane (1:1, molar ratio) of the anionic vesicles. The size of vesicles was controlled by extrusion methods (final pore size: 0.2 μm), with final mean diameters of approximately 200 nm (Table 1). The vesicles prepared without acidic lipids

t1.1 Table 1
t1.2 Diameter of prepared vesicles containing various amounts of acidic lipid

| Acidic lipid (mol%) | Mean diameter±SD (nm) | |
|---|---|---|
| | SA-vesicles | PG-vesicles |
| 0 (PC-vesicles) | 232±60 | 232±60 |
| 1 | 224±58 | 230±60 |
| 5 | 193±50 | 196±58 |
| 9 | 205±40 | 204±49 |
| 15 | 194±45 | 199±55 |
| 20 | 198±52 | 194±64 |

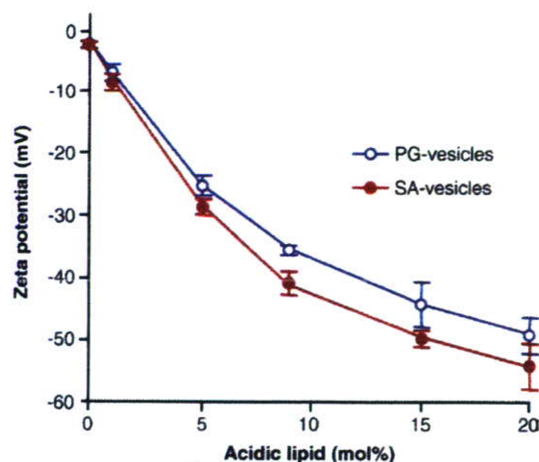


Fig. 2. Zeta potential of vesicles modified with SA (SA-vesicles) and PG (PG-vesicles) as a function of acidic lipid content. Zeta potentials were measured in 10 mM phosphate buffer (pH 7.4, NaCl; 20 mM) at 37 °C.

or with 1 mol% of acidic lipids tended to be slightly larger (ca 30 nm) than vesicles containing more acidic lipids. This effect of acidic lipids on the size of vesicles could be due to the improved dispersion stability of vesicles and electrostatic repulsion of the anionic surfaces. The vesicles without acidic lipids were observed to precipitate in a day, indicating poor dispersion stability. When the vesicles without acidic lipids contain a small amount of PEG-DSPE to prevent the aggregation of vesicles, the diameter of vesicles without acidic lipids was 202±49 nm. Therefore, we guess that the slightly large diameter of vesicles without acidic lipids or with 1 mol% of acidic lipids would be caused by the high aggregability of vesicles. The low aggregability of vesicles having a large zeta potential due to the electrostatic repulsive interaction between vesicles is an advantage of anionic vesicles as stable dispersions. 188

3.2. Zeta potential of vesicles 189

The zeta potential is the electrostatic potential at the hydrodynamic slip plane, and is characterized as having an electrical double-layer consisting of the Stern layer and the diffuse layer. Fig. 2 shows the zeta potential of prepared vesicles as a function of acidic lipid content at pH 7.4. Vesicles containing PC/CH (1:1, molar ratio) have an almost neutral surface (zeta potential: −2.22±0.62 mV), indicating that the surface is inactive for electrostatic events. The magnitude of the negative charge on the surface increased with the incorporation of SA or PG, indicating that the ionized groups of SA and PG act to characterize the vesicle surface depending on their content. The zeta potentials of SA-vesicles and PG-vesicles reached −54.2±3.68 mV and −49.0±2.89 mV for acidic lipids of 20 mol%, respectively, with the negative zeta potential of SA-vesicles being relatively higher compared to that of PG-vesicles at any concentration. In theory, the electrostatic potential is dependent upon distance from the membrane surface as well as surface charge density [22]. The slightly extended negative charge of SA from the surface shown in Fig. 1B, would reduce the distance between the change to slip plane, resulting in the higher negative zeta potential of SA-vesicles. 209

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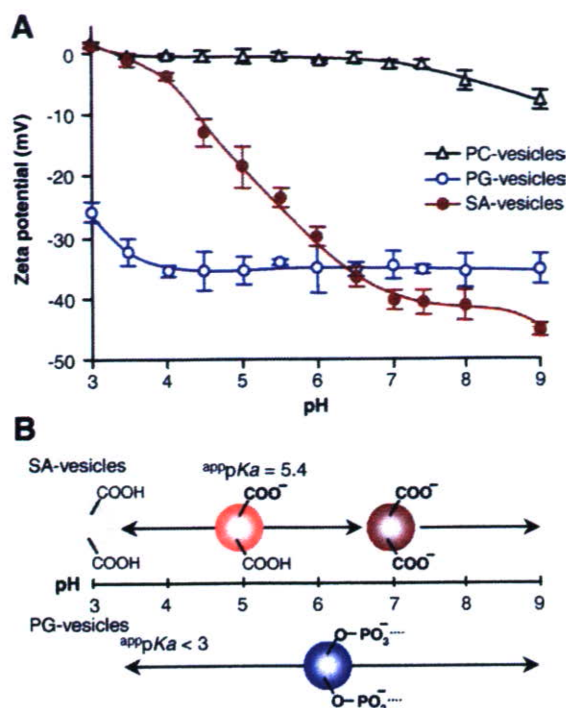


Fig. 3. Ionization state of acidic groups as a function of pH. (A) Zeta potential of vesicles (PC/CH, 1:1, molar ratio) (PC-vesicles), and vesicles containing 9 mol% of SA (SA-vesicles) or PG (PG-vesicles) at various pH. Zeta potentials were measured in 10 mM phosphate buffer (NaCl; 20 mM) at various pH (37 °C). (B) Schematic representation of the ionization state of acidic groups with pH. The apparent p$K_a$s of SA-vesicles and PG-vesicles were calculated to be 5.4 and <3.

This result demonstrated that the capacity of SA as an anionic component of vesicles is equal to acidic phospholipids at pH 7.4.

### 3.3. Ionization properties of acidic lipids

Stability of the ionization state conferred by acidity is an important characteristic of acidic compounds. We examined the ionization properties of acidic lipids as a function of pH using SA-vesicles and PG-vesicles containing acidic lipids of 9 mol% with zeta potentials of −40.7±2.09 mV and −35.4±0.61 mV (at pH 7.4), respectively. As shown in Fig. 3A, the zeta potential of SA-vesicles varied markedly depending on the environmental pH (pH 3–7). The change in the zeta potential of SA-vesicles is thought to reflect the ionization state of SA, as control vesicles without SA, namely PC-vesicles, maintained almost neutral surfaces irrespective of pH. The relationship between pH and the p$K_a$ of the acid is expressed using the well-known Henderson–Hasselbalch equation as follows:

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log\frac{[-\mathrm{COO}^-]}{[-\mathrm{COOH}]}. \quad (2)$$

When we analyzed the data shown in Fig. 3A using Eq. (2) and the assumption that the zeta potential was linearly correlated with the ionization acid, the p$K_a$ of the carboxyl group of SA was estimated as 5.4 (Fig. 3B). Above pH 7, the zeta potential of SA-vesicles was almost constant, indicating that the carboxyl group of SA would mostly be ionized above pH 7. The zeta potentials of PG-vesicles were almost constant in the range pH 4–9, indicating that the ionized form of the phosphoric acid moiety is stable in this range. The change in the zeta potential observed at a pH lower than pH 4, and its p$K_a$ would be lower than pH 3 [23,24]. Thus, we confirmed that the surface of SA-vesicles and PG-vesicles exhibited the characteristics of a weak acid with SA and a strong acid with PG, respectively, indicating that the individual characteristics of acidic groups are expressed on the surface of vesicles. We also observed that the magnitude of the negative electrostatic charges in SA-vesicles was equal to that observed in PG-vesicles at approximately neutral pH.

### 3.4. Electrostatic interactivity

$Ca^{2+}$ is found in biological fluids (normally 2–3 mM in plasma) and is known to mediate biological processes by binding to the anionic domains such as those involved in the specific binding of proteins to membranes [25,26]. Acidic phospholipids, such as PG and PS, are also known to bind $Ca^{2+}$ [27–29]. As shown in Fig. 4, the negative charge on anionic vesicles was suppressed by increasing the concentration of $Ca^{2+}$. When the concentration of $Ca^{2+}$ was increased to 3 mM, the zeta potentials of SA-vesicles and PG-vesicles were −16.4±1.9 mV and −13.8±1.4 mV, respectively. Recently, Hautala et al. reported that vesicles containing phosphatidic acid possess a specifically strong affinity for $Ca^{2+}$, and that the zeta potential of these vesicles changed from being strongly negative to positive after binding $Ca^{2+}$ [29]. These authors also showed that other acidic phospholipids, including PG, do not exhibit a strong affinity towards becoming cationic. Consequently, one aim of this experiment was to determine whether the binding of $Ca^{2+}$ is a specificity factor between SA-vesicles and PG-vesicles, and also whether the surface of SA-vesicles remained negative in the presence of $Ca^{2+}$. Our experiment showed that the surface of the SA-vesicles remained

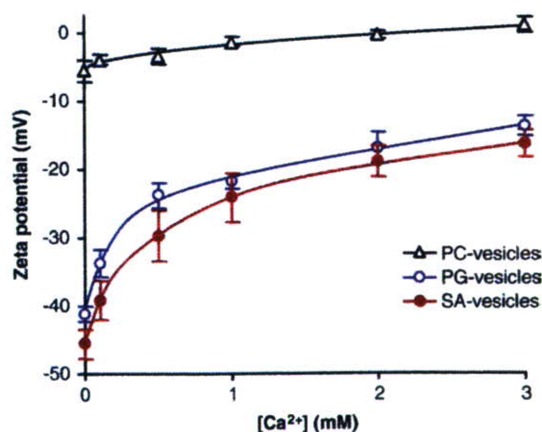


Fig. 4. Change in zeta potential of vesicles as a function of $Ca^{2+}$ concentration. Vesicles were dispersed at 100 μg/mL in 10 mM HEPES buffer (pH 7.4, at 37 °C) containing NaCl and $CaCl_2$ (total: 20 mM). PC-vesicles: PC/ CH (1:1, molar ratio), SA-vesicles: PC/CH/SA (1:1:0.2, molar ratio), and PG-vesicles: PC/CH/PG (1:1:0.2, molar ratio).

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268 negative in the presence of $Ca^{2+}$. In addition, comparisons of
269 SA-vesicles and PG-vesicles also showed that the specificity
270 of binding $Ca^{2+}$ was not observed.

271 Additional model-based studies of electrostatic interacti-
272 vity, pentalysine and poly-L-lysine (Mw. 15–30 kDa with a
273 repeating primary lysine amine) were also conducted. Oligo-
274 mers or polymers of lysine are often used to model basic
275 peptides or macromolecules and their electrostatic interactions
276 on membranes [30,31]. As shown in Fig. 5, the zeta potential
277 is a linear function of the concentration of pentalysine. The
278 lines, which represent the least-squares best fit, have slopes of
279 9.3 and 7.4 mV per decade for the pentalysine concentrations
280 in SA-vesicles and PG-vesicles. The slope for PC-vesicles was
281 as little as 0.26 mV per decade of pentalysine concentration
282 (data not shown), indicating that the negative charge of aci-
283 dic lipids mediate the interaction with basic pentalysine. A
284 change in the zeta potential is due to binding of basic pep-
285 tide [31] and a similar decay slope of the zeta potential would
286 indicate that the binding constant of a basic peptide to SA-
287 vesicles and PG-vesicles was similar. As shown in Fig. 6,
288 the change in the zeta potential of these vesicles due to the
289 interaction with poly-L-lysine increased drastically, changing
290 from a negative to a positive in presence of 1.5–2 μg/mL poly-
291 L-lysine. Conversely, the change in the surface potential of PC-
292 vesicles was negligible, indicating that the acidic lipids mediate
293 the interaction with basic macromolecules. This experiment
294 also demonstrates that the interactivity of SA-vesicles and PG-
295 vesicles to basic macromolecules is equal at pH 7.4, and that
296 within an electrostatic context, SA-vesicles and PG-vesicles
297 interact similarly with basic compounds at physiological pH.
298 It has been shown that electrostatic interactions are involved
299 in the binding of C1q to the surface of anionic vesicles con-
300 taining acidic phospholipid [6]. In the event that the negative
301 charge on the surface of vesicles is critical for complement
302 activation, both the SA-vesicles and PG-vesicles should be
303 capable of activating the complement system to similar deg-
304 ree. We therefore conducted animal experiments to clarify the
305 issue of complement activation by the anionic electrostatic
306 charge of vesicles.

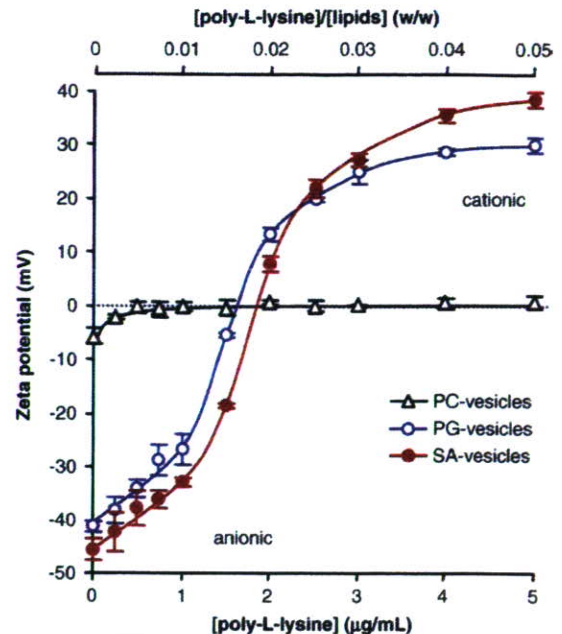


Fig. 6. Change in zeta potential of vesicles as a function of the concentration of basic macromolecule (poly-L-lysine, Mw. 15–30 kDa). Vesicles were dispersed at 100 μg/mL in 10 mM HEPES buffer (pH 7.4, at 37 °C, NaCl, 20 mM) containing various amount of poly-L-lysine. PC-vesicles: PC/CH (1:1, molar ratio), SA-vesicles: PC/CH/SA (1:1:0.2, molar ratio), and PG-vesicles: PC/CH/PG (1:1:0.2, molar ratio).

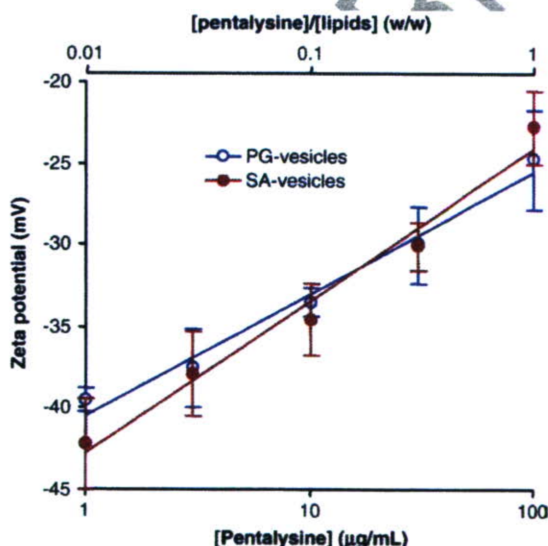


Fig. 5. Change in zeta potential of vesicles as a function of the concentration of basic oligomer (pentalysine). Vesicles were dispersed at 100 μg/mL in 10 mM HEPES buffer (pH 7.4, at 37 °C, NaCl, 20 mM) containing various amount of pentalysine. The lines have slopes of 9.3 and 7.4 mV per decade for the pentalysine concentrations in SA-vesicles and PG-vesicles. SA-vesicles: PC/CH/SA (1:1:0.2, molar ratio) and PG-vesicles: PC/CH/PG (1:1:0.2, molar ratio).

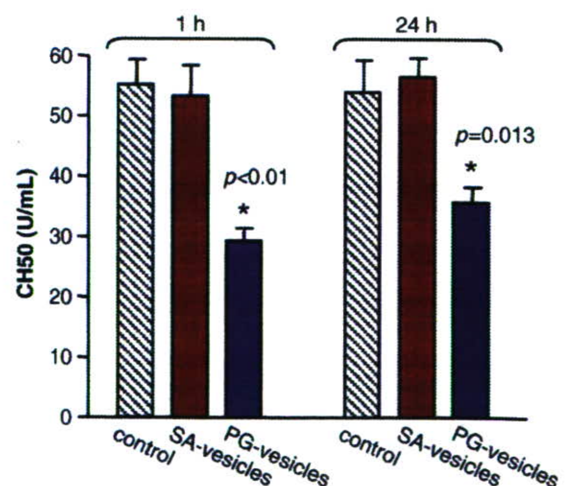


Fig. 7. Comparison of the 50% hemolytic unit of complement (CH50) in rat serum at 1 or 24 h after infusion of saline (control), SA-vesicles, or PG-vesicles. CH50 value for PG-vesicles was significantly lower than that of the control ($p<0.01$ at 1 h, $p=0.013$ at 24 h), indicating the complement consumption in serum after activation. Equal CH50 value for SA-vesicles with the control indicates that SA-vesicles failed to activate complement.

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### 3.5. Complement activation

Serum was collected at 1 and 24 h after the infusion of SA-vesicles or PG-vesicles in experimental rats to determine CH50 levels. The control group received saline as a vehicle. The values of CH50 in control rats, and SA-vesicles- and PG-vesicles-administered rats, were 55.1±4.1 U/mL, 53.2±4.8 U/mL, and 29.3±1.6 U/mL at 1 h after infusion of sample, respectively. At 24 h, the values of CH50 in control rats, and SA-vesicles- and PG-vesicles-administered rats, were 54.0±4.7 U/mL, 56.5±3.3 U/mL, and 35.8±2.3 U/mL, respectively (Fig. 7). The lower CH50 levels observed in the PG-vesicles-administered group in comparison with the control group indicate that complement consumption occurred after activation. These findings imply that significant complement activation is induced in rats receiving PG-vesicles compared to the control group ($p<0.01$ at 1 h, $p=0.013$ at 24 h). Complement consumption was not observed in rats administered SA-vesicles.

Since the negative charge and electrostatic interactivity of SA-vesicles were the same as in PG-vesicles (Figs. 2–6), the data obtained from the animal experiments indicates that the negative charge on the anionic vesicle is not a critical factor underlying the activation of complement. The first step in the activation of the classical complement pathway involves the binding of an activator to C1q, resulting in the activation of serine proteases C1r and C1s. It has been suggested that the negative charge of an activator such as PG-vesicles is involved in some way with the binding of the activator to C1q [1–8]. Assuming that the electrostatic interaction is non-specific, SA-vesicles should interact with C1q electrostatically. Since the action of complement proteases, which follows the binding of the activator to the C1q, is known to be highly specific [32,33], it seems likely that complement activation on anionic surface is limited to an activation step rather than a binding step. Such specific activation of complement by the anionic vesicles in the present study may be involved in the physiological regulation of complement activation on anionic biomembranes.

## 4. Conclusions

The carboxylic acid of SA and phosphoric acid of PG have equal capacity as anionic components of vesicles at neutral pH. The results presented in this investigation demonstrated that the negative electrostatic charge of anionic vesicles is not a critical factor in the activation of complement. Rather, the induction of complement activation by anionic vesicles is dependent on the structure of acidic lipids. This finding may facilitate development and various biological applications of anionic vesicles.

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## References

[1] H.C. Loughrey, M.B. Bally, L.M. Reinish, P.R. Cullis, The binding of phosphatidylglycerol liposomes to rat platelets is mediated by complement, Thromb. Haemost. 64 (1990) 172–176.

[2] A. Chonn, P.R. Cullis, D.V. Devine, The role of surface charge in the activation of the classical and alternative pathways of complement by liposomes, J. Immunol. 146 (1991) 4234–4241.

[3] J. Szebeni, The interaction of liposomes with the complement system, Crit. Rev. Ther. Drug Carr. Syst. 15 (1998) 57–88.

[4] S.M. Moghimi, I. Hamad, T.L. Andresen, K. Jorgensen, J. Szebeni, Methylation of the phosphate oxygen moiety of phospholipid-methoxy (polyethylene glycol) conjugate prevents PEGylated liposome-mediated complement activation and anaphylatoxin production, FASEB J. 20 (2006) 2591–2593.

[5] A.J. Bradley, D.E. Brooks, R. Norris-Jones, D.V. Devine, C1q binding to liposomes is surface charge dependent and is inhibited by peptides consisting of residues 14–26 of the human C1qA chain in a sequence independent manner, Biochim. Biophys. Acta 1418 (1999) 19–30.

[6] A.J. Bradley, E. Maurer-Spurej, D.E. Brooks, D.V. Devine, Unusual electrostatic effects on binding of C1q to anionic liposomes: role of anionic phospholipid domains and their line tension, Biochemistry 38 (1999) 8112.

[7] H. Jiang, B. Cooper, F.A. Robey, H. Gewurz, DNA binds and activates complement via residues 14–26 of the human C1q A chain, J. Biol. Chem. 267 (1992) 25597–25601.

[8] H. Jiang, D. Burdick, C.G. Glabe, C.W. Cotman, A.J. Tenner, beta-Amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain, J. Immunol. 152 (1994) 5050–5059.

[9] A.J. Bradley, D.V. Devine, S.M. Ansell, J. Janzen, D.E. Brooks, Inhibition of liposome-induced complement activation by incorporated poly(ethylene glycol)-lipids, Arch. Biochem. Biophys. 357 (1998) 185–194.

[10] E. Tsuchida (Ed.), Substitute: Present and Future Perspective, Elsevier Science, Amsterdam, 1998.

[11] S. Takeoka, T. Ohgushi, K. Terase, T. Ohmori, E. Tsuchida, Layer-controlled hemoglobin vesicles by interaction of hemoglobin with a phospholipid assembly, Langmuir 12 (1996) 1755–1759.

[12] H. Sakai, K. Hamada, S. Takeoka, H. Nishide, E. Tsuchida, Physical properties of hemoglobin vesicles as red cell substitutes, Biotechnol Prog. 12 (1996) 119–125.

[13] K. Sou, Y. Naito, T. Endo, S. Takeoka, E. Tsuchida, Effective encapsulation of proteins into size-controlled phospholipid vesicles using freeze-thawing and extrusion, Biotechnol Prog. 19 (2003) 1547–1552.

[14] H. Sakai, H. Horinouchi, M. Yamamoto, E. Ikeda, S. Takeoka, M. Takaori, E. Tsuchida, K. Kobayashi, Acute 40 percent exchange-transfusion with hemoglobin-vesicles (HbV) suspended in recombinant human serum albumin solution: degradation of HbV and erythropoiesis in a rat spleen for 2 weeks, Transfusion 46 (2006) 339–347.

[15] H. Saka i, Y. Masada, H. Horinouchi, E. Ikada, K. Sou, S. Takeoka, M. Suematsu, M. Takaori, K. Kobayashi, E. Tsuchida, Physiological capacity of the reticuloendothelial system for the degradation of hemoglobin vesicles (artificial oxygen carriers) after massive intravenous doses by daily repeated infusions for 14 days, J. Pharmacol. Exp. Ther. 311 (2004) 874–884.

[16] K. Sou, R. Klipper, B. Goins, E. Tsuchida, W.T. Phillips, Circulation kinetics and organ distribution of Hb-vesicles developed as a red blood cell substitute, J. Pharmacol. Exp. Ther. 312 (2005) 702–709.

[17] H. Abe, M. Fujihara, H. Azuma, H. Ikeda, K. Ikebuchi, S. Takeoka, E. Tsuchida, H. Harashima, Interaction of hemoglobin vesicles, a cellular-type artificial oxygen carrier, with human plasma: effects on coagulation, kallikrein-kinin, and complement systems, Artif. Cells Blood Substit. Biotechnol. 34 (2006) 1–10.

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[18] H. Abe, H. Azuma, M. Yamaguchi, M. Fujihara, H. Ikeda, H. Sakai, S. Takeoka, E. Tsuchida, Effects of hemoglobin vesicles, a liposomal artificial oxygen carrier, on hematological responses, complement and anaphylactic reactions in rats, Artif. Cells Blood Substit. Biotechnol. 35 (2007) 157–172.

[19] K. Sou, B. Goins, S. Takeoka, E. Tsuchida, W.T. Phillips, Selective uptake of surface-modified phospholipid vesicles by bone marrow macrophages in vivo, Biomaterials 28 (2007) 2655–2666.

[20] K. Sou, T. Endo, S. Takeoka, E. Tsuchida, Poly(ethylene glycol)-modification of the phospholipid vesicles by using the spontaneous incorporation of poly (ethylene glycol)-lipid into the vesicles, Bioconjug. Chem. 11 (2000) 372–379.

[21] H. Saka i, S. Hisamoto, I. Fukutomi, K. Sou, S. Takeoka, E. Tsuchida, Detection of lipopolysaccharide in hemoglobin-vesicles by Limulus amebocyte lysate test with kinetic-turbidimetric gel clotting analysis and pretreatment of surfactant, J. Pharm. Sci. 93 (2004) 310–321.

[22] S. McLaughlin, The electrostatic properties of membranes, Annu. Rev. Biophys. Biophys. Chem. 18 (1989) 113–136.

[23] A. Watts, K. Harlos, W. Maschke, D. Marsh, Control of the structure and fluidity of phosphatidylglycerol bilayers by pH titration, Biochim. Biophys. Acta 510 (1978) 63–74.

[24] J.F. Tocanne, J. Teissié, Ionization of phospholipids and phospholipid-supported interfacial lateral diffusion of protons in membrane model systems, Biochim. Biophys. Acta 1031 (1990) 111–142.

[25] J. Rizo, T.C. Sudhof, C2-domains, structure and function of a universal $Ca^{2+}$-binding domain, J. Biol. Chem. 273 (1998) 15879–15882.

[26] J.E. Murphy, D. Tacon, P.R. Tedbury, J.M. Hadden, S. Knowling, T. Sawamura, M. Peckham, S.E. Phillips, J.H. Walker, S. Ponnambalam, LOX-1 scavenger receptor mediates calcium-dependent recognition of phosphatidylserine and apoptotic cells, Biochem. J. 393 (2006) 107–115.

[27] A. Lau, A. McLaughlin, S. McLaughlin, The adsorption of divalent cations to phosphatidylglycerol bilayer membranes, Biochim. Biophys. Acta 645 (1981) 279–292.

[28] C.G. Sinn, M. Antonietti, R. Dimova, Binding of calcium to phosphatidylcholine-phosphatidylserine membranes, Colloids and Surfaces A: Physicochem. Eng. Aspects 282–283 (2006) 410–419.

[29] J.T. Hautala, M.L. Riekkola, S.K. Wiedmer, Anionic phospholipid coatings in capillary electrochromatography. Binding of $Ca^{2+}$ to phospholipid phosphate group, J. Chromatogr. A 1150 (2007) 339–347.

[30] D. Volodkin, V. Ball, P. Schaaf, J.C. Voegel, H. Mohwald, Complexation of phosphocholine liposomes with polylysine. Stabilization by surface coverage versus aggregation, Biochim. Biophys. Acta 1768 (2007) 280–290.

[31] D. Murray, A. Arbuzova, G. Hangyás-Mihályné, A. Gambhir, N. Ben-Tal, B. Honig, S. MaLaughlin, Electrostatic properties of membranes containing acidic lipids and adsorbed basic peptides: theory and experiment, Biophys. J. 77 (1999) 3176–3188.

[32] G.J. Arlaud, C. Gaboriaud, N.M. Thielens, M. Budayova-Spano, V. Rossi, J.C. Fontecilla-Camps, Structural biology of the C1 complex of complement unveils the mechanisms of its activation and proteolytic activity, Mol. Immunol. 39 (2002) 383–394.

[33] G. O'Brien, N.S. Quinsey, J.C. Whisstock, R.N. Pike, Importance of the prime subsites of the C1s protease of the classical complement pathway for recognition of substrates, Biochemistry 42 (2003) 14939–14945.

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