Effects of Hydrophobicity and Electrostatic Charge on Complement Activation by Amino Groups

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ABSTRACT Some studies have demonstrated that amino groups, acting as nucleophiles, are potent activators of the complement system, but others not. To clarify these contradictory results, we examined complement activation on two series of NH_2/CH_3 and NH_2/COOH mixed self-assembled monolayers (SAMs). NH_2/CH_3 mixed SAMs were not potent activators of the complement system regardless of the ratio of NH_2/CH_3 in mixed SAMs. Numerous serum proteins, such as albumin, were adsorbed onto those SAMs and formed a protein layer which inhibited access of C3b to amino groups. In contrast, much C3b and/or C3bBb were deposited on NH_2/COOH mixed SAMs with ~50−60% NH_2 density on the surface and SC5b-9 was found in serum exposed to this SAM, indicating activation of the complement system. These results suggest that C3b can easily access nucleophilic NH_2 groups because of the decrease in electrostatic interaction between negatively charged proteins and the NH_2 SAM surface.

KEYWORDS: complement activation • protein adsorption • self-assembled monolayers • blood compatibility

INTRODUCTION

When medical devices are implanted, various responses occur at the interface between living tissue and the artificial materials such as adsorption of proteins, activation of the complement and the coagulation systems, platelet activation, and cell adhesion. The interaction of plasma proteins with artificial materials is the first reaction in a series of events leading to thrombosis and inflammatory responses toward medical devices in contact with blood. These interactions should be controlled for the successful development of these devices. Thus, extensive studies have been performed to understand the interactions of body fluids, especially blood, with material surfaces.

The complement system is a cascade of enzymes consisting of approximately 30 fluid-phase and cell-membrane bound proteins, and plays an important role in the body's defense systems against pathogenic xenobiotics (1, 2). It is activated through three separate pathways: the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP). The development of the hemodialyzer has allowed the study of the activation of the complement system on artificial materials. This technique has clearly indicated that nucleophilic groups, such as hydroxyl groups on a dialysis membrane composed of regenerated cellulose (3, 4), strongly activate the complement system. In a previous study, we reported that a self-assembled monolayer of 11-mercaptoundecanol (OH-SAM) also acted as a strong activator of the complement system (5, 6). The amino group, another representative nucleophilic group, is expected to be a potential activator of the complement system through the alternative pathway, but few studies have demonstrated this result (7). Our earlier investigations found that a self-assembled monolayer of 11-amino-1-undecanethiol (NH_2−SAM) carrying amino groups at a high density could not effectively activate the complement system (8). We also observed a large amount of serum proteins deposited on the NH_2−SAM, which led us to speculate that amino groups on the SAM surface were masked by the adsorbed protein layer, and thus the amino groups could not activate the complement systems effectively. However, many aspects of this system remain to be elucidated. For example, the layer-by-layer method (9) has been introduced to prepare various biomaterials. A layer-by-layer membrane formed between poly-L-lysine (carrying primary amino groups) and alginic acid (carrying carboxylic acid groups) has been used to enclose islets of Langerhans (10). Activation of the complement by the capsule membrane is expected to be harmful for islets of Langerhans. Interactions between the complement system and amino groups neutralized with carboxylic acid groups remain to be elucidated.

In the present work, we examined the effects of surface densities of amino groups, hydrophobicity of surfaces, and electrostatic charges upon activation of the complement system on SAMs modified with amino groups. We employed two series of mixed SAMs, amino/methyl or amino/carboxy terminated alkanethiols, with different densities of surface

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amino groups as model surfaces and evaluated the occurrence of complement activation on those surfaces.

MATERIALS AND METHODS

Reagents and Antibodies. 1-Dodecanethiol (Wako pure chemical industries, Ltd., Osaka, Japan), 11-mercaptoundecanoic acid (Sigma-Aldrich Co., St. Louis, MO, USA) and 11-amino-1-undecanethiol hydrochloride ($C11NH_2$, Dojindo Laboratories, Kumamoto, Japan), barbital sodium, calcium chloride, magnesium chloride, ethanol (all purchased from Nacalai Tesque, Inc., Kyoto, Japan) and ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA; Dojindo Laboratories, Kumamoto, Japan) were of reagent grade and were used as obtained. Solutions of rabbit antihuman C3b antiserum (RAHu/C3b, Nordic Immunology, The Netherlands), sheep antihuman C1q antibody (PC020, The Binding Site Ltd., Birmingham, UK), rabbit anti-HSA antibody (55029, ICN Pharmaceuticals, Inc., USA), and goat anti-IgG antibody (109−005−088, Jackson ImmunoResearch Laboratories, Inc., PA, USA) were prepared and stored in accordance with supplier instructions.

Preparation of Serum and Buffers. All donors of blood enrolled in this research provided informed consent. The process was approved and accepted by the ethics review board of the Institute for Frontier Medical Sciences, Kyoto University. Blood was donated from 8 healthy volunteers who had consumed a meal at least 4 h before the donation. The preparation method for the serum has been described elsewhere (6, 8). Briefly, the collected blood was kept at ambient temperature for 30 min to induce blood coagulation, and centrifuged at 1100 × g for 30 min at 4 °C. The sera from 8 donors were then pooled in a bottle and mixed well; 1 mL aliquots were then divided into polypropylene vials in an ice bath and stored at −80 °C until use.

Complement functional activities of the pooled serum (NHS) for three pathways were assessed by Wieslab(R) Complement System Screen (Euro-diagnostica AB, Malmo, Sweden) in accordance with supplier instructions. Briefly, NHS specimens, which were diluted by diluents for the specific pathways (1/101 for the CP and LP and 1/18 for the AP), were applied to the wells for the specific pathways and incubated at 37 °C for 60 min. Each well was washed and applied with a solution of alkaline phosphatase conjugated anti C5b-9 neoantigen antibodies, and then washed again. Finally, the solution of coloring substrate was applied to each well, incubated at room temperature for 30 min and its absorbance at 405 nm was read on a microplate reader (Corona Electric Co., Ltd., Ibaraki, Japan). Complement functional activities of the NHS were 101 % (for CP), 98.4 % (for LP) and 97.6 % (for AP) of the positive reference sera supplied by Euro-diagnostica AB, respectively.

The dilute sera (1/101 for the CP and LP and 1/18 for the AP) were effectively activated on the surfaces for the specific pathways. In this studies, thus 10 % (v/v) NHS diluted by VB was employed for assessment of the complement activation on mixed SAMs, amino/methyl or amino/carboxy terminated alkanethiols.

Water was purified with a Milli-Q system (Millipore Co.). Veronal buffer (VB), composed of 5 mM sodium barbital, 142 mM NaCl, 3.7 mM HCl, 1.5 mM $CaCl_2$, and 10 mM $MgCl_2$ (pH 7.4), was prepared according to the protocol for CH50 measurement (11). For experiments under the inhibition of complement activation, EDTA was added to VB (without $CaCl_2$ and $MgCl_2$) for a final concentration of 10 mM (EDTA-VB).

Preparation of Self-Assembled Monolayer (SAM) Surfaces Carrying Different Functional Groups. Glass plates were coated with gold as previously reported (6). Ethanol was deoxygenized with nitrogen gas before use. The series of reaction solutions with different molar ratios of two thiols ($C11NH_2$/C10COOH or $C11NH_2$/$C11CH_3$) were prepared by mixing a 1 mM solution of each thiol and the total concentration of thiols was 1 mM in ethanol. The glass plates with a gold thin layer were immersed into the reaction mixtures at room temperature for 24 h to form two-component mixed SAMs. The glass plates were sequentially washed with ethanol and Milli-Q water three times each and then dried under a stream of dried nitrogen gas.

Surface Analyses of Glass Plates Carrying Mixed SAMs. Infrared (IR) adsorption spectra of sample surfaces were collected by the reflection-adsorption method (FTIR-RAS) using a Spectrum One (Perkin-Elmer, USA) spectrometer equipped with a Refractor (Harrick Sci. Co., NY) and a mercury−cadmium telluride (MCT) detector cooled by liquid nitrogen. Glass plates with a gold layer at 199 nm of thickness were used for FTIR-RAS analyses. Spectra were obtained using the p-polarized infrared laser beam at an incident angle of 75 degrees in the chamber purged with dry nitrogen gas for 128 scans at 4 cm^{-1} resolution from 4000 to 750 cm^{-1}. The areas of peaks at 2965−2966 cm^{-1} assigned to the asymmetric stretching mode of methyl groups were used to determine the surface concentrations of CH_3 groups, and the areas of peaks at 1719−1720 cm^{-1} or stretching mode of carboxyl groups were used to determine the surface concentrations of COOH groups (6).

Protein Deposition Observed by Surface Plasmon Resonance (SPR). The SPR apparatus employed in this study was a homemade apparatus (5, 6) prepared referring to the report by Knoll (12). The BK7 glass plate with the gold layer (49 nm in thickness) was coupled to a hemicylindrical prism with an immersion oil ($n = 1.515$, Cargille Laboratories, Cedar Grove, NJ). The sample surface was irradiated with a p-polarized He−Ne laser light ($\lambda = 632.8$ nm) through the prism. The intensity of the reflected light was monitored as a function of the incident angle. The incident angle, at which the reflectivity reached a minimum, was described as the SPR angle.

Human serum diluted with VB to 10 % (10 % NHS) was used in this study. A flow chamber with a sample plate was placed on a prism of the SPR apparatus, and VB was circulated at a flow rate of 3.0 mL/min in the flow chamber assembly. Reflectance was monitored during the flow of the liquid samples at an incident angle of 0.5° less than the SPR angle. Human serum was then introduced into the flow chamber assembly and circulated at a flow rate of 3.0 mL/min for 90 min. To clear serum from the sample surface, VB was introduced and circulated for additional 20 min. All experiments were performed at 37 °C.

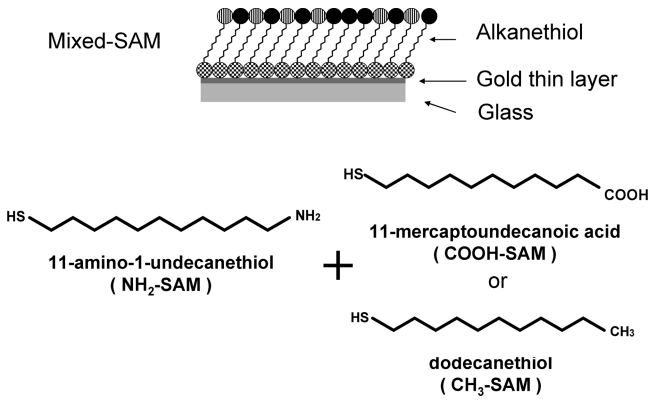
To identify proteins deposited on the surface, we flowed solutions of specific antibodies or antisera through the apparatus after exposure of the sample surface to human serum. Each of the antisera/antibodies, rabbit antihuman C3b, sheep antihuman C1q, rabbit anti-HSA, and goat anti-IgG diluted to 1 % with VB, was applied and circulated for 90 min and then VB was introduced for 20 min to wash out the serum solution. The thickness of the protein layer was calculated from the shift in the SPR angle (ΔSPR) using Fresnel fits for the system BK7/Cr/Au/SAM/protein/water, where the refractive indices of SAM and protein were assumed to be 1.45. The amounts of proteins adsorbed onto the surfaces were estimated from the thickness presuming that the density of the protein layer was 1 as follows (5)

$$\text{amount of adsorbed protein (ng cm}^{-2}) = 500 \times \Delta\text{SPR (deg)} \quad (1)$$

The change of the resonance angle was estimated to determine antibody immobilization.

Released Amounts of the Soluble Form of the Membrane Attack Complex, SC5b-9. We used a lab-made incuba-

Scheme 1. Schematic of the Sample Surfaces. Symbols Correspond to NH_2 (solid circles), either CH_3 or COOH (striped circles), and SH (checked circles) groups



tion chamber to examine release of SC5b-9 when different surfaces were exposed to 10% NHS. The chamber was composed of two glass plates carrying the same sample surfaces and a silicone gasket of 1 mm thickness with a hole of 20 mm in diameter. After 10% NHS were incubated in the chamber for 1.5 h at 37 °C, they were collected and EDTA was immediately added to a final concentration of 10 mM to stop further activation of the complement system. A commercial enzyme-linked immunosorbent assay (ELISA) kit (Quidel SC5b-9(TCC) EIA kit, Quidel Corp., CA) was used to determine the soluble form of membrane attack complex, SC5b-9 in the collected 10% NHS. The measurement procedure was performed in accordance with supplier instructions.

Statistical Analysis. Data from the experiments are expressed as the mean ± standard error of the mean. A one-way analysis of variance (ANOVA) was used to identify the statistical significance of the data. ANOVA was followed by posthoc pairwise *t*-tests adjusted using Holm's method and employing R language environment version 2.9.1 (13).

RESULTS

Surface Analysis. Mixed SAMs, which can be easily prepared by using two or more different kinds of alkanethiols with different terminal groups, can provide well-defined surfaces with serially varied surface properties. These SAMs have been used for studying the interaction between artificial materials and biological phenomena (6, 14). Glass plates with a 1 nm layer of chromium and a 49 nm gold layer were immersed into solutions of the series of reaction solutions with different molar ratios of two thiols, leading to the formation of the layers of mixed SAMs carrying various molar ratios of NH_2/CH_3 or NH_2/COOH on the gold layer (Scheme 1).

Compositions of two series of mixed SAMs were determined from the absorption intensity of FTIR-RAS spectra. The molar ratios of NH_2 in the two kinds of mixed-SAM surfaces are plotted against those of the reaction mixtures in Figure 1. For NH_2/CH_3 mixed SAMs, the surface concentrations of NH_2 gradually increased as the concentrations of amines increased in the reaction mixtures, but the surface concentration of NH_2 never reached the levels found in the reaction mixture across the whole concentration range. The same phenomena were previously observed (14). In contrast, for the preparation of NH_2/COOH mixed SAMs, the surface concentrations of NH_2 sharply increased as a function of the 11-amino-1-undecanethiol concentrations in the reaction mixtures and remained around 50% over a wide concentration range.

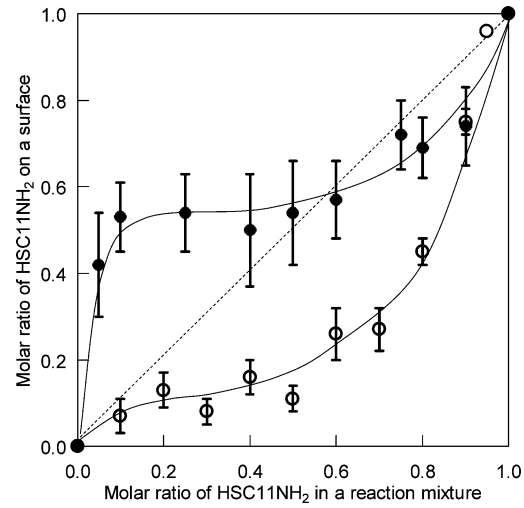


FIGURE 1. Surface compositions of SAMs formed in mixtures of 11-amino-1-undecanethiol (NH_2) and alkanethiols with methyl (CH_3. ○) or carboxy (COOH. ●) end groups. Error bars represent ± SEM (n = 3).

Interaction of Serum Proteins with NH_2/CH_3 Mixed SAMs. A comparison of the adsorption of serum proteins onto a series of NH_2/CH_3 mixed SAMs as a function of time shows that the SPR signals sharply increased during the initial few minutes and reached plateau around 20 min after exposure to 10% NHS (Figure 2A). C3b or C3bBb is expected to be immobilized on surface when the complement system is activated by the surface. After flushing out serum from the sensor chamber with VB, the application of 1% antihuman C3b antibody solution to determine the presence of C3b or C3bB resulted in only a modest increase in the SPR signals (Figure 2B).

Protein adsorption from 10% NHS was examined on a series of NH_2/CH_3 mixed SAMs and the protein composition in the adsorbed protein layers was examined using specific antibodies against C3b, C1q, immunoglobulin G (IgG) and serum albumin (HSA). Results are summarized in Figure 3. Although the amounts of total adsorbed serum proteins showed a small increase with increasing NH_2 density on the surfaces, over the range of 300 to 420 ng cm^{-2}, their differences were small. The amount of immobilized anti-HSA antibody surpassed that of any other antibodies examined, with values of ~400−600 ng cm^{-2}. Although maximum immobilization of anti-HSA was observed on a mixed SAM with NH_2/CH_3 = 35/65, no significant differences were observed between mixed SAMs with different surface NH_2/CH_3 compositions ($p = 0.2767$, ANOVA). Immobilized anti-IgG levels decreased with increasing surface NH_2 concentration. The amounts of immobilized anti-C3b antibody were comparable to that of anti-IgG antibody, less than 200 ng cm^{-2} at all examined points. C1q was hardly detected on any NH_2/CH_3 mixed SAMs using anti-C1q antibody.

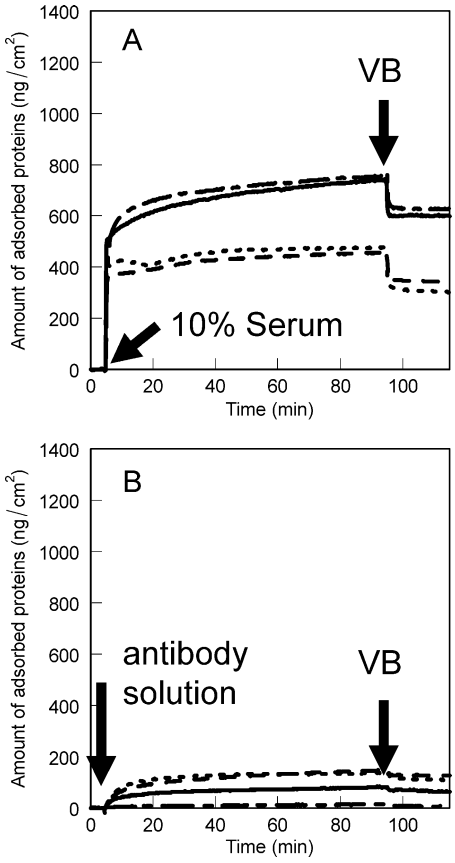


FIGURE 2. SPR sensorgrams during exposure of (A) 10% NHS and (B) 1% anti-C3b antiserum to a series of NH_2/CH_3 mixed SAM surfaces. (· · · · · ·) NH_2/CH_3 = 0/100; (- - -) NH_2/CH_3 = 45/55; (———) NH_2/CH_3 = 100/0; (· — · —) NH_2/CH_3 = 100/0 with 10 mM EDTA supplemented 10% NHS. A color version of this figure is available in the Supporting Information.

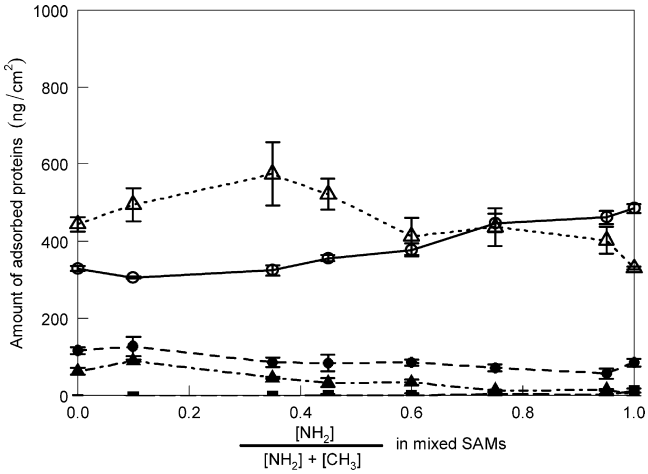


FIGURE 3. Amounts of adsorbed serum proteins (○) and anti-C3b (●), C1q (■), HSA (△), and IgG (▲) antibodies onto the protein layer adsorbed on NH_2/CH_3 mixed SAM surfaces. Error bars represent ± SEM (n = 3).

Interaction of Proteins with NH_2/COOH Mixed SAMs. Similarly as for NH_2/CH_3 mixed SAMs, we prepared time-course sensorgrams for a series of NH_2/COOH mixed SAMs to illustrate the protein adsorption from 10% NHS (Figure 4A) and antihuman C3b antibody immobilization onto the formed protein layer (Figure 4B). Initial sharp increases of SPR angles due to a difference of the reflective

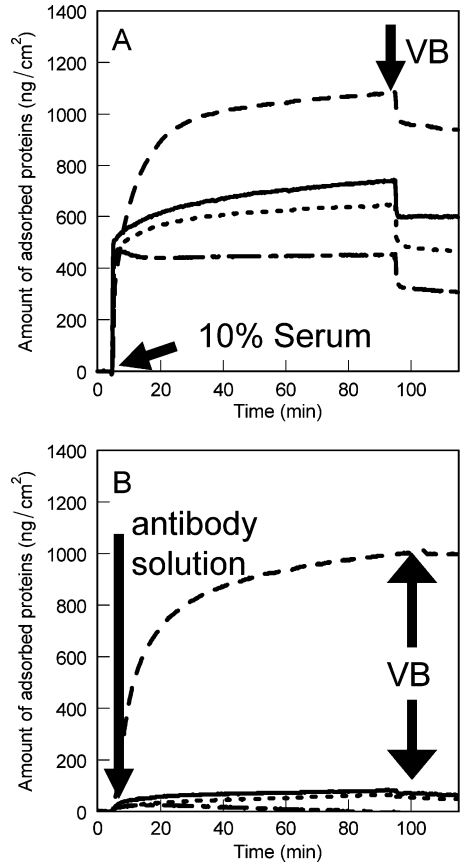


FIGURE 4. SPR sensorgrams during exposure of (A) 10% NHS and (B) 1% anti-C3b antiserum to a series of NH_2/COOH mixed SAM surfaces. (· · · · · ·) NH_2/COOH = 0/100; (- - -) NH_2/COOH = 55/45; (———) NH_2 100%; and (· — · —) NH_2/COOH = 55/45 with 10 mM EDTA supplemented 10% NHS. NH_2 100% was the same as for Figure 2. Error bars represent ± SEM (n = 3). A color version of this figure is available in the Supporting Information.

indexes caused by changing VB to 10% serum (Figure 4A) were followed by slow increases during 80 min. The largest increase was observed for the NH_2/COOH = 55/45 mixed SAM. After flushing out serum with VB, 1% antihuman C3b antibody solution was applied. A large increase of the SPR signal was observed for the protein layer formed on the mixed SAMs with NH_2/COOH = 55/45 and 65/35 (Figure 4B). Such an increase in the SPR signal was not observed on CH_3—SAM (Figure 2) or with 100% NH_2—SAM. When EDTA was added to the serum, no slow increase was observed in a SPR sensorgram, and no immobilization of antihuman C3b antibody was observed on NH_2/COOH = 55/45 mixed SAM (Figure 4B).

After the exposure of the surfaces to 10% serum, plentiful amounts of proteins were adsorbed on NH_2/COOH SAMs, regardless of the NH_2/COOH compositions (Figure 5). Treatment with serum protein antibodies revealed that the amounts of immobilized anti-HSA antibody and anti-C3b antibody varied inversely. Amounts of anti-C3b antibody increased with increasing surface NH_2 concentration, reaching the maximum at NH_2/COOH = 55/45 mixed SAM In contrast, the amounts of anti-HSA antibody showed a minimum at NH_2/COOH = 55/45 mixed SAM and then increased with increasing surface NH_2 concentrations. When the complement system is activated, the major component of

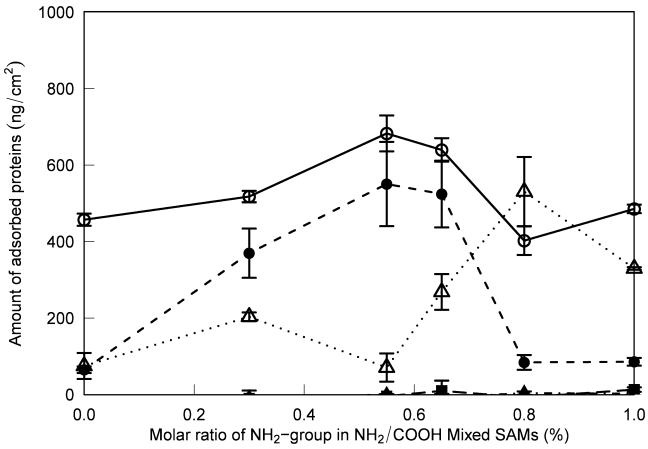


FIGURE 5. Amounts of adsorbed serum proteins (○) and anti-C3b (●), C1q (■), HSA (△), and IgG (▲) antibodies onto the protein layer adsorbed on NH_2/COOH mixed SAM surfaces. Error bars represent ± SEM ($n = 3$).

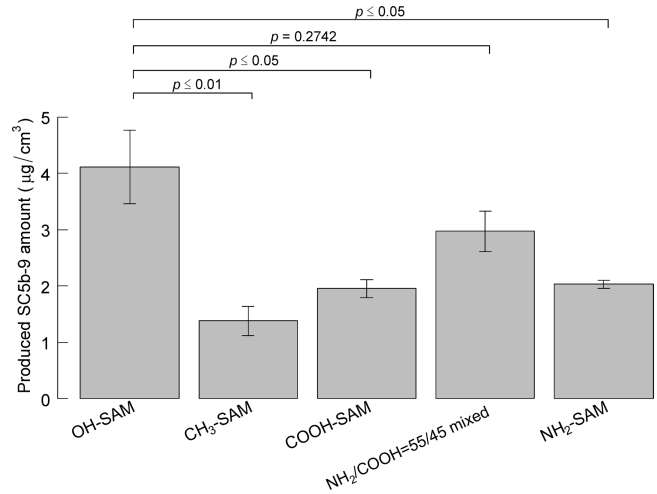


FIGURE 6. Release of SC5b-9 in 10% diluted serum exposed to OH-, CH_3-, COOH-, and NH_2-SAM, and NH_2/COOH = 55/45 mixed SAM. OH-SAM is used as a control surface. Experiments were repeated at least 3 times for each surface using the same pooled serum. Error bars a represent ± SD ($n = 3$). Amount of SC5b-9 in naïve 10% NHS and fully activated by incubation with zymosan were 0.026 ± 0.007 $\mu g/cm^3$ ($n = 2$) and 20 ± 4 $\mu g/cm^3$ ($n = 2$), respectively.

the protein layer is C3b or C3bBb. On the other hand, when the complement system is not activated, the major component of the protein layer is albumin or IgG reflecting protein concentration in serum. Immobilized anti-C1q and anti-IgG antibodies were hardly detected on any NH_2/COOH SAMs.

These results indicate that NH_2/COOH = 55/45 mixed SAM activate the complement system through the alternative pathway, but NH_2 or COOH SAMs are not activators.

Release of SC5b-9. As a consequence of activation of the complement system, a terminal complement complex, C5b-9, is generated by the assembly of C5 though C9. In the absence of a target cell membrane, for example, complement activation occurs on artificial materials, C5b-9 binds to regulatory S proteins, and is released into serum as a nonlytic SC5b-9 complex. We measure the concentrations of SC5b-9 complex released into serum exposed to SAMs to evaluate the activation of the complement (Figure 6). The amount of SC5b-9 from 10% NHS samples exposed to the OH-SAM surface was also included as the positive control. Although the released amount of SC5b-9 on the NH_2/COOH = 55/45 mixed surface tended to be larger than that on COOH SAM and NH_2 SAM, no statistical difference was observed among these three surfaces ($p = 0.356$ and 0.373, respectively). This result is partially due to large standard deviation of SC5b-9 amount on NH_2/COOH = 55/45 mixed SAM.

DISCUSSION

Numerous studies have investigated the interaction of the complement system with material surfaces. Craddock et al. (15, 16) reported that transient leukopenia during hemodialysis was induced through the complement activation on the hemodialysis membrane. Hydroxyl groups on a dialysis membrane made of regenerated cellulose (3, 4) cause activation of the complement system. An activation mechanism of the complement system on surfaces carrying OH group was proposed. C3 is hydrolyzed to C3a in plasma, and a thioester group in a C3b molecule is exposed to its surface and thus is able to react with nucleophilic hydroxyl groups on the surface. The bound C3b interacts with factor B, forming C3 convertase, and positions C3bBb onto the surface. This process amplifies the complement activation and triggers the complement loop activation, that is, the complement activation through the alternative pathway. This mechanism has long been accepted (17).

The amino group is also a nucleophilic group. Surfaces carrying amino groups are expected to be potential activators of the complement system through the alternative pathway (7). In our previous study (8), the NH_2−SAM and a polyethyleneimine(PEI)coated surface were employed as model surfaces to study the interactions between amino groups and the serum complement pathways. Although much protein was adsorbed from serum solutions on the two types of amino surfaces, C3b deposition on the surfaces was hardly detected. Only a small amount of sC5b-9 complex, which is produced when the complement system is activated, was detected in serum after exposure of serum to the amino surfaces. These results suggest that surfaces carrying amino groups at higher densities could not effectively activate the complement system (8). In recent years, several groups reported that the mechanism of the activation through the alternative pathway is more complex than reported previously (18−20) and have proposed more relevant models (17, 21−23). The behavior of the complement system in the presence of surfaces carrying amino groups is still poorly understood.

In the present work, we prepared several series of mixed SAMs, amino/methyl or amino/carboxy terminated alkanethiols, with different surface amino group densities as model surfaces for the examination of complement activation on those surfaces. On NH_2/CH_3 mixed SAMs, the amounts of adsorbed proteins slightly increased from 300 to 420 ng cm^{-2} with increasing surface NH_2 contents. Amounts of immobilized anti-HSA antibody on the protein layer exceeded that observed for other antibodies examined. Albu-

min is the major component of the protein layer. Immobilized amounts of anti-C3b antibody were less than 100 ng cm^{-2} at most of the examined points. Meanwhile, when NHS was exposed to OH-SAM, which is a strong activator of the complement system, immobilized amounts of anti-C3b antibody were greater than 1100 ng cm^{-2}, indicating that a major component of the adsorbed protein layer was C3b (5). These facts indicate that the NH_2/CH_3 mixed SAMs themselves could not activate the complement system. Nilsson et al. (17) reported that protein layers formed on artificial materials triggered the complement system through the alternative pathway. Our results did not indicate that the protein layer formed on NH_2/CH_3 mixed SAMs activate the complement system. Deposition of C1q was examined by using anti-C1q antibody to evaluate the contribution of the classical pathway. C1q was hardly detected on any NH_2/CH_3 mixed SAMs. These facts indicated that the complement system was not activated on any NH_2/CH_3 mixed SAMs through either the classical or the alternative pathway.

For the series of NH_2/COOH mixed SAMs, as shown in Figure 5, the amounts of anti-C3b antibody increased with increasing surface NH_2 concentration, with a maximum at NH_2/COOH = 55/45 to 65/35, whereas for the same density of surface NH_2 groups, immobilization of anti-HSA antibody showed a minimum and then increased with increasing surface NH_2 concentrations. As shown in Figure 6, amounts of SC5b-9 generated on CH3-, COOH-, and NH_2-SAMs were significantly less than that on the activator OH-SAM. These three single-composition SAMs are not an activator of the complement system. On the other hand, there is no statistical difference between amounts of SC5b-9 generated on OH-SAM and NH_2/COOH = 55/45 SAM. It indicates that the NH_2/COOH = 55/45 SAM may be a potent activator. Taking all of these facts into consideration, we figure that the complement system is activated on the SAMs of NH_2/COOH = 55/45 to 65/35.

Although amino groups are considered to be potent activators of the complement system through the alternative pathway (7), our recent study demonstrated that NH_2-SAM and PEI-coated surfaces are not effective activators of the complement system contradicted this idea. As discussed in our previous study, positively charged amino groups attract various negatively charged serum proteins, such as albumin, which strongly adsorb on the surfaces through an electrostatic interaction under physiological pH conditions. Surface coverage with the adsorbed protein layer interrupts access of C3b to the surface amino groups, and thus, C3 convertase could not be formed through interaction of surface-bound C3b with Bb. As similarly observed with NH_2−SAM and PEI coated surfaces, the same mechanism prevents activation of the complement system on NH_2/CH_3 mixed SAMs with any mixed ratios. On the series of NH_2/COOH mixed SAMs, activation of the complement system showed a maximum at around NH_2/COOH = 50/50 mixed SAM. Amino groups carry positive charge because of their protonation, and carboxylic acid groups carry negative charges because of their dissociation at physiological pH. These negative and positive charges cancel each other. Electrostatic interaction between surface charges and serum proteins weakens due to surface charge cancelation. Serum proteins cannot strongly adsorb onto the surfaces through an electrostatic interaction under physiological pH conditions. In a series of reactions, C3b formed in the liquid phase effectively approaches surface amino groups, becomes immobilized and forms C3 convertase with B, forming C3bBb, and ultimately triggers the complement activation through the alternative pathway.

CONCLUSION

Two series of mixed SAMs, NH_2-terminated/CH_3-terminated and NH_2-terminated/COOH-terminated, were employed to assess effects of hydrophobicity and electrostatic charge upon the behavior of the complement system on these surfaces. NH_2-terminated/CH_3-terminated mixed SAMs were not potent activators of the complement system at all examined points, and therefore the hydrophobicity is not related to the activation of the complement system onto the surface carrying the amino group. On the contrary, large amounts of C3b and/or C3bBb were deposited during exposure to 10% serum onto NH_2/COOH = 55/45 mixed SAM. These results suggest that complement proteins, mainly C3, can easily access the nucleophilic NH_2 groups because of the decrease in electrostatic interaction caused by the neutralization of the electrostatic charge on that surface.

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Supporting Information Available: Color versions of SPR sensorgrams, summary of contact angles, and FTIR spectra of the assessed mixed SAM (PDF). This material is available free of charge via the Internet at http://pubs.acs.org/.

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