Colloids and Surfaces B: Biointerfaces 161 (2018) 662–669

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Specific binding of human C-reactive protein towards supported monolayers of binary and engineered phospholipids

Tatsuro Goda*, Yuji Miyahara

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), 2-3-10 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan

ARTICLE INFO

Article history:
Received 25 July 2017
Received in revised form
13 November 2017
Accepted 14 November 2017
Available online 20 November 2017

Keywords:
C-reactive protein
Lysophospholipids
Anionic phospholipids
Inverted phospholipids
SPR
FRAP

ABSTRACT

Circulating C-reactive protein (CRP) recognizes altered plasma membranes and activates complements systems in the acute phase of inflammation and infection in human. We have shown previously the calcium-independent adsorption of CRP toward 1-palmitoyl-2-oleoyl-*sn*-*glycero*-3-phosphocholine (POPC) and lysophosphatidylcholine (LPC) on supported phospholipid monolayers. Here, we extended our study to other phospholipids and additives to elucidate the pattern recognition of CRP using a surface plasmon resonance biosensor. Surface density and lateral fluidity depended on the type of phospholipids in the monolayers as characterized by SPR and fluorescence recovery after photobleaching measurements. CRP recognized 1-palmitoyl-2-oleoyl-*sn*-*glycero*-3-phospho-L-serine (POPS) and 1-palmitoyl-2-oleoyl-*sn*-*glycero*-3-phospho-(1′-rac-glycerol) (POPG) in the supported POPC monolayers without calcium at pH 7.4 and 5.5. As opposed to LPC, CRP did not recognize 3-*sn*-lysophosphatidylethanolamine in the POPC monolayers in calcium-free conditions. While, the addition of 1-palmitoyl-2-oleoyl-*sn*-*glycero*-3-phosphoethanolamine (POPE) or sphingomyelin to supported POPC monolayers blocked CRP adsorption. Calcium-dependent CRP binding was observed only at pH 5.5 on supported monolayers of engineered phospholipids with inverted headgroups relative to POPC. The complement 1q (C1q) protein recognized the active form of CRP on the supported phospholipid monolayers. The discovery of CRP recognition with these phospholipids aids our understanding of the activation dynamics of CRP with phospholipid-based biomaterials when used during the acute phase.

1. Introduction

Innate immunity is the first line of host defense system against foreign challenge or tissue damage. The acute-phase reactant human C-reactive protein (CRP) in circulation increases by hundreds fold within 1 day from the onset of inflammation and infection and recruits complement molecules for the clearance of pathogens or cell debris [1,2]. The interaction of systemic CRP with altered plasma membranes determines the physiological roles of CRP [3–5]. For example, CRP adsorption onto a ligand in plasma membranes or lipoproteins leads to both pro-inflammatory and anti-inflammatory effects, depending on the ligand type and the ionic microenvironments [6–9]. The latest studies have revealed that structural transition of CRP from the original isopentamer to its protomer following target recognition triggers pro-inflammatory cascades [10–18]. Although CRP is beneficial in numerous physiological functions, it has some harmful effects especially in post-reproductive-age diseases such as atherothrombosis, autoimmune and other chronic inflammatory conditions [19]. Nowadays, a minor elevation in the systemic CRP level under non-acute conditions is recognized as a risk marker for cardiovascular diseases [16,20–22].

Fundamental understanding of the CRP interaction with various phospholipids is important, as many phospholipid-based materials from natural and synthetic origins are used in bioengineering researches, such as drug delivery systems (DDS), cell therapy, and biomedical devices. Phosphatidylcholine, phosphatidylserine, and phosphatidylinositol, have been used in combination with cholesterol and charged amphiphiles such as stearylamine or phosphatidic acid for making liposomes [23]. Recently, synthetic phospholipids with inverted headgroups have gained attention due to their unique physicochemical properties that make them amenable for applications in DDS [24–28]. Amphipathic polymers bearing the zwitterionic phosphorylcholine group were discovered to enter the cytoplasm across otherwise impermeable lipid bilayer barriers of plasma membranes [29,30]. Phospholipid-mimicking polymers have been developed for producing biologically inert functions to biomedical devices in *in vivo* situations [31,32]. Despite the prevalence of phospholipid-based biomaterials in nanomedicine, the interaction of these materials with CRP which

* Corresponding author.
E-mail address: goda.bsr@tmd.ac.jp (T. Goda).

https://doi.org/10.1016/j.colsurfb.2017.11.036
0927-7765/

is implicated in complement activation remains to be completely elucidated. The potential risk of phospholipid-based biomaterials for CRP-related complement activations and inflammation in post-reproductive-age diseases may not come into the open. This is because CRP level in non-acute phase is quite low for activating complement and inflammation. In addition, *in vivo* safety validation of these biomaterials are mostly performed using model animals such as mouse and rat where CRP is not an acute phase reactant [1,2,33].

To understand CRP/phospholipid interplay on molecular basis, we have been conducting researches using model biointerfaces and sensitive biosensors. Traditionally, CRP is known to recognize the zwitterionic phosphorylcholine headgroup in the presence of calcium [34]. To our knowledge, both calcium- and pH- dependent binding of human CRP onto phospholipid-mimetic materials was noted [9,35]. The amount of complement component C1q bound onto the active form of CRP on model surfaces was also enhanced under weakly acidic pH conditions, a common signature of inflammation site [36]. Moreover, we newly identified a calcium-independent interaction of CRP with a bioactive phospholipid, lysophosphatidylcholine (LPC), in model surfaces [37]. The glycerophosphate motif in LPC was responsible for the specific binding of CRP in the absence of calcium ions. To extend our study, we have examined the interaction of CRP with a series of bioactive or synthetic phospholipids that are frequently used in biomedical applications [23–28]. Surface density and lateral diffusivity of lipid molecules in supported monolayers were characterized to see those effects on the binding properties. Then, the complex formation between CRP on model surfaces with C1q was investigated. Surface plasmon resonance (SPR) was employed as a label-free biosensor for determining the binding kinetics and the amount of CRP adsorbed [38].

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phospho-L-serine (POPS), 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphoethanolamine (POPE), sphingomyelin (SM) from chicken egg yolk, 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phospho-(1′-rac-glycerol) (POPG), 2-((2,3-bis(oleoyloxy)propyl)dimethylammonio)ethyl hydrogen phosphate (DOCP), and 1-palmitoyl-2-(6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl)-*sn-glycero*-3-phosphocholine (NBD-PC) were obtained from Avanti Polar Lipids (Alabaster, AL). Tetradecanethiol, L-α-lysophosphatidylcholine (LPC) from soybean (Acyl chain length of 16:0 and 18:0), 3-*sn*-lysophosphatidylethanolamine (LPE) from egg yolk (acyl chain length of 16:0 and 18:0), cholesterol (3β-hydroxy-5-cholesten; Chol), CRP from human plasma, complement component C1q from human serum and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). D,L-α-phosphatidylcholine, dipalmitoyl (D,L-DPPC) was obtained from Santa-Cruz Biotechnology, Inc. (Dallas, TX). dodecyltrimethoxysilane was obtained from TCI Co. (Tokyo, Japan). All the other reagents of extra-pure grade were purchased from commercial sources and used as received. Chemical structure of phospholipids are shown in Fig. S1.

2.2. Preparation of supported phospholipid monolayers

Phospholipids in chloroform were mixed at desired compositions, followed by drying using a rotary evaporator under vacuum at 37 °C for 2 h. An aqueous buffer solution was then added to the dry lipid film to give a final concentration of 5 mM. The mixture underwent five freeze-thaw cycles using liquid nitrogen. Thereafter, the phospholipid suspension was passed 20 times through a polycarbonate filter with 100 nm pores using a mini-extruder kit (Avanti Polar Lipids). The extrusion process yields a suspension of unilamellar phospholipid vesicles with a homogenous size distribution.

A gold SPR sensor chip was soaked in 10 mM tetradecanethiol in ethanol for 2 h to form self-assembled monolayer (SAM) on gold, followed by rinsing with ethanol and water. The supported monolayer of phospholipids was formed on the SAM using the Biacore X100 (GE Healthcare Japan, Tokyo, Japan). The sensor chip was cleaned using 0.5 wt% sodium dodecyl sulfate (SDS) for 60 s, followed by flushing through buffer solution to obtain a stable baseline. The suspension of unilamellar phospholipid vesicle in a buffer solution (0.75 mM) was then injected under a flow rate of 5 or 30 μL min^{-1} for 18 min at 25.0 °C, followed by rinsing with 1 mM NaOH for 60 s or buffer solution for 60 min to remove any loosely adsorbed lipids from the surface. The lateral density (γ) of the phospholipids in the monolayers was determined by the SPR signal (ΔSPR) as:

$$(1)\gamma = \Delta SPR \cdot N_A / Mw$$

where N_A and Mw represent Avogadro's number and the molecular weight, respectively. The amount of phospholipids adsorbed was calculated based on the conversion of 1 resonance unit (R.U.) as 1 pg mm^{-2} [39].

2.3. FRAP measurements

A glass coverslip (No. 1S, Matsunami Glass, Osaka, Japan) was rinsed by ethanol and water for 5 min each. Then, the cleaned coverslip was silanized in a vapor of 10.0 vol% solution of dodecyltrimethoxysilane with toluene for 3 h at 100 °C [40]. The surface modified was washed by ethanol and water, followed by dried in air. Supported monolayers of phospholipids containing 0.5 mol% NBD-PC was prepared on dodecyltrimethoxysilane SAM by the vesicle fusion process. The lateral fluidity of supported monolayer was determined by fluorescence recovery after photobleaching (FRAP) measurements using a Nikon Eclipse Ti inverted microscope with a confocal laser scanning system (Tokyo, Japan). NBD-PC was excited with the 488 nm line of a diode laser (Nikon Lu−N4), and emission was detected at 525 nm using a standard PMT detector (Nikon C2-DU3). A 60 × water immersion objective lens (1.2 NA, Nikon) was used. The samples were measured at seven random locations. Initial intensities were recorded for 30 s. The bleaching was performed at a defined spot with the nominal radius (r_n) of 25 μm for 1 s at 100% laser power, and the recovery signal after photobleaching was recorded for 6 min. NIS-Elements software (Nikon) was used for determining the fluorescent intensity at the nominal bleach region of interest (ROI), the half time of recovery ($\tau_{1/2}$), and the spreading radius of photobleach profile (r_e) [41]. The following equation was used to determine bleaching depth parameter (K) and r_e from the fluorescence intensity profile across the bleach ROI from post bleach images:

$$f(x) = 1 - Kexp(-2x^2/r_e^2) \quad (2)$$

The diffusion coefficient (D_{FRAP}) was determined by the following equation [41]:

$$D_{FRAP} = (r_n^2 + r_e^2)/8\tau_{1/2} \quad (3)$$

2.4. CRP binding kinetics using SPR

The kinetic rate constants of CRP adsorption onto supported phospholipid monolayers was assessed using a Biacore X100 at

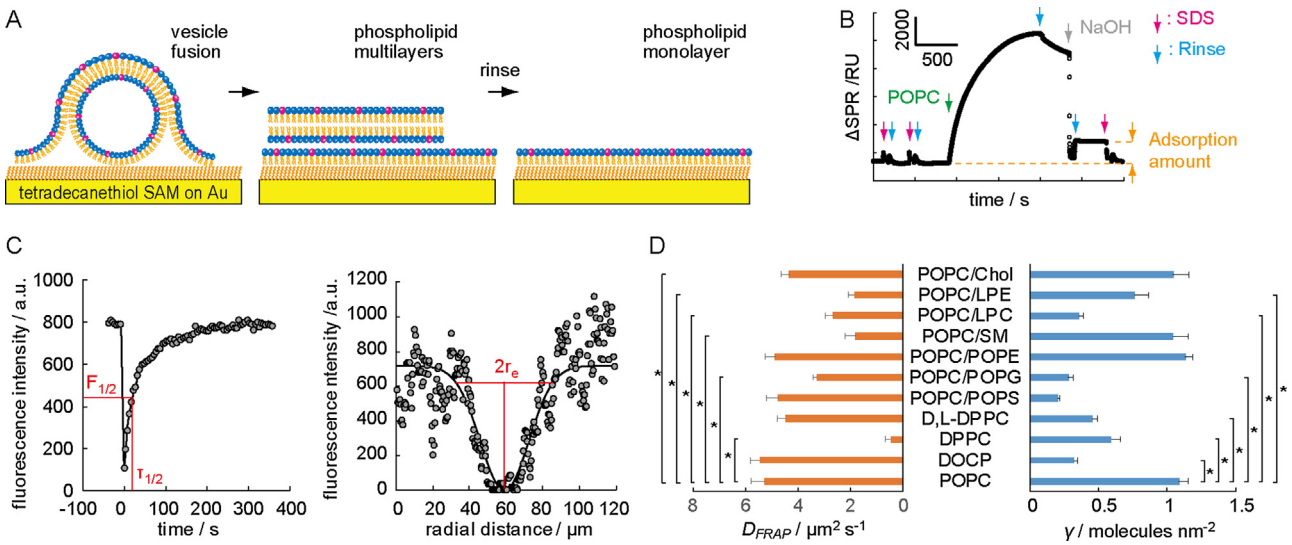

Fig. 1. Schematic of the formation of supported phospholipids monolayers. (A) Two-component phospholipid monolayers were formed by the vesicle fusion process. Hydrophobic fatty acids of the phospholipids face onto a gold surface that was modified with 1-tetradecanethiol SAM. (B) Time course of SPR sensorgram during the formation of the supported POPC monolayers. (C) FRAP data of NBD-PC in the supported POPC monolayers. (Left) Measurement of half time of recovery. (Right) Determination of r_e from photobleach profile along the diagonals of ROI by curve fitting. (D) Surface density and lateral fluidity of supported phospholipid monolayers. Data are shown in mean ± SD. *p < 0.05.

25.0 °C. To minimize the mass transfer limitation of CRP from the bulk phase, the flow rate was 30 μL min^{-1} which gives a mass transfer coefficient of 4.4 μm/s. The experiments were performed using buffered solutions (10 mM Hepes or MES; 100 mM NaCl; pH 7.4 or 5.5 for Hepes or Mes, respectively) in the presence or absence of 1 mM $CaCl_2$. Association and dissociation processes were for 180 s and 300 s, respectively. The CRP concentration was changed in the two times dilution series from 13.6 to 217 nM. The sensorgram was obtained in duplicate for each concentration. The set of measurements was performed three times. Kinetic rate constants are extracted from experimental data by an iterative process that finds the best fit for a set of equations describing the interaction using bundled software of Biacore X100. The bivalent binding mode was used for the fitting (Supplementary Materials) [37]. We determined global rate constant that applies to the whole data set of SPR sensorgrams of 10 independent sub-measurements. The model does not account for the mass transfer process of CRP from bulk phase due to the high flow rate used for the experiments.

2.5. Interaction of CRP with C1q

The C1q adsorption onto an active form of CRP on the supported monolayers was evaluated using the Biacore X100. Initially, supported monolayers were in contact with 217 nM CRP for 600 s, followed by rinsing with a buffer solution for 180 s. Then, CRP adsorbed was in contact with 100 nM C1q for 600 s, followed by rinsing with a buffer solution. Changes in the SPR signal from the baseline after incubation with CRP and C1q were recorded.

2.6. Statistical analysis

All values were expressed as mean with standard deviation (SD). Data were analyzed by ANOVA (one-way), followed by Tukey's test for multiple comparisons. $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Characterization of supported film

Supported phospholipid monolayers were prepared on the SAMs through hydrophobic interactions, with the acyl chains facing the surface (Fig. 1A). The surface density and lateral diffusion as determined by SPR and FRAP were relevant for most studies on vesicles and phospholipid layers (Fig. 1B–D) [42,43]. POPC, POPC/POPE, and POPC/Chol monolayers showed high γ and D_{FRAP} values. On the other hand, DOCP, POPC/POPS, POPC/POPG, and POPC/LPC resulted in low density with high fluidity. The decreased surface density implies that some phospholipids may reside by side-on orientation. A cone-shaped LPC causes the positive curvature of plasma membranes [44], thus disturbing the molecular packing in supported POPC/LPC monolayers. We understand that the low density with high fluidity for POPC/POPS, POPC/POPG and DOCP originates in electrostatic repulsion between the anionic phospholipids [45]. POPC/SM and POPC/LPE monolayers resulted in high density with low diffusivity, suggesting restricted mobility of SM or LPE due to strong hydrogen bonding [46]. L-DPPC had low D_{FRAP} because of the high melting temperature of 41 °C. Interestingly, racemic D,L-DPPC showed similar γ to L-DPPC, but dramatically increased the film fluidity. We understand the facilitated lipid mobility by disturbing stereospecific interaction between neighboring enantiomeric phospholipids [47]. All surfaces prevented nonspecific adsorption of BSA (Fig. S2), indicating the surfaces are adequately covered by the phospholipid molecules [48,49].

3.2. CRP binding experiments

CRP adsorption onto supported phospholipid monolayers was analyzed by SPR. Based on the hydrodynamic radius of pentameric CRP (Mw = 115 kDa) and the random sequential adsorption on a planar substrate, the surface concentration of CRP at full monolayer coverage is calculated to be 0.0102 molecules nm^{-2} or 1950 R.U (Supplementary Materials) [37]. Judged by the signal intensities (Fig. S3), CRP adsorption was in the regime of monolayer adsorption. The curve fit by the bivalent binding mode gave the binding constants (Table S1). The results are consistent with our previ-

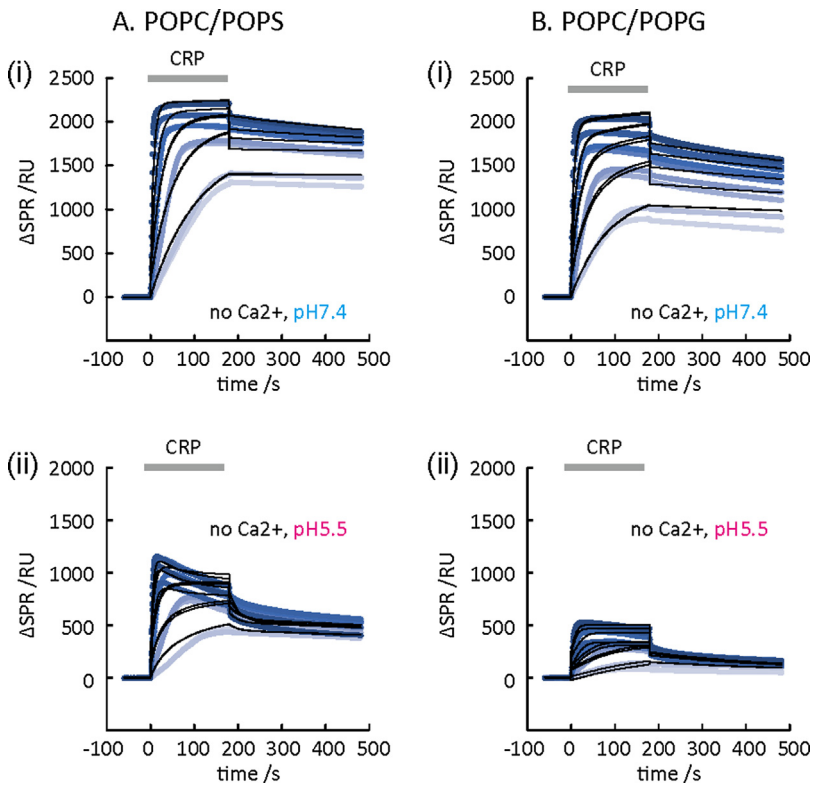

Fig. 2. SPR sensorgrams during association and dissociation processes of CRP at two-fold dilution series (13.6–217 nM) on the supported monolayers of POPC/POPS (5/1 mol/mol) (A) and POPC/POPG (5/1 mol/mol) (B) in the absence of calcium ion at pH 7.4 (i) and 5.5 (ii). Solid lines in black show the results of curve fitting by the bivalent binding model.

ous finding that pentameric CRP interacts with phosphorylcholine headgroups through multiple contacts [35,37]. The SPR signal owes to the specific CRP adsorption because the nonspecific BSA signals were weak at the same concentration range as CRP (Fig. S2).

3.3. Calcium-independent CRP binding by POPS and POPG

We screened CRP-active phospholipids through SPR study. Like in the case of LPC (Fig. S4), the addition of POPS into the supported POPC monolayers induced calcium-independent binding of CRP at pH 7.4 and 5.5 (Fig. 2A). Because POPC relies on calcium for CRP recognition (Fig. S3), POPS is responsible for this calcium-free binding. Similarly, the POPC/POPG monolayers displayed calcium-free CRP adsorption (Fig. 2B). The CRP binding mechanism for POPG and LPC is unlikely to be the same as that for POPS. In our previous study, the glycerophosphate is the element for calcium-free CRP binding [37]. The amount of CRP adsorbed on POPC/POPG in the absence of calcium ions was lower at pH 5.5 than pH 7.4, in agreement with the case of POPC/LPC (Fig. S4). The biding affinity of the calcium-free CRP adsorption onto POPC/POPS and POPC/POPG monolayers was orders of magnitude higher than the calcium-mediated CRP adsorption onto POPC monolayers at pH 7.4 (Table S1).

3.4. Inhibition of CRP binding by LPE, POPE, SM, and chol in POPC monolayers

On the contrary to LPC, the incorporation of lysophospholipid LPE into POPC monolayers blocked the calcium-dependent CRP binding at pH 5.5 (Fig. S5). Because of the net-charge neutrality of POPC (*pKa* of 1.0 for phosphate and 13.9 for amine) and LPE (*pKa* of 1.7 for phosphate and 11.2 for amine) at pH 5.5–7.4, we speculate that the protonation Glu, Asp, and His residues in CRP impaired the interaction with POPC/LPE at pH 5.5. We suggest that the glycerophosphate moiety in LPE did not give rise to calcium-independent CRP binding because the group in the center of molecule is buried in the hydrophobic domain of supported phospholipid monolayers. This is explained by more than 2-fold higher surface density of POPC/LPE than POPC/LPC (Fig. 1D). Our results indicates LPE is not involved in the complement activation, in agreement with physiological role in which LPE mediates cell signaling through G protein-coupled receptors (GPCRs). The incorporation of SM or Chol to POPC monolayers has the same effect as LPE (Fig. S5). SM is an abundant net-neutral sphingolipid which is found in the outer leaflet of peripheral and neuronal cells. SM and Chol are involved in the formation of lipid rafts and ordered domains. The relationship between the CRP repellency and lipid microdomain structures is intriguing, yet its molecular science is not clear. The monomeric form of CRP, which is a proinflammatory mediator, is known to anchor on lipid rafts on endothelial cells [12].

The incorporation of POPE to POPC monolayers inhibited the calcium-dependent CRP recognition at pH7.5 and 5.5 (Fig. S5). Phosphatidylethanolamine is a common phospholipid, particularly found in nervous tissues and bacterial membranes. Because surface density and lateral diffusivity remained unchanged by the POPE addition to POPC (Fig. 1), we interpret that the addition of POPE to POPC monolayers altered the two-dimensional patterns of binding groups, thereby reducing appreciable amount of CRP adsorption.

3.5. Effect of inverted headgroup and chirality of phospholipid

Calcium-dependent binding of CRP was much reduced on supported DOCP monolayers at pH 7.4 (Fig. 3A). The quaternary amine in DOCP is adjacent to the hydrophobic core of the phospholipid layer and the phosphate extends into the aqueous phase [25]. We

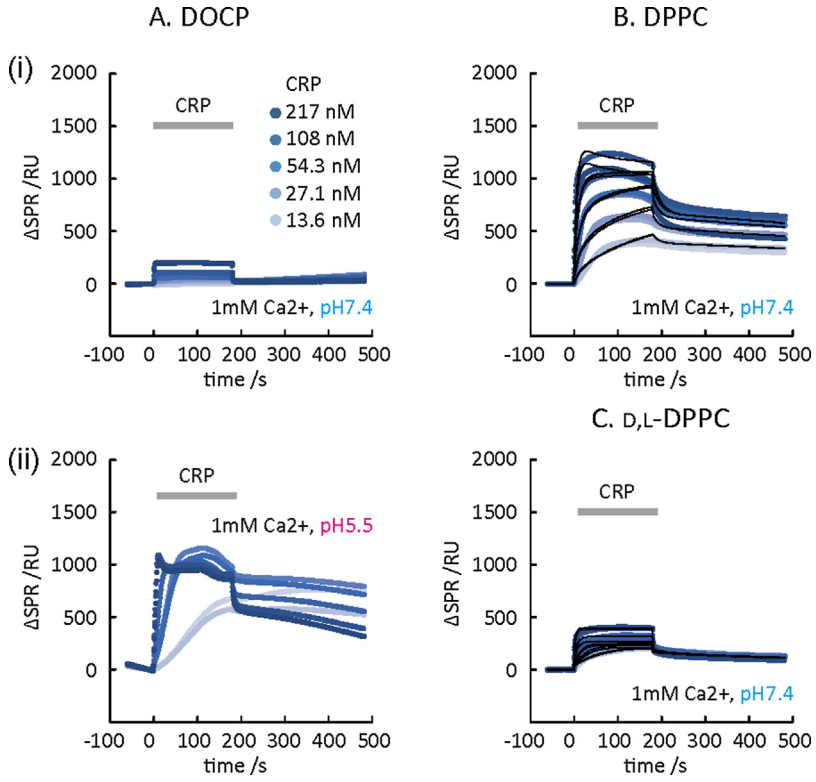

Fig. 3. SPR sensorgrams during association and dissociation processes of CRP at two-fold dilution series (13.6–217 nM) on the supported monolayers of DOCP at pH 7.4 (i) and 5.5 (ii) (A), L-DPPC at pH 7.4 (B) and D,L-DPPC at pH7.4 (C) at 1 mM [Ca^{2+}]. Solid lines in black show the results of curve fitting by the bivalent binding model.

hypothesize that ion-pairing of the divalent phosphate in DOCP with Ca^{2+} hampered the CRP recognition at pH 7.4. A noticeable binding of CRP was observed at pH 5.5 possibly due to the protonation of acidic residues in CRP as the charged state of DOCP is identical at pH 5.5–7.4 [24,25]. The sensorgrams were unable to fit by the bivalent model, indicating either desorption of DOCP together with CRP or replacement of DOCP by CRP molecules.

Next, supported DPPC monolayers were tested to see the effect of gel phase and chirality of supported phospholipid films on CRP recognition (Fig. 3B). Calcium-dependent CRP binding was blocked at pH 5.5 although this occurred at pH7.4. Furthermore, calcium-dependent CRP binding was blocked even at pH7.4 on supported monolayers of D,L-DPPC. The unprecedented repellency for CRP by racemic DPPC monolayers may be caused by the disturbance of chiral complex by changing the crystal dimension and unit cell parameters of DPPC (Fig. 1D) [47,50]. It is intriguing whether enhanced fluidity of supported D,L-DPPC monolayers is related to the CRP inertness or not. Another possibility for the poor binding of CRP to D,L-DPPC monolayers is the establishment of defined two-dimensional patterns of binding groups.

3.6. Binding of C1q to active form of CRP on supported phospholipid monolayers

Given unique CRP recognitions, an investigation was commenced on the complement activation by an active form of CRP on the supported monolayers by recognizing complement component C1q [9]. After incubation with CRP, an increment in the SPR signal was observed on POPC/LPC, POPC/POPS, and POPC/POPG surfaces by C1q (Fig. 4A–C). The signal following C1q injection corresponded to the formation of the C1q/CRP complex. The complex formation occurred without calcium ions. In contrast, there was no significant increase in the SPR signal following C1q incubation on CRP-exposed POPC under calcium-free conditions (Fig. 4D). This was due to the absence of CRP on the POPC monolayers (Fig. S3). The weak signal for CRP on the supported POPC/LPC monolayer was attributed to a continuous decrease in the baseline as mentioned earlier (Fig. S4) [37]. Our data suggest that the adsorption of acute-phase CRP to these phospholipids initiates the first binding steps of complement.

3.7. Discussion

POPS and POPG were newly identified as the ligand to be involved in the calcium-independent CRP binding, followed by C1q recognition (Figs. 2 and 4). Since they do not exist in the outer membrane of healthy cells, complement activation by the CRP adsorption with these marker is in accordance with host defense mechanisms. Anionic POPS is found in the inner leaflet and is typically exposed on the outer membranes of early apoptotic cells. POPS serves as an eat-me-signal for clearance by phagocytes [51,52]. The interaction of POPS with CRP supports the observation that CRP recognizes apoptotic cell membranes for enhancing opsonization and phagocytosis in association with the expression of the anti-inflammatory cytokines [6]. While, POPG is found in the cell membranes of bacteria and plants. Therefore, CRP interaction with POPG is a plausible mechanism for the clearance of foreign substances from the body. The calcium-free recognition of CRP with POPS, POPG, and LPC followed by C1q recruitment suggests the necessity of the clearance of dead cells or microbes through the CRP-mediated complement activation even at sites without calcium ions. The increased adsorption amount of CRP to POPS, POPG, and LPC in weakly acidic conditions may explain an enhanced mechanism of complement systems by decreased pH, a common microenvironment at the foci of inflammation and infection [9,36]. Moreover, alternations in the plasma membrane components in relation to diseases could be a cause of immunogenicity through the mediation of circulating CRP. In support of this statement, CRP binds to altered plasma membranes and phospholipids such as oxidized

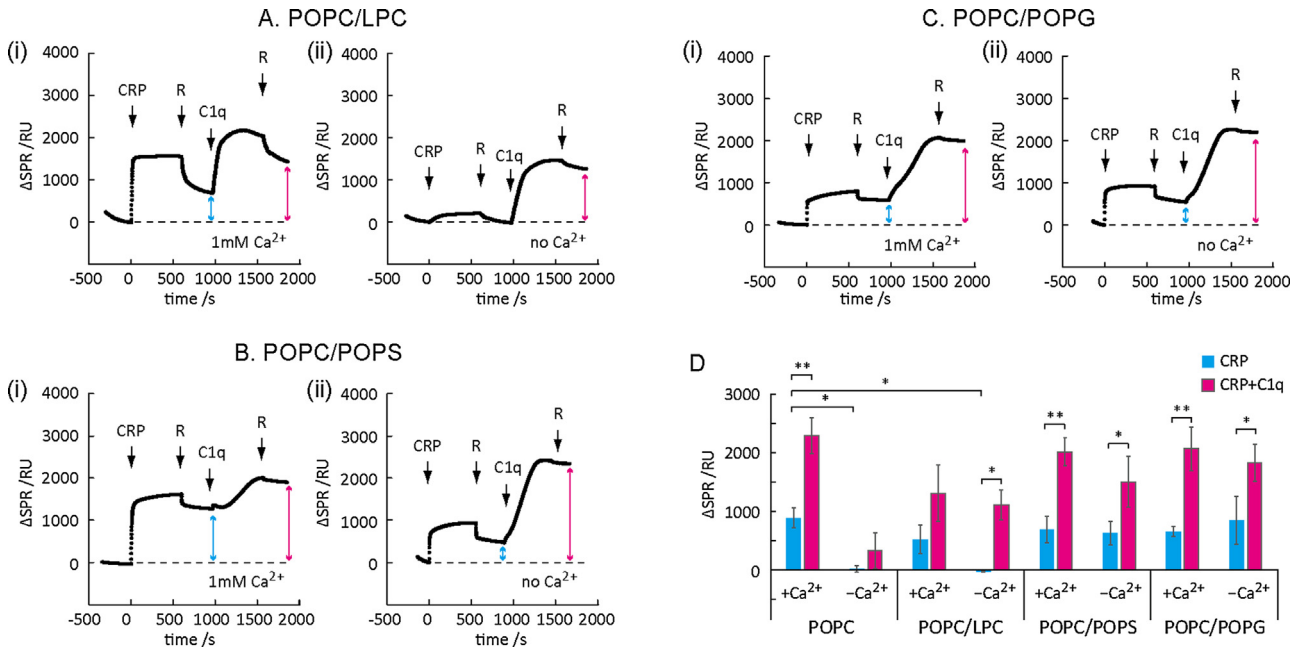

Fig. 4. (A–C) SPR sensorgrams showing the activation of C1q following the adsorption of CRP onto the supported monolayers of POPC/LPC (5/1 mol/mol) (A), POPC/POPS (5/1 mol/mol) (B) and POPC/POPG (5/1 mol/mol) (C) in the presence (i) or absence (ii) of 1 mM [Ca^{2+}] at pH 7.4. (D) SPR signal after incubation with CRP, followed by C1q in the presence or absence of 1 mM [Ca^{2+}]. $^{*}p < 0.05$, $^{**}p < 0.01$.

low-density lipoprotein (LDL), LPC, peroxidized phospholipids and apoptotic cells [3–5].

Physicochemical parameters, such as packing, mobility, curvature, and surrounding environments of the supported lipid monolayers have influence on the binding performance [35,53]. In the current study, the calcium-dependent CRP recognition on L-DPPC or D,L-DPPC monolayers was dramatically different (Fig. 3B). Similarly, calcium-dependent CRP binding onto POPC was suppressed by incorporating LPE, POPE, SM, or Chol in POPC monolayers (Fig. S5). Although the reason for the inhibition is not yet elucidated, these experiments imply that healthy plasma membranes of endothelial cells, which have complex lipid compositions, do not preferentially interact with circulating CRP even in the presence of calcium. A rigorous arrangement of the density, orientation, chirality, mobility, and domain structure of phospholipid in supported layers is necessary to elucidate the whole picture of CRP adsorption. Although supported phospholipid monolayers are the gold-standard platform that is easily prepared by the vesicle fusion process, they have limitations in elaborating the properties of interface.

It was difficult to identify that the compositions of phospholipid monolayers were consistent to those of the respective phospholipid vesicles. For binary lipid mixtures, factors that affect the composition in the supported bilayers are the interactions between a given lipid type and the underlying surface, surface-induced impeded lipid mobility, and an inherent heterogeneous distribution of lipids in the mixed liposomes [54]. A recent study shows that vesicles can display up to 10-fold variations in the relative lipid composition of individual liposomes within the same preparation [55]. A polydispersity in the physicochemical properties of the individual liposomes might lead to the different efficiency of vesicle fusion process, thereby yielding the compositions of the resultant phospholipid layers inconsistent to the original proportion of binary lipids. To minimize the above mentioned effects, supported phospholipid layers were prepared in the monolayer *via* hydrophobic interaction.

Taken together, these results provide the first compelling evidence for CRP-active phospholipids in the binary mixture with POPC on the basis of adsorption kinetics. To exclude any possible effects of all other plasma proteins from consideration, in the current study, CRP adsorption experiments was carried out from single protein buffered solutions. A question remains about the possibility of interactions between plasma proteins with either lipid monolayers or CRP. Other plasma proteins might prevail over CRP binding to phospholipid films if they are present. Investigations on the CRP binding kinetics in a complexed media would be necessary for elucidating the CRP dynamics with physiological relevance.

4. Conclusions

We investigated a ligand for CRP on supported monolayers of binary phospholipids using SPR. Anionic POPS and POPG in POPC monolayers were identified as new element responsible for calcium-independent CRP adsorption. While, CRP adsorption was completely blocked by adding POPE to the supported POPC monolayers or on mixed D,L-DPPC monolayers. The incorporation of LPE, Chol, or SM in supported POPC films blocked calcium-dependent CRP binding at pH 5.5. The inversion of the headgroup in the phospholipid monolayers dramatically altered CRP binding capability. Complement component C1q recognized any active forms of CRP on different types of phospholipid monolayers. The results provide fundamental aspects of CRP interplay on altered plasma membranes under various microenvironments. The molecular understanding of CRP dynamics is helpful for designing phospholipid-based biomaterials under CRP-rich conditions during the acute phase of inflammation and infection.

Conflict of interest

The authors have no conflict of interest to disclose.

Acknowledgements

The authors thank Prof. N. Yui at TMDU for offering the use of the SPR instrument. This research was financially supported by a Grant-in-Aid for Scientific Research on Innovative Areas “Nanomedicine

Molecular Science" (#26107705) from MEXT of Japan and the Tateishi Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.colsurfb.2017.11.036.

References

[1] M.B. Pepys, G.M. Hirschfield, C-reactive protein: a critical update, J. Clin. Invest. 111 (2003) 1805–1812.
[2] S. Black, I. Kushner, D. Samols, C-reactive protein, J. Biol. Chem. 279 (2004) 48487–48490.
[3] M.K. Chang, C.J. Binder, M. Torzewski, J.L. Witztum, C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: phosphorylcholine of oxidized phospholipids, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 13043–13048.
[4] M.K. Chang, K. Hartvigsen, J. Ryu, Y. Kim, K.H. Han, The pro-atherogenic effects of macrophages are reduced upon formation of a complex between C-reactive protein and lysophosphatidylcholine, J. Inflamm. 9 (2012) 42.
[5] D. Weismann, C.J. Binder, The innate immune response to products of phospholipid peroxidation, BBA–Biomembranes 1818 (2012) 2465–2475.
[6] D. Gershov, S. Kim, N. Brot, K.B. Elkon, C-reactive protein binds to apoptotic cells protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity, J. Exp. Med. 192 (2000) 1353–1363.
[7] M.V. Suresh, S.K. Singh, A. Agrawal, Interaction of calcium-bound C-reactive protein with fibronectin is controlled by pH: in vivo implications, J. Biol. Chem. 279 (2004) 52552–52557.
[8] D.J. Hammond Jr., S.K. Singh, J.A. Thompson, B.W. Beeler, A.E. Rusinol, M.K. Pangburn, L.A. Potempa, A. Agrawal, Identification of acidic pH-dependent ligands of pentameric C-reactive protein, J. Biol. Chem. 285 (2010) 36235–36244.
[9] T. Goda, P. Kjall, K. Ishihara, A. Richter-Dahlfors, Y. Miyahara, Biomimetic interfaces reveal activation dynamics of C-reactive protein in local microenvironments, Adv. Healthc. Mater. 3 (2014) 1733–1738.
[10] S.R. Ji, Y. Wu, L. Zhu, L.A. Potempa, F.L. Sheng, W. Lu, J. Zhao, Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: mCRP(m), FASEB J. 21 (2007) 284–294.
[11] S.U. Eisenhardt, J. Habersberger, A. Murphy, Y.C. Chen, K.J. Woollard, N. Bassler, H.W. Qian, C. von zur Muhlen, C.E. Hagemeyer, I. Ahrens, J. Chin-Dusting, A. Bobik, K. Peter, Dissociation of pentameric to monomeric C-reactive protein on activated platelets localizes inflammation to atherosclerotic plaques, Circ. Res. 105 (2009) 128–137.
[12] S.R. Ji, L. Ma, C.J. Bai, J.M. Shi, H.Y. Li, L.A. Potempa, J.G. Filep, J. Zhao, Y. Wu, Monomeric C-reactive protein activates endothelial cells via interaction with lipid raft microdomains, FASEB J. 23 (2009) 1806–1816.
[13] M. Mihlan, A.M. Blom, K. Kupreishvili, N. Lauer, K. Stelzner, F. Bergstrom, H.W.M. Niessen, P.F. Zipfel, Monomeric C-reactive protein modulates classic complement activation on necrotic cells, FASEB J. 25 (2011) 4198–4210.
[14] B. Molins, E. Pena, R. de la Torre, L. Badimon, Monomeric C-reactive protein is prothrombotic and dissociates from circulating pentameric C-reactive protein on adhered activated platelets under flow, Cardiovasc. Res. 92 (2011) 328–337.
[15] M.Y. Wang, S.R. Ji, C.J. Bai, D. El Kebir, H.Y. Li, J.M. Shi, W. Zhu, S. Costantino, H.H. Zhou, L.A. Potempa, J. Zhao, J.G. Filep, Y. Wu, A redox switch in C-reactive protein modulates activation of endothelial cells, FASEB J. 25 (2011) 3186–3196.
[16] J. Habersberger, F. Strang, A. Scheichl, N. Htun, N. Bassler, R.M. Merivirta, P. Diehl, G. Krippner, P. Meikle, S.U. Eisenhardt, I. Meredith, K. Peter, Circulating microparticles generate and transport monomeric C-reactive protein in patients with myocardial infarction, Cardiovasc. Res. 96 (2012) 64–72.
[17] J. Habersberger, A. Scheichl, F. Strang, S.U. Eisenhardt, N.M. Htun, K. Peter, The dissociation of pentameric to monomeric C-reactive protein (CRP) on microparticles drives inflammation in patients with myocardial infarction, Cardiology 126 (2013) 383.
[18] J.R. Thiele, J. Habersberger, D. Braig, Y. Schmidt, K. Goerendt, V. Maurer, H. Bannasch, A. Scheichl, K.J. Woollard, E. von Dobschutz, F. Kolodgie, R. Virmani, G.B. Stark, K. Peter, S.U. Eisenhardt, Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: in vivo proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy, Circulation 130 (2014) 35–50.
[19] M.B. Pepys, G.M. Hirschfield, G.A. Tennent, J.R. Gallimore, M.C. Kahan, V. Bellotti, P.N. Hawkins, R.M. Myers, M.D. Smith, A. Polara, A.J.A. Cobb, S.V. Ley, J.A. Aquilina, C.V. Robinson, I. Sharif, G.A. Gray, C.A. Sabin, M.C. Jenvey, S.E. Kolstoe, D. Thompson, S.P. Wood, Targeting C-reactive protein for the treatment of cardiovascular disease, Nature 440 (2006) 1217–1221.
[20] P.M. Ridker, N. Rifai, L. Rose, J.E. Buring, N.R. Cook, Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events, N. Engl. J. Med. 347 (2002) 1557–1565.
[21] T. Pischon, M. Mohlig, K. Hoffmann, J. Spranger, C. Weikert, S.N. Willich, A.F.H. Pfeiffer, H. Boeing, Comparison of relative and attributable risk of myocardial infarction and stroke according to C-reactive protein and low-density lipoprotein cholesterol levels, Eur. J. Epidemiol. 22 (2007) 429–438.
[22] S.K. Singh, M.V. Suresh, B. Voleti, A. Agrawal, The connection between C-reactive protein and atherosclerosis, Ann. Med. 40 (2008) 110–120.
[23] V.V. Ranade, Drug delivery systems 1. Site-specific drug delivery using liposomes as carriers, J. Clin. Pharmacol. 29 (1989) 685–694.
[24] J.P.M. Motion, J. Nguyen, F.C. Szoka, Phosphatase-triggered fusogenic liposomes for cytoplasmic delivery of cell-impermeable compounds, Angew. Chem. Int. Ed. 51 (2012) 9047–9051.
[25] E.K. Perttu, A.G. Kohli, F.C. Szoka, Inverse-phosphocholine lipids: a remix of a common phospholipid, J. Am. Chem. Soc. 134 (2012) 4485–4488.
[26] X.F. Yu, Z.H. Liu, J. Janzen, I. Chafeeva, S. Horte, W. Chen, R.K. Kainthan, J.N. Kizhakkedathu, D.E. Brooks, Polyvalent choline phosphate as a universal biomembrane adhesive, Nat. Mater. 11 (2012) 468–476.
[27] X.F. Yu, X.Q. Yang, S. Horte, J.N. Kizhakkedathu, D.E. Brooks, A pH and thermosensitive choline phosphate-based delivery platform targeted to the acidic tumor microenvironment, Biomaterials 35 (2014) 278–286.
[28] X.Q. Yang, N. Li, I. Constantinesco, K. Yu, J.N. Kizhakkedathu, D.E. Brooks, Choline phosphate functionalized cellulose membrane: a potential hemostatic dressing based on a unique bioadhesion mechanism, Acta Biomater. 40 (2016) 212–225.
[29] T. Goda, Y. Goto, K. Ishihara, Cell-penetrating macromolecules: direct penetration of amphipathic phospholipid polymers across plasma membrane of living cells, Biomaterials 31 (2010) 2380–2387.
[30] X.J. Lin, T. Konno, K. Ishihara, Cell-membrane-permeable and cytocompatible phospholipid polymer nanoprobes conjugated with molecular beacons, Biomacromolecules 15 (2014) 150–157.
[31] K. Ishihara, Bioinspired phospholipid polymer biomaterials for making high performance artificial organs, Sci. Technol. Adv. Mater. 1 (2000) 131–138.
[32] Y. Iwasaki, K. Ishihara, Cell membrane-inspired phospholipid polymers for developing medical devices with excellent biointerfaces, Sci. Technol. Adv. Mater. 13 (2012) 064101.
[33] M. Rassouli, H. Sambasivam, P. Azadi, A. Dell, H.R. Morris, A. Nagpurkar, S. Mookerjea, R.K. Murray, Derivation of the amino-acid-sequence of rat C-reactive protein from cdna cloning with additional studies on the nature of its dimeric component, J. Biol. Chem. 267 (1992) 2947–2954.
[34] J.E. Volanakis, K.W.A. Wirtz, Interaction of C-reactive protein with artificial phosphatidylcholine bilayers, Nature 281 (1979) 155–157.
[35] T. Goda, Y. Miyahara, Engineered zwitterionic phosphorylcholine monolayers for elucidating multivalent binding kinetics of C-reactive protein, Acta Biomater. 40 (2016) 46–53.
[36] A. Lardner, The effects of extracellular pH on immune function, J. Leukocyte Biol. 69 (2001) 522–530.
[37] T. Goda, Y. Miyahara, Calcium-independent binding of human C-reactive protein to lysophosphatidylcholine in supported planar phospholipid monolayers, Acta Biomater. 48 (2017) 206–214.
[38] P. Schuck, Use of surface plasmon resonance to probe the equilibrium and dynamic aspects of interactions between biological macromolecules, Annu. Rev. Biophys. Biomol. Struct. 26 (1997) 541–566.
[39] B. Liedberg, I. Lundstrom, E. Stenberg, Principles of biosensing with an extended coupling matrix and surface-plasmon resonance, Sens. Actuators B 11 (1993) 63–72.
[40] M.J. Zhu, M.Z. Lerum, W. Chen, How to prepare reproducible homogeneous, and hydrolytically stable aminosilane-derived layers on silica, Langmuir 28 (2012) 416–423.
[41] M. Kang, C.A. Day, A.K. Kenworthy, E. DiBenedetto, Simplified equation to extract diffusion coefficients from confocal FRAP data, Traffic 13 (2012) 1589–1600.
[42] J.M. Brake, M.K. Daschner, N.L. Abbott, Formation and characterization of phospholipid monolayers spontaneously assembled at interfaces between aqueous phases and thermotropic liquid crystals, Langmuir 21 (2005) 2218–2228.
[43] C. Huang, J.T. Mason, Geometric packing constraints in egg phosphatidylcholine vesicles, Proc. Natl. Acad. Sci. U. S. A. 75 (1978) 308–310.
[44] H.T. McMahon, J.L. Gallop, Membrane curvature and mechanisms of dynamic cell membrane remodelling, Nature 438 (2005) 590–596.
[45] P. Jurkiewicz, L. Cwiklik, A. Vojtiskova, P. Jungwirth, M. Hof, Structure dynamics, and hydration of POPC/POPS bilayers suspended in NaCl, KCl, and CsCl solutions, BBA–Biomembranes 1818 (2012) 609–616.
[46] J.C. Domingo, M. Mora, M.A. Demadariaga, Role of headgroup structure in the phase-behavior of N-acylethanolamine phospholipids – hydrogen-bonding ability and headgroup Size, Chem. Phys. Lipids 69 (1994) 229–240.
[47] M. Inagaki, M. Shibakami, S.L. Regen, Influence of phospholipid chirality on nearest-neighbor interactions within fluid bilayers, J. Am. Chem. Soc. 119 (1997) 7161–7162.
[48] A.L. Plant, Self-assembled phospholipid alkanethiol biomimetic bilayers on gold, Langmuir 9 (1993) 2764–2767.
[49] A.L. Plant, Supported hybrid bilayer membranes as rugged cell membrane mimics, Langmuir 15 (1999) 5128–5135.
[50] I. Sakurai, S. Iwayanagi, Phase-transitions in single-crystals of L-DPPC and DL-DPPC, Mol. Cryst. Liq. Cryst. 67 (1981) 745–755.

[51] V.A. Fadok, D.R. Voelker, P.A. Campbell, J.J. Cohen, D.L. Bratton, P.M. Henson, Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages, J. Immunol. 148 (1992) 2207–2216.
[52] S.J. Martin, C.P.M. Reutelingsperger, A.J. Mcgahon, J.A. Rader, R.C.A.A. Vanschie, D.M. Laface, D.R. Green, Early redistribution of plasma-membrane phosphatidylserine Is a general feature of apoptosis regardless of the initiating stimulus – inhibition by overexpression of Bcl-2 and Abl, J. Exp. Med. 182 (1995) 1545–1556.
[53] M.S. Wang, R.E. Messersmith, S.M. Reed, Membrane curvature recognition by C-reactive protein using lipoprotein mimics, Soft Matter 8 (2012) 7909–7918.
[54] T.K. Lind, M. Cardenas, Understanding the formation of supported lipid bilayers via vesicle fusion–a case that exemplifies the need for the complementary method approach (Review), Biointerphases 11 (2016) 031017.
[55] J. Larsen, N.S. Hatzakis, D. Stamou, Observation of inhomogeneity in the lipid composition of individual nanoscale liposomes, J. Am. Chem. Soc. 133 (2011) 10685–10687.