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Detection of polychlorinated biphenyls using an antibody column in tandem with a fluorescent liposome column
Effect of albumin on phospholipase A_2-catalyzed membrane leakage

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Abstract

Phospholipase A_2 (PLA_2)-catalyzed membrane leakage can be detected by immobilized liposomes containing a self-quenching fluorescent dye, 3,3-bis[*N*,*N*-di(carboxymethyl)aminomethyl]fluorescein (calcein). This enzymatic reaction was applied as signal amplification for biosensor detection of low concentrations of polychlorinated biphenyls (PCBs). In order to increase the fluorescent signal for improvement of PCBs detection, the effect of BSA on optimal lipid composition for PLA_2-catalyzed membrane leakage from fluorescent liposomes has been investigated in this report. Various kinds of calcein-entrapped liposomes were immobilized in Sephacryl S1000 gel beads using avidin–biotin binding. In a contrast, free calcein was removed by size exclusion chromatography on Sephacryl S300 for free liposome suspensions. The PLA_2-catalyzed membrane leakage was detected both in these gel-bead-immobilized liposomes and in free liposome suspensions. In both systems, the fluorescent release from the liposomes by PLA_2 hydrolytic action significantly increased with increasing albumin concentration. The most rapid and greatest membrane leakage by PLA_2 hydrolysis was found in anionic liposomes in the presence of albumin, both in free liposome suspensions and gel-bead-immobilized liposomes. Finally, the stabilities of various free liposomes and gel-bead-immobilized liposomes were monitored. Immobilized 1-palmitoy-2-oleoylphosphatidylcholine (POPC)/1-palmitoy-2-oleoylphosphatidylglycerol (POPG) liposome gel was chosen due to its excellent stability and large dye leakage by PLA_2. A concentration of PCBs as low as 0.1 ng/mL was detectable using this tandem column system.

Keywords: PCBs detection; Fluorescent; Liposome column; PLA_2; Albumin; Anionic liposomes; Immunoassay

1. Introduction

Polychlorinated biphenyls (PCBs) are persistent, bioccumulative and toxic pollutants widely spread in the environment. PCBs were used not only in the transformers of train carriages but also in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment, as plasticizers in paints, plastics and rubber products; in pigments, dyes and carbonless copy paper as well as in many other applications. Moreover, they were generated from combustion of chlorinated organic polymers. Therefore, they have been widely identified in the environment, in industry or in food [1,2]. The law for the promotion of environmentally sound destruction of PCBs waste has been enforced since July 2001 in Japan. Therefore, detection and remediation of PCBs as well as other environmental pollutants (e.g., dioxin) is an important issue today and a rapid and highly sensitive method is needed to detect PCBs for PCBs control.

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The task of detection of PCBs and related compounds in the environment can be complex, time-consuming and expensive. Many of the procedures for the detection of PCBs are based on GC/electron-capture detection (ECD) or GC/MS [3]. However, its high cost of hundreds of dollar per sample and requirement of specially trained operators usually taking 1–2 weeks to complete the analysis limit its applications for high-throughput screening of pollutants, although these methods achieve a low detection limit for PCBs. Thus, many immunoassays, such as enzyme-linked immunosorbent assay (ELISA) [2,4,5] and immunosensors [6] have been developed as simple and alternative methods for routine measurements of PCBs. This is generally performed using a competitive binding enzyme immunoassay in a mixture of PCBs, PCBs-binding antibody and a fixed amount of an enzyme-labeled PCBs analog. After competitive reaction during incubation of the mixture, the PCBs concentration in the sample is determined by measurement of the amount of the enzyme-labeled analog bound to the antibody. Recently, dye-entrapped liposomes have been used for signal amplification in competitive immunoassays [2,7,8]. A high concentration of fluorescent dye can be entrapped in liposomes and a small amount of the dye released upon antibody binding will result in a fluorescent signal, which can be detected. These advantages led to the development of liposome immunosensors to detect the herbicide alachlor and PCBs, based on immunocompetition and immunoaggregation between anti-PCB antibodies and analyte-tagged liposomes, respectively [9,10].

Immobilized liposome chromatography (ILC) columns have been developed for various chromatographic applications, such as those made of immobilized liposomes containing a self-quenching fluorescent dye, 3,3-bis[*N*,*N*-di(carboxymethyl)aminomethyl]fluorescein (calcein), for bioanalysis [11,12]. The advantage of the fluorescent liposome column is its excellent stability upon storage and chromatographic runs [11]. This combined with the immunoassay methods extend the liposome chromatography technique to the realm of detection of environmental pollutants. In our previous work, we have developed a tandem column system combining the liposome fluorescent column with an anti-PCBs antibody column for detection of PCBs [13], and using a negatively charged liposome column, a PCBs concentration as low as 0.5 ng/mL was detectable [14]. To further increase the fluorescent signal for sensitive PCBs detection, the effects of bovine serum albumin (BSA) on various liposome compositions for phospholipase A_2 (PLA_2)-catalyzed membrane leakage on the fluorescent liposome column have been investigated in this report. In this report, it is found that the fluorescent signal from liposomes hydrolyzed by PLA_2 can be amplified significantly using anionic liposomes in the presence of BSA. From comparison of the stability of free liposomes and gel-bead-immobilized liposomes, 1-palmitoy-2-oleoylphosphatidylcholine (POPC)/1-palmitoy-2-oleoylphosphatidylglycerol (POPG) liposome gel is used in the PCBs detection system due to its excellent stability and efficient fluorescent leakage from PLA_2-catalyzed membrane leakage.

2. Experimental

2.1. Materials

Sephacryl S-1000 (superfine) and HiTrap Protein G column (1 mL) were purchased from Amersham Pharmacia Biotech UK (Buckinghamshire, UK). Bio-Spin 6 was obtained from Nippon Bio-Rad Labs. (Tsukuba, Japan). Egg-white avidin was purchased from Pierce (Rockford, IL, USA). Egg yolk phosphatidylcholine (EPC, >99%), egg phosphatidylethanolamine (EPE, >99%), POPC (>99%), POPG (>99%), and 1,2-dioleoylphosphatidylethanolamine-*N*-(cap biotinyl) (biotin-cPE) were supplied from Avanti Polar Lipids (Alabaster, USA). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), and 3,3-bis(*N*,*N*-di(carboxymethyl)aminomethyl) fluorescein (calcein) came from Dojindo Labs. (Kumamoto, Japan). PLA_2 (EC 3.1.1.4) from bee venom and BSA were purchased from Sigma (St. Louis, MO, USA). 3,4-Dichloroaniline (Wako, Tokyo, Japan) and mouse anti-PCBs monoclonal IgG (Research Diagnostics, Flanders, USA) were all used as received. PCBs chemical product, KC-500, corresponding to Aroclor 1254 was purchased from GL Science (Tokyo, Japan). All other chemicals were of analytical grade.

2.2. Preparation of calcein-entrapped liposome gels and free liposomes

Phospholipids supplemented with 2 mol% of biotin-cPE were dried to a thin film by rotary evaporation. The lipid film was flashed with nitrogen gas, kept under high vacuum overnight, and dispersed in 100 mM calcein solution (pH 7.5) to form multilamellar vesicles. Large unilamellar vesicles (LUVs) were prepared by extrusion on two stacked polycarbonate filters of 100 nm pore size as described in the previous report [15]. The biotinylated LUVs used in this study were composed of POPG, POPC/POPG (molar ratio, 1:1), POPC, EPC, EPC/EPE (molar ratio, 1:1) or EPC/cholesterol (molar ratio, 2:1) were used in this studies. Sephacryl S-1000 gel was activated by 4-nitrophenyl chloroformate to a ligand density of 20–30 μmol/mL gel, and used to covalently couple avidin as described in [15]. Excess chloroformate in gels was blocked by further mixing with 1 M ethanolamine (pH 8.2) overnight at 4 °C or 2 h at 25 °C. The avidin-gels were stored at 4 °C in buffer H (10 mM HEPES, 150 mM NaCl, pH 7.5) supplemented with 3 mM NaN_3. For immobilization, the calcein-entrapped biotinylated liposomes were mixed with avidin-gels by gentle rotation for 2–3 h at 23 °C under nitrogen. Nonimmobilized liposomes together with nonentrapped calcein were then removed by washing with buffer H through a 10 μm filter. The immobilized calcein-entrapped liposomes

were packed into a 0.1 mL gel bed of an open column for the detection PLA_2-catalyzed membrane leakage except where otherwise stated. In the case of calcein-entrapped liposome suspensions, nonentrapped calcein was removed from the biotinylated LUVs by gel filtration on a Sephacryl S-300 column (3.5 cm × 2.0 cm I.D.) that had been equilibrate with buffer H.

2.3. Phosphorus and fluorescent determination

The phospholipid contents of the immobilized liposomes on the gel beads were determined as the phosphorus content of the gel beads by the method of Bartlett with modifications as described in detail by Yang et al. [16]. Every batch column contained the same lipid amount of 2 μmol except where otherwise stated. The calcein release was determined in a fluorescent spectrophotometer (F-4500, Hitachi, Tokyo, Japan) at excitation and emission wavelengths of 490 nm and 520 nm, respectively. After the enzymatic reaction, fractions of 2 mL were collected and the released calcein fluorescent content was measured.

2.4. Protocol of PCBs detection

3,4-Dichloroaniline–PLA_2 conjugate was prepared by rotation mixing of PLA_2 and 3,4-dichloroaniline in a reaction mixture containing *N*-hydroxysulphosuccinimide (NHS) and 1-ethyl-3-(3-diethylaminopropyl)-carbodiimide hydrochloride (EDC) at 4 °C for 24 h as described in our previous report [14]. A HiTrap Protein G column was used to immobilize anti-PCBs antibodies. Protein G is a type III Fc receptor that strongly binds to the Fc region of immunoglobulin G (IgG) by a nonimmune mechanism. One milliliter of anti-PCB monoclonal IgG (1 mg/mL) in buffer 1 (10 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.5) was coupled to the HiTrap Protein G column, and then washed with buffer 1. The solution eluted after injection and wash was collected and subjected to measurement of protein concentration using Bio-Rad Protein Assay (Bio-Rad Labs., Hercules, CA, USA).

A PCBs stock solution (1 μg/mL) in acetone was diluted by buffer 1 to obtain a series of PCBs concentrations in a range from 0.01 to 10 ng/mL. The mixture of PLA_2 conjugate (200 μl; final concentration, 1 μg/mL) and the PCBs solutions of various concentration, were applied to the 1 mL antibody column, and incubated in the column for 15 min at 25 °C. Then, unbound PLA_2 conjugate was eluted out using 3 mL of buffer 2 (10 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 100 M BSA, pH 7.5), and allowed to enter to the liposome column followed by elution with 10 mL of buffer 2 and fractionated at 2 mL each to measure the fluorescent intensity. The antibody column was regenerated using acetate buffer (pH 4.0), and the liposome column could be washed with buffer 2 to achieve a stable baseline with little fluorescence detectable as monitored by a fluorescent spectrophotometer.

3. Results and discussion

3.1. Effect of BSA on PLA_2-catalyzed membrane leakage in free liposome suspensions

PLA_2-catalyzed membrane leakage measurement can be facilitated by fluorescent online and offline analysis using immobilized liposomes [11,14]. We found that there was a low level of continuous fluorescent leakage from the PLA_2-hydrolyzed liposome column. It seems the PLA_2-hydrolyzed liposomal bilayers gradually decomposed. Considering the fact that BSA acts as a lipid carrier molecule [17–19], the effect of BSA on PLA_2-catalyzed membrane leakage was investigated. For a comparison with the immobilized liposome gels, a series experiments were done in various free liposome suspensions. First, POPC/POPG liposomes were selected to investigate the BSA concentration effect on PLA_2-catalyzed membrane leakage. As shown in Fig. 1, the fluorescent intensity increased, and then reached a plateau upon increasing the BSA concentration from 10 to 150 μM (filled squares, Fig. 1). BSA was able to extract the reaction products from the liposomes, resulting in immediate collapse the lipid bilayer structures. It should be noted that there is little fluorescent leakage when only BSA was added to the liposome suspensions. Therefore, a BSA concentration of 100 μM was used in the further studies. The effects of BSA on PLA_2-catalyzed membrane leakage were also systematically investigated in POPG, POPC, EPC, EPC/EPE and EPC/cholesterol liposomes (Fig. 2). It can be clearly seen that the fluorescent signal increased immediately as soon as BSA was added to the reaction solution. After a few minutes, the fluorescence level reached a plateau. In comparison to PLA_2-catalyzed hydrolysis of POPC/POPG liposomes in the absence of BSA (open diamonds, Fig. 2),

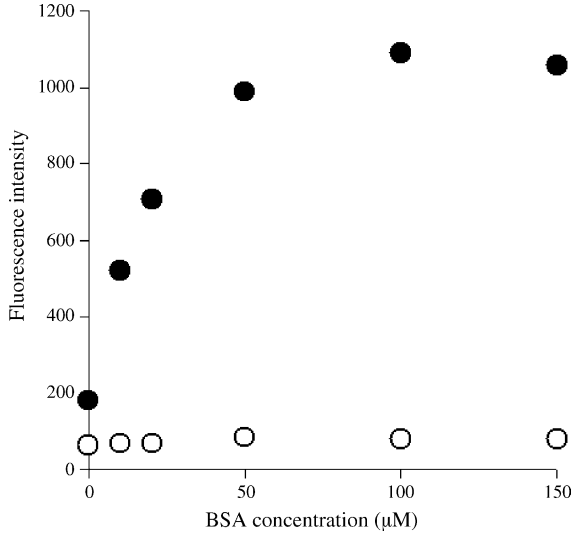

Fig. 1. Effect of BSA concentration on the calcein leakage from 40 μM POPC/POPG liposome suspensions hydrolyzed by 0.5 μg/mL PLA_2 (●) or in the absence of PLA_2 (○) for 30 min at room temperature.

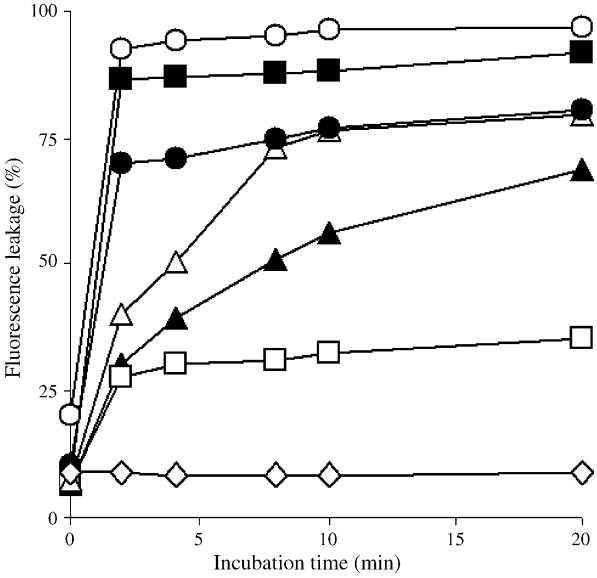

Fig. 2. PLA_2-catalyzed hydrolysis of various liosome suspensions: (◯) POPG, (●) POPC/POPG, (▲) EPC, (△) POPC, (□) EPC/Chol and (■) EPC/EPE in the presence of BSA, and (◊) POPC/POPG in the absence of BSA. PLA_2 (0.5 μg/mL) were applied to 40 μM free liposome suspensions in 2 mL reaction solutions. The fluorescence intensity was measured after 0, 2, 4, 8, 10 and 20 min incubation of the 2 mL reaction mixtures. The fluorescence leakage is expressed as a percentage of the total liposome solubilization with 100 mM *n*-octyl-β-D-glucopyranoside (β-OG, a non-ionic detergent).

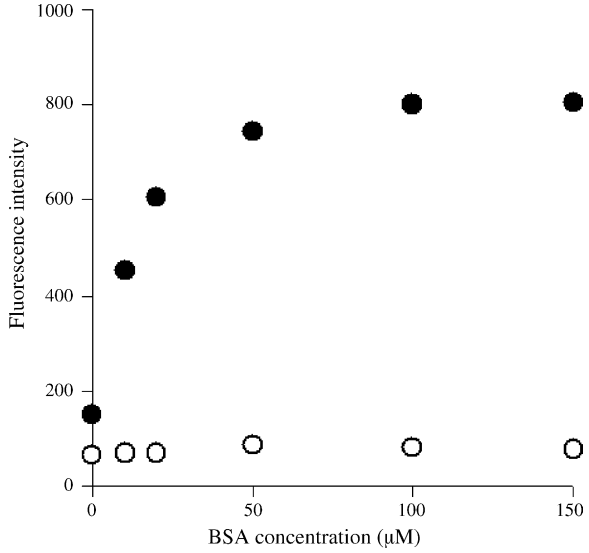

Fig. 3. Effect of BSA concentration on the calcein leakage from 0.1 μmol POPC/POPG liposome gels hydrolyzed by 0.5 μg/mL PLA_2 (●) or in the absence of PLA_2 (◯) for 30 min at room temperature.

the fluorescence level was five times higher in the presence of 100 μM BSA (filled circles, Fig. 2). This can be reasonably explained if BSA acts to remove products of the fatty acid from the liposomal bilayers after PLA_2 hydrolysis reactions on the liposomes. In each kind of liposome, the fluorescent signal caused by PLA_2-catalyzed membrane leakage was higher in the presence of BSA than in the absence of BSA, and there was almost no leakage if BSA alone was added to the liposomes (data not shown). For a comparison, the fluorescent leakages hydrolyzed by PLA_2 in the presence of 100 μM BSA in each kind of liposome are shown in Fig. 2. Clearly, the order of fluorescent leakage from the liposomes hydrolyzed by PLA_2 in the presence of BSA was POPG, EPC/EPE, POPC/POPG, POPC, EPC and EPC/cholesterol. Bee venom PLA_2 as a secreted enzyme has been reported to bind to anionic phospholipids in the 'scooting mode' [20–23]. This means that PLA_2 preferred the anionic liposomes due to several cationic residues on its interface binding surface, and the enzyme remains at the interface and catalyzes many reaction cycles. Therefore, PLA_2 slightly favored POPG over POPC/POPG (1:1) and POPC liposomes. PLA_2-catalyzed hydrolysis of the EPC/cholesterol liposomes showed the lowest fluorescent leakage due to the poor fluidity of incorporated cholesterol into the EPC liposomal bilayers. In general, PLA_2 catalyzes hydrolysis of compactly packed phospholipid are efficiently as loosely packed phospholipid [22].

3.2. *Effect of BSA on PLA_2-catalyzed membrane leakage in immobilized liposomes*

The effect of pH, incubation time and liposome compositions on PLA_2-catalyzed membrane leakage in avidin–biotin-immobilized liposomes were reported in our previous work [14]. To further find the largest fluorescent signal leaked from the liposome gels by PLA_2, the effects of BSA concentration on different liposomes were systemically investigated. BSA concentration effects on the PLA_2-catalyzed hydrolysis of the immobilized liposomes were performed on a POPC/POPG liposome column (Fig. 3). As in the results in free liposome suspensions (Fig. 1), the fluorescent signal increased and reached a plateau with increasing BSA concentration to 150 μM. It is also clearly seen that if only BSA of various concentrations was applied to the liposome column, the fluorescent intensity did not change and remained very low (open circles, Fig. 1). This suggested that there is no nonspecific adsorption between BSA and immobilized liposome gels. Similar experiments have been performed by Kim et al. [17], but they found that with higher BSA concentrations, a slight decrease in oleic acid release was observed, which was explained by the fact that more hydrophobic silica beads were exposed for nonspecific adsorption of BSA after BSA removed the breakdown products from the bead surface. However, in our study the hydrophobicity of Sephacryl S1000 gel beads is much lower [14], and even though BSA decomposed the PLA_2-hydrolyzed liposomes in the gel matrix, the gel surface was already blocked by the protein avidin. Therefore, the nonspecific binding of BSA to the gels should be lower. A very small amount of liposome gels, 0.1 μmol, was enough for hydrolytic reactions by an amount of PLA_2 100 times lower than that in our previous report in the absence of BSA [14]. Based on these results, 100 μM BSA and 0.1 μmol immobilized li-

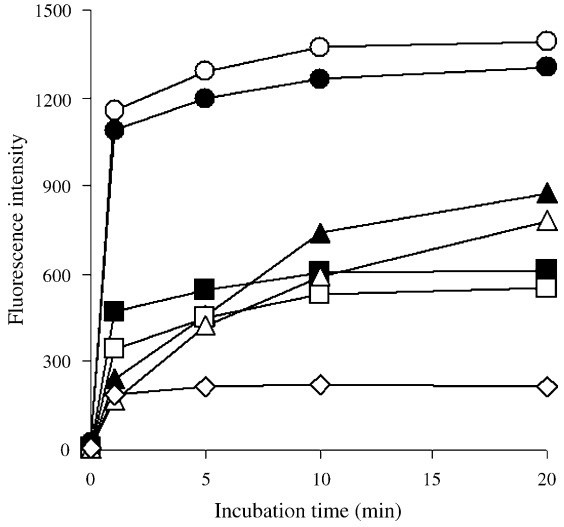

Fig. 4. PLA_2-catalyzed hydrolysis of Sephacryl gel-bead-immobilized liposomes (◯) POPG, (●) POPC/POPG, (▲) EPC, (△) POPC, (□) EPC/Chol and (■) EPC/EPE in the presence of BSA, and (◊) POPC/POPG in the absence of BSA. All liposome columns contain the same lipid amount of 0.1 μmol phospholipids and similar gel bed volumes of approximately 10 μl. Each fraction of 2 mL was collected and calcein release was measured after 0, 1, 5, 10, 20 and 30 min incubation time of PLA_2 with the different liposome columns.

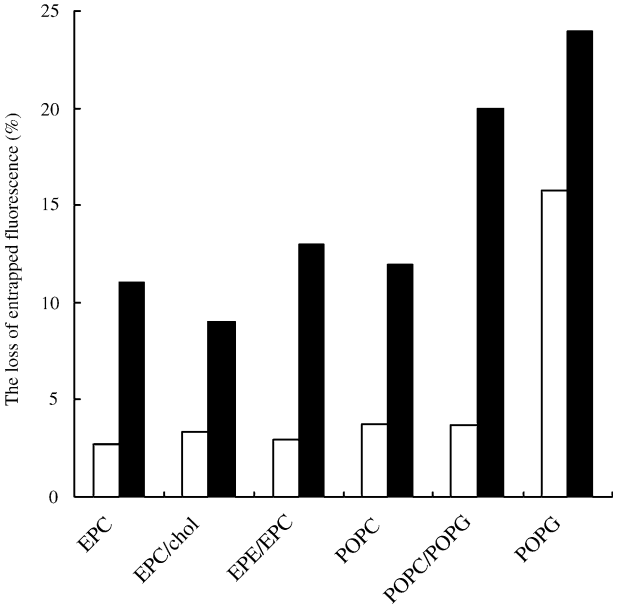

Fig. 5. Stability of various liposome gels (□) and liposome suspensions (■) upon storage at 4 °C after 60 days. The liposome-gel supernatant was removed by centrifugation at $700 \times g$ for 5 min with buffer. The loss of calcein is expressed as a percentage of the initial amount of entrapped calcein.

posome gels were used in the time courses of hydrolysis of different liposomes by PLA_2 (Fig. 4). The fluorescent leakage from POPC/POPG liposome gels by PLA_2 in the presence of BSA was five times higher than that in the absence of BSA (filled circles and open diamonds, Fig. 4), indicating addition of BSA efficiently collapses the immobilized liposomes as described for the free liposome suspensions mentioned in Section 3.1. Moreover, similar to the results in Fig. 2, the highest fluorescent leakage by PLA_2-catalyzed membrane leakage was observed on POPG liposome gels, and the lowest was on EPC/cholesterol liposome gels (Fig. 4). However, the fluorescent leakage behavior was different for PLA_2-catalyzed hydrolysis EPC/EPE liposomes in suspensions and immobilized gel beads. This can be explained by a small amount of EPC/EPE liposomes may covalently bind to the gel beads [16]. Therefore, the EPC/EPE liposomal bilayer packing became too compact to favor PLA_2-hydrolytic action, hence reducing the fluorescent leakage from the immobilized EPC/EPE liposomes such that it is lower than that in free liposome suspensions.

3.3. Stability of free liposome suspensions and liposome gels upon storage

In order to compare which kind liposome is better for efficient detection of fluorescent leakage due to PLA_2-catalyzed membrane leakage in free liposome suspensions and gel-bead-immobilized liposomes, we monitored their stability upon storage. Stability of the immobilized liposomes was excellent with a loss of 3–4% of the entrapped calcein after 60 days storage at 4 °C, except in POPG liposome gels where it was 15% (Fig. 5). In contrast, the free liposomes had poor stability with 9–25% of the entrapped calcein leaking after 60 days storage at 4 °C (Fig. 5). We have previously demonstrated that the avidin–biotin-immobilized liposomes have excellent stabilities for long-term use [12], which is the main advantage of the immobilized liposomes compared to free liposome suspensions. In a contrast to free-form liposomes, which were largely aggregated and precipitated upon storage, the liposomes immobilized in gel beads are protected from such aggregation by the gel matrix. The poorest stability was found in POPG liposomes of both free suspensions and immobilized in gel beads. Therefore, POPG is not suitable for measurement of fluorescence leakage even though it can cause the largest fluorescence leakage response due to PLA_2 hydrolytic action. POPC/POPG liposome gel should be chosen for further study because of its good stability and highly efficient fluorescence leakage catalyzed by enzymatic reaction.

3.4. PCB detection

Based on the above results, calcein-entrapped POPC/POPG liposome gels can be selected for efficient PLA_2-catalyzed membrane leakage measurement by offline fluorescent detection. The fluorescent intensity from the immobilized POPC/POPG liposomes hydrolyzed by PLA_2 increased with increasing PLA_2 concentration and showed a clear correlation (data not shown), indicating that the PLA_2-causing dye leakage from the liposomes could be developed as a molecular sensing system to detect PCBs. The principle schematics of the detection system were developed in our

previous study [14]. Briefly, after competitive immunoreactions between the analyte and conjugate of analyte-PLA_2 in the first column containing immobilized anti-PCB antibodies, the unbound PLA_2 conjugates were eluted. Then, the eluate from the first column is passed through to the second column containing fluorescent dye-entrapped gel-bead-immobilized liposomes. The eluted PLA_2 conjugates hydrolyze phospholipids in the gel-bead-immobilized liposomes on the gel support resulting in dye release. Accordingly, as PCB concentration increases, the amount of unbound PLA_2 conjugates increases resulting in greater fluorescent dye release.

As in our previous report, KC-500 was selected for PCB detection because the specificity of monoclonal mouse anti-PCBs to Aroclor 1254 (KC500, containing among other ~52% of pentachlorobiphenyls and ~1% of trichlorobiphenyls) is 100%, but 40% to Aroclor 1242 (KC300, containing among other 10% of pentachlorobiphenyls and 45% of trichlorobiphenyls). On the other hand, since the competitor should resemble the analyte as closely as possible [24], 3,4-dichloroaniline, which has a half domain of the PCB molecule, was selected as the competitor to mimic the moiety of PCBs. The activity of the PLA_2 conjugate was about 70% of the native PLA_2 (data not shown).

In our previous study, 0.01–10 μg/mL of PCBs was measurable on the two-column detection system [13]. Furthermore, the detection limit was improved to 0.5 ng/mL by increasing the fluorescent signal from the liposome column using negatively charged liposomes of EPC/EPG (7:3) [14] instead of neutral liposomes of EPC [13] for PLA_2-conjugate catalyzed membrane leakage. A clear correlation between the calcein release and the concentration of PCBs was also observed using anti-PCB monoclonal IgG immobilized column in tandem with the POPC/POPG fluorescent liposome column in the presence of BSA (Fig. 6). As shown in Fig. 6, the calcein release from the liposomes caused by the PLA_2 conjugate that eluted from the first column increased with increasing PCB concentration up to 10 ng/mL. There was no fluorescence increase when PCBs alone were applied to the columns, similar to our previous report [13]. This indicated that PCBs themselves did not cause dye release in the tested concentration range. Moreover, PLA_2 did not interact with the immobilized antibodies but competitive binding between free PCBs and hapten on the PLA_2 conjugate in the first column occurred successfully. Concentrations of PCB samples as low as 0.1 ng/mL were measurable using this system. Compared with the neutral liposomes gels in the absence of BSA (Fig. 6, open circles), the fluorescent signal from the anionic immobilized liposomes hydrolyzed by PLA_2 conjugate was significantly higher and the detection limit was 100 times lower. It should be noted that the amount of liposome gels used in this study is 10 times lower that that used in our previous studies [13,14] since BSA was added for efficient removal of the reaction products, resulting in rapid collapse of the liposomes.

In addition to the detection sensitivity, PCBs could be detected reproducibly using this tandem column system.

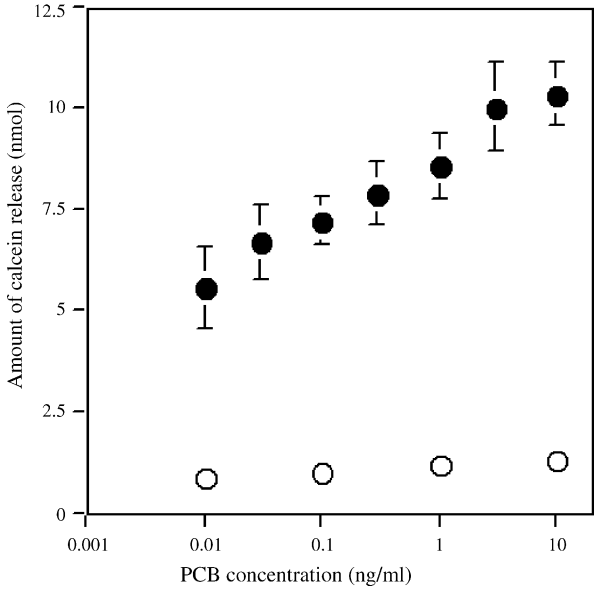

Fig. 6. Correlation between PCBs concentration and amount of released calcein using an anti-PCBs monoclonal IgG column in tandem with a 0.1 μmol POPC/POPG liposome column (●) in the presence of BSA and 1 μmol EPC liposome column (○) in the absence of BSA. All measurements were done with 1 μg/mL of 3,4-dichloroaniline–PLA_2 conjugate. The data in the graph are the averages of triplicate measurements.

The coefficients of variation in all measurement points were within 1–10% (Fig. 6). It takes 15 min for the first elution from the antibody column and 5 min for the second elution on the liposome fluorescent column. However, application of centrifugation or air pressure would bring about a reduction in time in the second column elution. Further detections of PCBs in environmental water and soil using this method are being carried out in our laboratory. For preliminary experiments, we found that over 20% of filtrated environmental water caused non-enzymatic dye leakage. Extraction from soil using ethanol disturbed the PLA_2 hydrolytic reaction, therefore, further clean up process of soil extraction will be needed for high sensitivity detection of PCBs. Moreover, when the antibody and liposome gel beads are packed on microtiter plates (e.g., a 96-well microtiter plate), this system should have potential use for high-throughput analysis (in progress in our laboratory).

4. Concluding remarks

The fluorescent leakage from the gel-bead-immobilized liposomes by PLA_2 hydrolytic actions significantly increased with increasing BSA concentration. The larger PLA_2-catalyzed membrane leakage was found on the immobilized anionic liposomes, POPC/POPG in the presence of BSA. The entrapped calcein was mostly retained in the immobilized liposomes until the liposomal membranes were hydrolyzed by a large amount of PLA_2 conjugate. Furthermore, the anionic liposome column could improve the detection of

PCBs to 0.1 ng/mL using the immunoassay combined with the immobilized liposome fluorescent assay system. This system is simple, sensitive, less time-consuming and less expensive than existing methods, and has the potential to be used as universal sensitization cartridges to determine other analytes.

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