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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