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A dimyristoyl phosphatidylcholine/polydiacetylene biomimetic assembly for the selective screening of progesterone

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ABSTRACT

We have reported a hybrid polymer composite, consisting of a supramolecular assembly of polydiacetylene (PDA) and phospholipid, that undergoes chromatic and fluorogenic changes after specific interactions with progesterone, the pregnancy hormone. Progesterone was selectively detected among 10 steroids by the mixed polymer of 1,2-dimyristoyl-sn-glycero-3-phosphocholine and PDA at an optimized ratio of 1:9. The triggering effect of progesterone on the membrane mimetic system were investigated using UV–vis, fluorescence, circular dichroism spectroscopy, Raman scattering, dynamic light scattering, transmission electron microscopy, and computational method. These results offer insight into self-assembly disruption and membrane targeting antibiotics as well as PDA-based sensing technology.

Introduction

Polydiacetylene (PDA) is a conjugated polymer in which closely packed diacetylene monomers form alternating ene-yne polymer chains, via 1,4 addition reactions, upon UV or γ irradiation [1]. The resulting supramolecule has a deep blue color and undergoes blue-to-red color and fluorescence transition in response to external stimuli, such as pH, temperature, solvent, mechanical stress, molecular recognition, and interfacial catalysis [2,3]. With their unique properties, PDA materials have proven to be effective sensing platforms; thus, they have attracted attention in the areas of pharmaceutical, industrial, and diagnostic applications [4,5]. Since the cellular membrane surface is heavily functionalized with recognition molecules serving as "antenna", integrating molecular recognition and signal transduction, modified PDA vesicles have been developed as a responsive nano-polymer for the detection of influenza virus [6], cholera toxin [7], and *Escherichia coli* [8].

In particular, PDA assemblies incorporating phospholipids have been used to construct artificial membrane systems, and phospholipid/PDA vesicles can be utilized as biosensors for antimicrobial peptides such as melittin, magainin, and alamethicin [9,10]. The colorimetric responses are closely related to biological association of the peptides with the phospholipid moieties within the membrane bilayer. The representative antimicrobial peptide, melittin is now commercially available with 1140 USD/mg in Sigma–Aldrich. Previous studies have demonstrated that steroid compounds interact with biomembranes through phospholipids [11]. In the present study, the membrane-mimetic supramolecule was systematically investigated for the membrane perturbation effect of steroids, including cholesterol, the major component of biomembranes.

Steroids are lipophilic compounds with four fused rings (three six-member cyclohexane rings and one five-member cyclopentane ring) that can intercalate into the bilayer of cellular membranes. Thereby, they can potentially modulate membrane fluidity or alter the role of membrane proteins during cellular activity. Specifically, *Helicobacter pylori*, a pathogen causing gastritc and duodenal diseases, also incorporates steroidal compounds into the cell membranes of via the mediation of phospholipids [12]. Progesterone, secreted mainly by the corpus luteum, is a steroid hormone and the preferred biomarker for the diagnosis of early pregnancy [13]. In addition, progesterone has various therapeutic uses at high

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concentrations (800 mg/day) for hormone replacement therapy, and treatment of osteoporosis and cysts [14–16]. Current techniques used to monitor progesterone levels include gas chromatography/liquid chromatography–mass spectrometry (GC/LC–MS), surface plasmon resonance (SPR), total internal reflection fluorescence (TIRF), and enzyme immunoassay (EIA) [17]. However, these methods require expensive equipment and time-consuming sample pretreatments, as well as trained operators. Accordingly, development of a rapid, low-cost, and simple method for the detection of progesterone remains a challenge.

In the present study, we evaluated the application of a biomimetic phospholipid/PDA supramolecule to study the interaction between progesterone and membrane lipids. Although the effect of cholesterol on lipid bilayers, and its applied research have been studied extensively [18–20], the potential role of progesterone has not been investigated. The selective colorimetric and fluorescent responsiveness of dimyristoyl phosphatidylcholine (DMPC)/PDA in the presence of progesterone was analyzed using UV–vis, fluorescence, and CD spectroscopy, Raman scattering, dynamic light scattering (DLS), transmission electron microscopy (TEM), and computational method. Furthermore, results showing the time dependence of the colorimetric response, the effects of progesterone concentration, and the optimized proportion of DMPC embedded in the PDA matrix are presented and discussed. The DMPC/PDA assembly can be used as a model system to study progesterone-membrane interactions and the related membrane-associated events. The investigation may provide the potential to develop new progesterone based medicines.

Experimental

Materials

10,12-Pentacosadiynoic acid (PCDA) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, Mo, USA). α-Cyclodextrin (α-CD) was obtained from the Microbial Carbohydrate Resource Bank (MCRB) at Konkuk University, Korea. 1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC), 1,2-dimyristoyl-sn-glycero-3-phosphorylglycerol sodium salt (DMPG), and 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid sodium salt (DMPA) were purchased from Avanti Polar Lipids. Estradiol, progesterone, cholesterol, 5α-cholestane, cortisone, prednisone, cholic acid, and sodium deoxycholate were obtained from Sigma–Aldrich Chemical Co. (St. Louis, Mo, USA). Testosterone and ergosterol were purchased from Tokyo Chemical Industry Co., Ltd.

Preparation of phospholipids/PDA supramolecules

To prepare the artificial membrane system, a mixture of PCDA (90%) and phospholipids (DMPC, DMPG, and DMPA; 10%) was dissolved in chloroform, and then dried under nitrogen gas. To optimize the DMPC portion within the PDA array, DMPC composition was also varied to comprise 5, 10, 20, or 40% of the total lipid content. To the thin lipid film, a HEPES buffer solution (5 mM, pH 8.0) was added to yield a concentration of 1 mM. The samples were then heated at 80 °C for 15 min and probe-sonicated for 12.5 min using a Sonics VC-505 instrument at 40% power [21]. The resulting solution was filtered through a 0.8 μm filter (Advantec, DISMIC-25CS, Toyo Roshi Kaisha Ltd.), and the milky

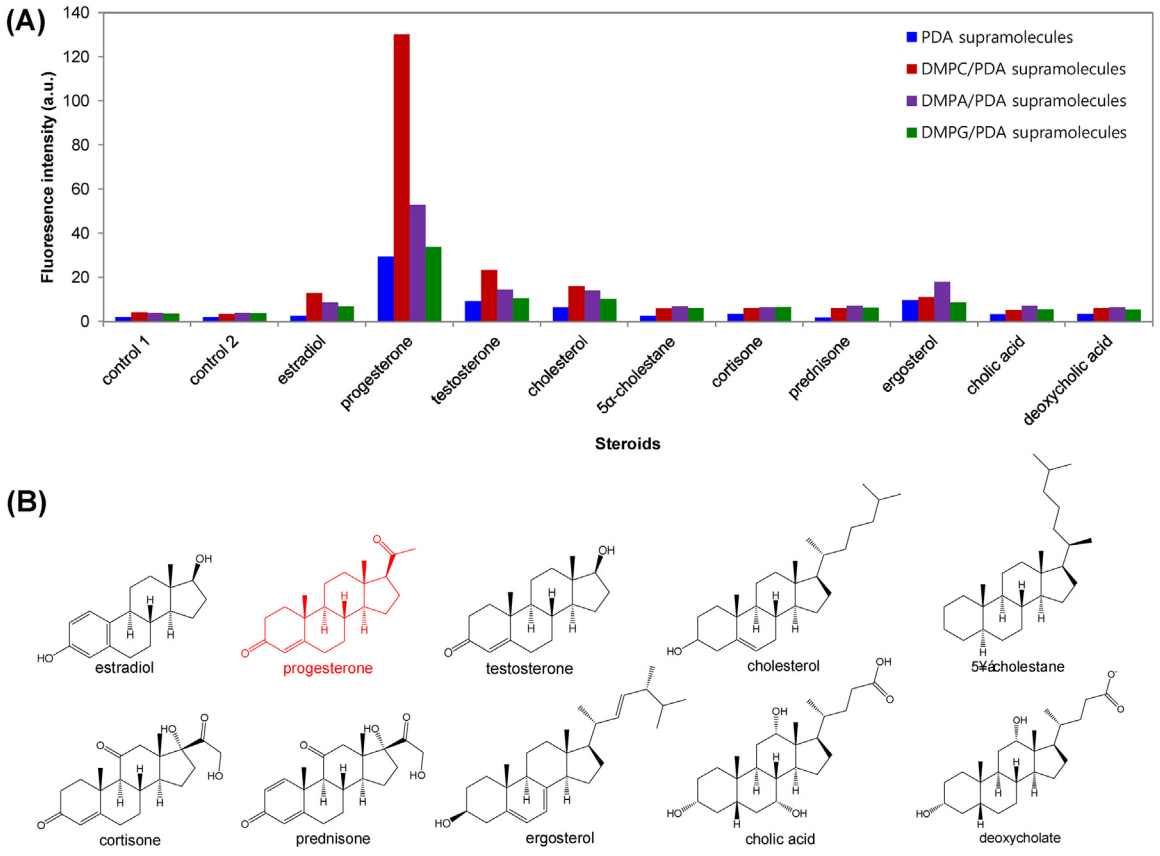

Fig. 1. (A) Screening of 10 steroids for the fluorescence response of phospholipid/PDA supramolecules. Phospholipids comprised 10% of the total lipid concentration, and the total steroid concentration was 1 mg/mL. (B) Chemical structures of the 10 steroid compounds used. The stock solution of steroids was prepared in DMSO or EtOH, and aliquots of steroid solutions were loaded onto the supramolecules. Control 1: DMSO, control 2: EtOH.

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