Solid-State Sol–Gel Biosensors

Immunoglobulin–Polydiacetylene Sol–Gel Nanocomposites as Solid-State Chromatic Biosensors

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Conjugated polydiacetylenes (PDAs) derived from the polymerization of 1,3-diacetylenic acid derivatives have attracted great interest in the domain of sensors by virtue of the intense bichromatic properties of their conjugated ene-yne backbones and the ease with which molecular recognition platforms can be coupled with PDA-mediated signal transduction to create colorimetric sensors.[1] Thus, bichromic self-assembled PDA structures, such as monolayers, bilayers, and liposomes displaying synthetic and biological receptors, undergo dramatic color changes corresponding to a transition from a highly conjugated "blue form" of the PDA to a less-conjugated "red form" in response to the molecular recognition event, without the requirement for any co-reagents.[1] Indeed, PDA-based systems have proved to be versatile and sensitive sensors for a wide range of analytes including cations, arenes, polyols, microbial toxins, oligopeptides, proteins, antibodies, and even bacteria and viruses.[2] However, most studies have utilized solution-phase sensing, which has necessarily limited the wider application of PDAs, as many sensor and array devices require solid-state materials which are stable, reusable, and amenable to large-scale fabrication.[3] Efforts have been made to achieve this goal by depositing PDA monolayers and bilayers onto glass, quartz, silicon, and polystyrene substrates as well as by entrapping them within silica sol–gels,[2h,j,l,4] but the resultant materials have been compromised by limited stability and processability, or long response times and reduced sensitivity to analytes, and as a result the need for a generic, stable, and fast-response solid-state PDA sensor platform remains.

Herein it is shown that highly responsive solid-state chromatic sensors can be produced by encapsulating PDA–phospholipid vesicles modified with immunoglobulin (IgG) in hybrid sol–gel materials composed of silica and functionalized siloxanes. The materials produced are rugged and processable and can be fabricated as monoliths, thick films, and microarrays, and furnish colorimetric biosensors that are sensitive and show short response times.

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In view of the efficient encapsulation of sensitive biological materials in functionalized, hydrophilic siloxane sol–gels,[3a–c,5] we explored whether such polymers could be used for the entrapment of liposomes composed of PDA and biological materials, and whether the trapped conjugates would efficiently undergo the required blue-to-red color transitions upon challenge with the corresponding analytes. For this purpose, IgGs including anti-human α-fetoprotein, anti-*E. coli* β-galactosidase, anti-bovine serum albumin (anti-BSA), and anti-yeast alkaline phosphatase were conjugated (reduced IgGs were coupled using *N*-sulfosuccinimidyl-4-(maleimidomethyl)cyclohexane-1-carboxylate) to liposomes composed of $21{:}2\Delta^{2a,4a}$, $23{:}2\Delta^{2a,4a}$, $22{:}2\Delta^{5a,7a}$, $23{:}2\Delta^{5a,7a}$, $24{:}2\Delta^{5a,7a}$, and $25{:}2\Delta^{2a,4a}$ acid 2′-aminoethylamides and various glyceryl, ethanolamine, and choline phospholipids (Scheme 1). The conjugates were polymerized to furnish blue-form IgG-PDA-phospholipid vesicles, and these were encapsulated in sol–gels composed of silica, 3-(3′-glyceroxy-2′-hydroxyprop-1′-oxy)propylsiloxane (GPPS), *N*,*N*-dimethyl-3-aminopropylsiloxane (DAPS), *N*,*N*′-bis(3-siloxypropyl)ethylenediamine (BSPE), and poly(vinyl alcohol) (PVA; Scheme 1). Silica was chosen for its mechanical rigidity and high encapsulation efficiency, while the functional siloxanes and PVA were selected for their high biocompatibility together with their ability to form highly mesoporous sol–gel polymer frameworks.[5,6]

Encapsulation of the IgG-PDA-phospholipid vesicles provided transparent blue, mesoporous composites which were deposited as thick films onto cellulose, nylon, polycarbonate, alumina, silica, and glass (Figure 1). Only slight color changes ($<7\,\%$) were observed upon sol–gel entrapment of the blue-form vesicles (Figure 2a), which indicates that encapsulation did not have a sufficient effect on the vesicle structure so as to trigger switching of the PDA conformation and that the liposomes were largely encapsulated in their native state. More importantly, exposure of the IgG-PDA sol–gels to antigens (α-fetoprotein, β-galactosidase, BSA, and phosphatase) resulted in the blue ($\lambda_{max}=620$–670 nm) to red ($\lambda_{max}=510$–560 nm) color changes characteristic of IgG-PDA-phospholipid solutions (Figure 1 and 2a), which demonstrates that both the biomolecular recognition of IgG and structural transition functions of PDA were preserved upon entrapment. In contrast, IgG-free PDA solutions and sol–gels underwent negligible ($<9\,\%$) color changes when exposed to antigens.

Libraries of 90–270 compositions were prepared for each immunosensor by varying the type and amount of diacetylene, phospholipid, and sol–gel precursor. These mixtures were then screened for the rate and extent of colorimetric response (CR), where CR is defined as $CR=\frac{(B_0-B_1)}{B_0}$, in which $B=\frac{A_{Blue}}{(A_{Blue}+A_{Red})}$, B_0 and B_1 are the pre- and postexposure values, and $A_{Blue/Red}$ is the blue/red absorbance.[2m] It should be noted that a CR value of 0.10–0.15 (namely, 10–15 % of the maximum possible colorimetric response) was usually readily discernible by the naked eye. Screenings showed that liposomes containing 40–60, 10–30, and 10 mol % of tricosa-2,4-diynoic acid ($23{:}2\Delta^{2a,4a}$) 2′-aminoethylamide, dimyristoylphosphocholine (DMPC), and linoleoylpalmitoylphosphati-

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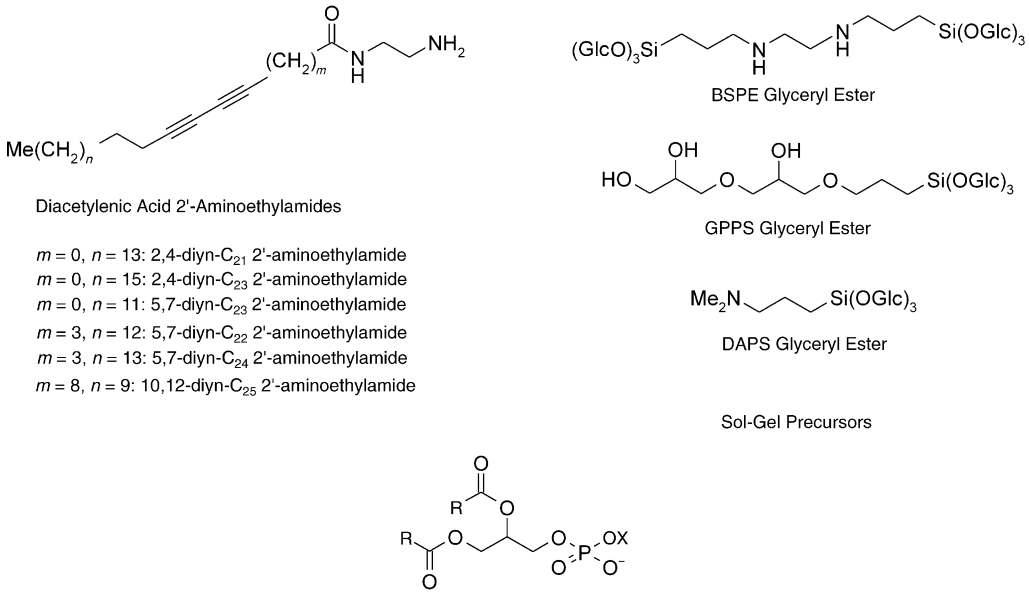

Scheme 1. Structures of lipids and sol–gel precursors used for forming IgG–PDA sol–gels.

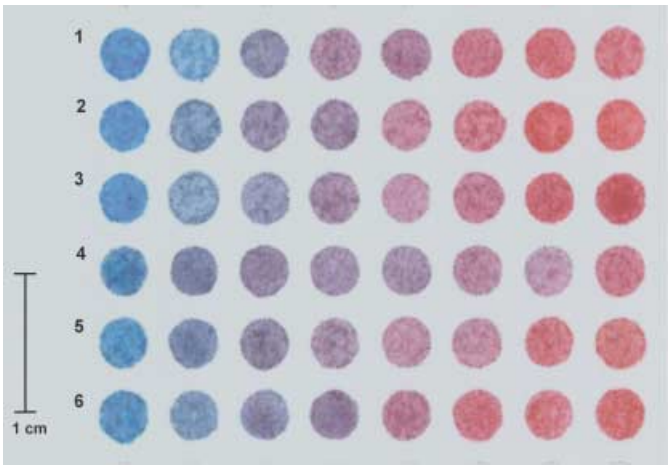

Figure 1. Anti-*E. coli* β-galactosidase IgG-PDA-liposome sol–gel spot array. Lanes 1–6 correspond to composites incorporating PDAs derived from 2,4-diyn-C_{21}, 2,4-diyn-C_{23}, 5,7-diyn-C_{22}, 5,7-diyn-C_{23}, 5,7-diyn-C_{24}, and 10,12-diyn-C_{25} acids, respectively. Vertical lanes (left to right) correspond to antigen concentrations of 0, 10, 20, 30, 40, 50, 70, and 100 ng mL^{-1}. Liposomes were formed from 60:30:10 mol% of diacetylenic 2′-aminoethylamide:DMPC:LPPE and were loaded with about 0.01 mg of IgG per mg of lipid. Liposomes were polymerized (UV, 254 nm, 1–2 min), mixed with sol–gel precursor, then spotted (ca. 100 μL) onto cellulose. The array was aged in a closed container at 4 °C for 40 h, then washed with phosphate buffer (50 mM, pH 7), before exposure to antigen (100 μL per spot) at 20 °C for 10 min. The sol–gels consisted of 40:10:10:30:10 (% w/w) silica-GPPS-DAPS-BSPE-PVA and held 0.78 mg of vesicles and 6.5 μg of IgG per spot.

dylethanolamine (LPPE), respectively, provided the most responsive and stable conjugates, and that a mixture of silica (30–40% w/w), GPPS (20–30% w/w), DAPS (5% w/w), BSPE (10–35% w/w), and PVA (10–15% w/w) gave the best balance of colorimetric response, stability, and mechanical properties for the final composites. The composites provided CR rates that were about two to threefold lower than those of the liposome solutions, which led to a CR value of 0.5 after about 1.1–1.6 min versus 0.5–0.6 min for the liposome solutions, and the maximal responses were about 68–76% compared to 89–92% (Figure 2b and 2c). Thus, encapsulation decreased the response rate as well as the total response, as expected from diffusional limitations and the steric restrictions which inevitably result from entrapment in sol–gel matrices.[5]

Despite encapsulation in a polymer framework, the recognition–transduction responses of the native IgG-PDA-phospholipid liposomes were preserved to a large degree in the composites, and antigens were readily detected down to 200–400 pg mL^{-1} with a corresponding response saturation at 1.0–1.4 ng mL^{-1}. This result compares favorably with the detection level of about 50–100 pg mL^{-1} and response saturation at 0.8–1.0 ng mL^{-1} that were observed for vesicle solutions (Figure 2d). Importantly, the IgG-PDA sol–gel materials showed little cross-reactivity, with CR values of less than 0.1 generally being observed upon challenge with proteins other than the corresponding antigens (Figure 2b and 2d). Also, interestingly, the performances of composites

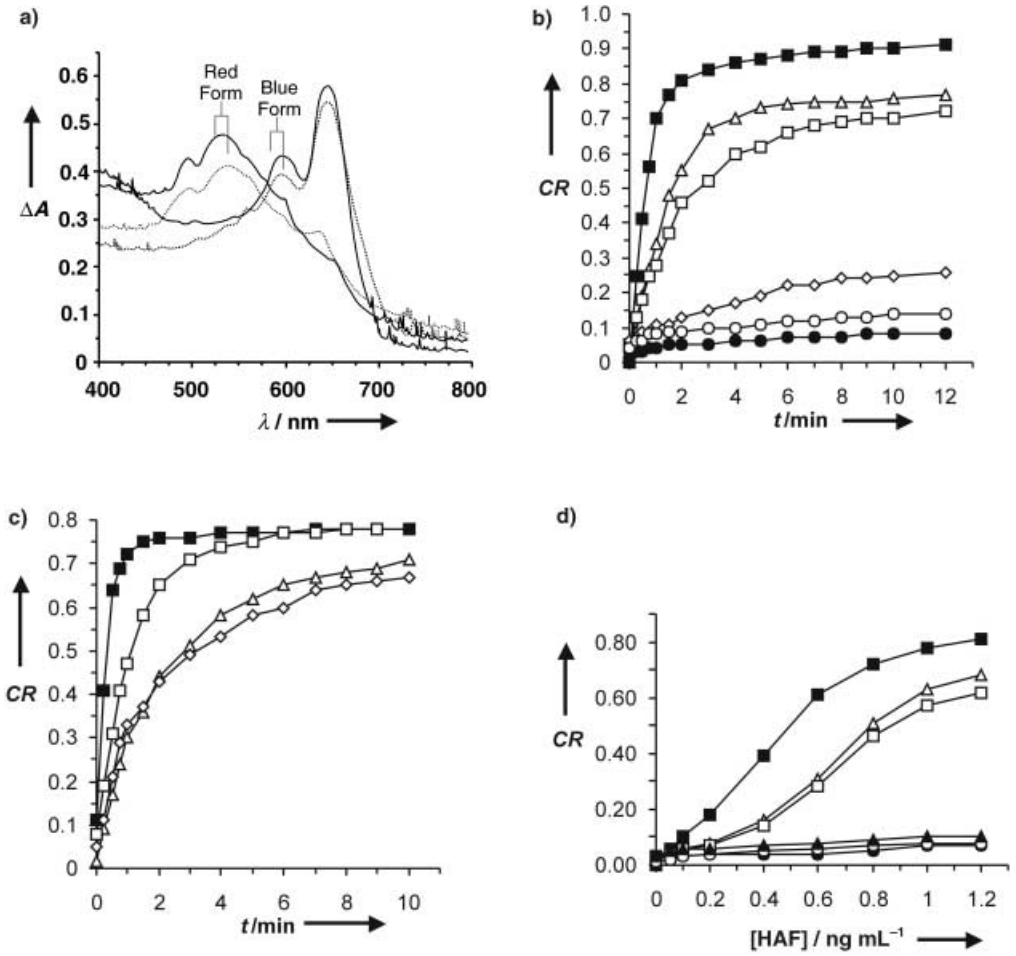

Figure 2. a) UV/Vis spectra of blue- and red-form IgG-poly(2,4-diyn-C_{23}) liposomes: aqueous (upper, solid lines) and sol–gel encapsulated (lower, broken lines). b) Colorimetric responses (*CR*) of aqueous and encapsulated anti-human α-fetoprotein IgG-poly(2,4-diyn-C_{23}) liposomes: ■: aqueous liposomes, exposed to α-fetoprotein at 1.5 ng mL^{-1}; liposomes in aged (closed container, 40 h, 4 °C); △: wet 35:20:5:30:10 (% w/w) silica-GPPS-DAPS-BSPE-PVA sol–gel, exposed to α-fetoprotein at 1.5 ng mL^{-1}; □: liposomes in sol–gels aged and dried (open container, 40 h, 4 °C), exposed to α-fetoprotein at 1.5 ng mL^{-1}; ◇: liposomes in aged, wet pure silica sol–gel, exposed to α-fetoprotein at 1.5 ng mL^{-1}; ○: liposomes in aged, wet 35:20:5:30:10 (% w/w) silica-GPPS-DAPS-BSPE-PVA sol–gel exposed to 15 ng mL^{-1} of BSA; ●: control PDA sol–gel without IgG. c) Colorimetric responses of IgG-poly(2,4-diyn-C_{23}) liposomes encapsulated in aged (closed container, 40 h, 4 °C), wet 35:20:5:30:10 (% w/w) silica-GPPS-DAPS-BSPE-PVA sol–gels: ■: anti-α-fetoprotein IgG-PDA liposomes; □: anti-*E. coli* β-galactosidase IgG-PDA liposomes; △: anti-BSA IgG-PDA liposomes; ◇: anti-alkaline phosphatase IgG-PDA liposomes. The materials were incubated with the corresponding antigens at 10 ng mL^{-1}. d) Colorimetric response of IgG-poly(2,4-diyn-C_{23}) liposomes to α-fetoprotein concentration, after 10 min incubation: ■: aqueous anti-human α-fetoprotein IgG-PDA liposomes; △: aqueous anti-human α-fetoprotein IgG-PDA liposomes in aged (closed container, 40 h, 4 °C), wet 35:20:5:30:10 (% w/w) silica-GPPS-DAPS-BSPE-PVA sol–gel; □: aqueous anti-human α-fetoprotein IgG-PDA liposomes in aged and dried (open container, 40 h, 4 °C) sol–gel; ▲: anti-BSA IgG-PDA in aged, wet 35:20:5:30:10 (% w/w) silica-GPPS-DAPS-BSPE-PVA sol–gel exposed to α-fetoprotein; ○: anti-β-galactosidase IgG-PDA in aged, wet 35:20:5:30:10 (% w/w) silica-GPPS-DAPS-BSPE-PVA sol–gel exposed to α-fetoprotein; ●: control PDA-sol–gel without IgG. Abbreviations: HAF, human α-fetoprotein.

that were aged and dried (at 4 °C, 40 h, ca. 9–14 % w/w water content) were not greatly diminished over those of the aged, wet composites (at 4 °C, 40 h, ca. 35–40 % w/w water content; Figure 2b and 2d). This result indicates the protective influence of the functional siloxane sol–gel network in providing an open hydrophilic framework that enables the efficient ingress and recognition of antigens at the IgG-PDA liposome surface, and also allows for the rapid and extensive structural transition of the PDA polymer to the red form. In contrast, entrapment of the vesicles in aged, wet pure silica sol–gels (aged at 4 °C, 40 h, ca. 40 % w/w water content) resulted in materials which were considerably less responsive, and whose maximal CR values were in the region of 0.2–0.35 (Figure 2b), thus underscoring the importance of the physicochemistry of the sol–gel matrix. It should also be noted that the hybrid composites furnished semi-rigid or flexible solids which were dimensionally stable and mechanically robust and could be cast as monoliths or applied as thick-film coatings on cellulose, nylon, polycarbonate, silica, alumina, and glass.

The successful fabrication of reagentless solid-state IgG-PDA sol–gel biosensors, and their ease of production, together with the availability of novel PDAs,[1c] facile interfacing of receptors with PDAs,[1,2] and recent advances

in silica–PDA composites[7] and hybrid and templated sol–gel polymers,[8] offers a potentially simple and generic route to solid-state colorimetric sensor and microarray platforms.

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