Short communication

Glass-supported lipid/polydiacetylene films for colour sensing of membrane-active compounds

Roman Volinsky a, Sofiya Kolusheva a, Tania Sheynis a, Mark Kliger b, Raz Jelinek a,∗

a Department of Chemistry and Ilse Katz Center for Nanotechnology, Ben Gurion University, Beer Sheva 84105, Israel
b Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI 48109, USA

Received 6 September 2006; received in revised form 9 November 2006; accepted 20 December 2006

Abstract

Glass-supported biomimetic lipid/polydiacetylene films were employed for colourimetric detection and analysis of amphiphilic and membrane-active molecules. The sensor films comprise lipid monolayers that constitute a biomimetic membrane platform, interspersed within polydiacetylene domains that function as the colour reporter. The optical detection scheme is based on visible blue–red transitions of polydiacetylene, induced by amphiphilic analytes interacting with the film. The colour transitions of the lipid/polydiacetylene films can be either detected by the naked eye, recorded spectroscopically, or registered through digital image analysis using conventional scanning devices. Digital image analysis, in particular, allows quantification of the colourimetric transformations. Detection threshold of micromolar concentration of a membrane-active cytolytic peptide is demonstrated.

Keywords: Langmuir–Schaefer films; Colour biosensors; Polydiacetylene; Chromatic polymers; Phospholipid films; Membrane peptides

1. Introduction

Two-dimensional assemblies such as monolayers and films deposited on solid substrates have been used extensively in varied sensor applications, such as surface plasmon resonance, quartz crystal microbalance, and others (Cush et al., 1993). Films that undergo colour changes in response to analytes or to biological processes are particularly attractive as simple sensing devices. Here, we describe the construction of mixed lipid/polydiacetylene (PDA) chromatic films deposited on glass substrates, and the use of such films for rapid colourimetric detection of amphiphilic and membrane-active molecules.

The sensor films comprise lipid monolayers as a biomimetic surface for docking and insertion of lipophilic and membrane-active species, and PDA as a module responsible for generation of colour signals. PDA exhibits unique chromatic properties. Following polymerization, PDA appears intense blue to the naked eye due to its conjugated ene-yne framework (Day and Ringsdorf, 1978). Furthermore, PDA systems undergo dramatic blue–red transformations induced by varied analytes and chemical processes, such as temperature elevation, ions in aqueous solutions, and increase in surface pressure (Rubner, 1986). The visible colour changes of PDA correspond to stress-induced structural transition of the conjugated polymer backbone, brought about by molecular perturbations of the pendant sidechains of the polymer (Tokura et al., 1986; Tomioka et al., 1989).

The incorporation of lipid molecules into PDA matrices has opened new avenues for biological sensing applications. Previous studies have shown that mixed vesicles comprising lipid molecules and PDA can be employed for studying membrane interactions of biological analytes (Evrard et al., 2001; Jelinek and Kolusheva, 2001; Kolusheva et al., 2000a). The chromatic transformations of PDA in such systems are induced by molecules that primarily interact with the lipid moieties, affecting the transformations of the polymer through the lipid/polymer interfaces (Kolusheva et al., 2001, 2000b). Specifically, previous studies have reported that molecular reorganization of the lipid bilayers in lipid/PDA systems following interactions of biological analytes induce the surface perturbations within the PDA moieties leading to the colourimetric transformations.
Lipid/PDA films condensed at the air/water interface (Langmuir films) were also utilized for studying membrane phenomena, such as lipid interactions of membrane-associated peptides (Volinsky et al., 2004, 2006). Practical applications of Langmuir films are limited, however, primarily because of the highly sensitive and technically demanding handling of films at the air/water interface. Furthermore, measuring colour transitions of Langmuir films is carried out by dedicated spectroscopy techniques such as UV–vis spectroscopy which require relatively large film coverage and substantial quantities of tested samples.

Here, we report on the usage of glass-supported lipid/PDA films for colourimetric sensing of biological amphiphilic and membrane-active species. In particular, we show that the colour transformations occurring within the solid-supported films can be measured and quantified through digital colour analysis of the scanned film surface using conventional scanners. This feature opens the way for using lipid/PDA films as colour sensors of varied amphiphilic and membrane-active species. The sensor films are particularly amenable to high throughput screening applications.

2. Materials and methods

2.1. Materials

The monomeric diacetylene 10,12-tricosadiynoic acid (TRCDA) was purchased from GFS Chemicals (Powell, OH). The compound was further purified by dissolving the monomer powder in chloroform, filtering through a 0.45 μm Nylon filter, and evaporating the solvent. Dimyristoylphosphatidylycholine (DMPC) and polymyxin-B (PMB) were purchased from Sigma.

DMPC and TRCDA were dissolved in chloroform and mixed at a total concentration of 2 mM (mole ratio was 9:1 between diacetylene and phospholipids). The water subphase used in the Langmuir trough was doubly purified by a Barnstead D7382 water purification system, producing distilled water having 18.3 MΩ resistance.

2.2. Film preparation

Glass slides were dipped in a cleaning (piranha) solution consisting of 70 mL of H2SO4 and 30 mL of H2O2 for 30 min at 70 °C, followed by sonication in the same solution for 10 min. Following the cleaning, the glass was rinsed thoroughly with pure water and dried at 70 °C. Prior to forming the self-assembled lipid/PDA films on the surface, the glass was immersed in a solution of 300 μL of octadecyltrithoxysilane (OTS) in 100 mL cyclohexane for 12 h (Kessel, 1991). Glass slides were then rinsed with cyclohexane to remove non-covalently bound OTS molecules.

All TRCDA/DMPC films were prepared in a computerized Langmuir trough manufactured by NIMA (model 622/D1 (7 cm × 50 cm), Nima Technology Ltd., Coventry, U.K.). The experiments were carried out at 22 °C. The surface pressure was monitored using a 1-cm-wide filter paper as a Wilhelmy plate. For each experiment, 35 μL of the lipid/diacetylene solution was spread on the water subphase (pH 6.3). Compression started after solvent evaporation (15 min) and was carried out at a constant barrier speed of 8 cm²/min⁻¹. The films were compressed up to 15 mN/m and then allowed to equilibrate at constant surface pressure for 10 min. The films were transferred onto the modified glass slides by a horizontal touch method (Langmuir–Schaefer method) (Gaines, 1966; Tschamer and McConell, 1981) and then irradiated at 254 nm to polymerize the TRCDA.

2.3. Atomic force microscopy

AFM measurements were performed at ambient conditions using a ThermoMicroscopes CP Research Instrument AFM mounted on an active antivibration table. Microfabricated Si-oxide ultralavers (Thermomicro) with integrated pyramidal tips were used. The 512 pixel × 512 pixel images were taken in a tapping mode with a scan rate of 1 Hz.

2.4. Ultraviolet-visible (UV–vis) spectroscopy

UV–vis measurements of the phospholipid/PDA films transferred onto the OTS-modified glass were carried out on a Jasco V-550 spectrophotometer. A quantitative value for the extent of blue–red transition as obtained from the UV–vis spectra is given by the percentage colourimetric response (%CR), which is defined as: (Charych et al., 1993).

\[ \text{CR} = \frac{PB_0 - PB}{PB_0} \times 100\% \]

where \( PB = A_{\text{blue}}/(A_{\text{blue}} + A_{\text{red}}) \) is the absorbance at either the “blue” component in the UV–vis spectrum (peak at 640 nm) or the “red” component (550 nm). (Note: “blue” and “red” refer to the visual appearance of the material, not its actual absorbance.) \( PB_0 \) is the red/blue ratio of the control blue sample, while \( PB \) is the value obtained after interaction is complete (colour change induced).

2.5. Colour scanning and image analysis

Aliquots of the tested solutions were placed on the surfaces of the films and incubated at 37 °C for several minutes. The films were sealed in a Petri dish to eliminate evaporation. Following incubation the drops were removed and the dry films were scanned on an Epson 4990 Photo scanner to produce high-resolution RGB images. The films were placed in a special film adaptor and scanned in transmitted mode at optical resolution 2400 dpi and colour depth of 24 bit. Digital colourimetric analysis (DCA) was carried out by cropping the sample spots in the scanned images, and the colour-change values were calculated using MATLAB® mathematical software.

3. Results and discussion

3.1. Colour transitions in lipid/PDA films

The colourimetric sensor films we have constructed comprise lipids and PDA. As depicted in Fig. 1, the lipids and PDA
molecules form distinct domains within the film rather than dispersed homogeneously (Gaboriaud et al., 2001; Volinsky et al., 2002). The AFM image and height profile in Fig. 1B show that the PDA domains are organized in the typical trilayer structures (Lio et al., 1996; Putman et al., 1992) interspersed within the fluid DMPC monolayer. The aligned PDA structure facilitates electronic delocalization within the polymer network and the corresponding blue colour of the films (Berman et al., 1995; Sasaki et al., 2000).

The thrust of the sensing mechanism described in this work is the visible colour transformation of PDA. Fig. 2 shows the typical colour changes observed in a DMPC/PDA film following irradiation with ultraviolet (UV) light (254 nm). Extended UV irradiation induces a structural transition of the PDA backbone, gradually transforming the PDA from the blue phase to the red phase (Hofmann and Peltonen, 2001; Kuriyama et al., 1996) as visually shown in Fig. 2A. The blue–red transformations of the film are also manifested spectroscopically, as featured in Fig. 2B. As the colour of the film becomes redder, the spectra in Fig. 2B show the gradual decrease in the intensity of the peak at around 650 nm (the “blue” component in the electromagnetic spectrum) and increase of the signal at around 550 nm (corresponding to the red colour).

The UV–vis spectra in Fig. 2B facilitate quantitative evaluation of the blue–red transitions (%CR, broken line in Fig. 2C). However, a simpler and readily available method that lends itself for direct quantification of the colour transitions utilizes colour scanning of the film (using conventional desk-top scanners) and

performing digital colourimetric analysis (DCA) of the scanned images.

DCA utilizes the standard “red–green–blue” (RGB) model for digital representation of colour. This widely used model essentially translates a visible power spectrum (any colour signal) into three distinct values corresponding to the intensities of red (R), green (G), and blue (B) colour channels. These values are obtained according to the following formulas (Pratt, 2001):

\[
R = \int_{0}^{\infty} P(\lambda) \tau(\lambda) \, d\lambda,
\]

\[
G = \int_{0}^{\infty} P(\lambda) \gamma(\lambda) \, d\lambda,
\]

\[
B = \int_{0}^{\infty} P(\lambda) \beta(\lambda) \, d\lambda,
\]

where \(P(\lambda)\) is the generalized power spectrum (transmittance spectrum), and \(\tau(\lambda)\), \(\gamma(\lambda)\) and \(\beta(\lambda)\) are the colour matching functions corresponding to each primary colour, and defined by the International Commission of Illumination (CIE): \(\tau(\lambda)\) is the red matching function around 600 nm, \(\gamma(\lambda)\) is the green function around 550 nm, and \(\beta(\lambda)\) the blue function at 450 nm (Fairman et al., 1997). Accordingly, the relative intensity of a particular RGB component in a picture or scanned image can be defined as the chromaticity level. For example, the red chromaticity level \(r\) can be calculated as (Pratt, 2001):

\[
r = \frac{R}{R + G + B}
\]

For the PDA-based films discussed here, we define a quantitative parameter denoted the red chromaticity shift (RCS) that represents the extent of the blue–red transition in a defined surface area:

\[
RCS = \frac{r_{\text{sample}} - r_0}{r_{\text{max}} - r_0} \times 100\
\]

in which \(r_{\text{sample}}\) is the average red chromaticity level of all pixels in the scanned surface, \(r_0\) is the average red level calculated in a blank surface (blue initially prepared prior to treatment), and \(r_{\text{max}}\) is the average red chromaticity level of the maximal blue–red transition—a film surface in which the greatest extent of blue–red transition occurred (positive control). In essence, RCS is the normalized change of the red chromaticity level in an entire examined film surface.

The solid line in Fig. 2C represents the DCA results calculated according to the above method for the scanned film surfaces shown in Fig. 2A. The colourimetric data are depicted in percentage representation, in which 100% corresponds to the maximal blue–red transformed film (the reddest film surface in Fig. 2A). Fig. 2C also presents (broken line) the quantitative determination of blue–red transitions of the film surfaces, calculated from the intensity ratios of the blue and red UV–vis signals in the spectra presented in Fig. 2B (%CR, see Section 2). The close correlation apparent in Fig. 2C between the colourimetric curve calculated using DCA (solid line) and through UV–vis spectroscopy (broken line) confirms that the image analysis methodology is a feasible tool for quantitative determination of the colour transformations in the lipid/PDA sensor film system. Previous reports have also correlated colour image analysis with chemical phenomena affecting the chromatic transformations (Suzuki et al., 2002).

### 3.2. Biosensing features

The colour transformations of lipid/PDA films occur also in biological contexts, making the technology a useful and sensitive biosensor. Fig. 3 depicts the colourimetric transitions induced in a DMPC/PDA film onto which small drops (15 µL volume) of decreasing concentrations of the antibacterial toxin polymyxin-B (PMB) (Danner et al., 1989; Tsubery et al., 2000) were placed. Following deposition of the PMB drops, the film was kept at 37°C for 10 min followed by removal of the solutions with a filter paper. The scanned image in Fig. 3A clearly shows the appearance of visible red spots in the locations where the PMB drops were placed. The extent of blue–red transitions in the spot...
areas was determined by DCA, and the data are represented graphically in Fig. 3B. Essentially, the dose response curve in Fig. 3B shows quantitatively the higher abundance and greater intensities of the red pixels in the scanned area as the concentration of PMB increases in the tested samples. The detection threshold apparent in Fig. 3B is in the micromolar range.

4. Conclusions

This study presents a colourimetric biosensing platform, based on glass-supported lipid/polydiacetylene films, applied for detection and analysis of membrane-active species. The transduction mechanism in the films rely on the PDA moiety undergoing dramatic blue–red transformations induced by binding of amphiphilic molecules onto the film surface. The colourimetric transitions are both visible to the naked eye as well as quantified spectroscopically. Importantly, conventional scanning of the films combined with digital colour analysis (DCA) can be employed for direct quantification of the colourimetric transformations. DCA confers significant advantages to the lipid/PDA system for practical biosensing applications. In particular, the technique allows analysis of extremely small sample quantities and very low sensitivity threshold, pointing to possible high throughput screening applications. The lipid/PDA platform exhibits other advantages for potential biosensing solutions; the films are stable in ambient conditions, and they retain their structural and chromatic characteristics for long time periods. Film stability is most likely ascribed to the scaffolding effect of the rigid polymer matrix, preventing collapse of the interspersed lipid layers and degradation of the films.

Acknowledgement

R.J. is grateful to the Human Frontiers Science Foundation for generous financial support.

References