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# Colorimetric Biosensor Vesicles for Biotechnological Applications.

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## ABSTRACT

Supramolecular assemblies of vesicles composed of natural lipids embedded in a matrix of polydiacetylene are shown to undergo blue-to-red color changes related to specific interactions between the lipids or recognition elements and biomolecules. The colorimetric transitions observed in the vesicles are due to structural modifications within the conjugated PDA polymer backbone. This colorimetric assay could serve as a membrane-mimicking environment for detection of interfacial processes, such as peptide-membrane interactions, antibody-antigen binding, enzymatic catalysis, and detection of physiological ions. The lipid/PDA mixed vesicles are robust and can be readily applied to diagnosis of physiological molecules and rapid screening of biological and chemical libraries.

## INTRODUCTION

PDA-based vesicles have been previously shown to undergo blue-to-red color transitions induced by a variety of interfacial processes. The color transitions observed in the vesicles are believed to originate from structural changes at the conjugate PDA polymer backbone [1]. Previous studies have shown that biological processes leading to structural perturbations at the PDA vesicle interface, including enzymatic catalysis [2], pH- changes [3], or ligand-receptor recognition [4], also induce blue-to-red transitions occurring in the vesicle assemblies.
We have recently demonstrated that PDA vesicles incorporating a high percentage of lipid molecules [Figure 1] similarly undergo colorimetric transitions and could be used for studying biological and chemical processes [5-8]. In the following we demonstrate some applications of the system.

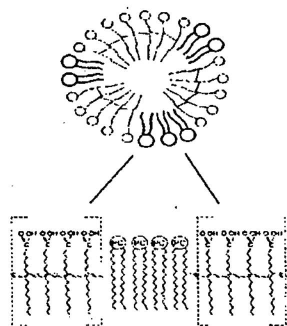

**Figure 1** Schematic representation of the lipid/PDA vesicles, indicating a part of the assembly of lipids and polydiacetylene. PC denotes the phosphatidylcholine headgroup.

## EXPERIMENTAL DETAIL

Mixed vesicles composed of physiological lipids and PDA were prepared using approximately 40% (mole ratio) lipids, such as dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG), and 60% polydiacetylene (PDA) lipids. Following sonication and polymerization, the lipids form organized assemblies that exhibit an intense blue color due to the conjugated backbone of the PDA polymer. The macrocyclic receptor homooxacalixarene triether [9] was inserted into the lipid moieties within DMPC/PDA following the polymerization step without disruption of the liposome organization or chromatic properties.

UV-vis measurements: samples were prepared by adding peptides to 0.4 mL vesicle solutions at 1 mM total lipid and 2 mM Tris. The pH in the solutions was 8.0 in all experiments. Examined peptides were dissolved in water, and the peptide-vesicle solutions were diluted to 1 mL prior to spectral acquisition. All measurements were carried out at 27 °C on a Hewlett-Packard 8452A diode-array spectrophotometer, using a 1cm optical path length cell.
Indolicidin ILPWKWPWWPWRR (CP10), and indolicidin analogue
ILKKWPWWPWRRK (CP11) have been chemically synthesised at the University of British Columbia.
A quantitative value for the extent of blue-to-red color transition is given by the *colorimetric response* (%CR), which is calculated from the visible absorbance spectra acquired for the vesicle solutions. The colorimetric response is defined:

$$\%CR=[(PB_0-Pb_I)/PB_0]*100$$

where $PB=A_{blue}/(A_{blue}+A_{red})$,

A is the absorbance at either the "blue" component in the UV-vis spectrum (640nm) or "red" component (500nm), $PB_0$ is the red/blue ratio of the control sample (before induction of color change), and $Pb_I$ is the value obtained for the vesicle solution after addition of the tested compound.

Fluorescence measurements: changes in tryptophan intrinsic emission were measured for 10μM peptide solution titrated with DMPG/DMPC/PDA vesicles incorporating the radical spin-probes 12-doxyl-stearic acid [12-DS] and 16-doxyl-stearic acid [16-DS] (Sigma).

## RESULTS AND DISCUSSION

### Peptide-membrane interactions

Interactions between peptides and lipid membrane play major roles in numerous physiological processes and the activities of antibacterial drugs. A variety of structural models describing interactions between short peptides and membranes and membrane permeation have emerged in recent years, such as the transmembrane channel aggregates, the "barrel-stave" model and the "carpet" mechanism [10]. The new colorimetric assay provides information upon peptide-membrane interactions between and could contribute to the elucidation of structural and

functional properties of membrane-associated peptides, and their organization in membrane environments.

The relationships between the observed colorimetric transitions in the vesicle solutions and the mechanisms of peptide-membrane interactions could be obtained from comparison between native membrane-peptides and their analogs. We have previously studied native melittin (L-melittin) and a melittin analogue in which certain L-amino acidc have been replaced with the corresponding D-amino acids (D-melittin) [8]. D-melittin is bound on the surface of the lipid assemblies and induces greater interfacial perturbations, and thus a more pronounced color change, compared to transmembrane insertion of a helical peptide such as native melittin [10].

We have applied the lipid/PDA colorimetric assay for studying indolicidin and its analogues. Figure 2 shows the colorimetric response of indolicidin (CP10) and its analogue CP11. According to our hypothesis, CP11, which clearly induces a greater blue-red color change compared to native indolicidin, causes more significant interfacial membrane perturbations compared with indolicidin, which inserts deeper to the membrane.

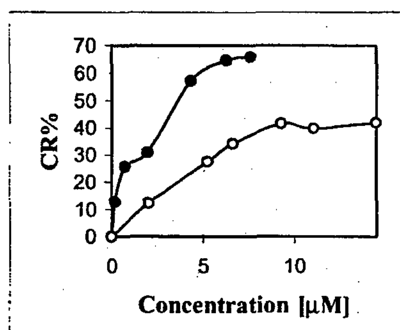


**Figure 2**: Tiration curves depiciting the change of CR% in DMPG/DMPC/PDA vesicles as a function of peptide concentration: (•) CP11 and (O) CP10.

In order to corroborate that conclusion we have examined liposomes incorporating the spin-labeled lipids 12-DS and 16-DS in order to estimate the average position of the tryptophan residues in the liposome, as spin-labels will cause a decrease in tryptophan fluorescence when in close proximity. Figure 3 shows that the decrease in the fluorescence emission of tryptophan in indolicidin (CP10) is greater than the corresponding decrease in CP11. This result confirms that indolicidin (CP10) penetrates deeper into the lipid core while CP11 bind to the surface of the lipids.

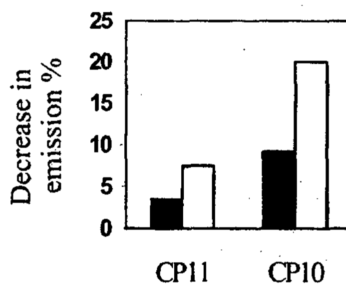


**Figure 3:** Decrease in tryptophan emission data of indolicidin (CP10) and indoliciand analogue (CP11) added to 1 mM DMPG/DMPC/PDA vesicles incorporated the spin-labeled lipids: (■)12-DS, (□) 16-DS.

## Ligand-receptor binding

The lipid/PDA system can also be chemically modified to allow for detection of selective guest-host or ligand-receptor binding events. We have examined the colorimetric properties of lipid/PDA vesicles incorporating the synthetic macrocyclic receptor homooxacalixarene triether, which exhibits selectivity among various neurotransmitters and ions [9,11]. The data presented in Figure 4 indicate that the receptor/lipid/PDA vesicle assembly exhibits distinct ligand selectivity. Specifically, the highest colorimetric response of the assay is observed when dopamine or sodium ions are added to the vesicle solution. Adrenaline or $K^+$ ions, on the other hand, give rise to significantly smaller blue-red color transitions. These results are similar to the complexation selectivity of homooxacalixarene triether in two phase system ($CH_2Cl_2/H_2O$) [9] and in PVC liquid membranes [11].

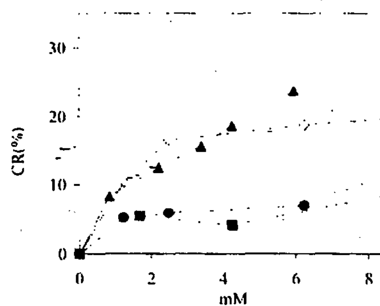


**Figure 4:** Colorimetric response for ligands added to DMPC/PDA vesicles incorporating homooxacalix arene triether: $Na^+$ ( ◊ ), $K^+$ ( ● ), dopamine ( ▲ ), adrenaline ( ■ ).

## CONCLUSIONS

Experiments carried out in our laboratory demonstrate that incorporation of natural lipids into the PDA matrix enables the vesicles to successfully mimic environments of cellular membranes, thus opening the way to a variety of biochemical applications. The lipid/PDA vesicle assembly exhibits rapid colorimetric transitions upon specific interactions with a variety of biological analytes in aqueous solutions, including membrane peptides, ions, and neurotransmitter ligands. The assay can be readily applied to diagnosis of physiological molecules and for rapid screening of chemical and biological libraries.

## REFERENCES

1.Tanaka, H., Gomez, M.A., Tonelli,A.E., Thakur, M. Thermochromic phase transition of a polydiacetylene, studied by high resolution solid- state 13C NMR. Polymer 22,1208-1215(1991).

2. Jelinek, R., Okada, S., Norvez, S., Charych, D.H. Interfacial catalysis by phspholipases at conjugated lipid vesicles: colorimetric detection and NMR spectroscopy, Chem. Biol. 5,619-629(1998).

3. Cheng, Q., and Stevens, R. C. Charge-Induced Chromatic Transition of Amino Acid-Derivatized Polydiacetylene Liposomes. Langmuir 14, 1974-1976(1998).

4. Charych, D.H., Nagy, J. O., Spevak, W., Bednarski, M. D. Direct colorimetric detection of a receptor-ligand interaction by a polymerized bilayer assemblyScience 261, 585-588(1993).

5. Kolusheva, S., Boyer, L., Jelinek, R. A colorimetric assay for rapid screening of antimicrobial peptides, Nature Biotechnology 18, 225-227 (2000).

6. Kolusheva, S., Kafri, R., Katz, M., Jelinek, R. Rapid Colorimetric Detection of Antibody-Epitope Recognition at a Biomimetic Membrane Interface, J. Am. Chem. Soc. 123(3) 417-422 (2001).

7. Kolusheva, S., Shahal, T., Jelinek, R. Cation – Selective Color Sensors Composed of Ionophore–Phospholipid– Polydiacetylene Mixed Vesicles, J. Am. Chem. Soc 122. 776-780 (2000).

8. Kolusheva, S., Shahal, T., Jelinek, R. Peptide-Membrane Interactions Studied by a New Phospholipid/Polydiacetylene Colorimetric Vesicle Assay. Biochemistry 39(51), 15851-15859 (2000).

9.Araki, K.; Inada, K.; Otsuka, H.; Shinkai, S. Conformational isomerism in and binding properties to alkali-metals and an ammonium salt of *O*-alkylated homooxacalix[3]arenes Tetrahedron *49*, 9465-9478(1993).

10. Oren, Ziv; Shai, Yechiel. Molecular mechanism of cell selectivity by linear amphipathic α-helical and diastereomeric antimicrobial peptides. Development of Novel Antimicrobial Agents: Emerging Strategies 183-204. (2001).

11. Odashima, K.; Yagi, K.; Tohda, K.; Umezawa, Y. *Bioorg. and Med.* Dopamine-selective response in membrane potential by homooxacalix[3]arene triether host incorporated in PVC liquid membrane. *Chem. Let.* 1999, *9*, 2375-2378.