

Detection and analysis of membrane interactions by a biomimetic colorimetric lipid/polydiacetylene assay

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Abstract

We describe applications of a colorimetric assay based on supramolecular assemblies of lipid–polydiacetylene vesicles for analysis and screening of membrane interactions of lipophilic enzymes, peptides, and ions and for study of the effects of lipid composition upon membrane properties. The lipid–polymer aggregates undergo visible and quantifiable blue-to-red transitions following interfacial interactions and perturbation by varied biochemical processes. Specifically, we show that the colorimetric assay can be tuned for selective detection of enzymes reacting with different lipid species. The experiments also demonstrate that the lipid/polymer platform facilitates screening of peptide–membrane interactions in multicomponent mixtures. The colorimetric vesicles can incorporate lipid species from different cellular sources facilitating analysis of the contribution of molecular components to membrane properties and lipid interactions.

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Development of bioanalytical assays for use in high-throughput screening of compound libraries is becoming increasingly important for drug discovery and identification of molecules having therapeutic promise. Specifically, molecules involved in membrane interactions, such as membrane peptides and lipophilic enzymes, are particularly attractive as pharmaceutical targets, given that such molecules play major roles in numerous physiological processes, including signaling, cytolysis, formation of ion-channels, and cellular recognition. We have recently demonstrated the application of a novel colorimetric assay based on lipid–polydiacetylene (PDA)¹ vesicles. Sonicated and UV-polymerized as-

semblies of lipid molecules and PDA form vesicular particles that exhibit blue color due to the electronic delocalization within the conjugated PDA backbone [1]. Furthermore, such systems undergo specific blue–red transitions induced by diverse biological and chemical processes [2–6]. The lipid molecules in the colorimetric vesicles essentially form “microdomains” within the PDA matrix and do not affect the polymerization and blue color of the vesicle solution [3]. Several reports have demonstrated that the lipid/PDA assemblies closely mimic lipid bilayer environments within cellular membranes [7–12]. This colorimetric lipid platform has been previously used for the study of diverse

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¹ Abbreviations used: PDA, lipid–polydiacetylene; DMPC, dimyristoylphosphatidylcholine; Sph, sphingomyelin; CB, cerebroside; BSA, bovine serum albumin; PMB, polymixin-B; IPTG, isopropylthiogalactoside; LB, Luria–Bentane; PLA₂, phospholipase A₂; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; DG galactosyldiaclyglycerol.

membrane-associated processes, including phospholipase cleavage [13], membrane disruption by antimicrobial peptides [7,8], ion sensing [9], antibody–epitope recognition [10], and the activities of membrane penetration enhancers [11].

In the following, we demonstrate practical bioanalytical applications of the colorimetric assay. The experiments presented herein have been designed to examine whether the colorimetric platform exhibits sufficient selectivity and sensitivity for revealing the activities of lipophilic and membrane-active species. Further experiments have been carried out to probe the capability for “tuning” the selectivity of the assay through modification of the lipid content of the chromatic vesicles and for applications of the colorimetric platform for studying the effect of membrane composition upon its properties.

Materials and methods

Materials

The diacetylene monomer tricosadienoic acid was purchased from GFS Chemicals (Powell, OH). Dimyristoylphosphatidylcholine (DMPC), sphingomyelin (Sph), cerebroside (CB), phospholipase A₂, galactosidase, sphingomyelinase, bovine serum albumin (BSA), polymixin-B (PMB) sulfate, isopropylthiogalactoside (IPTG), and melittin were purchased from Sigma (St. Louis, MO).

Cell growth

Escherichia coli, B/r H-266 strain, was grown in liquid culture in Luria–Bertani (LB) standard medium overnight at 37 °C. Strain HDL1001 cells (IPTG dependent) were grown overnight at 37 °C in LB medium supplemented with 100 μM IPTG, kanamycin (50 μg/ml), and tetracycline (25 μg/ml). The cells were then harvested, excess IPTG was removed by several washes with LB medium, and the cells were diluted 1:10⁴ (relative to the overnight culture) into fresh medium lacking additional IPTG for 4 h. At 45 min after cell arrest, the culture was divided into two parts. One part was grown without additional IPTG (HDL1001⁻), while the other part was grown in the presence of 100 μM IPTG (HDL1001⁺). Both cultures were grown for another 24 h at 37 °C [14].

Haloferax volcanii DS2 was obtained from the American Tissue Culture Collection and grown aerobically at 40 °C as previously described [15]. Lipids from *E. coli* (strain B/r H-266, generously provided by Dr. Y. Fishov, BGU), *H. volcanii*, and red microalgae were extracted by the following procedure: Cultures were harvested and resuspended in brine and chloroform/

methanol (1:1 v/v). The mixture was gently shaken for 1 h and refrigerated overnight. Following refrigeration, the chloroform phase was separated, and the remaining methanol/water solution was reextracted with chloroform. The two chloroform phases were then recombined, the solvent was removed under reduced pressure at room temperature, and the remaining lipid-containing fraction was freeze-dried. Lipids were stored at –20 °C until use.

Thin-layer chromatography (TLC)

TLC analyses were carried out on 10 × 10-cm glass plates coated with silica gel-60 (Merck). Two-dimensional separation was achieved using TLC tanks (Sigma), in which the first solution was chloroform:methanol:water (65:25:4 volume ratio) and the second solution consisted of chloroform:methanol:acetic acid (65:25:10 volume ratio) [14]. Following elution, the lipids were visualized by exposure to iodine vapor or by sulfuric acid charring. Lipids containing amino groups were detected by a ninhydrin test and phospholipids were detected by molybdenum blue reagent [16].

Lipid transmethylation and fatty acid analysis

Samples of total extracted lipids from the different sources and spots scraped off from the TLC plates (not stained by sulfuric acid charring) were transmethylated with 2 ml of methanol/sulfuric acid (1:50) [17]. Heptadecanoic acid was added as an internal standard, and the mixtures were sealed in a light-protected Teflon-lined vial under argon atmosphere and heated at 80 °C for 1 h.

Gas chromatographic analysis was performed with a Supelcowax 10 fused-silica capillary column (30 m × 0.32 mm) at 200 °C (injector and flame ionization detector temperatures 230 °C, split ratio 1:100). Fatty acid methyl esters were identified by co-chromatography with authentic standards (Sigma Chemical Co., St. Louis, MO).

Gel filtration

BSA, melittin, and PMB (200 μg each) were dissolved in 0.3 ml of 50 mM Tris–HCl, pH 7.8. The mixture was applied to a Sephadex G-100 (Sigma) column (10 × 0.5 cm) using Tris–HCl as the mobile phase; 96 fractions (0.1 ml) were collected into microwell plates containing 0.1 ml DMPC/PDA (2:3 mole ratio) liposome solutions (pH 8.2) in each well.

Sample preparation

Preparation of vesicles containing lipids and PDA (2:3 molar ratio) has been described previously [7].

Briefly, the lipid constituents are dried together in vacuo followed by addition of deionized water and probe sonication at 70 °C. The vesicle solution is cooled, kept at 4 °C overnight, and then polymerized by irradiation at 220 nm for a few seconds. The resulting solution exhibits a blue appearance. Samples were prepared at concentrations of 1 mM total lipid, 2 mM Tris–base, pH 8.2.

Vesicles containing natural lipids (i.e., extracted lipids from different microorganisms) were prepared at 1:1 mass ratio and at concentration of 0.41 mg/ml total lipid, 2 mM Tris–base, pH 8.0–8.2. The various enzymes and peptides were mixed with the vesicles prior to addition of the Tris buffer.

Determination of peptide–vesicle partition coefficients

The colorimetric analysis of peptide–lipid interactions (see below) correlated the blue–red changes with vesicle-bound peptide concentration. Accordingly, for each vesicle composition we have determined the concentration of bound peptide (i.e., partition coefficients). A calibration graph correlating peptide concentration with UV absorbance at 220 nm was initially constructed and used to determine the concentration of soluble, unbound peptide. Increasing peptide quantities were added to aqueous lipid/PDA vesicle solutions (0.5 mM, Tris buffer 25 mM at pH 8); the solutions were left at room temperature for a few minutes to allow equilibration and followed by ultracentrifugation at 30,000 rpm for 40 min to precipitate the vesicle–peptide aggregates. The concentration of soluble [unbound] peptide in the supernatant was determined from the calibration curve and subtracted from the initial concentration to yield the amount of vesicle-bound peptide. Concentrations of unbound peptides were confirmed using the Lowry method [18].

UV-vis spectroscopy

All spectroscopic measurements were carried out at 27 °C (except the variable temperature experiments) using a Jasco V550 UV-vis spectrophotometer, with a 1-cm-optical-path cell.

Quantitation of the extent of blue–red color transition is given by the colorimetric response (%CR) [7], defined by

$$\%CR = \frac{[PB_0 - PB_1]}{PB_0} \times 100$$

$$\text{and } PB = \frac{A_{\text{blue}}}{[A_{\text{blue}} + A_{\text{red}}]}$$

where A is the absorbance measured at either the “blue” component (640 nm) or the “red” component (500 nm) of the visible spectrum. PB_0 is the red/blue ratio of the control sample (i.e., before induction of color change), while PB_1 is the value obtained for the vesicle solution

after occurrence of the color change. All reported %CR values were averages of six or seven independent measurements, carried out for different sample batches and on different days.

Results

Colorimetric detection of enzyme cleavage

The lipid/PDA assemblies allow for incorporation of a variety of lipid species [12,19]. This versatility facilitates the application of the colorimetric assay for the detection and analysis of diverse biological membrane processes. Such applications are exemplified by experiments which depict chromatic transitions induced by interfacial enzymatic cleavage (Fig. 1). Fig. 1 depicts the percentage colorimetric response (%CR—see Materials and methods) of solutions containing vesicles composed

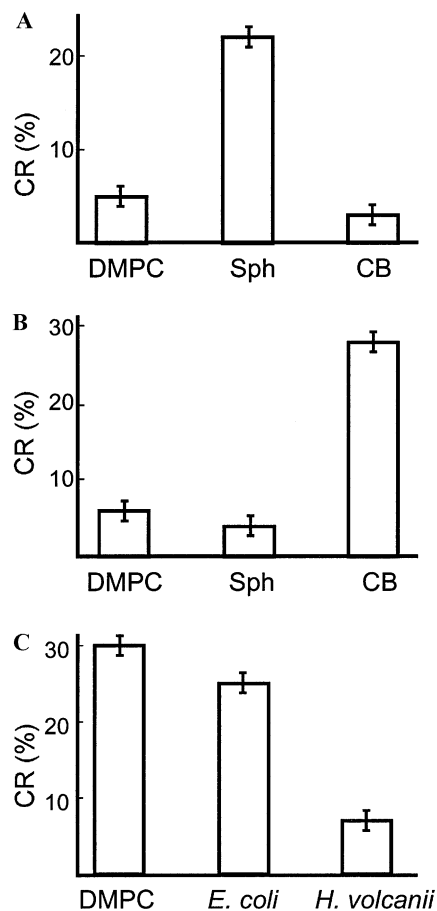


Fig. 1. Selective colorimetric response induced by enzymatic lipid cleavage. Colorimetric response (%CR) calculated from the visible absorption spectra of solutions of PDA vesicles containing different lipids (DMPC/PDA, Sph/DPA, CB/PDA, *E. coli*/PDA, *H. volcanii*/PDA) mixed with enzymes: (A) sphingomyelinase, (B) galactosidase, (C) phospholipase A2. Enzyme quantities were 20 units per sample of 30 μ l vesicles in Tris buffer (see Materials and methods). Color changes occurred instantly after addition of enzymes at 25 °C.

of PDA and various lipid molecules following treatment with lipid- and glycolipid-degrading enzymes, including phospholipase A2, sphingomyelinase, and galactosidase. Previous colorimetric and nuclear magnetic resonance studies have confirmed that interfacial enzymatic catalysis induces colorimetric transitions within DMPC/PDA vesicles [13].

Fig. 1 reveals the high specificity of the colorimetric transitions in response to membrane-associated enzyme–substrate recognition events. The %CR values shown in Fig. 1 reflect the extent of blue–red transitions observed (see Materials and methods). Essentially, high %CR is indicative of a more pronounced red appearance of the solution, while low %CR corresponds to a more blue color (the %CR of the initial blue solution prior to induction of the colorimetric transition is defined as zero). The experimental data shown in Fig. 1 (and other colorimetric experiments reported below) are averages of at least six independent measurements. The error bars indicated in Fig. 1 confirm the reproducibility of the colorimetric analysis.

Fig. 1A shows that a significantly more enhanced red color (i.e., higher %CR) is induced when sphingomyelinase is added to vesicles containing Sph/PDA compared to DMPC/PDA or CB/PDA solutions. Similarly, a considerably higher %CR is recorded when galactosidase is mixed with CB/PDA particles as compared to DMPC/PDA or Sph/PDA (Fig. 1B). The blue–red transitions induced by lipid-degrading enzymes have been ascribed to the cleavage reactions occurring at the surface of the lipid/PDA vesicles, events that perturb the vesicle interface [13,20].

The experiments described in Fig. 1C address the application of the colorimetric assay for analysis of lipid mixtures extracted from cellular membranes, depicting the colorimetric responses induced upon addition of phospholipase A2 (PLA₂) to mixed vesicles containing PDA and total lipid extracts from a bacterium (*E. coli*) or an archaeon (*Haloferax volcanii*). In archaea such as *H. volcanii*, membrane phospholipids are composed of repeating isoprenyl subunits linked to a glycerol backbone via ether linkages, rather than through the ester bonds found in bacterial and eukaryal phospholipids [21]. Accordingly, the chromatic data clearly show that PLA₂, which cleaves ester bonds that link fatty acyl groups to the glycerol backbone, induces only a small blue–red transition in the PDA assemblies incorporating the archaeal lipids (Fig. 1C). Conversely, the strong colorimetric response induced upon the reaction between PLA₂ and vesicles containing PDA and the membrane lipids of *E. coli* is to be expected, given the ester linkages present in the bacterial lipids. This significant colorimetric transition is similarly consistent with the blue–red transformation observed in the case of vesicles containing PDA and DMPC, in which ester linkages are also present.

Screening of peptide–membrane interactions

The sensitivity of the colorimetric transitions of the lipid/polymer vesicles to membrane interactions facilitates application of the assay as a tool for functional screening of biological mixtures. Fig. 2 depicts spectroscopic data obtained by combining the lipid/PDA vesicle assay with gel chromatography for the separation and detection of membrane-interacting species in a mixture containing albumin, melittin [22], and polymyxin B [23]. In the experiment, the solution containing the peptide mixture was passed through a Sephadex G-100 column, and each eluted fraction was added to a separate well within a standard 96-well plate containing DMPC/PDA vesicle solution.

The application of the colorimetric assay as a functional screening tool for membrane interactions is graphically shown in Fig. 2A, which depicts the intensity of the visible absorption peak at 500 nm recorded as a function of fraction number. The peak at 500 nm is essentially a measure of the red appearance of the vesicle solution, induced by binding of a membrane-active species in the eluted solution. The appearance of two distinct peaks in Fig. 2A is consistent with the presence of the two membrane-active peptides in the mixture, namely melittin and PMB, which differ in their molecular weights. Fig. 2B records the elution of the three biopolymers in the mixture as detected at 214 nm, with the three peaks corresponding to the three peptides present. Importantly, the first peptide eluted after approximately 20 fractions (Fig. 2B) does not appear in the graph outlined in Fig. 2A. This peptide is most likely albumin, bearing the greatest molecular weight in the peptide mixture. Albumin is not expected to significantly interact with lipid membranes [24] and thus would not

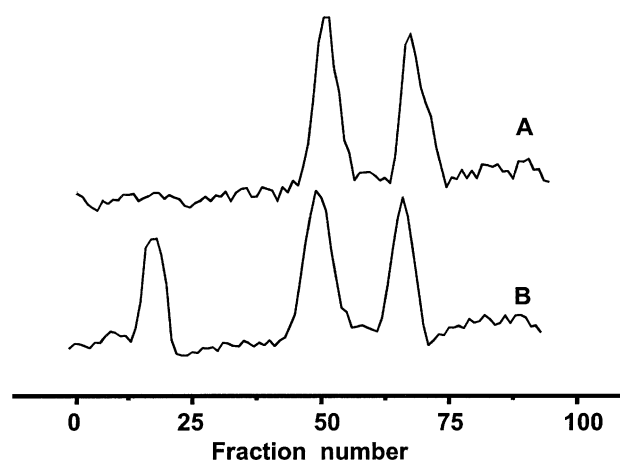


Fig. 2. Conjugation of the colorimetric assay with size chromatography. Eluted fractions of a mixture of albumin, melittin, and PMB were added to wells containing DMPC/PDA vesicle solutions. Graph depicts the relationship between eluted fraction number and the absorption at (A) 500 nm (red band in the visible spectrum) and (B) 214 nm.

give rise to a blue–red transition. The two other peaks detected in Fig. 2B appear in the same fraction numbers as those in Fig. 2A and confirm that the colorimetric transitions observed by the vesicle assay (Fig. 2A) are specifically induced by the two membrane-perturbing peptides.

Dependence of peptide–membrane interactions upon lipid composition: ratio of negative:neutral phospholipids

We have previously demonstrated that the lipid bilayer domains within the lipid/PDA vesicle assembly can be composed of a variety of synthetic and natural phospholipids, glycolipids, lipopolysaccharides, cholesterol, and other molecules, and that different lipid compositions do not adversely affect the chromatic properties of the assay [13]. Earlier studies have demonstrated that diacetylenic fatty acids could also undergo polymerization while incorporating complete membrane fragments of microorganisms grown in culture [19]. Figs. 3 and 4 examine the application of the colorimetric vesicle assay for studying the dependence of peptide–lipid interactions upon variations of the lipid bilayer composition.

Fig. 3 depicts results of experiments designed to investigate the relationship between the lipid–bilayer interactions of the membrane-active peptides melittin and PMB and the ratio between negative and neutral lipids within the vesicles. Specifically, in the experiments summarized in Fig. 3 the PDA matrix contained the total lipid composition extracted from the membrane of an *E. coli* strain (HDL1001 [14]) in which the ratio between zwitterionic phospholipids (phosphatidylethanolamine (PE)) and negative phospholipids (phosphatidylglycerol (PG) and cardiolipin (CL)) can be regulated by induction of the *lac* operon [14]. We have incorporated in the colorimetric vesicles lipids extracted from two bacterial cultures: cells grown without induction with IPTG (HDL1001[−]) and IPTG-induced cells (HDL1001⁺) (see Materials and methods). The TLC images of the lipids extracted from the two cultures (Fig. 3A) clearly show that HDL1001⁺ exhibits a higher concentration of PG and CL.

The colorimetric data in Fig. 3B demonstrate that vesicle interactions and degree of membrane permeation of melittin and PMB depend upon lipid composition. The %CR values presented in Fig. 3B correspond to the colorimetric transitions induced by identical vesicle-bound peptide concentrations, taking into account the respective partition coefficients of each peptide (see Materials and methods). Importantly, comparison between biophysical membrane interactions of the peptides is meaningful only upon taking into account the concentration of vesicle-bound peptides since the %CR depends upon the concentration of attached peptide and not the peptide in aqueous solution (due to the partition coefficient; see Materials and methods).

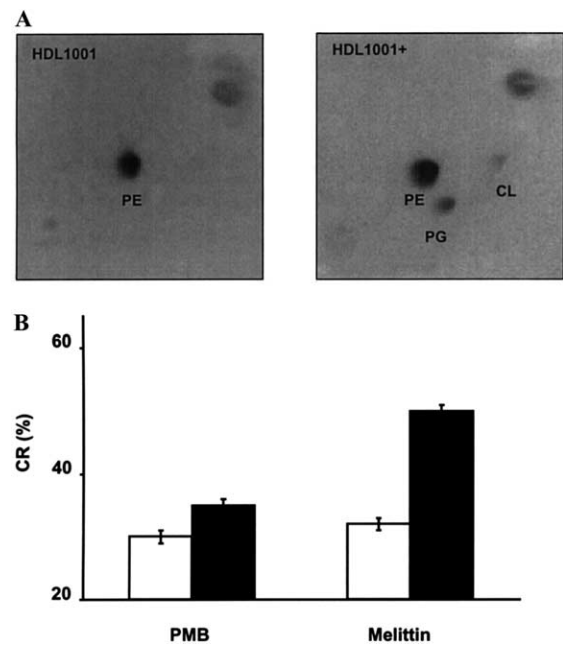


Fig. 3. Peptide–membrane binding probed by the colorimetric assay: higher affinity of melittin to negative phospholipids. (A) Two-dimensional thin-layer chromatograms of chloroform extraction of HDL1001[−] (left) and HDL1001⁺ (right). The phospholipids were visualized by exposure to molybdenum blue reagent. PG, phosphatidylglycerol; PE, phosphatidylethanolamine; CL, cardiolipin. (B) Colorimetric response (%CR) of vesicle solutions at concentrations of 2 μM melittin and 2 μM PMB. The vesicles examined incorporated total lipid extract [HDL1001⁺]/PDA (black column) and total lipid extract [HDL1001[−]]/PDA (white column). Weight ratio of lipids:PDA was 1:1.

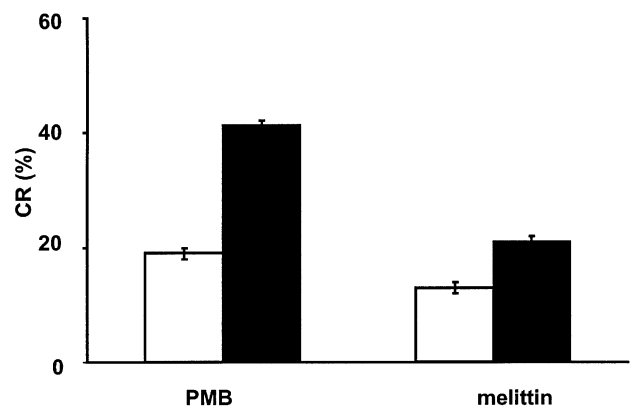


Fig. 4. Dependence of peptide–membrane binding on fatty-acid unsaturation and headgroup specificity. Peptide-binding assay diagrams depicting the colorimetric response (%CR) of vesicle solutions after addition of 1 μM melittin and 1 μM PMB. The vesicles examined were total lipid extract (*P. cruentum*, high cell density)/PDA (black column) and total lipid extract (*P. cruentum*, low cell density)/PDA (white column). Weight ratio of lipids:PDA was 1:1.

The colorimetric response induced by melittin or PMB (at concentrations of 1 μM) was similar—at approximately 30%—when the lipid/PDA vesicles contained

lipids extracted from HDL1001⁻ (no IPTG added) which has a higher proportion of zwitterionic lipids vs negative lipids in the membrane. However, in the case of HDL1001⁺, in which the PG and CL contents were increased almost eightfold [14], melittin induced a higher blue–red transition than PMB (Fig. 3B). This result indicates a higher surface localization of melittin in bilayers containing negatively charged lipid constituents, confirmed in several previously published studies [25,26]. Similarly, PMB does not exhibit specific binding to the negatively charged headgroups of phospholipids within cellular membranes, consistent with the colorimetric data shown in Fig. 3B.

Dependence of peptide–membrane interactions upon degree of fatty acid unsaturation and headgroup specificity

In addition to the dependence of peptide–membrane interactions upon the proportion of negative phospholipids (Fig. 3), we have examined the relationship between the colorimetric response and the relative degree of unsaturation of lipid acyl chains (relative abundance of carbon–carbon double bonds) within cellular membranes (Fig. 4). The experiments depicted in Fig. 4 have utilized PDA vesicles containing total lipid extracts from the red algae *Porphyridium cruentum* strain 1380.1 d in which the relative unsaturation of fatty acid chains was affected by the concentration of cells during growth [17]. The membrane of *P. cruentum* contains approximately 80% galactosyldiacylglycerol (DG) derivatives, and previous studies have demonstrated that the degree of DG chain unsaturation could be regulated through the growth conditions [27]. Specifically, the two cell cultures used for extractions differed in the ratio between the 20:4 and 20:5 chains in the algal membrane (Table 1).

The %CR data in Fig. 4 indicate that melittin and PMB induced higher colorimetric response when the vesicles contained lipids from *P. cruentum* that was grown at a higher cell density and in consequence had fewer total number of double bonds in the acyl residues (Table 1). PMB, for example, induced approximately 40% CR in the vesicles containing lipids from the high-concentration cell growth vs 20% in lipid/PDA vesicles incorporating lipids extracted from algae grown at low concentration. The corresponding values for melittin

were 23 and 15%, respectively (Fig. 4). These significant differences in chromatic response correspond to the lower fluidity within lipid bilayers having less unsaturation (lower abundance of double bonds within the alkyl chains of the fatty acids) [28]. Increased rigidity of the lipid moieties would reduce penetration of the peptides into the lipid bilayers [29,30], instead resulting in peptide binding at the lipid headgroup region within the lipid/PDA vesicles. Indeed, interfacial peptide binding generally leads to a higher degree of blue–red transitions (higher %CR induced within the vesicle solutions) since such surface interactions induce greater perturbation of the pendant polymer side chains within the PDA matrix [20].

Fig. 4 further shows that PMB induces a significantly higher %CR than melittin within vesicles containing total lipid extracts from *P. cruentum* grown at higher cell density conditions (approximately 40% CR for PMB vs 20% CR induced by melittin). This result is consistent with the high affinity of PMB to sugar moieties at bacterial cell surfaces [31]. Melittin, on the other hand, does not exhibit specific affinity to glycolipids or sugar headgroups. The difference between the colorimetric responses induced by PMB and melittin in the lipid/PDA vesicle solutions is again due to the dependence of peptide–lipid interactions upon lipid composition.

Colorimetric detection of ion–lipid interactions

Interaction of the membrane with ions is critical to its functionality and stability. We have thus examined the applicability of the colorimetric assay for studying ion–lipid interactions (Figs. 5 and 6). Fig. 5 depicts the colorimetric responses induced by different metal cations in lipid/PDA vesicles containing lipids extracted from *E. coli* HDL1001 strain in which the ratio between zwitterionic and negative lipids in the membrane has been regulated through addition of IPTG (see above). The lipids were extracted from bacteria grown at two different conditions [14]: without induction of the *lac* operon with IPTG (higher ratio of PE to PG + CL), and with addition of IPTG (lower ratio of PE to PG + CL) [14]. The colorimetric data in Fig. 5 clearly show more pronounced blue–red transitions when the ions were added to vesicles containing a higher proportion of negatively charged lipids within the PDA framework. This result most likely arises from the greater electrostatic affinity between the positive cations in the aqueous solution and the negative headgroups of the phospholipids incorporated within the colorimetric vesicles.

Fig. 5 further indicates a direct effect of the size of the cations upon the colorimetric transitions. Specifically, larger cations induce higher %CR in both lipid/PDA vesicle compositions. In 10 mM ion solutions of vesicles containing total lipid extracted from HDL1001⁺ bacteria Na⁺ induces approximately 15% CR (Fig. 5A), K⁺

Table 1
Fatty acid composition of *Porphyridium cruentum* vs cell density at growth

Cell density	% Composition				
	16:0	18:2	20:4ω6	20:5ω3	Others
Low	34.8	6.4	16.6	34.0	8.2
High	30.3	20.9	28.8	7.7	12.3

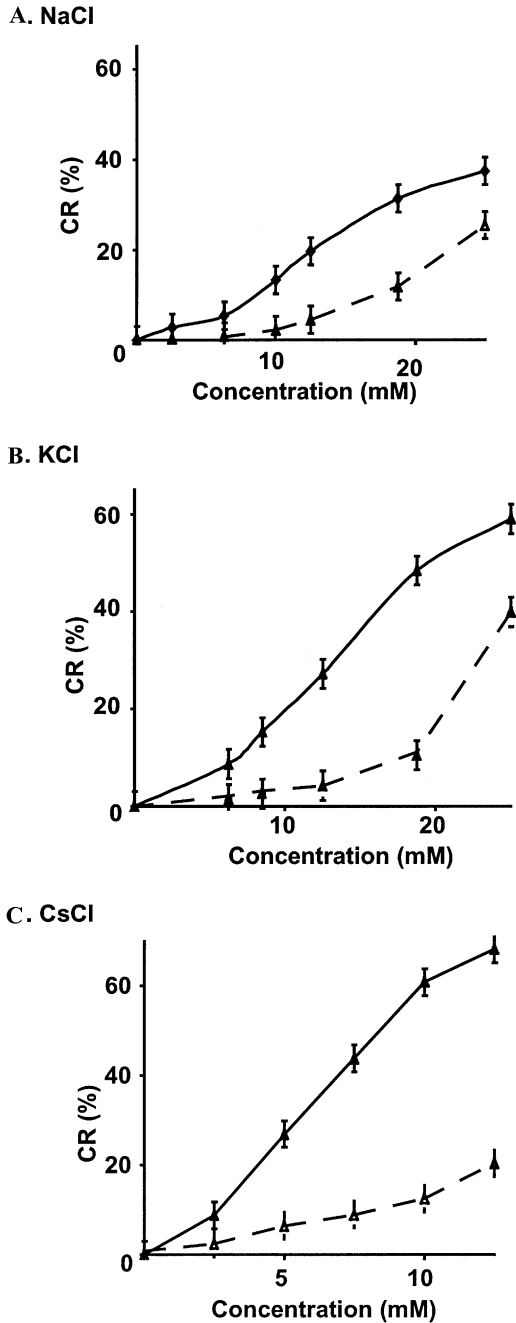


Fig. 5. Colorimetric transitions induced by metal cations: stronger binding to negative phospholipids and ion size effect. Curves depicting the %CR calculated from the visible absorption spectra of lipid/PDA vesicle solutions vs ion concentration of (A) NaCl, (B) KCl, and (C) CsCl. The vesicles examined were total lipid extract (HDL1001⁺)/PDA (solid lines) and total lipid extract (HDL1001⁻)/PDA (broken lines). Weight ratio of lipids:PDA was 1:1.

around 20% (Fig. 5B), while Cs⁺ gives rise to a %CR approaching 65% (Fig. 5C). A similar effect is observed for vesicles in which HDL1001⁻ was the lipid source (Fig. 5). The observed increase in the %CR most likely arises from higher perturbation of the lipid-headgroup moieties as the size of the cation becomes larger.

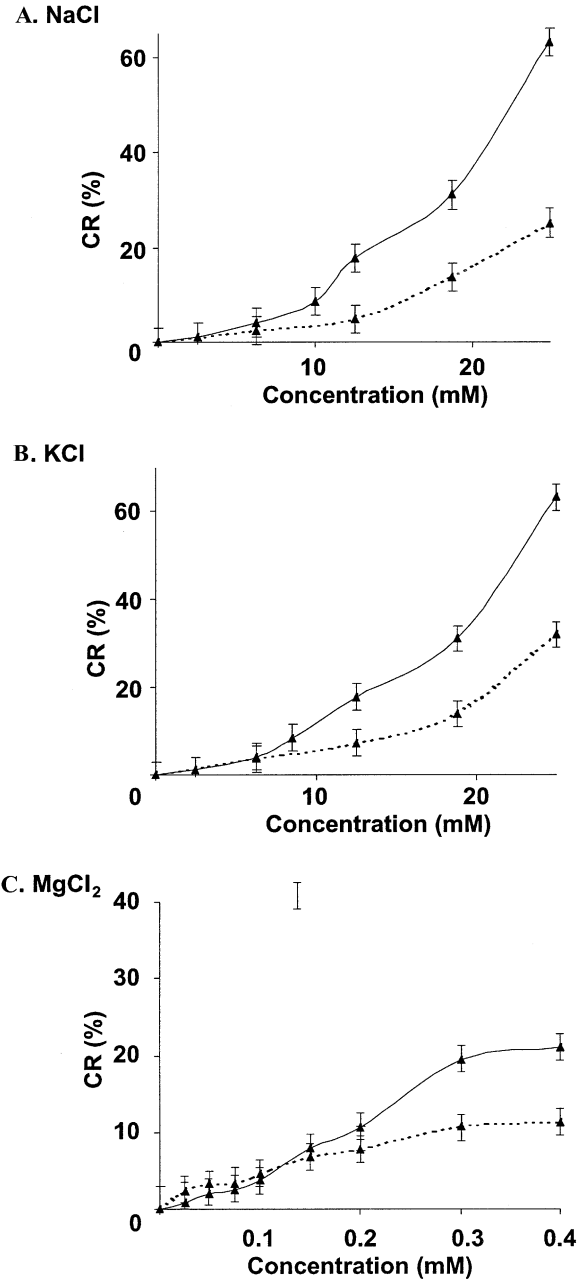


Fig. 6. Colorimetric transitions induced by metal cations in vesicles containing PDA and bacterial vs archaeal lipids: higher response of PDA vesicles containing bacterial lipids. Curves depicting the %CR calculated from the visible absorption spectra of lipid/PDA vesicle solutions vs concentration of (A) NaCl, (B) KCl, and (C) MgCl₂. The vesicles examined were total lipid extract (*E. Coli*)/PDA (solid lines) and total lipid extract (*H. volcanii*)/PDA (broken lines). Weight ratio of lipids:PDA was 1:1.

The colorimetric assay has been additionally applied to examine ion interactions with lipids extracted from distinctly different microorganisms (Fig. 6). Fig. 6 portrays the chromatic transitions induced by metal cations in particles composed of PDA matrices incorporating total lipid extracts from Bacteria (*E. coli*) or Archaea

(*H. volcanii*). *H. volcanii* thrive in highly saline environments approaching saturation. Fig. 6 demonstrates that sodium, potassium, and magnesium all induce smaller colorimetric transitions in the archaeal lipid/PDA assemblies, as compared to particles containing the *E. coli* lipids. This observation suggests that the haloarchaeal lipid membranes are less perturbed by salts. Previous work has shown that haloarchaeal liposomes remain Na⁺- and H⁺-tight under high-salt conditions [32].

Discussion

This paper describes bioanalytical applications of the newly developed lipid/PDA colorimetric platform as a practical tool for analysis of lipid binding and membrane interactions. The data presented in Figs. 1–6 indicate that the colorimetric transitions could reveal both the presence of membrane-active analytes in solution, and the degree of lipid–bilayer perturbation by membrane-interacting species. The latter information is related to the dependence of the blue–red transitions upon the degree of interface disruption of the lipid moieties within the vesicles [7–12]. Importantly, the induced color changes within the lipid/PDA solutions occur rapidly, in most cases within seconds after interaction with the analyte, and the assay can be easily applied for different cellular membrane models.

The results demonstrate that the colorimetric technique can be used for detection of enzymatic lipid cleavage, lipid bilayer permeation by antimicrobial peptides, and lipid–ion interactions. The experiments indicate that the colorimetric platform, combined with conventional chromatography separation techniques, can identify membrane binding of different molecular species in a mixture. Thus the assay could serve as a powerful tool for both biochemical separation and functional analysis.

The experiments demonstrated that the colorimetric assay can be used for investigating the relationship between lipid composition and membrane processes and properties. Fig. 1, for example, demonstrated that the induced color changes are directly correlated to the presence of specific enzymatic lipid substrates within the vesicles. Figs. 3–6 further showed that the colorimetric response induced by interfacial lipid perturbation by membrane-active peptides or metal cations is dependent upon the lipid constituents within the biomimetic vesicles. This study indicates that information on membrane processes and interactions can be obtained through selection or “tuning” of the molecular composition of the lipid/PDA particles.

The PDA assemblies can incorporate both specific lipid molecules and entire lipid extracts from varied microorganisms while retaining the chromatic properties

and selective colorimetric responses of the vesicles. This versatility could be utilized for identifying important biochemical parameters affecting membrane properties. The presence of archaeal lipids in the vesicles, for example, reduces their colorimetric sensitivity toward metal cations (Fig. 6), which echoes the viability of halophilic Archaea in highly saline environments. The colorimetric assay is easily amenable to practical bioanalytical applications; the vesicle platform can be used in conjunction with a 96-well microplate, allowing rapid functional screening of compound libraries.

Application of the colorimetric assay yields reproducible data with experimental errors of generally less than 5% (for %CR measurements). The lipid/PDA vesicles are quite robust and chromatic responses are reproduced for different vesicle batches on different days. The colorimetric assay can be applied within varied buffer conditions, particularly with regard to physiological pH and salinity. Extension of the assay for studying other biological membrane processes, such as signaling and membrane fusion, is straight forward and is currently under investigation in our laboratories.

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