Biomimetic lipid/polymer colorimetric membranes: molecular and cooperative properties

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Abstract Characterization of membranes and of biological processes occurring within membranes is essential for understanding fundamental cellular behavior. Here we present a detailed biophysical study of a recently developed colorimetric biomimetic membrane assembly constructed from physiological lipid molecules and conjugated polydiacetylene. Various analytical techniques have been applied to characterize the organization of the lipid components in the chromatic vesicles and their contributions to the observed blue-to-red color transitions. Experiments reveal that both the polymerized units as well as the lipids exhibit microscopic phases and form domains whose properties and bilayer organization are interdependent. These domains are interspersed within mixed lipid/polymer vesicles that have a size distribution different from those of aggregates of the individual molecular constituents. The finding that fluidity changes induced within the lipid domains are correlated with the chromatic transitions demonstrates that the colorimetric platform can be used to evaluate the effects of individual molecular components, such as negatively charged lipids and cholesterol, upon membrane fluidity and thermal stability.—Kolusheva, S., E. Wachtel, and R. Jelinek. **Biomimetic lipid/polymer colorimetric membranes: molecular and cooperative properties. *J. Lipid Res.* 2003.** 44: **65–71.**

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Elucidation of the structure and organization of biological membranes is essential for the understanding of various cellular functions that rely on biochemical processes occurring within membranes, such as response to environmental conditions, signaling mechanisms, cell-cell communication, cell motility, or protein transport. Parameters affecting membrane properties include the molecular composition of the membrane, the structure and fluidity of the lipid bilayer, and the interactions of membrane components with the cell environment. Such membrane properties have been investigated by a variety of analytical and structural methods (1). To better detect and analyze diverse membrane processes, we have recently developed a novel colorimetric lipid assay (2–7). This assay has been used to examine various aspects of membrane biology, including enzymatic cleavage of lipids (2), screening of antimicrobial peptides (3), peptide-membrane interactions (4), ion sensing (5), antibody-epitope recognition (6), as well as assessing the activities of membrane penetration-enhancers (7). This colorimetric platform consists of supramolecular aggregates of physiological lipid molecules embedded within a chromatic polydiacetylenic (PDA) lipid matrix (8). Conjugated polydiacetylenes have been shown to exhibit rapid blue-red colorimetric transitions upon interfacial disruption of the pendant alkyl side-chains (8). Our results have demonstrated that lipid/PDA systems similarly exhibit unique bio-chromatic transitions attributed to specific interfacial perturbation of the incorporated lipid domains. Such interactions indirectly affect the conjugated network of the adjacent PDA framework, thereby inducing colorimetric transitions (2–7).

In the present work we probe in detail the structures and organization of the lipid and polymer components within the chromatic particles. In addition, experiments were conducted to evaluate the applicability of the colorimetric system for studying the effects of specific lipid components, such as cholesterol or cardiolipin, upon the rigidity and thermal stability of membranes. Understanding the molecular properties of the lipid/PDA system is essential for evaluation of whether the colorimetric platform can indeed serve as a reliable model for studying cellular membrane processes.

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Abbreviations: DLS, dynamic light scattering; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DSC, differential scanning calorimetry; ESR, electron spin resonance; PDA, polydiacetylene; PE-(NBD), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiol-4-yl); SAXS, small angle X-ray scattering; uv, ultra-violet.

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MATERIALS AND METHODS

Materials and sample preparation

The diacetylene monomer tricosadiynoic acid was purchased from GFS Chemicals (Powell, OH). Dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG), cardiolipin, and cholesterol were purchased from Sigma (St. Louis, MO). Preparation of vesicles containing lipids and PDA has been described previously (4). Briefly, the lipid constituents were dissolved in cholesterol/ethanol and dried together in vacuo, followed by addition of deionized water and probe-sonication for 2–3 min at 70°C. The vesicle solution was cooled and kept at 4°C overnight, and then polymerized in an ultra-violet (uv) oven (cross-linker) by irradiation at 220 nm for 10–20 s. The resulting solutions exhibited an intense blue color. Non-sonicated multilamellar suspensions (300 mM total lipids) for the small angle X-ray scattering (SAXS) and differential scanning calorimetry (DSC) measurements (see below) were prepared by dissolving the dry lipid and diacetylene constituents in distilled water.

UV-visible spectroscopy

Samples were prepared at concentrations of 1 mM total lipid. Measurements were carried out at 27°C (except the variable temperature experiments) using a Jasco V550 uv-visible spectrophotometer, with a 1 cm optical path cell.

Quantification of the extent of blue-red color transition is given by the colorimetric response (%CR), as defined by (8):

$$\%CR = [(PB_0 - PB_I)/PB_0] \times 100\% \qquad (Eq.\ 1)$$

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

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 blue-red ratio of the control sample (i.e., before induction of color change), while PB_I is the value obtained for the vesicle solution after appearance of the color change.

Small angle X-ray scattering

Samples were inserted into 1.5 mm diameter thin wall quartz X-ray capillaries. Scattering experiments were performed at room temperature using Ni-filtered Cu radiation (0.154 nm) from an Elliott GX6 rotating anode X-ray generator operating at a power rating of 1.2 kW. The X-radiation was further monochromated and collimated by a 20 cm Franks mirror and a series of slits and height limiters. The scattering was measured by a linear position-sensitive detector of the delay-line type and histogrammed into 256 channels with a Z80 microprocessor. The camera parameters were calibrated using anhydrous cholesterol. Typical exposure time was 1 h.

Differential scanning calorimetry

Samples were weighed into the aluminum pans of the calorimeter, which were subsequently sealed. Molar concentrations of the different suspensions were adjusted in order to obtain approximately equivalent quantities of the lipids. The DSC experiments were performed on a DuPont 990 Thermal Analyzer (DuPont Instruments, Wilmington, DE). Distilled water served as a blank. Scan rates of 5°C/min and sensitivity of 1/5 were used. Each sample was scanned three times.

Dynamic light scattering

Vesicle solutions were prepared in pure water as described above (Materials and sample preparation section) and were filtered (0.2 μm) or centrifuged to remove aggregates and dust when necessary. Each solution sample was then injected into a thin-walled cylindrical borosilicate glass cuvette (1 cm diameter) and placed in a vat filled with toluene as the index matching fluid. During the course of the measurements, the vat temperature was kept at room temperature. The light source was an argon ion laser (Spectra Physics, $\lambda = 514.5$ nm) and photons scattered by the sample were collected by a photomultiplier tube mounted on the goniometer arm at 90° to the direction of the incident radiation. The photoelectron count-time autocorrelation function was measured with a BI 2030AT (Brookhaven Instruments) digital correlator and analyzed using the constrained regularization algorithm, CONTIN (9). Applying the Stokes-Einstein equation to the translational diffusion coefficients provides an intensity-weighted distribution of hydrodynamic sizes (10).

Chromatography and fluorescence measurements

Liquid chromatography analysis of the vesicles in an aqueous solution was carried out using cross linked Sepharose 4B as the stationary phase (350 × 5 mm). The conjugated lipid-fluorescent marker 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiol-4-yl) [PE-(NBD), Avanti Polar Lipids, Alabaster, AL] was incorporated into the vesicles (phospholipid-probe ratio of 40:1). Fluorescence emission spectra were acquired at 27°C on an Edinburgh F920 spectrofluorimeter using excitation at 463 nm and emission at 536 nm. Excitation and emission slits were both set on 8 nm. Total sample volumes were 1 ml, and the solutions were placed in a quartz cell having a 1 cm optical path length. Light scattering from the vesicles was confirmed to account for less than 5% of the emission intensity.

Electron spin resonance

Samples for electron spin resonance (ESR) experiments were prepared using the spin-probe 5-doxyl-stearic acid [5-DS, Sigma]. The spin-probe fatty acid was added to the vesicle samples after the polymerization step, in a molar ratio of 100:1 (phospholipid-spin-probe). Samples were placed in a 20 mm length, 1 mm id quartz capillary and ESR spectra were recorded using a Bruker EMX-220 digital X-band spectrometer at 25°C. The amplitudes of 12.5 kHz and 100 kHz, modulation, and the microwave power level were selected at sub-critical values (0.5 G and 20 mW, respectively) to reach the best signal-to-noise ratio. Processing of the ESR spectra (digital filtering, double integration, etc.) was carried out using Bruker WIN-EPR software.

RESULTS AND DISCUSSION

Structure and phase separation

The colorimetric platform we have developed for studying membrane properties consists of a mixed assembly of membrane lipids and other membrane constituents (such as cardiolipin, or cholesterol), combined with polymerized diacetylenic lipids (denoted polydiacetylene, or PDA) (4, 5). Lipid/PDA mixed vesicles and multilamellae are generally prepared by mixing the lipids and diacetylene monomers in the desired molar ratios. Following sonication and polymerization, organized lipid/polymer assemblies are formed that exhibit an intense blue color due to the conjugated backbone of the PDA polymer (11). Earlier studies have demonstrated that polymerization of the diacetylenic units could be achieved even when they are associated with native membranes of various microorganisms grown in cell culture (12). Previous work has shown that the lipids and PDA are interspersed within the mixed assemblies, most likely forming distinct domains. Here we characterize the

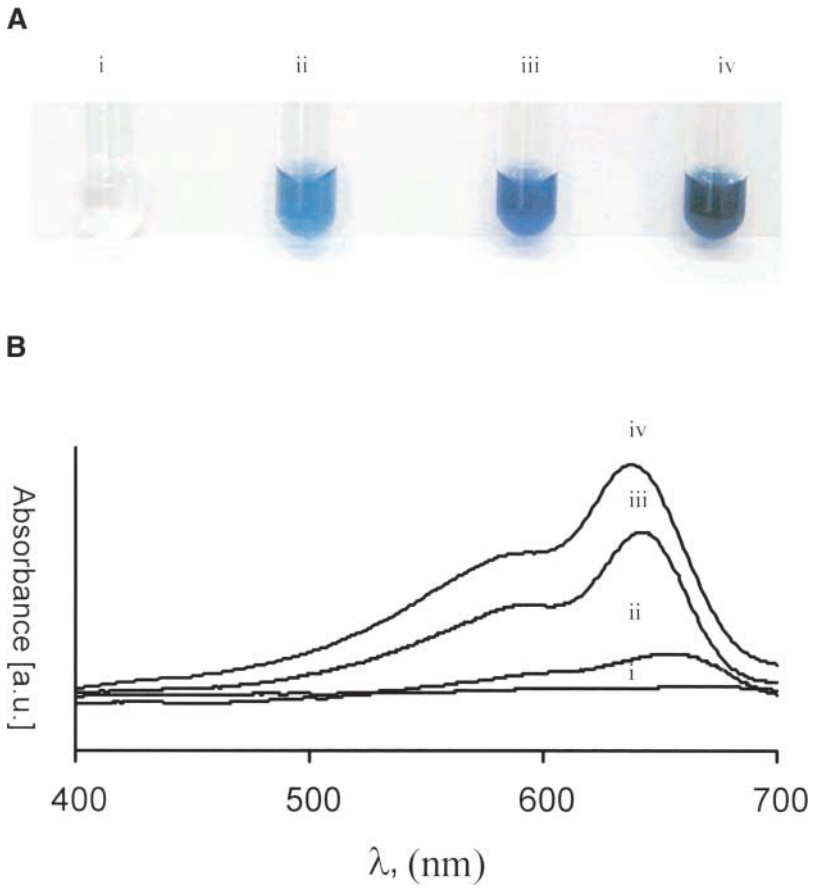

Fig. 1. A: Photograph depicting the appearance of vesicles with different dimyristoylphosphatidylcholine-polydiacetylene (PDA) molar ratios: *i*) 3:2; *ii*) 1:1; *iii*) 2:3; and *iv*) pure PDA. B: Visible spectra of the vesicle solutions shown in A. *i*) 3:2 (DMPC-PDA molar ratio); *ii*) 1:1; *iii*) 2:3; *iv*) pure PDA.

organization and association between the lipids and polymer and define their cooperative properties.

Figure 1 presents a photograph depicting the appearance and uv-vis spectra of aqueous solutions of vesicles having different molar ratios of DMPC and PDA. Figure 1 clearly shows that the intensity of the blue color and the corresponding absorbance spectrum are closely related to the lipid-polymer ratio within the vesicles. Indeed, construction of vesicles with relatively high mol% of DMPC yields completely colorless vesicles [Fig. 1A(i), B(i)]. The dependence of the colorimetric property of the vesicles upon the lipid-PDA molar ratio indicates that the two components are interspersed and do not form two separate vesicle phases in the solution. Formation of two distinct vesicular species would give rise to similar conjugation and blue appearance of the solutions, without any relationship to the relative quantities of lipid and polymer used, a result that is not observed here.

DSC experiments, shown in **Fig. 2**, have been carried out in order to further explore the phase separation and effects of association between DMPC and PDA in mixed assemblies. As noted in the Materials and Methods section, the DSC measurements were carried out for lipid/PDA multilamellar mixtures; importantly, such multilamellar suspensions exhibit biochromic responses similar to those of the vesicles utilized in previous studies (2–7). Figure 2 features the thermograms of suspensions of pure DMPC [Fig. 2(i)], pure PDA [Fig. 2(ii)], and mixed DMPC/PDA [Fig. 2(iii)]. The DSC traces of the pure components exhibit the respective endothermic transitions, at 24°C for DMPC [Fig. 2(i)] (13) and 53°C for PDA [Fig. 2(ii)] (14). A pretransition at 18°C is furthermore apparent in the thermogram of the phospholipid [Fig. 2(i)].

A significantly different result is detected, however, for mixed DMPC/PDA multilamellar vesicles, [Fig. 2(iii)]. In

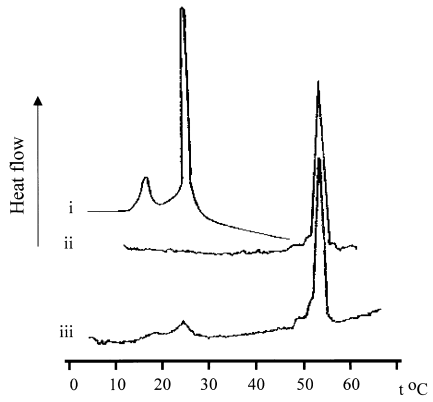

Fig. 2. DSC thermograms of *i*) DMPC; *ii*) PDA; and *iii*) DMPC-PDA (2:3 molar ratio).

this case the enthalpy of the phospholipid melting transition at around 24°C is substantially reduced. Overall, although the DSC data suggest that the thermal properties of the PDA matrix are not markedly perturbed by the phospholipids, the phospholipid assemblies are strongly affected by the polymer. Thus, the cooperative behavior observed in the DSC analysis points to a phase separation between the two components that is not macroscopic. The apparent interactions between the phospholipid and the polymer framework, which affect the size and long-range ordering of the lipid units, could account for the color changes induced within the PDA framework by biological and chemical processes that occur exclusively within the lipid domains of mixed lipid/PDA vesicles (4, 5).

To further evaluate the degree of local ordering and molecular organization within the PDA matrix and the lipid domains, SAXS analysis was performed. SAXS has been extensively used to study lipid ordering within membrane assemblies. **Figure 3A** depicts SAXS diffraction profiles of PDA alone and in mixtures with different phospholipid molecules as well as cholesterol. Importantly, the appearance of distinct peaks in the diffraction patterns indicates that the lipid/PDA aggregates contain at least partially ordered structures.

Figure 3A(i) features the SAXS profile of pure PDA aggregates. A repeat distance of ~45Å (0.025 $Å^{-1}$) is observed. The diffraction pattern of the DMPC-PDA assembly (2:3 molar ratio) [Fig. 3A(ii)] again features the reflection at around 45Å (0.025 $Å^{-1}$) plus an additional reflection at 67Å (0.015 $Å^{-1}$). This second peak is attributed to phase-separated phospholipid. In both profiles, the small number of diffraction peaks and their large line width shows that the aggregates are not well ordered (15). The interlamellar spacing of DMPC has been observed to vary from 60Å to 74Å depending on the temperature and the presence of additives (16).

More intense and narrower diffraction peaks appear in the SAXS profiles of DMPG/DMPC/PDA (at 60Å, 45Å, and 30Å) [Fig. 3A(iii)] and cholesterol/DMPC/PDA (65Å, 45Å, and 35Å) [Fig. 3A(iv)]. Significantly, the smaller line width of the peaks in these scattering profiles points to more ordered lamellar lipid structures, as compared with DMPC/PDA. Enhanced long-range ordering is further indicated by the appearance of the second order of the phospholipid lamellar diffraction pattern at ~30Å [Fig. 3A(iii)]. It is worth noting that the sharpening of the diffraction peak assigned to PDA in parallel with those of the lipids is evidence for interactions between the lipid and the PDA domains, which affect the organization of the polymerized matrix. The reflection at around 35Å [Fig. 3A(iv)] is assigned to cholesterol crystallites. It is known that cholesterol begins to phase separate as crystallites in mixtures with DMPC above mole fraction 0.45 (17). Furthermore, the strong diffraction peak of cholesterol crystallites, at 34Å, is readily observed for phospholipid/cholesterol mixtures when crystallization has occurred (18, 19). The scattering data presented in Fig. 3A(iii, iv) are in agreement with the findings that both cholesterol and negatively charged phospholipids contribute to the stabilization of bilayer structures (20). Cholesterol is known to constrain the

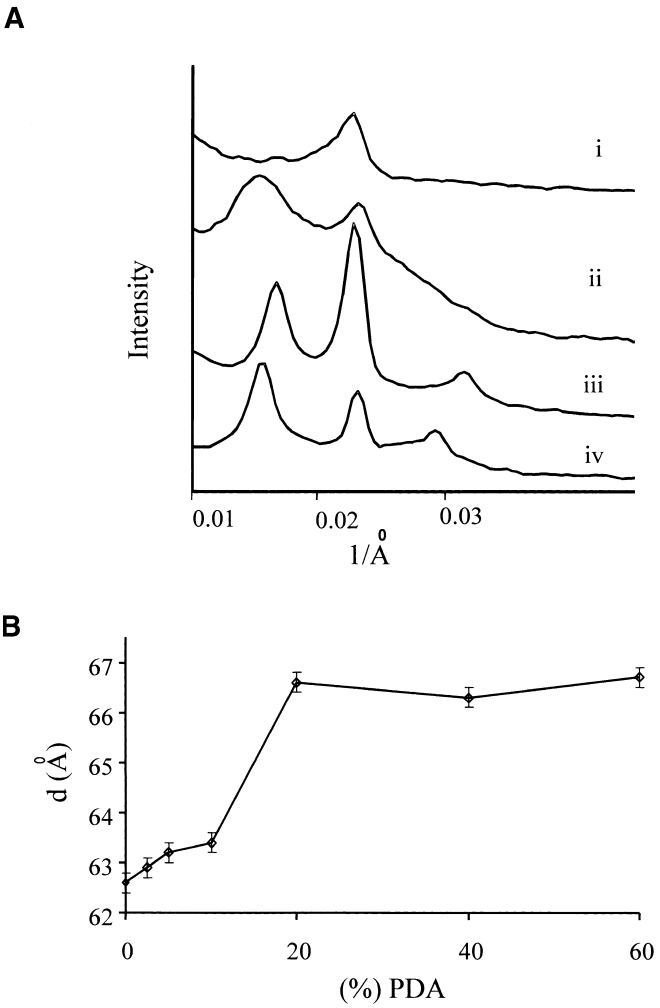

Fig. 3. A: Small angle X-ray scattering (SAXS) patterns of multilamellar vesicles composed of *i*) PDA (100%); *ii*) DMPC-PDA (molar ratio 2:3); *iii*) dimyristoylphosphatidylglycerol-DMPC-PDA (1:1:3); *iv*) cholesterol-DMPC-PDA (1:1:3). B: Graph depicting the interbilayer spacing determined from the SAXS pattern of DMPC/PDA multilamellar vesicles as a function of mol%age of PDA.

conformational freedom of melted hydrocarbon chains (16, 19). Similarly, the influence of PDA on the DMPC molecules may be to promote organization into bilayers with more extended chains. The observation that the membrane lipids in the mixed lipid/PDA assemblies appear to adopt bilayer structures is important, since it demonstrates, for the first time, that the lipid domains within the colorimetric vesicles exist in the fundamental organizational unit found in cellular membranes.

SAXS data presented in Fig. 3B further validate these results, and demonstrate the effect of the interactions between the lipids and PDA. The graph in Fig. 3B summarizes the interlamellar repeat distance of the phospholipids in DMPC/PDA vesicle suspensions at different lipid-polymer molar ratios, as calculated from the diffraction patterns [i.e. Fig. 3A(ii), and other data not shown]. The interlamellar distance for DMPC increases from 62.5Å in the absence of PDA and reaches a plateau at 66.5Å at around 20 mol% PDA. These values may be compared with literature values for the inter-bilayer spacing of

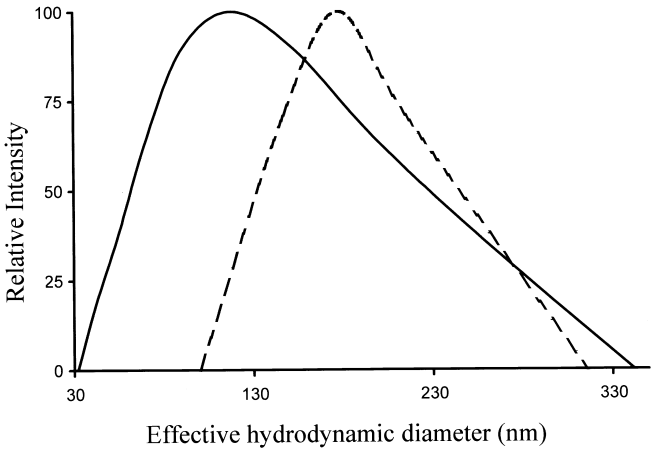

Fig. 4. Intensity weighted size distribution of vesicles as obtained in the dynamic light scattering experiment: DMPC-PDA (2:3 molar ratio) (solid line); PDA (dashed line).

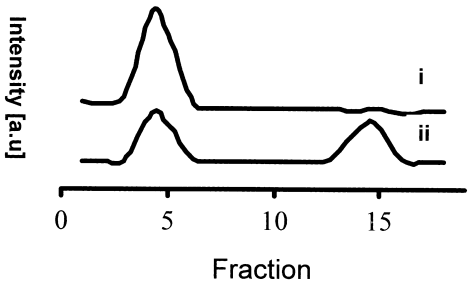

Fig. 5. Size-exclusion chromatography of a suspension containing DMPC vesicles and DMPC/PDA vesicles. The fluorescent marker 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiol-4-yl) [PE-(NBD)] was added to both components. *i*) Visible absorbance at 640 nm; *ii*) fluorescence intensity (536 nm) as a function of eluted fraction.

DMPC multilamellar vesicles, specifically 61 Å in the liquid crystalline state and 66Å at 20°C (16). The spacing of ~67Å in Fig. 3A(ii) is comparable to that observed for liquid crystalline phase DMPC multilamellar vesicles in the presence of 25 mol% cholesterol (16). It is interesting to note that there is a percolation threshold of the polymer framework at around 20 mol% PDA, in which a long-range conjugated network is formed. The plateau apparent in Fig. 3B, at which addition of PDA no longer influences the interlamellar spacing of DMPC most likely signifies the coexistence of distinct domains of phospholipids and polydiacetylene within the mixed vesicles.

Vesicle size analysis

The DSC and SAXS experiments presented in Figs. 2–3 employed multilamellar lipid/polymer suspensions that retain the bio-chromatic properties previously observed for sub-micron vesicles. Evidence that the phospholipid/polymer vesicles adopt distinct organization and morphologies compared with vesicles formed from the pure components is obtained from dynamic light scattering (DLS) and chromatography measurements shown in **Figs. 4** and **5**. The DLS data shown in Fig. 4 were recorded for sonicated vesicles of both pure PDA as well as mixed DMPC/PDA. The mixed DMPC/PDA vesicles are smaller on average [~110 nm] than the PDA particles (~190 nm). Clearly the association between the phospholipids and the polymer modifies the size distribution of the vesicles. TEM experiments [data not shown] have shown similar size distribution of lipid/PDA particles. Previous reports have demonstrated that the sizes and morphologies of PDA-based aggregates are indeed strongly dependent upon their composition (21).

In the size-exclusion chromatography analysis shown in Fig. 5, visible and fluorescence spectroscopies have been employed to probe the size differences of polymerized DMPC/PDA vesicles and pure DMPC vesicles, both containing the fluorescence marker PE-(NBD). The fluorescence spectrum in Fig. 5(ii) clearly demonstrates the existence of two populations. In particular, the coincidence between the first eluted peak in the fluorescence spectrum [Fig. 5(ii)] and visible absorbance at 640 nm [Fig. 5(i)] indicates that the DMPC is indeed associated with the PDA in the mixed vesicles, since the PE-(NBD) marker is incorporated within lipid assemblies and not the polymer. The appearance of two peaks in the elution profile confirms that the mixed DMPC/PDA vesicles are larger on average than the pure DMPC vesicles, thus are eluted faster from the column as depicted in Fig. 5.

Biophysical properties

The bilayer organization of the phospholipids in the mixed lipid/PDA vesicles, as inferred from the SAXS experiments, is an essential requirement for the utilization of the system for studying membrane processes. An important question, however, concerns the sensitivity of the platform and its colorimetric responses to the inclusion of different lipids and membrane species. In **Figs. 6** and **7**, we examine the physical and chromatic properties of PDA matrices containing various molecular components. Figure 6 depicts the ESR spectra of lipid/PDA aggregates incorporating 5-DS labeled stearic acid. Spin-labeled fatty acids and lipid probes have been extensively used as tools for probing the organization and local motion within lipid membranes (22–24). Previous ESR analysis has confirmed that 5-DS is incorporated within the lipid domains and not inside the PDA matrix (4). Furthermore, the 100:1 (phospholipid/spin-probe) molar ratio employed in these experiments does not alter the colorimetric properties and organization of the lipid/PDA vesicles.

Figure 6 shows the outer hyperfine splitting of 5-DS within several vesicle environments including pure DMPC (Fig. 6A), DMPC/PDA (Fig. 6B), cholesterol/DMPC/PDA (Fig. 6C), and cardiolipin/DMPC/PDA (Fig. 6D). The hyperfine splitting of hydrophobic spin-probes such as 5-DS is primarily affected by the mobility of the probe within the lipophilic membrane environment (22). Accordingly, the data presented in Fig. 6 demonstrate that the outer hyperfine splitting of the 5-DS ESR signal strongly depends upon the composition of the lipids associated with PDA. While the splitting in pure DMPC vesicles is around

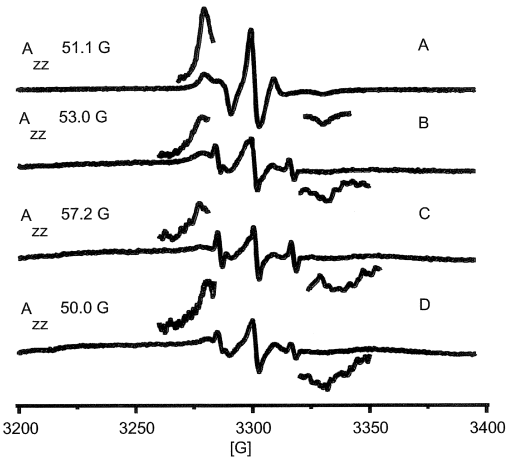

Fig. 6. Electron spin resonance (ESR) spectra of 5-DS incorporated within (A) DMPC vesicles, (B) DMPC-PDA (molar ratio 2:3), (C) cholesterol-DMPC-PDA (1:1:3), and (D) cardiolipin-DMPC-PDA (1:1:3). The hyperfine splittings calculated from the spectra are indicated.

51G (Fig. 6A), it significantly increases to ~53G in DMPC/PDA (Fig. 6B). Incorporation of cholesterol into the vesicles further reduces the fluidity with resultant splitting of around 57G (Fig. 6C), while in cardiolipin/DMPC/PDA the observed splitting is 50G indicating substantially higher mobility within the lipid domains (Fig. 6D).

Based on the ESR data in Fig. 6 we may consequently conclude that the fluidity of the lipid domains within the lipid/PDA assemblies is strongly affected by their molecular composition. Cholesterol, for example, reduces fluidity within the lipid moieties, as compared with DMPC/PDA, a result that is consistent with its known capacity to induce membrane consolidation (25, 26). Cardiolipin, on the other hand, is known to induce higher membrane fluidity (27), an effect that is clearly apparent in Fig. 6D. The ESR data therefore provide further evidence that the lipid domains exhibit biologically relevant behavior within the lipid/PDA aggregates, and complement the SAXS patterns shown in Fig. 3, which demonstrated the sensitivity of lipid ordering to molecular composition.

Further experiments have been carried out in order to determine the effect of lipid composition upon the chromatic properties of the vesicles. Figure 7 describes the dependence of colorimetric and dynamical properties of different vesicles upon temperature. Figure 7A features curves depicting the percentage colorimetric response (CR) induced by varying the temperature of solutions containing DMPC/PDA and cholesterol/DMPC/PDA vesicles. Higher CR values indicate more pronounced color transitions, i.e., more reddish appearance of the solutions (8). The data in Fig. 7A indicate that lipid composition clearly affects the thermal sensitivity (i.e., thermochromism) of the vesicles. The DMPC/PDA assemblies undergo stronger blue-red transitions as compared with cholesterol/DMPC/PDA vesicles. Furthermore, the stability and degree of polymerization of as-prepared cholesterol-containing vesicles is enhanced compared with DMPC/PDA vesicles. The insert above Fig. 7A shows a photograph of the blue parent lipid/PDA vesicle solution, and the color changes induced following heating the cholesterol/DMPC/PDA (purple) and DMPC/PDA (red) solutions to 50°C. The colorimetric data depicted in Fig. 7A are in agreement with the observed differences in fluidity (Fig. 6) and in the degree of ordering (Fig. 3) of the lipid assembly, and confirm that the presence of cholesterol confers increased rigidity to the colorimetric membrane-model, thus reducing its temperature sensitivity. Earlier studies of cholesterol in lipid vesicles have pointed to similar conclusions (24).

To further probe the mechanism of the thermochromic transitions occurring in the lipid/PDA particles, the effect of increased temperature was examined by ESR spectroscopy. The diagram depicted in Fig. 7B features the outer hyperfine splitting measured from the ESR spectra of the 5-DS spin-probe in DMPC/PDA and cholesterol/DMPC/PDA solutions at room temperature and after incubation of few minutes at 50°C. The results clearly show that the increased temperature induces higher membrane fluidity (i.e., smaller hyperfine splitting) in both assemblies, while

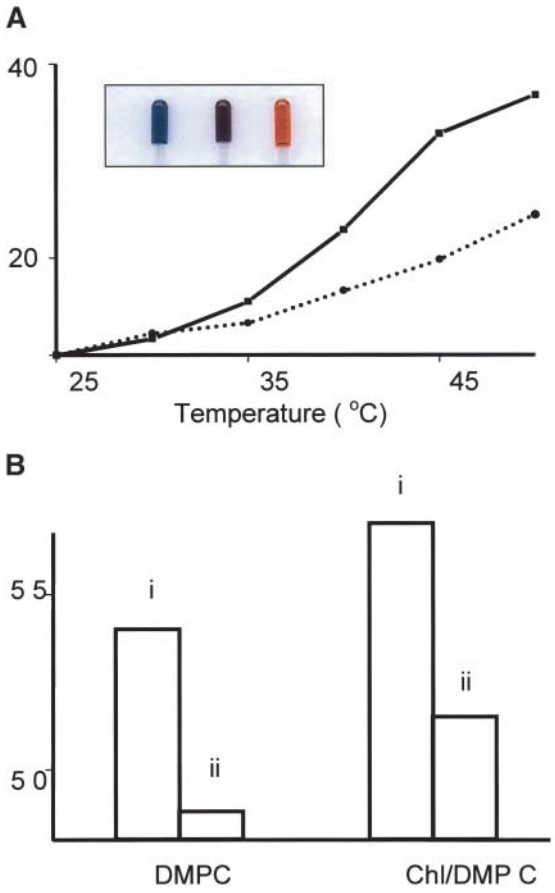

Fig. 7. A: Graph depicting the colorimetric response (CR) calculated from the visible absorption spectra of DMPC-PDA (molar ratio 2:3; solid line) and cholesterol-DMPC-PDA (molar ratio 1:1:3; broken line) as a function of the solution temperature. The insert shows a photograph of glass tubes containing the solution (either DMPC/PDA or cholesterol/DMPC/PDA) before heating (blue), cholesterol/DMPC/PDA at 50°C (purple), and DMPC/PDA at 50°C (red). B: Outer hyperfine splitting in the ESR spectra of 5-DS incorporated in DMPC-PDA vesicles (molar ratio 2:3) and cholesterol-DMPC-PDA (1:1:3), respectively. *i*) T = 25°C (room temperature); *ii*) T = 50°C.

the greater rigidity of the cholesterol-containing domains is retained. It is thus likely that the increased mobility of the lipid molecules is a primary factor responsible for the structural transformation of the adjacent polymer, resulting in the observed color changes. Earlier studies have also correlated changes of lipid fluidity in lipid/PDA assemblies with the observed colorimetric transitions (7).

The data presented in Figs. 6 and 7 indicate potential applications of lipid/PDA assemblies as probe for fluidity modifications within membranes. The colorimetric assay, in conjunction with other spectroscopic techniques, such as ESR fluorescence techniques and others, could provide insight into the effects of both membrane components as well as membrane-interacting substances upon lipid mobility. In that regard, an advantage of the colorimetric assay as a diagnostic tool is its robustness and the observation of rapid color transitions correlating with the fluidity alterations.

CONCLUSIONS

This work presents a biophysical characterization of a recently developed colorimetric lipid assay for the study of membrane properties. The colorimetric platform is based upon particles composed of conjugated polydiacetylene together with cellular membrane components, such as phospholipids or cholesterol. An important goal of this study has been to investigate structural features of the lipid/PDA particles and to determine whether these assemblies can indeed serve as valid models for cellular membranes. The experimental data indicate that the lipids and polydiacteylene most likely form interspersed, interacting microscopic phases. The phospholipids incorporated within the PDA matrix adopt a bilayer structure, the dominant lipid organization within cellular membranes. Additional results point to the contribution of changes in fluidity within the lipid domains in inducing the blue-red transitions.

The experiments described in this work shed light upon the effects of external environmental parameters, such as temperature, upon structural and dynamical properties of the organized lipid assemblies. The new colorimetric platform also facilitates elucidation of the contribution of distinct membrane components, such as cholesterol or cardiolipin, toward shaping membrane properties. This capability could open the way for application of the assay for detailed analyses of the roles played by particular molecules, such as glycolipids and proteins, in determining membrane functions and properties.

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