Imaging membrane processes in erythrocyte ghosts by surface fusion of a chromatic polymer

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Red blood cells (RBCs1 or erythrocytes) have been common targets for studying cellular membranes and for assessing the effects of biological and pharmaceutical compounds on human cells [1,2]. We present here a generic platform for microscopic visualization and spectroscopic analysis of membrane-perturbing events through incorporation of polymeric diacetylene (polydiacetylene, PDA) onto the membrane of erythrocyte ghost cells (resealed erythrocytes of which the hemoglobin interior was discarded [3]). PDA assemblies mimic lipid bilayers and also exhibit unique chromatic properties; following polymerization, PDA appears intense blue to the naked eye due to the conjugated (ene–yne) backbone [4]. The polymer can also undergo remarkable blue–red colorimetric changes following conformational transitions in the conjugated backbone, induced by external structural perturbations [4]. PDA also exhibits intriguing fluorescence properties; no fluorescence is emitted by the blue-phase PDA, whereas the transformed red-phase PDA fluoresces strongly at 560 and 640 nm [5]. PDA systems can also be employed as a biological tool; we have demonstrated that vesicles comprising phospholipids and PDA constitute a useful biomimetic platform for studying membrane processes [6–9]. Here we show that phospholipid/PDA vesicles can be fused onto the surface of ghost cells and that such assemblies can be used for studying membrane perturbations and morphological changes of erythrocyte ghosts.

Ghost cells were prepared according to conventional methods [3] and then incubated for 30 min at 25°C with dimyristoylphosphatidylethanolamine (DMPE)/dimyristoylphosphatidylglycerol (DMPG)/PDA (1.8:0.2:3.0 molar ratio) vesicles, constructed according to previously published methods [8,9]. The ghost–vesicle assemblies were then polymerized by short (10–20 s) irradiation at 254 nm. The resulting solution exhibited a strong blue appearance. A high degree of lipid fusion between the vesicles and the ghost membrane was confirmed through application of fluorescence resonance energy transfer (FRET) (data not shown).

Fig. 1 depicts microscopy experiments designed to examine whether interactions with membrane-active pharmaceutical substances and modifications of the ghost surface induce localized fluorescence transformations of the membrane-incorporated PDA. Fig. 1 presents differential interference contrast (DIC) (top palettes) and fluorescence (bottom) microscopy images of the effects of several membrane-active compounds on the ghost cells. The as-prepared PDA-labeled ghosts did not emit any fluorescence since the unperturbed polymer exists in the initial blue phase—which is non-fluorescent [5] (Fig. 1A). Note that the circular shape of the erythrocyte ghost, apparent in the phase-contrast microscopy picture, was not affected by surface incorporation of the polymer patches (Fig. 1A).

The microscopy images in Fig. 1(B–D) clearly demonstrate that addition of molecules that interacted with the cell membrane and affected its structure induced fluorescence from the PDA patches on the ghost surface. Furthermore, the appearance of fluorescent domains in the PDA-labeled ghosts was different for each of the three compounds tested (Fig. 1B–D). Pyridoxal, a slow cell-penetrating pharmaceutical substance [10], gave rise to weakly-fluorescent patches on one side of a PDA-labeled ghost—for which the typical spherical appearance was altered (Fig. 1B). Other unperturbed parts of the ghost surface did not emit fluorescence.

Imipramine, on the other hand, an amphiphilic antidepressant drug causing membrane destabilization [11], induced severe membrane disruption at a much lower concentration, leading to intensely fluorescent domains localized at the disrupted membrane of the ghost cell (Fig. 1C). Quinidine, a Na⁺ channel inhibitor [12], also gave rise to fluorescent PDA domains distributed upon the ghost surface (Fig. 1D). Similar to the other compounds tested, the fluorescence induced by quinidine seems to track areas in which the cell surface was altered. The concentrations employed in the microscopy experiments depicted in Fig. 1 were similar to biologically-active values reported in the literature.

In addition to fluorescence microscopy imaging, the new PDA-labeled ghost platform could be exploited for colorimetric screening of membrane interactions (Fig. 2). Fig. 2A depicts the aqueous suspension of the polymer-labeled ghosts placed in wells of a standard 96-well plate. The blue appearance of the control suspension (left well in Fig. 2A) corresponds to the conjugated framework of the surface-incorporated PDA. The addition of quinidine (0.45 mM) to the PDA-labeled ghosts induced a clearly visible purple color (middle well, Fig. 2A), whereas imipramine (45 μM) gave rise to a reddish suspension (Fig. 2A, right well).

Visible spectroscopic analysis (Fig. 2B) further attests to the bioanalytical capabilities of the new PDA-labeled ghosts for colorimetric screening of membrane-active compounds. A particularly important goal of carrying out the experiments shown in Fig. 2B was to explore whether the colorimetric transformations in the new erythrocyte system indeed corresponded to membrane interactions and/or surface perturbations within the ghost cell or whether the color changes were induced by nonspecific direct binding of the interacting compounds with the surface-attached polymeric vesicles (the latter obviously would be less interesting from a biological point of view).

Fig. 2. Colorimetric data. (A) Scanned picture of an aqueous suspension of PDA-labeled ghost cells before (left well) and after the addition of 0.45 mM quinidine (middle well) or 45 μM imipramine (right well). (B) Visible absorbance spectra of PDA-labeled ghosts before (solid line) and 0.5 h after (broken line) the addition of 3 mM DMPE vesicles. (C) Cell-free DMPE/DMPG/PDA vesicles before (solid line) and 0.5 h after (broken line) the addition of 3 mM DMPE.

Fig. 2B depicts the colorimetric transformations induced by mixing the PDA-labeled ghost cells with multilamellar vesicles of DMPE that are expected to undergo fusion with the ghost membrane bilayer [13]. The visible absorbance spectra in Fig. 2B demonstrate that the addition of the
DMPE vesicles to the PDA-labeled ghosts increased the intensity of the signal at around 520 nm (hence a red appearance of the PDA-labeled ghost suspension). This spectral change stands in contrast to the apparent absence of colorimetric response when DMPE was added to a cell-free suspension of DMPE/DMPG/PDA vesicles (i.e., polymerized phospholipid/diacetylene vesicles prepared without the presence of ghost cells (Fig. 2C). This result indicates that interactions with the erythrocyte membrane, rather than nonspecific interactions between the soluble analyte (here DMPE) and the surface-fused chromatic vesicles, play the pivotal role in inducing the colorimetric transitions.

The fluorescence and colorimetric experiments depicted in Figs. 1 and 2 demonstrate the use of the new polymer-labeled ghost cells for studying physiological processes such as surface interactions of pharmacological compounds and membrane fusion. Specifically, the fluorescence microscopy images in Fig. 1 showed that correlation exists between the induction of fluorescence within the membrane-incorporated PDA patches and the occurrence of localized surface alterations of the ghost cell. The appearance and distribution of fluorescent areas in the PDA-labeled ghosts varied among the three compounds tested, but overall they evidently were related to local membrane disruption and structural changes of the cell surface (Fig. 1B).

The visual image and visible spectroscopy data in Fig. 2 point to the use of the colorimetric properties of the PDA-labeled ghost system for gleaning information on membrane interactions in ghost cells. The color image in Fig. 2A also demonstrates the feasibility of using the new system in conventional high-throughput formats. The distinct colors observed by the naked eye after the addition of the pharmacological compounds to the PDA-labeled ghosts (Fig. 2A) most likely correspond to the different mechanisms of membrane disruption affected by the compounds, specifically the extent of bilayer structural modification by the compounds [5–9]. The colorimetric data attest to the simplicity and usefulness of the assay as a bioanalytical tool.

In summary, we have constructed new chemically engineered erythrocyte ghosts through the attachment of chromatic polydiacetylene patches onto the cell membrane. These hybrids facilitated visualization and investigation of cell surface processes. The new chromatic ghost platform is generic by nature and conceptually different from other cellular imaging techniques. In essence, the fluorescent/colorimetric polydiacetylene patches do not report on specific biomolecular targets within the cell surface but rather respond to processes and membrane interactions that give rise to structural and dynamic modifications of the membrane. This approach makes possible microscopic imaging and quantitative spectroscopic analyses of biological events occurring on actual membranes rather than on model bilayer systems. The PDA-labeled ghosts are robust and can be maintained for long time periods. The new platform could find wide applicability in studying membrane fusion processes and high-throughput drug screening and in deciphering biochemical processes affecting the shapes and morphologies of erythrocytes.

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References