Membrane Fluidity Measurements Using UV Fluorescence Polarization and the POLARstar Omega

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- Membrane fluidity is measured in both liposomes and mitochondrial membranes via fluorescence polarization
- UV FP optics are used on the POLARstar Omega to measure DPH (1,6-diphenyl-hexa-1,3,5-triene) fluorescence
- The effects of dibucaine and propranolol on membrane fluidity are measured

Introduction

Membrane fluidity and other properties of lipids are commonly assessed using various fluorogenic membrane probes and fluorescence polarization (FP) measurements. The basic principle is that alterations in lipid packing (e.g. temperature-dependent lipid phase transitions) change the mobility of a membrane-bound fluorophore. The latter parameter (specifically, "rotational relaxation") can be monitored by exciting the fluorophore with a polarized light and measuring the emitted light in two planes - parallel and perpendicular to the polarization plane of the excitation light. FP is defined as the following ratio: $I_{\parallel} - I_{\perp} / I_{\parallel} + I_{\perp}$, where $I_{\parallel}$ and $I_{\perp}$ are fluorescent intensities measured in the parallel and perpendicular channels, respectively.

Membrane fluidization increases mobility of the dye and decreases the intensity of the emitted parallel component. Accordingly, FP is reciprocally related to the membrane fluidity. DPH is one of the classical probes used for FP measurements both in native membranes and artificial membrane systems (such as synthetic liposomes)[1]. Optical characteristics of DPH strongly depend on the environment; the dye is almost non-fluorescent in aqueous solutions, while binding to the hydrophobic region of the membrane results in a sharp increase in the fluorescence signal (with an excitation maximum in UV range). In addition to the standard FP optics provided with the POLARstar Omega, BMG LABTECH offers an optional pair of UV FP optics suitable for probes with excitation maxima less than 400 nm. This note describes the use of these optics for measurements of relative changes in lipid fluidity of DPH-labeled membranes.

Fig. 1: BMG LABTECH's POLARstar Omega multidetection microplate reader.

Materials and Methods

☐ Phospholipids from Avanti Polar Lipids
☐ Mitochondria isolated from rat liver[2]
☐ Opaque-walled, clear bottom 96-well plates from Costar
☐ POLARstar Omega microplate reader from BMG LABTECH, equipped with UV FP optics

Membrane fluidity measurements were performed in liposomes and isolated mitochondria. Samples were placed in an opaque-walled, clear-bottomed 96-well plate. Sample volume was 100 µl. A 355 nm excitation filter and two identical 430 nm emission filters were installed in the corresponding filter wheels (emission filters were positioned 180° apart).

To validate the method, FP measurements were taken using liposomes with defined gel-to-liquid crystalline phase transition temperature. Unilamellar liposomes were prepared from 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (MPPC). The transition temperature for this lipid is in the 35 - 37°C range (according to Lipid Data Bank, http://www.caffreylabs.ul.ie/). For fluorescent labeling, MPPC liposomes were pre-incubated with 10 µM DPH (Sigma) at 45°C for 30 min in a KCl-based buffer (150 mM KCl; 10 mM HEPES, pH 7.4; 2 mM EGTA). Steady-state FP measurements were taken at 25 - 45°C. Temperature on the Omega's incubator was increased by 2°C and FP measurements were taken after equilibrating the samples for 10-15 minutes. Data were acquired in endpoint mode.

A more complex model was investigated, native mitochondrial membranes. Isolated rat liver mitochondria (0.2 mg/ml) were preincubated with 10 µM DPH at room temperature for 40 minutes. Temperature in the Omega's incubator was increased by 2°C and the samples were equilibrated for 10 minutes at each given temperature before FP measurements were taken. Mitochondria used in these experiments were in a deenergized (non-respiring) state. Lastly, the effects of membrane active drugs dibucaine and propranolol on membrane fluidity in liposomes and mitochondria were determined.

Liposomes were formed from the following lipid mixture mimicking mitochondrial membranes: 47% phosphatidylcholine (PC), 28% phosphatidylethanolamine (PE), 9% phosphatidylinositol, 9% phosphatidylserine (PS), and 7% cardiolipin (CL). Data were acquired in kinetic mode.

Results and Discussion

As shown in Figure 2, increasing the temperature from 25 to 35°C did not change the FP values in MPPC liposomes. This reflects the expected high ordered gel state of the lipid in this temperature range. Increasing the temperature from 35 to 39°C results in a sharp, large-scale decrease in FP values corresponding to the phase transition. In the liquid phase, FP values continued to decline as the temperature increased to 45°C. The apparent transition temperature of ~37°C agrees well with earlier calorimetric measurements on phase-transition in this lipid system[3].

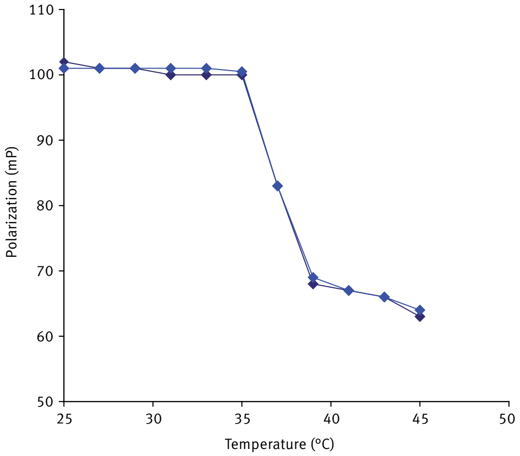

Fig. 2: Effect of temperature on FP measured in DPH-labeled MPPC liposomes. Target FP value was set to 100 mP for the DPH-labeled liposome sample equilibrated at 25 °C. Data shown are replicate samples. Note the onset of the lipid phase transition at the temperature above 35 °C.

Unlike MPPC vesicles, no rigid (temperature-insensitive) state or large-scale phase transitions were observed in the mitochondrial membranes (Fig. 3). A gradual decrease in FP in the 25 - 45°C temperature range is seen, which is consistent with previously published data on membrane fluidity measurements in DPH-labeled deenergized mitochondria[4]. Relative FP values in whole mitochondria and purified outer mitochondrial membranes (OMM) were also compared. The patterns of temperature-dependence were similar in both membrane systems, but the relative FP values in OMM were significantly higher than those obtained in whole mitochondria (Fig. 3). This result can be readily explained by the higher cholesterol level in the OMM compared to the inner mitochondrial membrane[5].

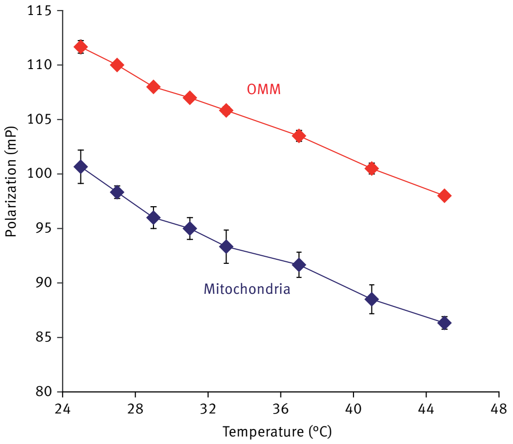

Fig. 3: Effect of temperature on FP measured in DPH-labeled isolated mitochondria and purified outer mitochondrial membranes (OMM). Target FP value was arbitrary set to 100 mP for the DPH-labeled mitochondrial sample equilibrated at 25°C. Data shown are average of 3 replicates.

Finally, the effects of propranolol and dibucaine on liposomes and isolated mitochondria were examined. Depending on concentrations, these drugs can increase membrane fluidity[6,7] or rigidify some membranes[8]. They are also known to have multiple effects on mitochondrial function. Figure 4 shows that the addition of propranolol to liposomes (panel A) results in a rapid decrease in relative FP values. A similar effect was observed in isolated mitochondria (panel B). The propranolol-induced decrease in FP was smaller in comparison to the dibucaine-induced changes, but nonetheless was readily detected. The concentrations of the drugs (40-80 µM) were in the same range as those affecting mitochondrial activities[9]. The data suggest that some of the effects of the drugs on mitochondrial function may be due to an increase in membrane fluidity.

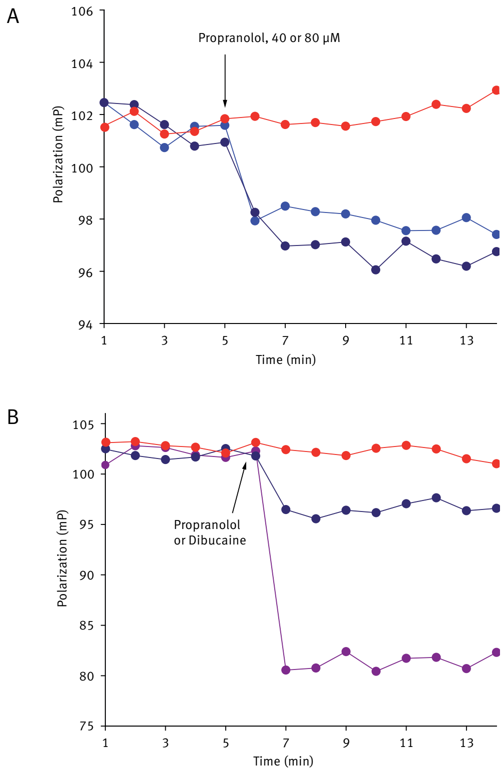

Fig. 4: Effect of propranolol and dibucaine on FP measured in DPH-labeled PC/PE/PS/PI/CL liposomes (A) and isolated mitochondria (B). Arrows indicate the addition of drug - 40 µM propranolol (light-blue line), 80 µM propranolol (dark-blue line), 80 µM dibucaine (purple line), control (red line). Red lines – control samples. Incubation conditions are the same as in Fig. 2 and 3.

Conclusion

This study demonstrates the suitability of the POLARstar Omega microplate reader with UV FP optics for measurements on membrane fluidity in a microplate-based format. The method allows for the monitoring of large scale alterations in membrane fluidity (such as phase transitions), as well as more subtle changes in lipid dynamics. This approach can be used as a high-throughput screening assay in the search for compounds altering lipid fluidity.

References

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