Application Note
FP-0004

Fluorescence Depolarization Measurement of a Liposome

Introduction

Fluorescence depolarization or fluorescence anisotropy can be observed when a fluorophore emits light of different intensities depending on the axes of polarization. When a molecule is excited by the absorption of light from its ground state to a transition state and relaxes to a stable vibrational state, it will then return back to its ground state by radiating fluorescence light. In this process there is a directionality, described by a transition dipole moment and determined by molecular structure, which is closely related to the direction of polarization of the excitation and fluorescence light.

FP-6500 Spectrofluorometer

For example, when fluorescence is emitted before a molecule rotates, the fluorescence light will be strongly polarized towards the direction of the excitation light's polarization. If the light is emitted after the rotation of the molecule in a completely random direction, the fluorescence will be no longer polarized.

When measuring fluorescence polarization, the following factors will affect the molecular movement: (1) molecular size, (2) viscosity of the molecule's environment, and (3) strength and degrees of freedom of a bound molecule. Larger molecules and more viscous or populated conditions require more energy in order for a molecule to move.

This application note will demonstrate the use of the FP-6500 spectrofluorometer and its automatic polarizers to monitor the temperature-dependent polarization of diphenyl hexatriene (DPH) upon addition to a lipid bilayer.

Keywords

FP-6500, Fluorescence, Polarization, ADP-303

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications

Measurement Conditions			
Excitation Wavelength	357 nm	Emission Wavelength	430 nm
Excitation Bandwidth	3 nm	Emission Bandwidth	3 nm
Response Time	2 sec	Data Interval	0.1 °C
Temperature Gradient	20°C/hr	Measurement Mode	Fluorescence

Experimental

The fluorescence polarization spectrum can be measured using the automatic polarizers. By placing the excitation polarizer in the vertical position and the emission polarizer in the horizontal and vertical positions, both fluorescence intensities, I_{VH} and I_{VV}, are measured, respectively. In order to correct for the sensitivity difference of the emission detector, the spectrum of rhodamine B is measured by setting the excitation polarizer in the horizontal position and setting the emission polarizer in the vertical and horizontal positions. The instrument grating factor is then defined as the following:

$$G = \frac{I_{VV}}{I_{VH}}$$

and multiplied by I_{VH} to obtain an instrumental correction factor for the emission optics. The degree of polarization (P) is given by the following equation:

$$P = \frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + G \cdot I_{VH}}$$

Results

Figure 1 shows the temperature-dependent fluorescence anisotropy of 0.6 µM of DPH in 130 µM of phosphatide. The spectrum indicates that DPH is oriented between the lipid bilayer and is restricted from movement at a low temperatures. The phase transition of the membrane at 40°C reorients the membrane, allowing DPH to move freely and decreasing the polarization.

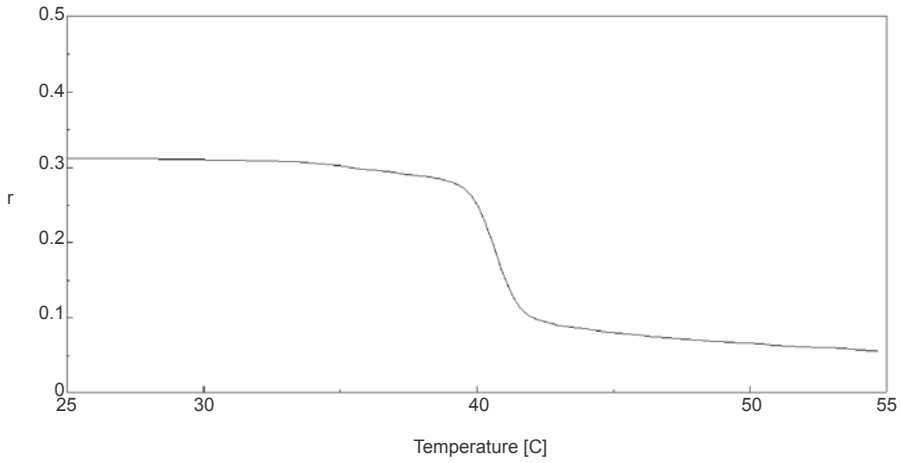

Figure 1. Fluorescence polarization measurement of DPH added to phosphatide as a function of temperature.

Conclusion

This application note demonstrates that the FP-6500 can be used with automatic polarizers to obtain information regarding the mobility of fluorophores under different environmental conditions.

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications