Application Note

FP-0004

Fluorescence depolarization measurement for Liposome by using FP-6500

1. Fluorescence polarization and depolarization

The molecule is excited (S_0 --> S_1) by the absorption of light from ground state to transition state and stabilized in the most stable vibrational state by its molecular relaxation process, and then it returns again (S_1 --> S_0) to the ground state by radiating fluorescence light.In the process of S_0 --> S_1 or, S_1 --> S_0, there is a directionality (transition moment), determined by molecular structure, that is closely related to polarization direction of excitation and fluorescence light.

(1)Absorption process: With exciting by linearly polarized light, probability of excitation of each molecule is expressed as below.
$P(\alpha) = P_0\cos^2\alpha$ (α: Angle between polarization direction and transition moment, P_0: proportional constant)
When vector of electric field of excitation light and transition moment are in parallel condition, theprobability of excitation will become maximum.
(2) Radiation process: Fluorescence in the process of S_1 --> S_0 has polarizing direction in conformity with the direction of transition moment. Fluorescence light from the molecule excited by polarized light has polarization property depending on the polarizing direction of excitation light. Moreover, such polarization property of the fluorescence light will be affected also by the rotation of the molecule due to Brownian motion, etc. until the fluorescence is radiated. In other words, when fluorescence is radiated before the rotation of its molecule, fluorescence light will be strongly polarized towards direction of the polarization of excitation light, but on the other hand, when fluorescence is radiated after rotation of its molecule in completely random direction, the fluorescence light will be no longer polarized. This is called “depolarization”.

2. Measurement method and measured example for fluorescence depolarization

In order to measure the fluorescence depolarization by FP-6500 spectrofluorometer, automatic polarization measurement unit and its software are needed.
By setting the Excitation (Ex) side polarizer in the vertical position(V), both fluorescence intensities(I_{VV} and I_{VH}) are measured by setting the fluorescence(Em) side polarizer in the horizontal position(H) and V. In order to correct the sensitivity difference of the Em side detector against polarization, the spectrum is measured by setting the Ex side polarizer in H position and setting the Em side polarizer in V and H position to obtain instrumental function, $G(=I_{HV}/I_{HH})$, and the correction is applied multiplying I_{VH} by G factor. Accordingly, each parameter is expressed by the following formula.

Total fluorescence intensity (F): $I_{VV} + 2G \cdot I_{VH}$

Fluorescence anisotropy (r): $\dfrac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2G \cdot I_{VH}}$

Polarization degree (P): $\dfrac{I_{VV} - G \cdot I_{VH}}{I_{VV} + G \cdot I_{VH}}$

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

As an example, measurement was made using 0.22 mg/L rhodamine B in glycerin solution.

[Measurement conditions]

Automatic polarization measurement unit:	ADP-303 Water-cooled Peltier thermostatted automatic polarization measurement unit.
Measurement mode:	Fluorescence
Excitation wavelength:	550 nm, Emission
Wavelength:	600 nm
Excitation bandwidth:	5 nm
Emission bandwidth:	5 nm
Response:	0.1 sec
Measurement temperature:	25 degrees C

Chart 1 Fluorescence polarization of 0.22 mg/L rhodamine B in glycerin solution

No.	I_{VV}	I_{VH}	G	F	P	r
1	244.1098	30.737	2.833	418.2683	0.4741	0.3754
2	244.3634	30.7426	2.833	418.553	0.4745	0.3757
3	243.714	30.6777	2.833	417.5363	0.4743	0.3755
4	243.3629	30.6519	2.833	417.0391	0.474	0.3753
5	242.9584	30.5799	2.833	416.2262	0.4743	0.3756
Ave.	243.7017	30.6778	2.833	417.5246	0.4742	0.3755
S.D.	0.5639	0.067	0	0.9398	0.0002	0.0002
C.V.	0.2314	0.2184	0	0.2251	0.0353	0.0419

3. Fluorescence polarization measurement of Liposome

Fluorescence polarization depends on the time from excitation of the molecule to its radiation and on how long polarization property of the initially excited molecule is kept without some movement of the molecule. Therefore, in other words, by measuring the degree of fluorescence polarization, it can be known how difficult the molecule can move. The factors related to molecule movement are as follows.

(1) Molecular size (The larger molecule is harder to move.)
(2) Viscosity of environment where molecule exists (In higher viscous condition, molecule is harder to move.)
(3) Strength and degrees of freedom of molecule in bondage.

The above factors are taken into consideration mainly for measurement of biological samples, and especially are utilized for analysis of the size and conformation change for biological polymer and the local conformation. Here is a result of polarization degree measured under the condition that diphenyl hexatriene (DPH) is added to liposome(lipid bilayer) that is generated by dispersion of the phosphatide in water and the temperature is changed from 25 degrees C to 55 degrees C.

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

[Measurement conditions]

Automatic polarization measurement unit:	ADP-303 Water-cooled Peltier thermostatted automatic polarization measurement unit.
Excitation wavelength:	357 nm
Emission wavelength:	430 nm
Excitation bandwidth:	3 nm
Emission bandwidth :	3 nm
Response:	2 sec
Data import interval:	0.1 degrees C
Temperature gradient:	20 degrees C/hour
Phosphatide:	130 µM
DPH:	0.6 µM

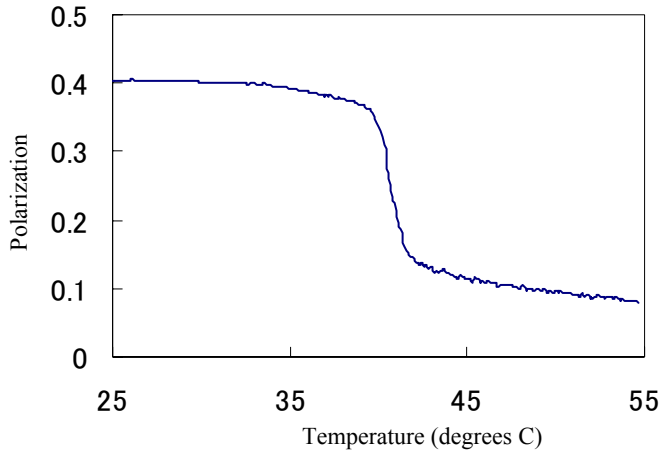

Figure 1 Temperature gradient measurement for fluorescence polarization of DPH added to liposome

DPH is held in oriented form between lipid bilayer and is restricted to move at a low temperature, but when membrane drastically flickers by the phase transition of lipid bilayer at 40 degrees C, the movement of DPH is activated, and depolarization is clearly observed.

JASCO INTERNATIONAL CO., LTD

4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512