FLUORESCENCE ANISOTROPY STUDIES ON BODIPY (PYRROMETHENE 546) DYE AS A NOVEL THERMAL PROBE

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1. INTRODUCTION

Fluorescence anisotropy is a well-known technique in the field of biological science, where researchers use this method to find the folding states of proteins [1]. In our study, we want to use fluorescence anisotropy to investigate the fluid temperature in a microchannel using a temperature-sensitive fluorophore. Solution's temperature is measured using fluorescence anisotropy r based on the combination of Stokes-Einstein and Perrin's equation [2], given by:

$$r = \frac{r_0}{1 + \frac{\tau k_B T}{V \eta}}, \tag{1}$$

where r_0 is the limiting anisotropy (anisotropy without any molecular rotation, also known as freezing anisotropy), τ is the fluorescent lifetime, k_B is the Boltzmann constant, T is the temperature of the solution, V is the molecular volume, and η is the solution viscosity, respectively. In the present work, BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dye has been employed as a working probe because BODIPY has many advantages over other commercially available dyes like fluorescein; for example, high extinction coefficient, high fluorescence quantum yield, and high pH stability [3]. Therefore, BODIPY has a potential to be a good temperature probe for the solutions. Two types of BODIPY dyes are used in this work, one is $C_{14}H_{17}BF_2N_2$ (or pyrromethene 546), and the other is $C_{22}H_{33}BF_2N_2$ (or pyrromethene 597). The chemical structures of these dyes are shown in Fig. 1.

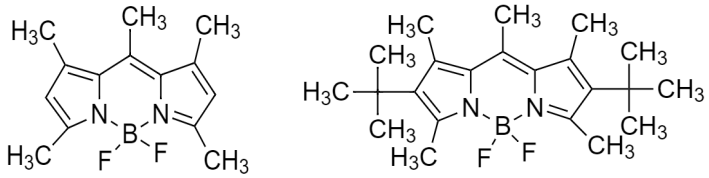

Fig. 1 Chemical structures of $C_{14}H_{17}BF_2N_2$ (pyrromethene 546; left), and $C_{22}H_{33}BF_2N_2$ (pyrromethene 597; right).

The aim of this work is to reveal whether these BODIPY dyes are available as a temperature probe using the fluorescent anisotropy. In this work, the temperature-dependent fluorescence properties of pyrromethene 546 were investigated. Additionally, since Pyrromethene 546 and pyrromethene 597 have a difference of -8CH_2 groups, it was also investigated whether -8CH_2 groups affect fluorescence properties or not.

2. EXPERIMENTAL PROCEDURE

BODIPY dye ($C_{14}H_{17}BF_2N_2$ or pyrromethene 546) was mixed in dimethyl sulfoxide (DMSO) with a concentration of 10 mM (hereinafter "pyrromethene 546 solution"). After this, pyrromethene 546 solution was diluted with methanol with a ratio of 1:1000, and then fluorescence properties were measured. Another BODIPY dye ($C_{22}H_{33}BF_2N_2$ or pyrromethene 597) was also used to compare the fluorescence properties when we increase -8CH_2 groups. Pyrromethene 597 in DMSO is thereinafter called as "pyrromethene 597 solution". Fluorescence anisotropy, emission spectra, and absorption spectra were measured using both spectrofluorometer and spectrophotometer, respectively.

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3. RESULTS AND DISCUSSION

Figure 2(a) shows the absorption and emission spectra of pyrromethene 546 and pyrromethene 597 solutions in MeOH, while Fig. 2(b) shows the fluorescence anisotropy for the same.

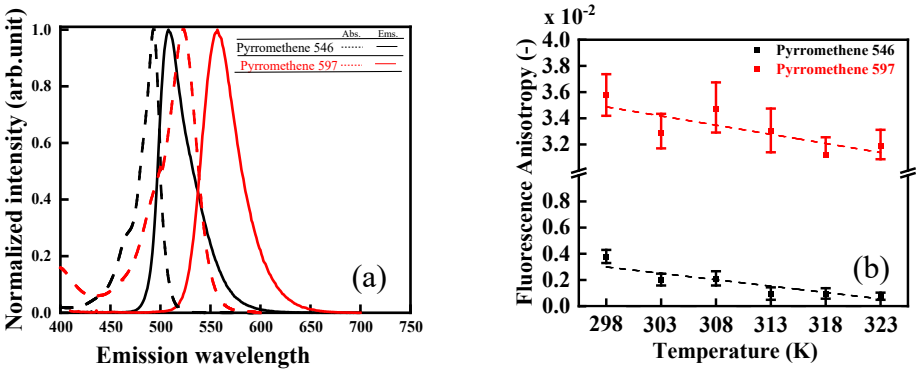

Fig. 2 (a) Absorption and emission spectra and (b) fluorescence anisotropy of pyrromethene 546 and pyrromethene 597 solutions in MeOH.

From Fig. 2, it is seen that -8CH_2 groups affect fluorescence properties. Pyrromethene 597 has high emission and absorption wavelength. Apart from this, pyrromethene 597 shows fluorescence anisotropy, one-order higher than pyrromethene 546. The relationship between fluorescence anisotropy r and polarization p is indicated as [2]:

$$r = \frac{2p}{(3-p)} \tag{2}$$

It is shown by Tatsumi et. al., that higher value of polarization is more preferred for temperature measurement [4]. Since polarization and anisotropy are related using Eq. (2), it can be said that higher value of anisotropy is also preferred. The linear fitting (shown by dash line) of anisotropy shown in Fig. 2(b) shows that the overall trend is: as the temperature increases, anisotropy decreases; with the larger dependence of pyrromethene 597 (dr/dT = -1.39x10^{-4} K^{-1}) on temperature as compared to pyrromethene 546 (dr/dT = -9.76x10^{-5} K^{-1}). Hence, it is possible to say that the temperature sensitivity of pyrromethene 597 on temperature is also good. From these facts, pyrromethene 597 could be useful and reliable as a temperature probe for solutions.

4. CONCLUSION

In the present work, the fluorescence properties of BODIPY dye of pyrromethene 546 was investigated and they were compared with that of pyrromethene 597. From measurement results, it is clear that -8CH_2 groups affect the fluorescence properties greatly. Hence, we succeeded to indicate that the BODIPY dye of pyrromethene 597 would be a good temperature probe.

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