Photophysical Properties of 2-Hydroxytryptanthrin Analog as a Near-Infrared Dye for Fluorescent Imaging

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2-Hydroxytryptanthrin analog (2-(2-hydroxy-12-oxoindolo[2,1-*b*]quinazolin-6(12*H*)-ylidene)malononitrile, **DCM-T2OH**) was synthesized as a near-infrared (NIR) dye for fluorescent imaging. In high-polarity aprotic solvents (acetonitrile, *N,N*-dimethylformamide, and dimethyl sulfoxide), protic solvents (methanol and ethanol), and an aqueous solution containing 20% acetonitrile, both the absorption and fluorescence bands of **DCM-T2OH** were observed at the NIR region (650–900 nm), which is referred to as the “optical window” of cells and tissues.

Key words: Near-infrared fluorescence, Fluorescent dye, 2-Hydroxytryptanthrin

1. INTRODUCTION

Fluorescent analysis reagents such as fluorescent chemosensors and fluorescent probes that are based on organic fluorescent dyes are simple and highly versatile and in particular, they have become indispensable as molecular tools for understanding biological phenomena in life sciences [1, 2]. We have been exploring the photophysical properties of tryptanthrin derivatives as novel fluorescent dyes [3–10]. Tryptanthrin (**T**, Fig. 1a) is a weakly basic alkaloid present in several plant species [11] and shows antimicrobial activity against diverse pathogenic bacteria and fungi [12]. When an amino group or another electron-donating substituent is introduced at the 2-position of tryptanthrin, intramolecular charge transfer (ICT) occurs between the electron-withdrawing carbonyl group at its 6-position and the amino group resulting in strong fluorescence and positive fluorescence solvatochromism [3a, 5, 9]. The fluorescence wavelengths of the fluorescent solvatochromic dyes vary according to the surrounding environment (polarity difference); therefore, a variety of life phenomena can be observed via the fluorescent color change. The fluorescent tryptanthrin derivatives have widespread potential applications in diverse fields such as labeling reagents, chemosensors, laser dyes, photosensitizers, and fluorescent organic devices. These dyes must be able to absorb and emit at long wavelengths for their various applications. We expect them to be of particular interest as organic fluorescent dyes for fluorescent imaging probes in biological research. The near-infrared (NIR) region (650–900 nm) is referred to as the “optical window” of cells and tissues because of the lack of efficient endogenous absorbers in this spectral range and the subsequent high penetration depth (of the order of a few millimeters) in most tissues [13]. Organic fluorescent dyes that emit NIR fluorescence are preferred for applications in biological systems because they can reduce auto-fluorescence and photodamage to living cells. Previously, 2-hydroxytryptanthrin (**T2OH**, Fig. 1b) was synthesized as a NIR dye for fluorescent imaging [6]. The absorption maxima ($\lambda_{a, \max}$) of **T2OH** in a pH range from 1.3 to 7.2 and from 8.5 to 10.6 were *ca.* 410 nm and *ca.* 495 nm, respectively. Regardless of the pH range, the

fluorescence maximum ($\lambda_{f, \max}$) was *ca.* 660 nm. The $\lambda_{f, \max}$ was in the optical window of 650 nm or more, but the $\lambda_{a, \max}$ was 500 nm or less. Therefore, we planned to shift the $\lambda_{a, \max}$ and $\lambda_{f, \max}$ of **T2OH** to longer wavelengths by accelerating the ICT. We synthesized 2-hydroxytryptanthrin analog (2-(2-hydroxy-12-oxoindolo[2,1-*b*]quinazolin-6(12*H*)-ylidene)malononitrile, **DCM-T2OH** (Fig. 1c), in which the dicyanomethylene group was a better electron acceptor than the carbonyl group of **T2OH** as a NIR fluorescent dye for fluorescent imaging. In our study, we report on the spectral characteristics of **DCM-T2OH**.

Fig. 1 Structural formulas of (a) **T**, (b) **T2OH**, and (c) **DCM-T2OH**.

2. EXPERIMENTAL

2.1 Synthesis

The synthesis of **DCM-T2OH** was performed as follows (Scheme 1) [14]. **T2OH** [6] (0.1013 g, 0.3836 mmol) was dissolved in 5 ml of dimethyl sulfoxide (DMSO) and malononitrile (0.1336 g, 2.022 mmol) was added into the solution. After adding two drops of concentrated hydrochloric acid, the mixture was stirred for 6 h at 90°C under an argon atmosphere. The reaction mixture was poured into ice/water, and the crude product was collected. Column chromatography on silica gel with

ethyl acetate/dichloromethane (1/4, v/v) as the eluent yielded pure **DCM-T2OH** as a red solid (0.0856 g, 71.50%). The electron-spray ionization (ESI) mass spectrum of **DCM-T2OH** showed the corresponding molecular ion peak, and the proton NMR spectrum showed that the compound was correctly synthesized.

DCM-T2OH: ^1H NMR (500 MHz, CDCl_3) δ 6.19 (1H, s, OH), 7.34 (1H, dd, H_3 , $J = 3.0$ Hz, $J = 8.6$ Hz), 7.45 (1H, t, H_8 , $J = 7.6$ Hz, $J = 8.2$ Hz), 7.76 (1H, ddd, H_9 , $J = 1.2$ Hz, $J = 7.6$ Hz, $J = 8.4$ Hz), 7.79 (1H, d, H_1 , $J = 3.0$ Hz), 7.92 (1H, d, H_4 , $J = 8.6$ Hz), 8.47 (1H, d, H_7 , $J = 8.2$ Hz), 8.60 (1H, d, H_{10} , $J = 8.4$ Hz); ESI-MS m/z ($\text{M}+\text{H}$) calcd 313.0726, found 313.0591.

2.2 Measurements

Stock solutions of **DCM-T2OH** were prepared by dissolving a weighed amount of each compound in different solvents. The ultraviolet and visible UV-vis spectra (between 300 nm and 900 nm) of the resulting solutions were recorded at room temperature with a Jasco V-670 spectrophotometer. The fluorescence spectra were measured between 450 nm and 850 nm with a Hitachi F-4500 fluorometer using an excitation wavelength (λ_{ex}) at the absorption band on the longest wavelength side. The concentration of all the samples was 10 μM ($\text{M} = \text{mol dm}^{-3}$), and no intermolecular interactions were observed.

3. RESULTS AND DISCUSSION

3.1 Absorption and fluorescence spectra of **DCM-T2OH**

Absorption spectroscopy and fluorescence spectroscopy were performed using low-polarity aprotic solvents, high-polarity aprotic solvents, and protic solvents. The low-polarity aprotic solvents used were 1,4-dioxane (DOX, 2.10), toluene (PhMe, 2.43), chloroform (CF, 4.89), and dichloromethane (DCM, 9.02). The numerical values in parentheses were the relative permittivities (ϵ). The high-polarity aprotic solvents used were acetonitrile (MeCN, 36.00), dimethyl-formamide (DMF, 37.06), and dimethyl sulfoxide (DMSO, 47.00). The protic solvents used were ethanol (EtOH, 25.00) and methanol (MeOH, 32.35). The absorption and fluorescence spectra of **DCM-T2OH** and the photophysical properties of **DCM-T2OH** are shown in Fig. 2, 3 and Table 1. In the low-polarity aprotic solvents, absorption bands of **DCM-T2OH** were observed at *ca.* 500 nm, as shown in Fig. 2a. These absorption bands are from undissociated **DCM-T2OH**. On the other hand, in the high-polar aprotic solvents, absorption bands were observed at *ca.* 700 nm as well as at *ca.* 500 nm, as shown in Fig. 2b. These absorption bands on the long wavelength side are from ion-dissociated **DCM-T2O⁻**. In DMF and DMSO, which are more polar than MeCN, the molar extinction coefficients of the absorption bands on the short wavelength side are smaller, and those of the absorption bands on the long wavelength side are larger. This result shows that there are more ion-dissociated **DCM-T2O⁻** than undissociated **DCM-T2OH** in the solvents, which are

more polar. In protic solvents, the absorption bands were observed at *ca.* 650 nm along with absorption bands at *ca.* 500 nm, as shown in Fig. 2c. The absorption of ion-dissociated **DCM-T2O⁻** on the long wavelength side in the protic solvents was observed on the shorter wavelength side than in the high-polarity aprotic solvents because the ground state was stabilized by hydrogen bonding. In the low-polarity aprotic solvents, fluorescence bands of **DCM-T2OH** were observed at *ca.* 550 nm, as shown in Fig. 3. These fluorescence bands are from undissociated **DCM-T2OH**. However, in the high-polarity aprotic solvents and protic solvents, fluorescence bands of ion-dissociated **DCM-T2O⁻** were observed at *ca.* 750 nm and *ca.* 770 nm, as shown in Fig. 3. These results showed that in both high-polarity aprotic solvents and protic solvents, the ion-dissociated **DCM-T2O⁻** absorbs and fluoresces within the “optical window.” In the aromatic

Fig. 2 UV-vis absorption spectra of **DCM-T2OH** in (a) low-polarity solvents, (b) high-polarity solvents, and (c) protic solvents.

Fig. 3 Fluorescence spectra of **DCM-T2OH** in solvents with different polarities.

Table 1. Absorption maxima ($\lambda_{a, \max}$), Molar absorbance coefficients (ϵ), Emission maxima ($\lambda_{f, \max}$), Fluorescence quantum yields (Φ_f), and Stokes shifts of **DCM-T2OH**.

Solvents	$\lambda_{a, \max} / \text{nm}$	$\log(\epsilon_{a, \max} / \text{dm}^{-3} \text{mol}^{-1} \text{cm}^{-1})$	$\lambda_{f, \max} / \text{nm}$	$\Phi_f^{b)}$	Stokes shift / cm^{-1}
DOX	497	4.23	552	0.08	2005
PhMe	499	4.20	539	0.08	1487
CF	507	4.26	575	0.10	2333
DCM	496	4.19	551	0.04	2012
MeCN	746 ^{a)}	4.41	768 ^{a)}	0.04	384
DMF	759 ^{a)}	4.84	778 ^{a)}	0.02	322
DMSO	759 ^{a)}	4.82	782 ^{a)}	0.02	388
EtOH	655 ^{a)}	3.96	753 ^{a)}	0.03	1987
MeOH	616 ^{a)}	3.33	748 ^{a)}	0.03	2865

a. The value of ion-dissociated **DCM-T2O⁻**.

b. The Φ_f values were determined at room temperature relative to the absolute Φ_f of 2-(*N,N*-dimethylamino)tryptanthrin in dichloromethane ($\Phi_f = 0.89$) [5] using solutions of matched absorbance ($0.1 \text{ L mol}^{-1} \text{ cm}^{-1}$) at the excitation wavelength.

solvent toluene (PhMe), a shoulder is observed around 570 nm, as shown in Fig. 3. This shoulder could be due to intermolecular interactions such as exciplex formation between solvent and solute molecules.

3.2 pH dependence of **DCM-T2OH**

UV-vis absorption and fluorescence spectra of **DCM-T2OH** in a MeCN/H₂O (1/4, v/v) solution containing 0.1 M HEPES at different pH are shown in Fig. 4. The pH was modulated by adding 0.1 M HCl or 0.1 M NaOH in a MeCN/H₂O (1/4, v/v) solution containing 0.1 M HEPES. At a pH of 7.1, the absorption of undissociated **DCM-T2OH** at *ca.* 500 nm and the absorption of ion-dissociated **DCM-T2O⁻** at *ca.* 650 nm were observed. As the pH decreased, the absorption on the longer wavelength side disappeared (Fig. 4a-1). At a pH of 2.8, only short wavelength absorption from undissociated **DCM-T2OH** was observed. In a pH range from 2.8 to 7.1, fluorescence bands were observed at *ca.* 750 nm, as shown in Fig. 4b. This result occurs because of fluorescence by ion-dissociated chemical species, **DCM-T2O⁻**, in the excited state as in the case of **T2OH** [6]. We expected that in an alkaline solution, the absorption at *ca.* 650 nm from ion-dissociated **DCM-T2O⁻** would increase and the absorption at *ca.* 500 nm from undissociated **DCM-T2OH** would disappear. However, in a pH range from 7.1 to 11.5, contrary to results expected, the absorption bands of ion-dissociated **DCM-T2O⁻** on the longer wavelength side disappeared as the pH increased (Fig. 4a-2). This result suggests that alkaline hydrolysis of **DCM-T2OH** to **T2OH** was suspected. Actually, the shape of the absorption spectrum at a pH of 11.5 was very similar to that of the proton-dissociated tryptanthrin-2-olate anion (**T2O⁻**) [6]. In a pH range from 8.0 to 11.5, fluorescence spectra were observed at *ca.* 650 nm, as shown in Fig. 4b. The shape of these fluorescence spectra was also very similar to that of **T2O⁻**. When acid was added to an alkaline solution of **DCM-T2OH** and neutralized and ESI-MS was measured, the $[\text{M}+\text{H}]^+$ ion peak of **T2OH** was observed. This result confirmed that **DCM-T2OH** hydrolyzed to **T2OH** in the alkaline solution.

4. CONCLUSION

DCM-T2OH was synthesized as a NIR dye for fluorescent imaging. In high-polarity aprotic solvents (MeCN, DMF, and DMSO), protic solvents (MeOH and

Fig. 4 (a) UV-vis absorption and (b) fluorescence spectra of **DCM-T2OH** in a MeCN/H₂O (1/4, v/v) solution at different pH values.

EtOH), and an aqueous solution containing 20% acetonitrile, both the absorption and fluorescence bands of **DCM-T2OH** were observed at the NIR region (650–900 nm), which was referred to as the “optical window” of cells and tissues. In the acidic solution, ion-dissociated **DCM-T2O[−]** regardless of pH fluoresced at *ca.* 750 nm, while in the alkaline solution, ion-dissociated **T2O[−]** by hydrolysis of **DCM-T2O[−]** fluoresced at *ca.* 650 nm.

5. ACKNOWLEDGEMENTS

This work was supported by JSPS KAKENHI Grant Number 16K05805 and 19K05518. The authors acknowledge Shared Facility Center for Science and Technology, Hirosaki University (SFCST) for Proton NMR and ESI mass spectrometry measurements. The authors would like to thank Enago (www.enago.jp) for the English language review.

6. REFERENCES

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(Received September 30, 2019; Accepted January 15, 2020;
Published Online February 1, 2020)