Journal of Photochemistry and Photobiology A: Chemistry 332 (2017) 25–31

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Photo-switchable and self-erasable fluorescent nanoprobe

Keitaro Sou[a,b,*], Li Yan Chan[c], Chi-Lik Ken Lee[c]

[a] Waseda Bioscience Research Institute in Singapore (WABIOS), 11 Biopolis Way #05-02 Helios, Singapore 138667, Singapore
[b] Comprehensive Research Organization, Waseda University, 513 Wasedatsurumaki-cho, Shinjuku-ku, Tokyo 162-0041, Japan
[c] Department for Technology, Innovation and Enterprise (TIE), Singapore Polytechnic, 500 Dover Road, Singapore 139651, Singapore

ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 25 July 2016
Accepted 2 August 2016
Available online 8 August 2016

Keywords:
Fluorescence
Imaging
Molecular assembly
Nanoparticles
Photochromism

ABSTRACT

Optically switchable fluorescent molecule is useful for developing photo-functional materials. However, most of the conventional fluorescent molecule is in a class of "always-on" type, which does not possess a fluorescence switching function. In this work, switchable fluorescent nanoparticles based on self-assembly of amphiphilic derivative of rhodamine B is synthesized and evaluated for its optical properties. The fluorescent nanoparticles functioned as a photo-switchable and self-erasable nanoprobe by reversible intramolecular ring opening cyclization of the rhodamine B derivative in dispersion and free-standing hydrogel.

1. Introduction

Photochromic materials have attracted increasing interest because of their potential applications in optical memory and optical data storage [1–3], optical molecular switches [4,5], and sensors [6–8]. In particular, fluorescent photochromic materials allow higher visibility and sensitivity compared to the photochromic molecules, which undergo visible color changes upon photo irradiation. An approach to design the *on*/*off* fluorescence switching system is based on the quenching and fluorescence resonance energy transfer (FRET) concept, by controlling the intra- or intermolecular electron transfer and energy transfer [9–12]. Several fluorescent switching molecules that responded to the pH [13–16], light [17], metal ions [18], and biomolecules, such as DNA and proteins [19,20], have been developed.

Light source has several advantages to control the *on*/*off* fluorescence switching. For example, the light irradiation can achieve rapid *on*/*off* switching, long range control, wavelength specificity, and precise spatial control. These advantages allow on demand remote control for super resolution imaging [21–23], manipulation of cell functions [24,25], technology for controlled drug release [26–28], and high-speed optical rewritable system [29]. Consequently, the development of molecules which allow fluorescence *on*/*off* switching by light irradiation is having an increasing interest in the broad science field. Nonetheless, the availability of this type of fluorescence molecules remains scarce.

Rhodamine B (RhB) is one of the "always-on" type fluorescent probes. Several reports have indicated that chemically modified RhB, rhodamine spiroamide, exhibits photochromic property by intramolecular ring opening cyclization reaction [30–34]. Herein, we report a unique amphiphilic RhB derivative which possesses chromism property and fluorescence *on*/*off* switching capability in aqueous dispersion and hydrogel by sensing pH change and UV light (Scheme 1). The amphiphilic structure permits the spontaneous incorporation of the RhB derivative into nanoparticle molecular self-assemblies in aqueous media.

2. Materials and methods

2.1. Materials

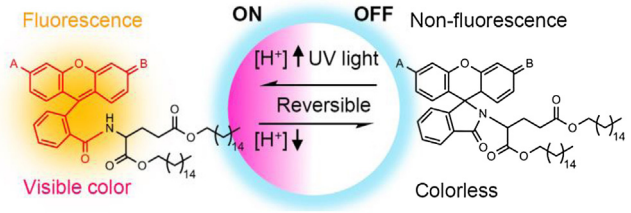
Rhodamine B, L-glutamic acid, 1-hexadecanol and gellan gum (Phytagel™) were purchased from Sigma-Aldrich Corp. (St. Louis, MO). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[monomethoxy poly(ethylene glycol) (2000)] (PEG-DSPE) was purchased from NOF Corp. (Tokyo, Japan).

2.2. General procedures

The NMR spectra in $CDCl_3$ were recorded on a Bruker 400 Ultrashield (400-MHz) spectrometer (Bruker, Fällanden,

* Corresponding author at: Waseda Bioscience Research Institute in Singapore (WABIOS), 11 Biopolis Way #05-02 Helios, Singapore 138667, Singapore.
E-mail address: soukei@aoni.waseda.jp (K. Sou).

http://dx.doi.org/10.1016/j.jphotochem.2016.08.001
1010-6030/



Scheme 1. Conceptual scheme showing the reversible change in optical properties of amphiphilic rhodamine chromophore based on ring opening cyclization reaction.

Switzerland). Analytical TLC was performed on commercial plates coated with silica gel (TLC MERK, silica gel 60 F254). MS spectra were measured with a liquid chromatograph-mass spectrometer (LCMS-2020; Shimadzu, Kyoto, Japan).

2.3. Synthesis of compounds

L-glutamic acid (2.96 g, 20 mmol), hexadecyl alcohol (11.62 g, 48 mmol) and *p*-toluenesulfonic acid (monohydrate, 4.56 g, 24 mmol) were dissolved in benzene (150 mL). The mixture was refluxed for 14 h at 110 °C with a Dean-Stark receiver. The solvent was evaporated and the residue was dissolved into chloroform (150 mL). The chloroform solution was washed with saturated $NaHCO_3$ aqueous solution (2 times), followed by distilled water (2 times). Na_2SO_4 (5 g) was added to the chloroform solution and kept at room temperature for 12 h. After removing the Na_2SO_4 by filtration, the solvent was evaporated and the residue was dissolved into hot methanol (350 mL). After recrystallization from methanol on ice, 1, 5-dihexadecyl-L-glutamate was obtained as a white solid (7.7 g, yield 65%). TLC (silica gel) chloroform/methanol (4/1): Rf 0.90. ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 0.88 (t, 6H, $-CH_3$); 1.26 (s, 52H, $-CH_2-CH_2-$); 1.62 (m, 4H, $-CO-O-C-CH_2-$); 1.83, 2.07 (m, 2H, glu β-CH_2); 2.46 (m, 2H, glu γ-CH_2); 3.46 (m, 1H, glu α-CH); 4.07, 4.11 (t, 4H, $-CO-O-CH_2-$).

Rhodamine B (240 mg, 0.5 mmol) and *N,N′*-dicyclohexylcarbodiimide (DCC) (103 mg, 0.5 mmol) were dissolved in chloroform (5 mL). The mixture was stirred for 4 h at room temperature. 1, 5-Dihexadecyl-L-glutamate (226 mg, 0.38 mmol) and DMAP (46 mg, 0.38 mmol) were added to the mixture and stirred for 14 h at room temperature. The reaction mixture was purified by column chromatography (Silicagel 60, mobile phase; chloroform/methanol, 8/1) and amphiphilic rhodamine B derivative (RhB-G16) was obtained (200 mg, yield 52%). TLC (silica gel) chloroform/methanol (8/1): Rf 0.94. ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 0.88 (t, 6H, $-CH_3$); 1.15 (t, 12H, $-CH_3$); 1.26 (s, 52H, $-CH_2-CH_2-$); 1.44, 1.51 (m, 4H, $-CO-O-C-CH_2-$); 1.90, 2.03 (m, 2H, glu β-CH_2); 2.17 (m, 2H, glu γ-CH_2); 3.32 (q, 8H, $-CH_2-$), 3.69 (t, 1H, glu α-CH); 3.85 (m, 4H, $-CO-O-CH_2-$); 6.22–6.54 (m, 6H, ArH); 7.11 (m, 1H, ArH); 7.47 (m, 2H, ArH); 7.90 (m, 1H, ArH). ^{13}C NMR ($CDCl_3$, 400 MHz, δ ppm): 12.54, 12.64, 14.12, 22.69, 24.96, 25.39, 25.63, 25.80, 25.90, 28.36, 28.59, 29.27, 29.32, 29.37, 29.56, 29.63, 29.67, 29.71, 31.07, 31.93, 33.97, 44.34, 44.41, 54.32, 64.26, 65.12, 65.87, 77.22, 97.39, 97.78, 104.49, 105.80, 107.53, 108.24, 122.88, 124.05, 128.12, 129.33, 130.79, 131.89, 132.48, 148.83, 148.86, 152.81, 153.56, 153.82, 167.32, 170.11, 172.90. MS (ESI) *m/z* (M^+) 1021.

2.4. Preparation and characterization of self-assemblies

RhB-G16 and PEG-DSPE were dissolved in benzene at 1/10, molar ratio and the solution was freeze-dried. The obtained mixture of RhB-G16 and PEG-DSPE was stored in freezer (−20 °C). The mixture was dispersed in phosphate buffered saline (PBS) with vortex mixer for 5 min. The diameters of the resulting assemblies were ascertained using a particle size analyzer based on dynamic light scattering (Zeta-Sizer Nano ZS; Malvern Instruments, Ltd., Malvern, Worcestershire, UK). The average diameter ± standard deviation (SD) was calculated.

2.5. Effect of pH on the optical properties of the nanoparticle dispersion

The mixture of RhB-G16 and PEG-DSPE (1/10, molar ratio) was dispersed in PBS (pH 1–10) ([RhB-G16] = 10 μM). UV–vis spectra of the dispersion were recorded with UV–vis spectrophotometer (V-670; JASCO Co., Tokyo, Japan). The fluorescence intensity of the dispersion was measured with fluorescence spectrophotometer (λ_{ex} = 556 nm, λ_{em} = 575 nm, F-2700; Hitachi Ltd., Tokyo, Japan). Signal-to-background ratio (S/B) in fluorescence intensity at various pH was plotted where the background was the fluorescence intensity at pH 7.4.

2.6. Measurement of fluorescence quantum yield

According to a previous reported procedure [35], the fluorescence quantum yields of the mixture of RhB-G16 and PEG-DSPE (1/10, molar ratio) were measured using Eq. (1).

$$\Phi = \Phi_{st} \cdot \frac{A_{st}}{A} \cdot \frac{I}{I_{st}} \quad (1)$$

where Φ, A, and I are fluorescence quantum yield, absorbance and integral fluorescence intensity of the samples, and Φ_{st}, A_{st}, and I_{st} are fluorescence quantum yield, absorbance and integral fluorescence intensity of the reference. Rhodamine B was used as the reference, of which Φ_{st} is 0.31 in water [36]. The UV–vis spectra were recorded with UV–vis spectrophotometer (V-670; JASCO Co., Tokyo, Japan), and the A and A_{st} values were measured at λ = 510 nm. Fluorescence emission spectra were obtained using the fluorescence spectrophotometer (λ_{ex} = 510 nm, F-2700; Hitachi Ltd., Tokyo, Japan), and then I and I_{st} values were integrated.

2.7. Fluorescence on/off switching by UV irradiation

The mixture of RhB-G16 and PEG-DSPE (1/10, molar ratio) was dispersed in PBS (pH 7.4, [RhB-G16] = 10 μM). The dispersion was put in a square quartz cell. The UV light (λ = 302 nm) was irradiated to the dispersion on a transilluminator (LAS-1000 UV mini; Fujifilm, Tokyo, Japan). The spectral change was measured by UV–vis spectrophotometer (V-670; JASCO Co., Tokyo, Japan) and fluorescence spectrophotometer (λ_{ex} = 556 nm, λ_{em} = 575 nm, F-2700; Hitachi Ltd., Tokyo, Japan). Increase of fluorescence emission intensity at 575 nm by continuous excitation at 302 nm was measured to determine the rate constant of fluorescence *on* from *off* form (k_{on}). Decrease of fluorescence emission intensity at 575 nm (λ_{ex} = 556 nm) after UV irradiation (λ = 302 nm) for 1 min was measured to determine the rate constant of fluorescence *off* from *on* form (k_{off}).

2.8. Fluorescence imaging in hydrogel

1.5% gellan gum hydrogel (75 × 75 × 1.4 mm) containing the mixture of RhB-G16 and PEG-DSPE (1/10, molar ratio) was prepared (PBS, pH 7.4, [RhB-G16] = 50 μM). The hydrogel was masked and then exposed to short-wave UV light (λ = 302 nm) on a transilluminator (LAS-1000 UV mini; Fujifilm, Tokyo, Japan) for 30 s. The fluorescence images were acquisitioned on the transilluminator under long-wave UV light (λ = 365 nm).