Fluorescence Enhancement in 7-Hydroxyquinoline Analogs by Methyl Substitution and Their Spectroscopic Characteristics in Aqueous Solution

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Significant enhancement of the photosensitivity of 7-hydroxyquinolines in aqueous buffer solution has been achieved simply by introducing two methyl groups at 2 and 4 positions. The product of the molar extinction coefficient and fluorescence quantum yield ($\varepsilon \cdot \Phi_f$) for 7-hydroxy-2,4-dimethylquinoline is about 13-fold greater than that of original 7-hydroxyquinoline.

Photoinduced excited state proton transfer (ESPT) of 7-hydroxyquinoline (**HQ**) is a unique reaction system. In the presence of water for example, **HQ** is proposed to form a cyclical complex with water molecules,[1] and undergoes excited state proton transfer through a proton-relay system mediated by water.[2] The produced excited state species (**Z***) emits a large Stokes shifted fluorescence to give the ground state tautomer followed by fast back proton transfer. Various models have been proposed so far for solvent-assisted ESPT of **HQ**. Fang investigated triple proton transfer within cyclic complexes based on ab initio calculations.[3] Kohtani et al. confirmed the formation and 1:2 stoichiometry of **HQ**–alcohol complexes in hexane on the basis of spectral analysis and semiempirical MO calculations.[4] Kwon et al. reported the intrinsic proton shuttling dynamics of cyclically **HQ**–alcohol complexes which undergo stepwise ESPT with the assistance of solvent fluctuations.[5] Park et al. recently reported the Grotthuss-type proton-transport mechanism of water in cyclical **HQ**–water complex in diethyl ether.[6]

In a neutral aqueous solution, **HQ** is in the equilibrium configuration of four prototropic species: a normal form (**N**), an anion form (**A**), a cation form (**C**), and a zwitterion form (**Z**) as shown in Figure 1.[7] **HQ** exist mostly as **N** (67%) and **Z** (29%) with minor species of **C** (3%) and **A** (1%) at pH 7.[7b] In the excited state, the pK_a of the 7-hydroxy group decreases, whereas that of the quinolinium nitrogen increases. Therefore the 7-hydroxy group in **HQ** acts as a proton donor and the nitrogen acts as a proton acceptor in the excited state.[8]

The fluorescence of many traditional fluorescent probes[9] for bio-imaging is usually very weak in water, but conjugation to hydrophobic biomolecules such as a protein, DNA, or plasma membrane, enhances their fluorescence. Very recently, we have reported the new **HQ**-based fluorescent dye, Tsukuba-Green (**TG**), 4-ethoxycarbonylmethyl-7-hydroxy-2-methylquinoline (Figure 2).[10] The photochemical behavior of **TG** might be quite characteristic and distinctive when **TG** is used as a fluorescence probe for live-cell imaging. Unlike those traditional probes, **TG** emits green fluorescence ($\lambda_{max} = 509$ nm, $\Phi_f = 0.15$) in aqueous solution, that dramatically changes to blue fluorescence peaking around 360–380 nm in hydrophobic media, due to the similarity of the excited-state dynamics of **TG** to **HQ**. In addition, **TG** is found to be delocalized in the cells. Fortunately, the photosensitivity, the product of the molar extinction coefficient and fluorescence quantum yield ($\varepsilon \cdot \Phi_f$) of **TG** in neutral buffer solution is 4–5-fold greater than that of the parent compound **HQ**, suggesting that **TG** possesses advantages as a fluorescent probe compared to **HQ**. These results let us assume the significant effect of substituent on its photosensitivity of **HQ**. The main factor for the improvement of the photosensitivity seems to be the methyl substituent. However, since **TG** is the first **HQ**-based fluorescent probe for living cells, an improvement of the fluorescence efficiency of **HQ** families for biological use has not been explored yet. In order to reveal the highly important properties of **TG** as a fluorescent probe, we have studied the effect of methyl substitution on the photochemical properties of **HQ** as a model system. In this paper, we report the substituent effect on the fluorescence efficiency using **HQ** analogs, 7-hydroxy-2-methylquinoline (**HMQ**) and 7-hydroxy-2,4-dimethylquinoline (**HDMQ**) (Figure 2). pH Dependence of **HQ**, **HMQ**, and **HDMQ** on their photochemical behavior is also investigated.

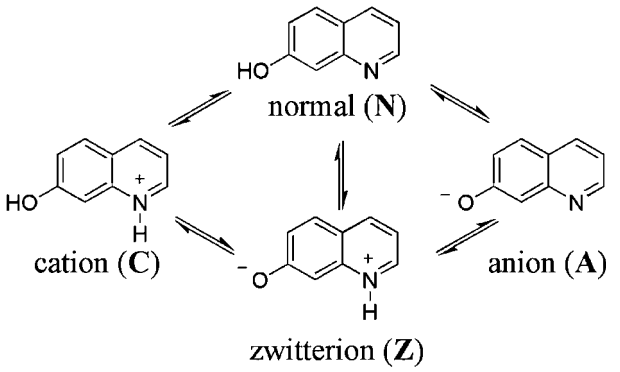
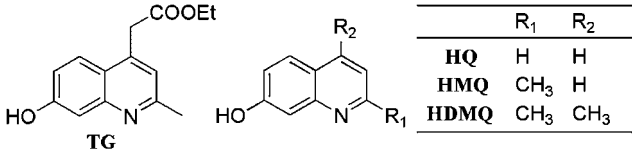


Figure 1. Equilibrium among the prototropic species of 7-hydroxyquinoline (**HQ**) in water.



	R_1	R_2
HQ	H	H
HMQ	CH_3	H
HDMQ	CH_3	CH_3

Figure 2. Chemical structures of 4-ethoxycarbonylmethyl-7-hydroxy-2-methylquinoline (**TG**), 7-hydroxyquinoline (**HQ**), and its analogs.

HMQ and **HDMQ** were prepared according to a previous report.[11] The photochemical data of **HQ** have been reported elsewhere.[10] Figure 3a shows the steady-state absorption spectra of **HQ**, **HMQ**, and **HDMQ**, respectively, in water (1% DMSO). The lowest absorption bands of prototropic

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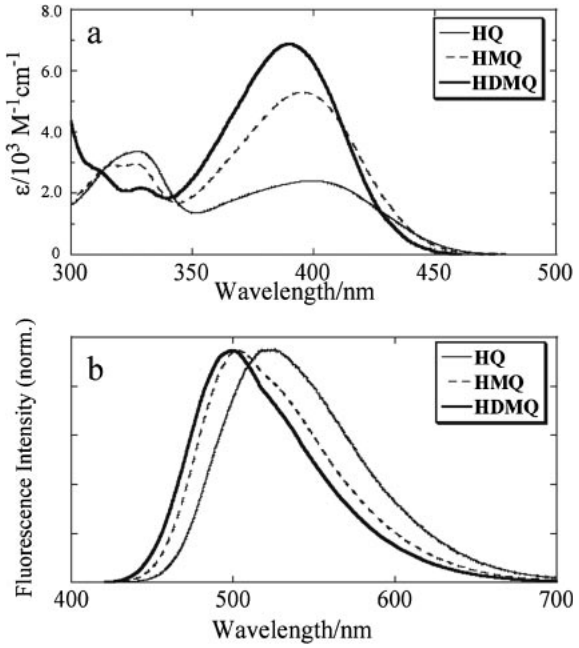


Figure 3. Absorption (a) and fluorescence spectra (b) of **HQ** (thin line), **HMQ** (dash line), and **HDMQ** (solid line) in water (1% DMSO).

equilibrium species of **HQ** in water are spectrally distinguishable. The bands peaking around 330 and 400 nm shown in Figure 3a are mainly due to the lowest electronic transitions of the **N** and **Z** species, respectively.[7b] The lowest absorption peaks of **C** and **A** species appeared around 340 and 360 nm, respectively (shown later). The spectral shape and the ε value apparently vary depending on the structure. In the spectrum of **HQ**, the absorption band of **N** is more intense than that of **Z**. Slight sharpening and remarkable intensification of the band of **Z** in **HMQ** and **HDMQ** indicate that the equilibrium between **N** and **Z** shifted to the **Z** side in **HMQ** and **HDMQ**. The ε value at the absorption band of **Z** is 2400 M^{-1} cm^{-1} in **HQ**, which increased more than twice to 5300 M^{-1} cm^{-1} in **HMQ**, and 6900 M^{-1} cm^{-1} in **HDMQ**, respectively. The enhancement of **Z** proportion in **HMQ** and **HDMQ** may be related to the increase of basicity at quinolinium nitrogen that makes cyclical water complex more rigid and that suppresses nonradiative process in the excited state. The pK_a values of quinolinium nitrogen and 7-hydroxy group were estimated from the pH profile of the absorbance to be 5.6 and 9.8 for **HQ**, 6.2 and 9.6 for **HMQ**, 6.5 and 10.1 for **HDMQ**, respectively.

As shown in Figure 3b, each compound shows a single fluorescence band around 500 nm, ascribed to emission from **Z***.[8h] The observed fluorescence slightly shifted to shorter wavelength from 526 nm for **HQ** to 503 and 500 nm for **HMQ** and **HDMQ**, respectively. More importantly, the quantum yield of fluorescence of **HMQ** ($\Phi_f = 0.17$) and **HDMQ** ($\Phi_f = 0.26$) are about 3–4 times higher than that of **HQ** ($\Phi_f = 0.06$). The fluorescence lifetime of **HMQ** ($\tau = 5.0$ ns) and **HDMQ** ($\tau = 6.8$ ns) is also longer compared to that for **HQ** ($\tau = 2.7$ ns). In each compound, the fluorescence spectra by excitation at different wavelength were almost identical, and all three compounds showed a single fluorescence decay curve. This indicates that the emission state should be uniform for each compound in aqueous solution. The photosensitivity, of **HMQ** ($\varepsilon \cdot \Phi_f = 900$ M^{-1} cm^{-1}) and **HDMQ** ($\varepsilon \cdot \Phi_f = 1800$ M^{-1} cm^{-1}), is about 6- and 13-fold greater than that of **HQ** ($\varepsilon \cdot \Phi_f = 140$ M^{-1} cm^{-1}) (Table 1). The absorption bands of **Z** (400 nm) are used for excitation, which is a less harmful wavelength to the cells compared to that of **N** (325 nm).

Table 1. Absorption Maxima, Molar Extinction Coefficient, Fluorescence Maxima, Fluorescence Quantum Yield, and Fluorescence Lifetime of 7-Hydroxyquinoline and Its Analogs

	λ_{abs} /nm	ε /M^{-1} cm^{-1}	λ_{flu} /nm	Φ_f	$\varepsilon \cdot \Phi_f$ /M^{-1} cm^{-1}	τ /ns
HQ	400	2400	526	0.06	140	2.7
HMQ	394	5300	503	0.17	900	5.0
HDMQ	389	6900	500	0.26	1800	6.8

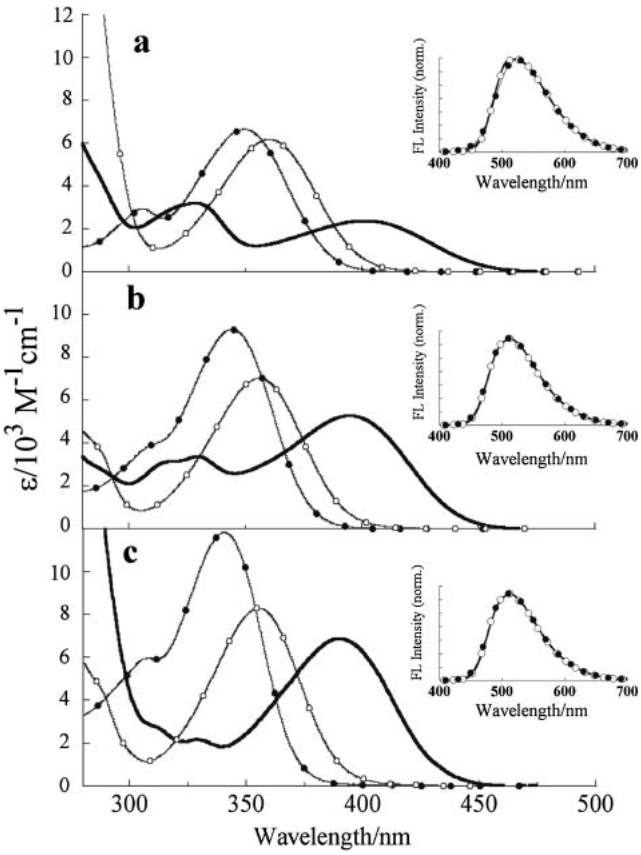
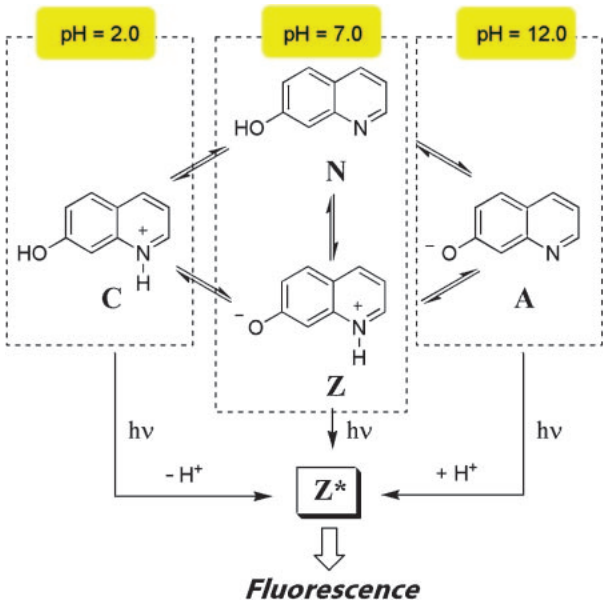


Figure 4. pH Dependence of the absorption spectra of **HQ** (a), **HMQ** (b), and **HDMQ** (c) at pH 2.0 (closed circle), pH 7.0 (solid line), and pH 12.0 (opened circle), respectively. Inset of each figure shows the normalized fluorescence spectra at pH 2.0 (closed circle), pH 7.0 (solid line), and pH 12.0 (opened circle), respectively.

The absorption spectra of all three compounds changed depending on pH. The apparent absorption spectra of **HQ**, **HMQ**, and **HDMQ** in aqueous buffer solution at various pH are shown in Figure 4. The fluorescence excitation spectra are also changed with changing pH in similar manner (Figure S1). The absorption spectrum of all three compounds at pH 2.0 is dominated by **C**, and the bands at pH 12 are due to the production of **A**, as depicted in Scheme 1. The ε values of both **C** and **A** are



Scheme 1.

also largest in **HDMQ**: ε values are 6500 (**C**) and 6200 (**A**) $M^{-1}\,cm^{-1}$ for **HQ**, 9300 (**C**) and 7000 (**A**) $M^{-1}\,cm^{-1}$ for **HMQ**, and 12000 (**C**) and 8300 (**A**) $M^{-1}\,cm^{-1}$ for **HDMQ**, respectively. Unlike the pH dependence observed in the absorption and excitation spectra, no change was observed in the fluorescence spectra of each compound (Inset of Figure 4) measured at pH 2, pH 7, and pH 12. In addition, the fluorescence quantum yield of all compounds did not depend on pH. This indicates that the structure of the excited-state tautomer of all three compounds is independent from pH at least between pH 2 and pH 12, because the excitation of these **HQ** analogs render the 7-hydroxy group more acidic and the quinolinium nitrogen become more basic. The schematic representation of the structural change of **HQ** at different pH is shown in Scheme 1.

In summary, significant enhancement of the photosensitivity of **HQ** in aqueous buffer solution has been achieved using 2-methyl or 2,4-dimethyl analogs of 7-hydroxyquinoline. The product of the molar extinction coefficient and fluorescence quantum yield ($\varepsilon \cdot \Phi_f$) for **HMQ** and **HDMQ**, respectively, is about 6- and 13-fold greater than that of **HQ**. The lifetime of tautomer fluorescence increased with increasing fluorescence quantum yield from 2.7 ns in **HQ** ($\Phi_f = 0.06$) to 6.8 ns in **HDMQ** ($\Phi_f = 0.26$). The pH dependence of the absorption spectra was observed for all compounds, while fluorescence spectra and fluorescence quantum yield was not affected by pH (from pH 2 to 12) suggesting the same emission species despite the different ground-state structure depending on pH. These results presented in this paper indicate that the photosensitivity of **HQ** in physiological conditions can be controlled simply by introduction of substituent group(s). In addition, **HDMQ** can be a potential candidate as a new fluorescent probe for live cell imaging, and the study on this line is now in progress.

Experimental

Materials. 7-Hydroxyquinoline (**HQ**) was purchased from Acros Organics and used as received. **HMQ** and **HDMQ** were prepared according to previously reported methods (see Supporting Information).[11]

Measurement. ^{1}H NMR spectra were measured with a JEOL EX-270 (270 MHz for ^{1}H NMR) or a Bruker ARX-400 (400 MHz for ^{1}H NMR) spectrometer using DMSO-d_6 as a solvent with tetramethylsilane as an internal standard. UV absorption and fluorescence spectra were recorded on a Shimadzu UV-1600 spectrophotometer and on a Hitachi F-4500 fluorescence spectrometer, respectively. Fluorescence decay measurements were performed using time-correlated single-photon counting.

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Supporting Information

Synthetic procedure and characterization data for **HMQ** and **HDMQ**, fluorescence excitation spectra of **HQ**, **HMQ**, and **HDMQ** (Figure S1), and photograph of irradiated sample of **HQ** and **HDMQ** (Figure S2). This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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