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Importance of the orthogonal structure between porphyrin and aniline moieties on the pH-activatable porphyrin derivative for photodynamic therapy

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ABSTRACT

The photo-induced side effects of photodynamic therapy of cancer can be suppressed using pH-activatable porphyrin derivatives, which have been developed using an aniline moiety as a pH-responsive quencher. The acid dissociation coefficient of a pH-activatable photosensitizer increased by replacing a dimethylamino with a diethylamino group. Based on this diethylamino derivative, we studied the importance of the intramolecular orthogonal structure between porphyrin and aniline moieties on the pH-activatable properties. The character of the S_1 state of the non-orthogonal derivative (Por-NEt_2) was different from that of the orthogonal derivative (Por-Me-NEt_2), but the derivatives possessed the ON/OFF switching functions for both fluorescence and singlet oxygen sensitization. However, the maximum quantum yields of Por-Me-NEt_2 were much larger than those of Por-NEt_2, and the ON/OFF switching ratio of Por-Me-NEt_2 was higher than that of Por-NEt_2. This is due to the suppressed rotation of the aniline moiety, indicating that the intramolecular orthogonal structure is important for the high ON/OFF switching performances.

1. Introduction

Photosensitizers, which show fluorescence and produce singlet oxygen ($^1O_2$), have received considerable attention as a drug for photodynamic therapy (PDT) and photodynamic diagnosis (PDD). [1] In PDT and PDD, the tumor specificity is very important to minimize erroneous diagnoses and photo-induced side effects. There are two major strategies to improve the tumor specificity. One is to improve the selective accumulation efficiency of photosensitizers to the tumor tissue. In this regard, many types of nano-carriers [2–4] and ligands such as peptides [5–9], sugars [10–14], and antibodies [15–19] have been studied. The other is to develop activatable photosensitizers [20]. The fluorescence and $^1O_2$ sensitization efficiencies of activatable photosensitizers are inefficient in normal tissues. However, a certain trigger in the tumor tissue can modify the photosensitizer's chemical properties, increasing the fluorescence and $^1O_2$ sensitization efficiencies. These inactive and active states are called "ON" and "OFF" states, respectively. This type of activation functions have been well developed in the field of fluorescence probes [21–28]. In many cases, a fluorophore is combined with a quencher unit, and fluorescence is in the OFF state due to quenching. A trigger can change the chemical property of the quenching unit, loosening the quenching ability. As triggers for the tumor selective activation, low-pH condition [21,23–25], hypoxic condition [29], tumor specific biomolecules, such as enzyme [26,27,30], are proposed. Applying these ideas to photosensitizers, pH- [31–40], hypoxia- [41,42], and biomolecule-activatable photosensitizers [31,43–47], have been developed. These activatable photosensitizers can minimize erroneous diagnoses and photo-induced side effects.

A porphyrin derivative is one of the most promising photosensitizers for PDT and PDD, [1,44,48–52] because some porphyrin analogues have already been applied clinically. We have developed pH-activatable porphyrins that can be activated in low-pH conditions [53]. These pH-activatable porphyrins comprise a porphyrin moiety as a photosensitizer and aniline moiety(ies) as pH-responsive quencher(s). The charge separation from the aniline to porphyrin moieties efficiently quenched the first excited singlet (S_1) state of the pH-activatable porphyrin, and therefore quantum yields of fluorescence and $^1O_2$ sensitization were low in neutral conditions. In contrast, these quantum yields can be increased by adding an acid because of the protonation of the aniline moiety. These pH-activatable porphyrins containing aniline moiety(ies) showed a high ON/OFF switching ratio and a high quantum yield of $^1O_2$ sensitization in acidic conditions. [53] Therefore, this type of pH-activatable porphyrins are considered to be promising pH-

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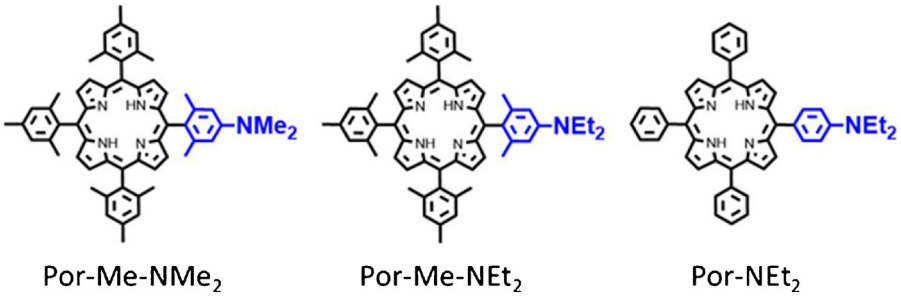

Fig. 1. Molecular structures of pH-activatable photosensitizers.

activatable photosensitizers.

For the clinical application of these pH-activatable porphyrins, additional chemical modifications are indispensable because of their low water solubility and other factors. The previous pH-activatable porphyrin, such as Por-Me-NMe_2, contains methyl groups at the 2′ and 6′ positions of the aniline moiety, Fig. 1. These methyl groups induce steric hindrance between methyl groups and porphyrin ring to maintain intramolecular orthogonality of porphyrin and aniline moieties, which minimizes the ground state interaction. However, the presence of these methyl groups limits the molecular design of pH-activatable porphyrins. Therefore, it is very important to clarify whether the methyl groups are irreplaceable, prior to developing pH-activatable porphyrins. In this paper, we studied the effects of the orthogonal structure on the pH-activatable functions and discussed necessity of the orthogonal structure for the pH-activation functions or not. We also modified the amino group of the aniline moiety to improve pH-response for the clinical application.

2. Experimental

2.1. Materials

Por-Me-NEt_2 and Por-NEt_2 were synthesized according to the similar procedure reported previously. [53] Dimethyl sulfoxide (DMSO) and water were used as solvents. Hydrochloric acid was used as an acid.

2.2. Measurements

UV–vis absorption spectra were recorded on a Hitachi U3310 spectrophotometer. Fluorescence emission and excitation spectra were measured using a Horiba FluoroMax 4p fluorescence spectrometer. The fluorescence quantum yields were determined using an absolute photoluminescence quantum yield spectrometer (Hamamatsu C9920-02) and Horiba FluoroMax 4p fluorescence spectrometer. For the phosphorescence measurements of singlet oxygen, 355-nm light from a Nd^{3+}: YAG laser (Tokyo Instruments Lotis II, 1.0 mJ/pulse, pulse width 8 ns, repetition rate 10 Hz) was used as the excitation light source. Phosphorescence was detected using a photomultiplier tube for the NIR region (Hamamatsu R5509-42) after dispersion with a monochromator (Ritsu MC-10 N, blaze wavelength: 1250 nm, slit width 0.7 mm). The signals from the photomultiplier tube were fed to a digitizing oscilloscope (Tektronix, TDS-380 P). The decay time profile of the phosphorescence of singlet oxygen was recorded at 1270 nm. Because the lifetime of singlet oxygen was independent of HCl concentrations (Fig. S1), the quantum yield of singlet oxygen photosensitization (Φ_Δ) was determined by comparing the initial phosphorescence intensities for the sample and a standard. The initial phosphorescence intensities were estimated by the curve fitting of the decay. Perinaphthenone is used as standard (quantum yield of $^1O_2$ sensitization is 0.98). [54] The absorbance of the sample solutions at the excitation wavelength (355 nm) was set to 0.1.

2.3. Quantum chemical calculations

Quantum chemical calculations (DFT) were performed using Gaussian 09 at the B3LYP level with the 6–31 G(d) basis set.

3. Results and discussions

3.1. Improvement of pK value

To apply the previously developed pH-activatable porphyrin derivative Por-Me-NMe_2 [53] to the biological system, the acid dissociation constant pK_1 of the mono-protonated aniline moiety has to be increased. As established, the pK_1 values of the protonated aniline derivative strongly depends on the substituents on the amino nitrogen. For example, the pK_1 values of the protonated *N*,*N*-dimethylaniline and *N*,*N*-diethylaniline were reported to be 5.06 and 6.56, respectively [55]. A diethylamino group is also known as a better substituent for a pH-activatable fluorescence probe [21] and a pH-activatable photosensitizer [35] for biological applications. Therefore, we replaced a dimethylamino group of Por-Me-NMe_2 with a diethylamino group (Por-Me-NEt_2, Fig. 1).

Figure S2a shows the UV–vis absorption spectra of Por-Me-NMe_2 [53] and Por-Me-NEt_2 in DMSO-H_2O (9:1 v/v). The absorption spectra are independent of the alkyl groups. The fluorescence spectra of Por-Me-NEt_2 in DMSO-H_2O (9:1 v/v) were obtained in the absence of HCl (Fig. 2a). In neutral conditions, fluorescence was hardly observed, indicating that the S_1 state of Por-Me-NEt_2 was efficiently quenched by the charge separation from the aniline to porphyrin moieties, as in the case of Por-Me-NMe_2 [53]. The fluorescence quantum yield Φ_f of Por-Me-NEt_2 in the neutral condition was estimated to be 0.004 (Table 1), which is similar to that of Por-Me-NMe_2 (0.002 [53]). The quantum yield of $^1O_2$ sensitization Φ_Δ of Por-Me-NEt_2 was also determined to be 0.040 in neutral DMSO-H_2O (9:1 v/v) by monitoring the near-IR phosphorescence of $^1O_2$ (Table 1). This Φ_Δ value is also similar to that of Por-Me-NMe_2 (0.03 [53]). By adding HCl, UV–vis absorption spectrum of Por-Me-NEt_2 did not change up to − log[HCl] = 1.0, as shown in Fig. S2b. The absorption spectral change observed below − log [HCl] = 1.0 is due to the protonation of pyrrole nitrogen as in the case of Por-Me-NMe_2 [53]. Fluorescence spectra were obtained at various concentrations of HCl (Fig. 2a). The fluorescence intensity increased without any change in the spectral shape. This indicates that the acid protonates the aniline moiety, suppressing the quenching. Therefore, the pH-activatable function is preserved even though the alkyl groups on the amino nitrogen are replaced. Fig. 2b shows the detailed acid concentration dependence of the Φ_f value for Por-Me-NEt_2 in DMSO-H_2O (9:1 v/v) against that for Por-Me-NMe_2 [53]. The increase of the Φ_f value is observed at around − log[HCl] = 5.0, which is higher than that of Por-Me-NMe_2 (about 3.5 [53]). This indicates that the increased pK_1 value is attained by replacing a dimethylamino group with a diethylamino group, i.e., the ON/OFF switching of Por-Me-NEt_2 is possible at a higher pH region (near neutral conditions). Similar dependence is also obtained for the Φ_Δ of Por-Me-NEt_2 (not shown) as for Por-Me-