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A Reversible Photoacid Functioning in PBS Buffer under Visible Light

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Supporting Information

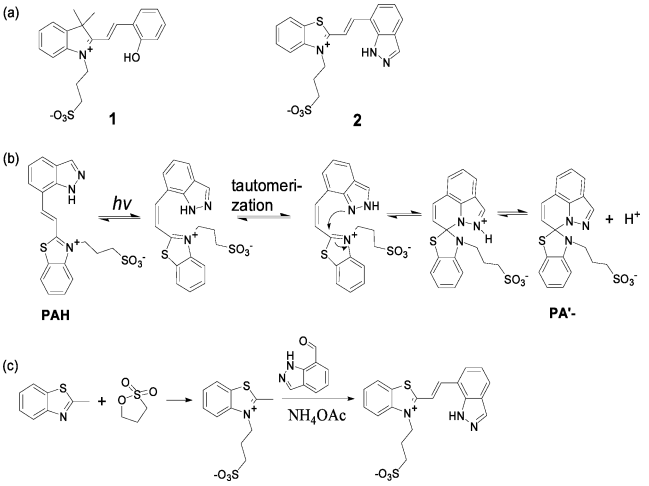
ABSTRACT: A metastable-state photoacid that can reversibly release a proton in PBS buffer (pH = 7.4) under visible light is reported. The design is based on the dual acid−base property and tautomerization of indazole. The quantum yield was as high as 0.73, and moderate light intensity (10^2 μmol·m^2·s^{-1}) is sufficient for the photoreaction. Reversible pH change of 1.7 units was demonstrated using a 0.1 mM aqueous solution. This type of photoacid is promising for control of proton-transfer processes in physiological conditions and may find applications in biomedical areas.

Photoacids are molecules that transform into strong acids upon irradiation. They can be utilized for remote, spatial, and temporal control of proton concentration and proton-transfer processes. The irreversible photoacid generators (PAGs) have been extensively studied as photo initiators for cationic polymerization and applied to photolithography.[1−5] Excited-state photoacids have been studied since the 1970s and have been utilized to investigate fast proton-transfer processes.[6−11] Hydroxyazobenzenes have also been utilized for photogeneration of pH jump.[12] The recently discovered metastable-state photoacids (mPAH) can produce a large proton concentration with high efficiency and good reversibility.[13−15] In addition, visible light with moderate intensity, e.g., LED and sunlight, can be used to activate mPAHs. Therefore, mPAHs can be conveniently incorporated in different systems to control various proton-transfer processes. Several applications of mPAHs have been demonstrated recently including control of acid-catalyzed reactions, volume change of hydrogels, polymer conductivity, bacteria killing, odorant release, and color change of materials.[13,16−19] They have also been utilized to control supramolecular assemblies,[20] molecular switches,[21] microbial fuel cells,[22] and cationic sensors.[23] Given that proton transfer is involved in the mechanisms of many enzymes and abnormal cellular pH is related to many diseases including cancer, cardiovascular diseases, and Alzheimer's disease, etc., mPAHs have a lot of potential in the biomedical area. However, the bioapplications of mPAH are limited by the fact that none of the previously reported mPAH works at a pH of 7.4, which is the common physiological pH. Herein, we report a novel mPAH, which functions in 1× PBS buffer (pH = 7.4) under visible light.

Metastable-state photoacids are generally designed by linking an electron-accepting moiety and a weakly acidic nucleophilic moiety with a double bond. Photoinduced trans−cis isomerization of the double bond allows a nucleophilic cyclization reaction to occur between the two moieties, which generates a highly acidic metastable form.[13] For example, compound **1**, which is the first mPAH reported and has been used in several applications, is shown in Scheme 1. This type of mPAH is related to the photoreaction of spiropyrans, which has been utilized to control proton transfer.[24] All the previously reported mPAHs have phenol derivatives as the nucleophilic moiety.[13,15] Plain phenol has a pK_a of 10. The pK_a is lowered when it is linked to a strong electron-accepting moiety. Due to the acidity of the phenol moiety of the mPAHs, proton dissociation occurs at a pH of 7.4 in the dark. The deprotonation also shifts the thermal equilibrium to the cyclized acidic form. Consequently, a large portion of the mPAHs release protons in the dark, which is the reason that none of the previously reported mPAH can function at a pH of 7.4. Figure 1 (left) shows the UV−vis spectrum of **1** in the PBS buffer. Approximately 83% of **1** changed to the deprotonated ring-opening form (PA^-) or cyclized form (PA'^-) in the dark. The dark acidity of mPAH may be lowered by using a nucleophilic moiety with a weaker acidity than that of phenol. However, in the mechanism of mPAH, the nucleophilic cyclization involves a proton transfer or a proton release.[13] A low acidity of the nucleophilic moiety may retard the cyclization reaction.

In this work, a novel mPAH (**2** in Scheme 1) using indazole as the nucleophilic moiety was designed and synthesized.

Scheme 1. (a) Structures of the mPAH 1 and 2, (b) Proposed Mechanism of the Photoreaction of 2, and (c) Synthesis of 2



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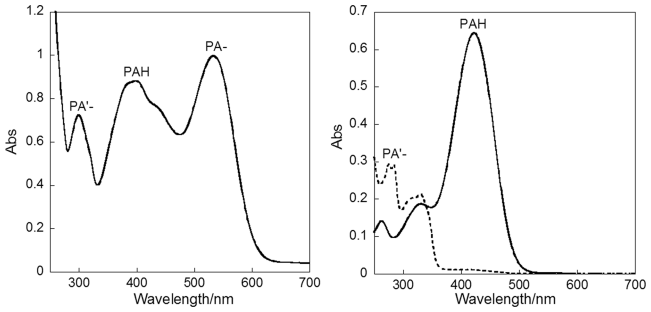

Figure 1. UV−vis spectrum of mPAH 1 in PBS buffer in the dark (left) and that of 2 before and after irradiation. (PAH is the protonated form, PA^- is the deprotonated form of PAH, and PA'^- is the deprotonated cyclized acidic form.)

Indazole is both a weak acid and a weak base. As described above, its acidity determines the dark acidity of the corresponding mPAH. The pK_a of its NH acidity is 13.86,[25] which is nearly 4 units higher than that of phenol. The photoinduced acidity of the corresponding mPAH is limited by its basicity since the proton released from the photogenerated acid can bind to the indazole moiety. Therefore, the pK_a of the photogenerated acid is close to that of the conjugate acid of indazole, which is 1.25. This pK_a is more than 6 units lower than 7.4 and thus is enough for releasing essentially all the protons at this pH. Indazole has two tautomers, i.e., 1*H*-indazole and 2*H*-indazole. Although 1*H* tautomer is more stable than 2*H* tautomer, the energy difference is very small (15 kJ/mol).[26] The photoinduced cyclization of **2** may occur without releasing a proton before the nucleophilic reaction since the 2*H* tautomer can act as a nucleophile (Scheme 1).

The synthetic route of **2** is shown in Scheme 1. Heating a mixture of 1,3-propane sultone and 2-methyl-benzothiazole yielded 2-methyl-1-(3-sulfonatepropyl)-benzothiazolium, which was reacted with 1*H*-indazole-7-carbaldehyde in ethanol to yield **2** as an orange precipitate. A small amount of ammonium acetate was used as a catalyst in the second step to increase the yield. Given the photosensitivity, workup needs to be done in the dark. The procedures are given in the Supporting Information (SI). The photoacid **2** has a low solubility in water and alcohols but can be dissolved in DMSO.

To study the behavior of **2** at a pH of 7.4, **2** was dissolved in a 1× PBS buffer containing 1% of DMSO. The UV−vis spectrum of the solution is shown in Figure 1. A strong absorption band with a λ_{max} at 422 nm (molar absorptivity 2.9×10^4 L $mol^{-1}cm^{-1}$) was observed, which is assigned to the protonated photoacid (PAH). The λ_{max} is close to that of **1** (424 nm). The absorption of the deprotonated form (PA^-) was studied using a pH jump experiment, which is described in the SI. The λ_{max} was determined to be 498 nm. Unlike the UV−vis spectrum of **1** in PBS buffer, no absorption peak was observed at the λ_{max} of the deprotonated form in the spectrum of **2** (Figure 1). To confirm that the protonated form is predominant, a small volume of concentrated HCl was added to break the buffer and change the solution to be strongly acidic (pH ∼ 1). A previous study showed that the protonated form of mPAH is predominant in strongly acidic conditions.[14] Therefore, comparing the amount of the protonated form in PBS buffer with that in the acidic solution allows us to estimate the portion of the protonated form in PBS buffer. After acidification, no significant change of the absorbance was observed (after considering the volume change due to the addition of HCl solution), indicating that the protonated form is predominant. Therefore, **2** does not release its proton at a pH of 7.4 in the dark. The stability of **2** in PBS buffer was also studied by monitoring the decrease of the UV−vis absorption with time. These data were fitted to a first-order equation, and the half-life was calculated to be ∼48 h, which is long enough for some bioapplications. The details are described in the SI.

The photoactivity of **2** was studied by irradiating a PBS solution (1% DMSO) of **2** using a 470 nm LED. The UV−vis spectrum obtained after 3 min irradiation is shown in Figure 1. The peak at 422 nm disappeared after irradiation, and multiple peaks between 350 and 250 nm appeared, which are assigned to the cyclized acidic form. Besides the disappearance of the peak at 422 nm, the most pronounced change is that the dip at 284 nm in the spectrum before irradiation changed to a strong peak after irradiation. After the light was turned off, the reformation of PAH was monitored using UV−vis spectroscopy (Figure S5). Since the reverse reaction in the PBS buffer is slow, we studied whether irradiation with UV light (254 and 365 nm) could accelerate the reverse reaction. We found that irradiating a solution of PA′H with 365 nm light slowed down the reverse reaction because PAH also substantially absorbs at this wavelength (Figure 1). When 254 nm light was used, the UV−vis spectrum was different from that of PAH indicating a different photoreaction occurred.

The photoreaction was also studied by NMR analysis. A deuterated DMSO solution of **2** (∼3 mM) was irradiated for 10 min and then scanned by NMR. The obtained spectrum showed the expected photoproduct (PA'^-) together with substantial amount of cis-PAH and small amount of trans-PAH (Figures S2 and S3). Increasing the irradiation time did not change the spectrum, which indicates that the mixture was due to the reverse reaction that occurred during the NMR measurement (∼8 min including time for setup and scan) rather than an uncompleted photo reaction. In fact, when the sample was kept in the dark for 30 min after irradiation, peaks for trans-PAH became predominant and very little cis-PAH and PA'^- can be observed. After 1 h, the spectrum became the same as that of the unirradiated sample except that the NH peak was broader than before.

To confirm that **2** indeed releases its proton upon irradiation, photoinduced pH change of a solution of **2** in deionized water containing 10% DMSO was studied. The concentration was about 0.1 mM, which is nearly the maximum concentration. The pH change was measured using both a pH meter and pH indicators. The pH of the solution was 6.0 in the dark. This pH is due to the acidity of deionized water but not the photoacid. To confirm this, the ground-state pK_a was experimentally determined to be 10.1. (The details are described in the SI.) A 0.1 mM solution of **2** in pure water should result in a pH close to 7 instead of 6. Deionized water often has a pH ∼ 6 due to absorption of CO_2. The solution of **2** was irradiated using a 470 nm LED light for 5 min. The pH after irradiation was 4.3, which was 1.7 units lower than the initial pH. After the irradiated solution was kept in the dark for 48 h, the pH returned to the original level.

The activity of the proton released from the mPAH is demonstrated by protonation of an acridine dye (Figure 2). This acridine dye changes from orange to blue upon protonation, and its λ_{max} changes from 440 to 604 nm.[19] The protonated form has a pK_a of 4.7. A solution of **2** (0.25 mM), and the dye (0.25 mM) in DMSO was irradiated for 5 min. The color of the solution changed from orange to dark green

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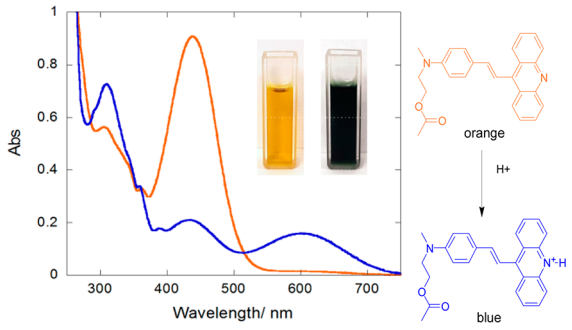

Figure 2. UV−vis spectra (taken using a 1 mm cuvette) of a solution of **2** and an acridine dye before (orange) and after (blue) irradiation (left) and the structures of the acridine dye and its protonated form (right).

(Figure 2). Before irradiation, UV−vis spectrum showed a strong absorption at 438 nm, which is an overlap of the absorption of **2** and the dye. After irradiation, this absorption decreased due to the photoreaction of **2** and protonation of the dye. The protonated dye showed a peak at 601 nm. The dark green color of the solution is a combination of blue and orange. The protonation was reversible. After 8 h, the absorption at 438 nm recovered to 98% of the original level.

The quantum yield of the photoreaction was measured by irradiating a solution of **2** in DMSO. The good solubility of **2** in DMSO allows a relatively high concentration to be used. Given that the quantum yield of **1** was also measured in DMSO, the quantum yield of **2** in DMSO can be compared with that of **1**. Solutions of **2** with concentrations near 0.06 mM (absorbance >1.5) were irradiated for 3 s using a 470 nm LED. The intensity of light was measured by an Apogee quantum meter. The photon flux was set to be ∼90 μmol·m^{-2}·s^{-1} by adjusting the distance between the sample and the LED. UV−vis spectra were quickly taken after irradiation. The amount of the reacted mPAH was calculated from the decrease of the UV−vis absorbance. The quantum yield was calculated to be as high as 0.73, which is higher than that of **1** (∼0.30).

In summary, a novel mPAH **2** was designed and synthesized. It can keep the protonated form in PBS buffer and reversibly release its proton under visible light with a high quantum yield of 0.73. While the design is based on the dual acid−base property and tautomerization of indazole, the detailed mechanism deserves further spectroscopic and theoretical study. This type of photoacid is promising for control of proton-transfer processes in physiological conditions and finding applications in biomedical areas.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b06218.

Details of synthesis, NMR spectra, stability measurement, ground-state pK_a measurement, determination of the UV−vis absorption of the deprotonated form (PDF)

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Notes

The authors declare no competing financial interest.

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