Fluorescence Modulation

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Photoreversible Fluorescence Modulation of a Rhodamine Dye by Supramolecular Complexation with Photosensitive Cyclodextrin**

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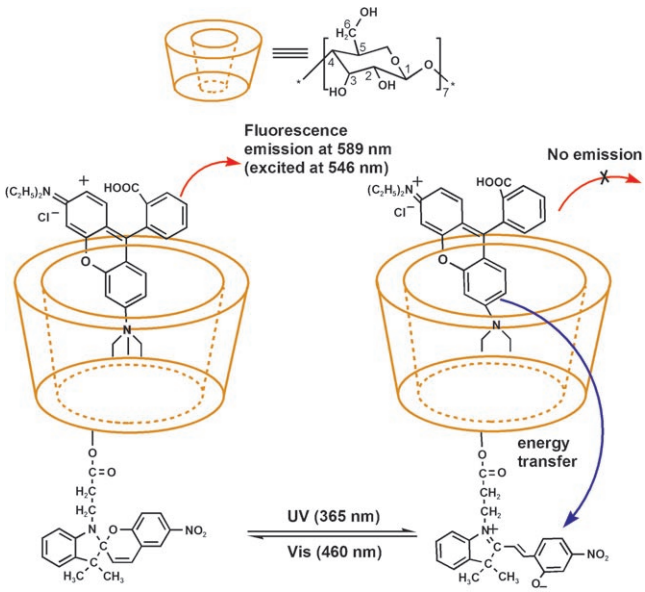
Materials with properties that can be optically modulated are of high interest in many scientific areas, including biotechnology and optics.[1] Photosensitive—in particular photochromic—compounds can be used to achieve this purpose, as they can be converted reversibly by light between two states with different spectroscopic properties.[2–5] This attribute can then be used to modulate or switch various functions at the molecular or supramolecular level by using light as a trigger. Spiropyran, a promising photochromic compound, undergoes reversible structural transformations in response to external inputs, such as light.[4,6,7] This special property of the spiropyran molecules was used to construct complex, optically controlled integrated logic gates and molecular switches composed of dyad or triad molecules with spiropyran as one of their units.[8–10] In this dyad or triad method, the modulation of various photonic or optoelectronic processes was usually realized by fluorescence quenching through energy transfer. In this approach to a light-controlled molecular switch or logic gate, several issues still need to be addressed: Firstly, the specific fluorophore must be covalently linked to the spiropyran molecule (or linked to it through a spacer) to form the dyad or the triad; hence, in this approach, only one dyad or triad can be used for a specific application. Secondly, self-quenching is still observed as a result of the self-aggregation of the fluorophore moieties. Thirdly, the dyads or triads are usually hydrophobic, which limits their applications in hydrophilic environments; in addition, they lack the necessary biocompatibility for applications in the fields of biotechnology.

It is well known that cyclodextrin (CD) can form supramolecular inclusion complexes with small-molecule chromophore compounds that fit into its 5–8 Å cavity;[11–13] in this way, the fluorescence intensity, biocompatibility, and photostability of the guest chromophore compounds can be enhanced.[14,15] Herein, we synthesized photosensitive cyclodextrin (CDSP) by covalently attaching spiropyran moieties onto β-CD. Rhodamine B[16–18] (RhB) was used as a model fluorophore to form a supramolecular complex with the CDSP, and photoreversible fluorescence modulation was thus realized. This strategy is easily achievable, and the guest rhodamine molecules can be readily replaced by other chromophore compounds. In addition, the self-quenching of RhB can be decreased by supramolecular complexation. The strategy could provide a facile method for reversible fluorescence modulation, controlled by light, for a wide range of chromophores, which is essential in applications such as reversible bioimaging. At the same time, the chromophore complexes will have the necessary biocompatibility and hydrophilicity for applications in the field of biotechnology because of the presence of the oligosaccharide cyclodextrin.

Photosensitive β-cyclodextrin was synthesized and based on mass spectrometry (MS), high-performance liquid chromatography (HPLC), NMR spectroscopy, and elemental analysis (see the Supporting Information), it was estimated that two spiropyran moieties were present per β-cyclodextrin molecule; the compound was thus designated as CDSP-2. The structure and reversible fluorescence modulation of the supramolecular complex formed by CDSP-2 and RhB is shown in Scheme 1.

The spiropyran molecules can adopt one of two stable states, namely, the open-ring state, which is known as the protonated merocyanine (McH) form (upon UV light irradiation), and the closed-ring state, which is known as the spiro (SP) form (upon visible-light irradiation).[19,20] The as-pre-



Scheme 1. Supramolecular complex (composed of photosensitive β-cyclodextrin and RhB) and photoreversible fluorescence modulation. The position of RhB—included in the β-cyclodextrin cavity—was estimated according to 2D-NMR measurements.

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pared CDSP-2 solution in the dark (that is, the SP form of the photosensitive cyclodextrin) exhibited an absorption band at about 340 nm. After UV irradiation (at 365 nm), a new absorption band at 550 nm appeared as a result of the formation of the McH form (see the Supporting Information).

The formation of a complex between RhB and the CDSP-2 was verified by 2D NMR experiments (see the Supporting Information). Rotating-frame Overhauser-effect spectroscopy (ROESY) of an equimolar mixture of photosensitive β-CD and T-shaped guest RhB molecules displays clear nuclear Overhauser effect (NOE) correlations between the H-3 and H-5 protons of β-CD and the methyl protons of the diethylamino groups in RhB as well as NOE correlations between the same protons of β-CD and the aromatic protons of the diethylaminophenyl group in RhB. Since both the H-3 and H-5 protons are located inside the β-CD cavity, and the H-5 protons are found near the primary (narrow) side of this cavity while the H-3 protons are located near its secondary (wide) side, these NOE correlations indicate that the RhB molecule is accommodated inside the β-CD cavity.

The complexation between RhB and the photosensitive β-CD was further demonstrated by means of absorption and fluorescence spectra of RhB in the absence and presence of CDSP-2 (see the Supporting Information). Complexation of RhB by CDSP-2 causes a sharpening and a slight bathochromic shift of the absorption band. The latter effect is characteristic for the immersion of the guest in a less polar environment. The shift in fluorescence is not pronounced, which suggests that the relaxation of the dye through geometrical and solvent effects is smaller, as expected for inclusion in a less polar and more confined environment.[14] However, at the same concentration of RhB, the fluorescence intensity of the complex is stronger than that of a pure RhB solution, which is probably because the supramolecular complexation between RhB and CDSP-2 reduces the self-quenching of RhB resulting from self-aggregation. A widely used method[21] was employed to determine the complexation stoichiometry, and a complexation ratio of 1:1 for RhB and CDSP-2 was found (see the Supporting Information).

The fluorescence modulation for the stoichiometric RhB–CDSP-2 complex upon exposure to UV or visible light is illustrated in Figure 1. After irradiating the complex solution with UV light (at 365 nm, 12 W) for five minutes, it was immediately moved into the fluorescence spectrometer to perform the emission measurements (at an excitation wavelength of 546 nm); in this case, the characteristic fluorescence emission of RhB (at 589 nm) was significantly quenched. However, after irradiation with visible light (at 460 nm, 15 W) for ten minutes, the intensity of this fluorescence signal was recovered (see Figure 1 a). During the fluorescence measurements, 546-nm light was used to excite the RhB molecules; this light could also convert the McH forms of spiropyran into SP forms. However, the rate of the ring-closure reaction from McH to SP (on the ten-minute scale)[22] is much lower than that of the energy-transfer process (on the nanosecond scale). In a one-minute fluorescence measurement carried out under weak irradiation (at 546 nm), only a small portion of McH spiropyran could be converted into SP spiropyran. The remaining McH form of spiropyran quenched the RhB

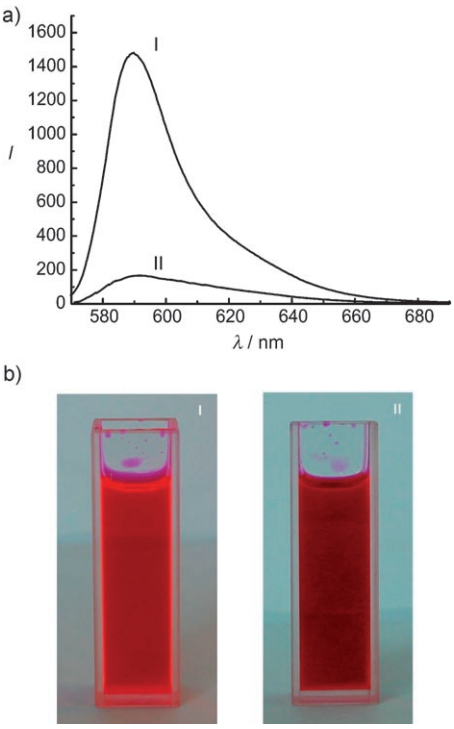

Figure 1. a) Fluorescence-emission spectra of the RhB–CDSP-2 solution (in ethanol/DMSO 4:1, v/v; concentration: 3×10^{-5} M, slit: 2.5 nm, $\lambda_{ex}=546$ nm) . b) Photographs of the RhB–CDSP-2 complex solution after: I) ten minutes of visible-light irradiation and II) five minutes of UV irradiation.

emission, while the SP form caused a background fluorescence (Figure 1 a, sample II).

In Figure 1 b, the appearance of the RhB–CDSP-2 solution after visible-light irradiation is clearly different from that after UV irradiation. Bright fluorescence can be seen in the former case under ambient light (which includes the excitation wavelength of RhB).

The reversible nature of the fluorescence modulation in the RhB–CDSP complexes upon exposure to alternating cycles of UV and visible light is illustrated in Figure 2. In an ethanol/dimethyl sulfoxide (DMSO) solution, five minutes of UV irradiation and ten minutes of visible-light irradiation can reversibly turn "on" and "off" the fluorescence of RhB.

From the above results, it is clear that the closed-ring form of the spiropyran moieties caused no change in the fluorescence of RhB, while the open-ring (McH) form did. The energy of the first-excited-singlet state of RhB was estimated to be 2.18 eV from the average frequencies of the absorption and emission bands. Similar calculations, based on absorption and emission maxima,[23] gave first-excited-singlet-state energies of 3.65 eV ($\lambda_{max}=340$ nm) for the SP-form spiropyran moieties of CDSP-2, and of 2.10 eV ($\lambda_{max}=552$ nm, $\lambda_{em}=630$ nm) for the McH-form moieties. These results suggest that energy transfer from RhB to the SP form of CDSP-2 is impossible, while transfer to the McH form of the compound may occur. Therefore, no fluorescence quenching of RhB could be observed before ring opening of the spiropyran moieties. However, upon UV illumination, these moieties

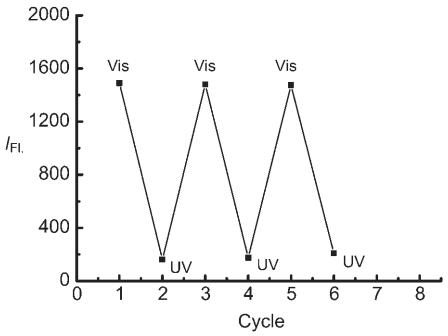

Figure 2. Fluorescence-emission intensity of the RhB–CDSP-2 solution (in ethanol/DMSO 4:1, v/v; concentration: 3×10^{-5} M, slit: 2.5 nm, $\lambda_{ex}=546$ nm) recorded at 589 nm immediately after several UV and visible-light irradiation cycles.

adopted the open-ring (McH) form, thus making energy transfer from the RhB molecules to the spiropyran centers more efficient; this resulted in an clear fluorescence quenching of rhodamine.

There are several requirements on the energy-transfer process:[24] In this case, there is an excited-state energy-level match between RhB and the open-ring spiropyran moieties, and a spectral overlap between the fluorescence emission of RhB ($\lambda_{em}=589$ nm, from 570 to 650 nm) and the absorption of the open-ring spiropyran moieties ($\lambda_{max}=552$ nm, from 500 to 610 nm). In addition, the distance between RhB (which is located inside the CDSP-2 cavity) and the open-ring spiropyran moieties is estimated to be 1.1–1.4 nm based on the bond lengths, the position of the RhB molecule included in CDSP-2, and the height of the CD molecule; this distance is within the Förster radius.[24] All these conditions can meet the requirements for a fluorescence resonance energy transfer (FRET) process.[24] To investigate whether the fluorescence quenching is a result of electron transfer, the electrochemical potentials of CDSP-2 were measured by means of cyclic voltammetry. No differences were observed in the oxidation or reduction waves when the SP-form spiropyran moieties were photoisomerized into the McH form (see the Supporting Information), which suggests that the reduction and oxidation potentials were not affected by the photoinduced structural changes. These results are consistent with previous reports on similar spiropyran derivatives.[23,25] Therefore, fluorescence quenching of RhB through electron quenching is unlikely; we believe that it could probably be the result of a FRET process instead.

In summary, we can use light to reversibly switch "on" and "off" the fluorescence emission of a rhodamine dye by including the dye molecules in the cavity of a spiropyran-modified β-cyclodextrin. Energy transfer could be the reason for fluorescence quenching by the McH form of CDSP-2. This study could open up possibilities for achieving facile fluorescence modulation—controlled by light—for various chromophore compounds.

Experimental Section

β-CD, *N*,*N*′-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), RhB, tetra-*n*-butylammonium hexafluorophosphate (TBAHFP), and 3-iodopropanoic acid were purchased from Sigma–Aldrich. 2,3,3-trimethylindolenine was received from Acros Organics. *N*,*N*-dimethylformamide (DMF), DMSO, ethyl methyl ketone, ethanol, and methanol were analytically pure solvents (and were distilled before use).

During the synthesis process, all the reaction vessels were wrapped with aluminum foil to ensure that the reaction proceeded in the dark. The carboxy-containing spiropyran l-(β-carboxyethyl)-3′,3′-dimethyl-6-nitrospiro(indoline-2′,2 [2*H*-1] benzopyran) (SpCOOH) was synthesized according to the literature method.[26] 2,3,3-trimethylindolenine (0.06 mol), 3-iodopropanoic acid (0.06 mol), and ethyl methyl ketone (5 mL) were heated under nitrogen at 100 °C for three hours. The resulting solid was dissolved in water, and the solution was washed with chloroform. Evaporation of water gave l-(β-carboxyethyl)-2,3,3- trimethylindolenine iodide (75 % yield). This iodide (0.04 mol), 5-nitrosalicylaldehyde (0.04 mol), and piperidine (0.04 mol) were dissolved in ethyl methyl ketone, and the solution was heated under reflux for three hours. After allowing the solution to stand over night, the product precipitated as a yellow crystalline powder, which was collected by filtration and washed with methanol to give the product SpCOOH (78 % yield).

CDSP-2: SpCOOH (8 mmol) and β-cyclodextrin (3 mmol) were added to anhydrous DMF (30 mL) in the presence of DCC (0.021 mol) and DMAP (0.0021 mol) and stirred at 25 °C for 24 h. The mixture was then filtered, and the filtrate was precipitated in cold methanol. Afterwards, the product was dissolved in DMF and re-precipitated in methanol (several times). Then, the product was washed with large amounts of deionized water, and the material was further purified by means of silica-gel chromatography [using ethyl acetate/petroleum ether (2:8, v/v) and then acetonitrile/water (10:1, v/v) as eluents] (28 % yield).

^{1}H NMR, ^{13}C NMR, and 2D-NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. UV/Vis spectra were recorded on a Hitachi U-3010 UV/Vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. The cyclic-voltammetric experiments were carried out with a CHI 660 setup (CH Instruments Inc.).

For the fluorescence and UV/Vis absorption measurements, RhB was dissolved in ethanol/DMSO (4:1, v/v) and mixed with an equimolecular amount of photosensitive cyclodextrin in ethanol/DMSO (4:1, v/v). The mixture was stirred for 48 h in the dark. The solvent was then evaporated, and the mixture was washed with deionized water and dried under vacuum at 65 °C for 100 h. The resulting powder was dissolved in deuterated DMSO for the 2D-NMR measurements.

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[1] M. Bossi, V. Belov, S. Polyakova, S. W. Hell, *Angew. Chem.* **2006**, *118*, 7623 – 7627; *Angew. Chem. Int. Ed.* **2006**, *45*, 7462 – 7465.
[2] M. Irie, S. Kobatake, M. Hirochi, *Science* **2001**, *291*, 1769 – 1772.
[3] J. Andréasson, Y. Terazono, B. Albinsson, T. A. Moore, A. L. Moore, D. Gust, *Angew. Chem.* **2005**, *117*, 7763 – 7766; *Angew. Chem. Int. Ed.* **2005**, *44*, 7591 – 7594.
[4] G. K. Such, R. A. Evans, T. P. Davis, *Macromolecules* **2006**, *39*, 9562 – 9570.

[5] S. Just, A. Aemissegger, P. Guntert, O. Zerbe, D. Hilvert, *Angew. Chem.* **2006**, *118*, 6445 – 6448; *Angew. Chem. Int. Ed.* **2006**, *45*, 6297 – 6300.
[6] N. Shao, J. Y. Jin, S. M. Cheung, R. H. Yang, W. H. Chan, T. Mo, *Angew. Chem.* **2006**, *118*, 5066 – 5070; *Angew. Chem. Int. Ed.* **2006**, *45*, 4944 – 4948.
[7] P. Belser, L. D. Cola, F. Hartl, V. Adamo, B. Bozic, Y. Chriqui, V. M. Iyer, R. T. F. Jukes, J. Kuhni, M. Querol, S. Roma, N. Salluce, *Adv. Funct. Mater.* **2006**, *16*, 195 – 208.
[8] J. Andreasson, S. D. Straight, G. Kodis, C. D. Park, M. Hambourger, M. Gervaldo, B. Albinsson, T. A. Moore, A. L. Moore, D. Gust, *J. Am. Chem. Soc.* **2006**, *128*, 16259 – 16265.
[9] X. Guo, D. Zhang, G. Yu, M. Wan, J. Li, Y. Liu, D. Zhu, *Adv. Mater.* **2004**, *16*, 636 – 640.
[10] J. L. Bahr, G. Kodis, L. Garza, S. Lin, A. L. Moore, *J. Am. Chem. Soc.* **2001**, *123*, 7124 – 7133.
[11] W. Saenger, *Angew. Chem.* **1980**, *92*, 343 – 361; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344 – 362.
[12] N. Benkirane-Jessel, P. Schwinte, P. Falvey, R. Darcy, Y. Haikel, P. Schaaf, J.-C. Voegel, J. Ogier, *Adv. Funct. Mater.* **2004**, *14*, 174 – 182.
[13] P. Falvey, C. W. Lim. R. Darcy, T. Revermann, U. Karst, M. Giesbers, A. T. M. Marcelis, A. Lazar, A. W. Coleman, D. N. Reinhoudt, B. J. Ravoo, *Chem. Eur. J.* **2005**, *11*, 1171 – 1180.
[14] J. Mohanty, W. M. Nau, *Angew. Chem.* **2005**, *117*, 3816 – 3820; *Angew. Chem. Int. Ed.* **2005**, *44*, 3750 – 3754.
[15] R. Dondon, S. Fery-Forgues, *J. Phys. Chem. B* **2001**, *105*, 10715 – 10722.
[16] J. Y. Ying, C. P. Mehnert, M. S. Wong, *Angew. Chem.* **1999**, *111*, 58 – 82; *Angew. Chem. Int. Ed.* **1999**, *38*, 56 – 77.
[17] V. Böhmer, *Angew. Chem.* **1995**, *107*, 785 – 818; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713 – 745.
[18] Y. Yang, K. Yook, J. Tae, *J. Am. Chem. Soc.* **2005**, *127*, 16760 – 16761.
[19] G. Berkovic, V. Krongauz, V. Weiss, *Chem. Rev.* **2000**, *100*, 1741 – 1754.
[20] T. Suzuki, T. Kato, H. Shinozaki, *Chem. Commun.* **2004**, 2036 – 2037.
[21] G. Pistolis, A. Malliaris, *J. Phys. Chem.* **1996**, *100*, 15562 – 15568.
[22] A. K. Chibisov, H. Gorner, *J. Photochem. Photobiol. A* **1997**, *105*, 261 – 267.
[23] L. Zhu, R. F. Khairutdinov, J. L. Cape, J. K. Hurst, *J. Am. Chem. Soc.* **2006**, *128*, 825 – 835.
[24] K. E. Sapsford, L. Berti, I. L. Medintz, *Angew. Chem.* **2006**, *118*, 4676 – 4704; *Angew. Chem. Int. Ed.* **2006**, *45*, 4562 – 4588.
[25] J. F. Zhi, R. Baba, K. Hashimoto, A. Fujishima, *J. Photochem. Photobiol. A* **1995**, *92*, 91 – 97.
[26] A. Fissi, O. Pieroni, G. Ruggeri, F. Ciardelli, *Macromolecules* **1995**, *28*, 302 – 309.

