ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 10874–10876

www.rsc.org/chemcomm

COMMUNICATION

Fluorescence photoswitching based on a photochromic pK_a change in an aqueous solution†

Tuyoshi Fukaminato,* Emi Tateyama and Nobuyuki Tamaoki*

Received 15th August 2012, Accepted 19th September 2012
DOI: 10.1039/c2cc35889a

Reversible fluorescence photoswitching of RSA-AZO dyad 1 was clearly demonstrated in an acidic aqueous solution. The fluorescence photoswitching mechanism is based on the reversible ring opening/closing reactions of the RSA unit induced by a photochromic pK_a change along with the photoisomerization of the AZO unit.

Fluorescent photochromic small molecules and proteins have attracted increasing interest owing to their potential applications in optical data storage and molecular switches.[1,2] In addition, biological applications such as bio-markers or super-resolution fluorescence microscopes are becoming one of the trends in this field.[3] For the latter applications, the fluorescence photoswitching property in aqueous conditions is indispensable. Although some fluorescence photoswitching in aqueous conditions including intracellular environments has been demonstrated by utilizing assembled systems such as nanoparticles or vesicles containing photochromic molecules,[4] (non-assembled) water-soluble fluorescent photochromic molecules are still rare[5,6] and it is desirable to improve their fluorescence properties and functionalities.

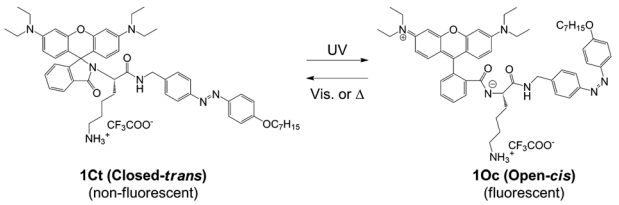
Rhodamine spiroamide (RSA) fluorophores[6] are some of the prominent functional groups of water-soluble fluorescent photoswitchable molecules for super-resolution microscopic applications because of their high fluorescence quantum yields and robust photochemical stability in aqueous conditions. The derivatives undergo photoinduced ring-opening and thermally induced ring-closing reactions. The closed-state is transparent in the visible region and is practically non-fluorescent. Upon irradiation with UV light, the open-state, which absorbs in the green region and emits at around 580 nm, is produced. Although the open-state is highly fluorescent and enables the detection of a fluorescence signal even at the single-molecule level, the fluorescent state cannot be maintained for a long time due to the rapid thermal back reaction (in a few milliseconds in polar solvents).[6a] The fast thermal recovery restricts its photoswitching application to only super-resolution fluorescence microscopy. On the other hand, the ring opening/closing processes of RSA depend on the pH or the polarity of the surrounding environment, that is, the open- and the closed-states of RSA exist in equilibrium depending on the local pH or polarity.[6b,d] With decreasing pH of the solvent, the ratio of the fluorescent open-state increases, and vice versa. In this study, we focused on this pH dependence of ring-opening/closing reactions of RSA. It is expected that ring opening/closing reactions of RSA can be indirectly induced by connecting a photochromic unit, which enables reversible control of the local conditions such as polarity or acidity along with its photoisomerization. This approach may allow us to keep the photo-induced fluorescent open-state of the RSA unit stable, which makes it possible not only to increase the detectable photon number in super-resolution microscopic applications but also to utilize the derivative for other applications such as fluorescent photoswitchable bio-markers. Here we report on the molecular design and the synthesis of a water-soluble fluorescent photoswitchable RSA derivative based on photochromic pK_a switching.

In order to prepare our desired fluorescent photoswitchable molecules, it was necessary to avoid spectral overlap between the absorption bands of both isomers of a photochromic unit and the fluorescence band of the RSA unit to deter fluorescence quenching due to an intramolecular energy transfer. Azobenzene (AZO) derivatives are one of the most popular photochromic compounds and have been extensively used for the photocontrol of biomolecular structures and biological functions.[7] AZOs show reversible *cis-trans* photoisomerization upon alternate irradiation with appropriate UV and visible light even in aqueous conditions and both isomers have an absorption band within relative short-wavelength regions ($\lambda < 500$ nm). In addition, it is well known that AZOs reversibly change molecular polarity or basicity along with the photoisomerization.[8,9] Taking these advantages into account, we selected AZOs as a suitable photoswitching unit in our molecular design.

It has been challenging to develop a highly fluorescent molecule to connect with the AZO unit, since the pico-second photoisomerization dynamics of AZO can easily quench the excited state of a fluorophore and therefore AZOs are commonly utilized as efficient fluorescence quenchers.[10] Eshow *et al.*[11] recently reported that highly fluorescent properties can be achieved for AZO-connecting fluorescent molecules by carefully selecting a fluorescence unit, whose absorption and fluorescence bands are far from the absorption band of the AZO unit. According to their strategies, we designed and synthesized a RSA-AZO dyad **1** (Scheme 1), in which an AZO derivative is

Research Institute for Electronic Science, Hokkaido University, N20, W10, Kita-ku, Sapporo 001-0020, Japan. E-mail: tuyoshi@es.hokudai.ac.jp, tamaoki@es.hokudai.ac.jp; Fax: +81-11-706-9350; Tel: +81-11-706-9350

† Electronic supplementary information (ESI) available: Detailed synthetic procedure of dyad **1**, HPLC analyses of **1** upon irradiation with 365 nm light and absorption spectra of dyad **1** in other pH conditions. See DOI: 10.1039/c2cc35889a



Scheme 1 Photoisomerization of RSA-AZO dyad **1**.

connected to the Rhodamine B fluorophore through an amino-acid lysine spacer. In this molecular design, the lysine spacer plays an important role in the separation of electronic states of both RSA and AZO units and the high water-solubility. Dyad **1** was prepared according to Scheme S1 in the ESI.† Synthetic and structural characterization details are provided in the ESI.†

The left sides of Fig. 1a and b show absorption and fluorescence spectral changes of **1** upon alternate irradiation with UV (365 nm) and visible (490 nm) light in an aqueous solution (pH 3.8 ± 0.1). The weak absorption band at 563 nm and the pronounced absorption bands around 300–400 nm, which correspond to the absorption band of the open-state of the RSA unit and the π–π* transition band of the AZO unit, are observed. The absorption spectrum suggests that the equilibrium between the open- and the closed-state of the RSA unit almost leans towards the closed-one before photo-irradiation. Upon irradiation with 365 nm light to the **1Ct** (Closed-*trans*) solution, the absorption band at around 300–380 nm is gradually decreased and the characteristic absorption band at 563 nm is dramatically increased and the solution color changes from nearly colorless to pink, as shown in the right side of Fig. 1a. These spectral and color changes suggest that the ring-opening reaction of the RSA unit takes place and the equilibrium between the open- and the closed-state of the RSA unit leans towards the open one. From HPLC analyses, the conversion ratio from the *trans*- to the *cis*-isomer of the AZO unit of dyad **1** upon 365 nm light irradiation at the photostationary state (PSS) was estimated to be >95% (see Fig. S1 in ESI†). Upon irradiation with 490 nm light, the absorption spectrum of the PSS solution nearly recovers to the initial one (Fig. 1a). The rate constant of thermal back-relaxation (k) for **1Oc** (PSS under irradiation with 365 nm light) was determined to be $2.2 \pm 0.1 \times 10^{-4}$ min^{-1} at 25 °C by plotting the absorbance at 563 nm (Fig. 1c) (a detailed explanation is described in ESI†). The small k value indicates that the photo-induced open-state of RSA-AZO dyad **1** can be maintained for a reasonably long time in comparison with the photoinduced open-state of conventional RSA derivatives.[6a]

As shown in the left side of Fig. 1b, reversible fluorescence intensity changes were also observed along with the photo-isomerization. Before photoirradiation, very weak orange fluorescence centred at 580 nm was observed under excitation with 520 nm light. When the solution reached the PSS under irradiation with 365 nm light, the fluorescence intensity increased to around 15 times the initial intensity and a strong orange emission was readily visible (right side of Fig. 1b). The fluorescence intensity decreased again upon visible (490 nm) light irradiation. These absorption and fluorescence spectral changes were repeatable for at least more than five-cycles (Fig. 1d). Fluorescence quantum yield (Φ_f) for the PSS state

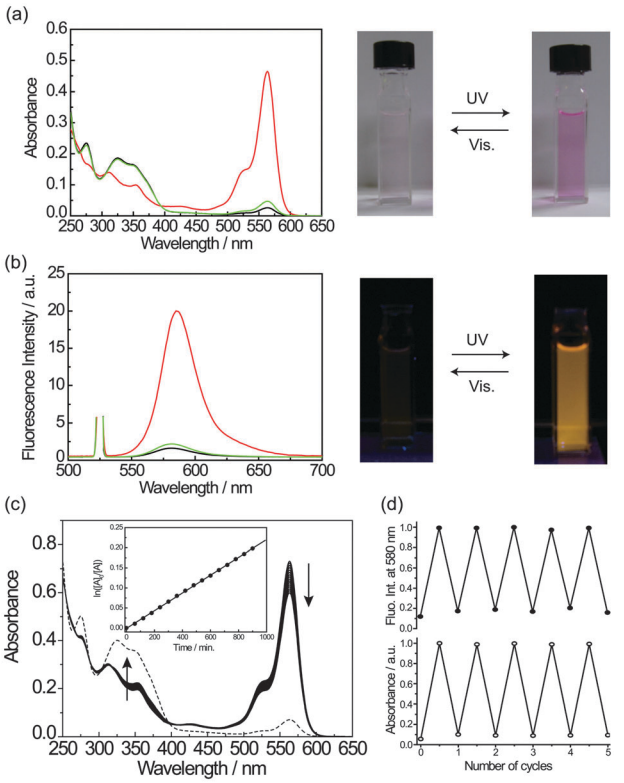


Fig. 1 (Left): (a) absorption and (b) fluorescence spectral changes in an aqueous solution ([C] = 8.8×10^{-6} M, pH 3.8 ± 0.1) upon alternate irradiation with UV (365 nm) and visible (490 nm) light; before photoirradiation (**1Ct**, black-line), PSS under 365 nm light irradiation (red-line), PSS under 490 nm light irradiation (green-line). (Right): photographs of the color and fluorescence changes along with photoisomerizations. (c) The change of absorbance at 563 nm of a PSS solution (pH 3.8 ± 0.1, 25 °C) under dark conditions; the dashed-line corresponds to the absorption spectrum before photoirradiation and each line is monitored at 1 hour intervals. Inset: the thermal fading of **1Oc** in an aqueous solution (pH 3.8 ± 0.1) at 25 °C under dark conditions. (d) Photoswitching cycles of **1** in an aqueous solution (pH 3.8 ± 0.1) upon alternate irradiation with UV (365 nm) and visible (490 nm) light. The changes of absorbance at 563 nm (open-circle) and fluorescence intensity at 580 nm (solid-circle) were monitored in an aqueous solution (pH 3.8 ± 0.1).

under 365 nm light irradiation was estimated to be 0.37 ± 0.05 in an aqueous solution (pH 3.8) (see "General" in ESI†), which is almost similar to that of typical RSA derivatives.[6d] Therefore, this result indicates that the quenching effect of the AZO unit on the fluorescence of the RSA unit was negligible.

These reversible absorption and fluorescence spectral changes in dyad **1** may be attributed to the change of the equilibrium constant between the open- and the closed-states of the RSA unit along with the photoisomerization of the AZO unit. In order to confirm the origin of the photoinduced ring-opening/closing reaction of the RSA unit, pH dependences of absorption spectra of RSA were measured for both *cis*-AZO and *trans*-AZO isomers. In an aqueous solution of pH 5.0, the absorption band at 563 nm was not clearly observed (see Fig. S2a in ESI†), while a characteristic strong absorption band at 563 nm was observed in a solution of pH 3.0 (see Fig. S2b in ESI†). In both conditions, however, the

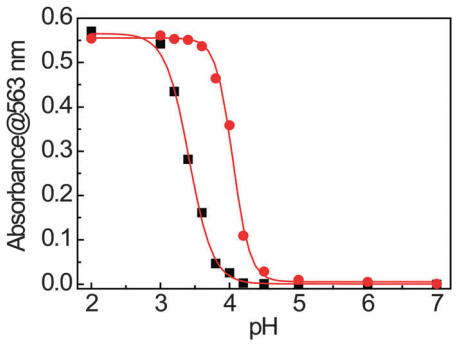


Fig. 2 Titration curves of the *trans*-AZO isomer (black-square) and *cis*-AZO isomer (PSS under 365 nm light irradiation) (red-circles). The absorption change at 563 nm was monitored in the pH range from 7.0 to 2.0 in aqueous solutions.

absorption spectral change at 563 nm band was not remarkably observed along with the photoisomerization of the AZO unit. The titration curves of an absorbance at 563 nm against the pH value of the solvent were plotted for both **1Ct** and **1Oc** solutions (Fig. 2). From these titration curves, pK_a values of the protonated open-form of RSA were estimated to be 3.4 for the *cis*-AZO and 4.1 for the *trans*-AZO isomer, respectively. Therefore, the useful pH range for the fluorescent photoswitchable molecule **1** can be estimated to be around 3.5–4.0. These results suggest that the observed ring-opening and closing reactions of the RSA unit upon alternate irradiation with UV and visible light are induced by pK_a changes along with the photoisomerization of the AZO unit.[9]

In conclusion, a water-soluble fluorescent photoswitchable molecule, RSA-AZO dyad **1**, was designed and synthesized, and the reversible fluorescence photoswitching was successfully demonstrated in aqueous solution. The mechanism in the fluorescence photoswitching of dyad **1** is based on the reversible change of molecular pK_a induced by the photoisomerization of the AZO unit. It is clearly confirmed that the fluorescence switching mechanism based on a photochromic pK_a change is useful for the design of water-soluble fluorescent photoswitchable molecules. Optimization of the switching efficiency and the useful pH range in this system are under progress.

This work was supported by JSPS KAKENHI; Grant-in-Aid for Challenging Exploratory Research (No. 23655111) and Young Scientist (A) (No. 24685021).

Notes and references

1 Reviews: (*a*) M. Irie, *Chem. Rev.*, 2000, **100**, 1685–1716; (*b*) F. Raymo and M. Tomasulo, *J. Phys. Chem. A*, 2005, **109**, 7343–7352; (*c*) M. Heilemann, P. Dedecker, J. Hofkens and M. Sauer, *Laser Photonics Rev.*, 2009, **3**, 180–202; (*d*) A. Bianco, S. Perissinotto, M. Garbugli, G. Lanzani and C. Berrelli, *Laser Photonics Rev.*, 2011, **5**, 711–736; (*e*) T. Fukaminato, *J. Photochem. Photobiol., C*, 2011, **12**, 177–208.

2 (*a*) R. M. Dickson, A. B. Cubitt, R. Y. Tsien and W. E. Moerner, *Nature*, 1997, **388**, 355–358; (*b*) M. Irie, T. Fukaminato, T. Sasaki, N. Tamai and T. Kawai, *Nature*, 2002, **420**, 759–760; (*c*) S. Habuchi, R. Ando, P. Dedecker, W. Verheijen, H. Mizuno, A. Miyawaki and J. Hofkens, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 9511–9516; (*d*) V. Adams, H. Mizuno, A. Grichine, J.-i. Hotta, Y. Yamagata, D. Bourgeois and J. Hofkens, *J. Biotechnol.*, 2010, **149**, 289–298.

3 (*a*) R. Ando, H. Mizuno and A. Miyawaki, *Science*, 2004, **306**, 1370–1373; (*b*) M. Bates, T. R. Blosser and X. Zhuang, *Phys. Rev. Lett.*, 2005, **94**, 108101; (*c*) S. W. Hell, *Science*, 2007, **316**, 1153–1158; (*d*) P. Dedecker, C. Flors, J.-i. Hotta, H. Uji-i and J. Hofkens, *Angew. Chem., Int. Ed.*, 2007, **46**, 8330–8332; (*e*) T. Grotjohann, I. Testa, M. Leutenegger, H. Bock, N. T. Urban, F. Lavoie-Cardinal, K. Willing, C. Eggeling, S. Jakobs and S. W. Hell, *Nature*, 2011, **478**, 204–208.

4 (*a*) M.-Q. Zhu, L. Zhu, J. J. Han, W. Wu, J. K. Hurst and A. D. Q. Li, *J. Am. Chem. Soc.*, 2006, **128**, 4303–4309; (*b*) Y. Zou, T. Yi, S. Xiao, F. Li, C. Li, X. Gao, J. Wu, M. Yu and C. Huang, *J. Am. Chem. Soc.*, 2008, **130**, 15750–15751; (*c*) Y. Osakada, L. Hanson and B. Cui, *Chem. Commun.*, 2012, **48**, 3285–3287.

5 (*a*) M. Heilemann, E. Margeat, R. Kasper, M. Sauer and P. Tinnefeld, *J. Am. Chem. Soc.*, 2005, **127**, 3801–3806; (*b*) G. Marriott, S. Mao, T. Sakata, J. Ran, D. K. Jackson, C. Petchprayoon, T. J. Gomez, E. Warp, O. Tulyathan, H. L. Aaron, E. Y. Isacoff and Y. Yan, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 17789–17794; (*c*) J. R. Nilsson, S. Li, B. Önfelt and J. Andréasson, *Chem. Commun.*, 2011, **47**, 11020–11022.

6 (*a*) J. Fölling, V. Belov, R. Kunetsky, R. Medda, A. Schönle, A. Egner, C. Eggeling, M. Bossi and S. W. Hell, *Angew. Chem., Int. Ed.*, 2007, **46**, 6266–6270; (*b*) V. N. Belov, M. L. Bossi, J. Folling, V. P. Boyarskiy and S. W. Hell, *Chem.–Eur. J.*, 2009, **15**, 10762–10776; (*c*) H. Aoki, K. Mori and S. Ito, *Soft Matter*, 2012, **8**, 4390–4395; (*d*) H. Montenegro, M. D. Paolo, D. Capdevila, P. F. Aramendia and M. L. Bossi, *Photochem. Photobiol. Sci.*, 2012, **11**, 1081–1086.

7 (*a*) M. Banghart, K. Borges, E. Isacoff, D. Trauner and R. H. Kramer, *Nat. Neurosci.*, 2004, **7**, 1381–1386; (*b*) S. Muramatsu, K. Kinbara, H. Taguchi, N. Ishii and T. Aida, *J. Am. Chem. Soc.*, 2006, **128**, 3764–3769; (*c*) B. Schierling, A.-J. Noël, W. Wende, L. T. Hien, E. Volkov, E. Kubareva, T. Oretskaya, M. Kokkinidis, A. Römpp, B. Spengler and A. Pingoud, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 1361–1366; (*d*) A. A. Beharry and G. A. Woolley, *Chem. Soc. Rev.*, 2011, **40**, 4422–4437; (*e*) T. Kamei, T. Fukaminato and N. Tamaoki, *Chem. Commun.*, 2012, **48**, 7625–7627.

8 (*a*) A.-L. M. Le Ny and C. T. Lee Jr., *J. Am. Chem. Soc.*, 2006, **128**, 6400–6408; (*b*) A. Estévez-Torres, C. Crozatier, A. Diguet, T. Hara, H. Saito, K. Yoshikawa and D. Baigl, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 12219–12223.

9 M. V. Peters, R. S. Stoll, A. Kühn and S. Hecht, *Angew. Chem., Int. Ed.*, 2008, **47**, 5968–5972.

10 S. Sando and T. Kool, *J. Am. Chem. Soc.*, 2002, **124**, 2096–2097.

11 (*a*) L.-H. Liu, K. Nakatani, R. Pansu, J.-J. Vachon, P. Tauc and E. Ishow, *Adv. Mater.*, 2007, **19**, 433–436; (*b*) L.-H. Liu, K. Nakatani and E. Ishow, *New J. Chem.*, 2009, **33**, 1402–1408; (*c*) A. Jacquart, R. M. Williams, A. M. Brouwer and E. Ishow, *Chem.–Eur. J.*, 2012, **18**, 3706–3720.