COMMUNICATION

Stabilization of the Closed-Ring Isomer of Spiropyran by Amide Naphthotube in Water and Its Application in Naked-Eye Detection of Toxic Paraoxon

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Abstract: The thermodynamically unstable, colourless closed-ring isomer of spiropyran can be stabilized in water by the anti-configurational isomer of amide naphthotube. The influence of the binding on the thermodynamics and kinetics of the spiropyran have been studied. The complex was further used to prepare a test paper that allows naked-eye detection of toxic paraoxon.

Photoswitchable molecules, such as azobenzene, spiropyran, and diarylethene, have recently received increasing attention due to their applications in photoresponsive materials, molecular machines and devices, molecules sensing and other fields.[1] Among these molecules, spiropyran[2] is unique because of their rich responsive properties. For example, the closed-ring spiro isomer (**2-SP** in Figure 1a) of spiropyran is colourless, while the open-ring merocyanine isomer (**2-MC**) possesses a large π conjugated system and absorbs visible light. These two isomers can be interconverted by UV/visible light or heating. Thermodynamic stability of the two isomers largely depends on the environment.[2] In organic solvents, the spiro isomer is more stable; on the hand, the merocyanine isomer is more stable in water presumably due to the extensive hydrogen bonds with surrounding water molecules.[3] However, majority of the merocyanine isomer can be photochemically converted into the spiro isomer under environment light, producing a far-from-equilibrium state with a mixture of both spiro and merocyanine isomers in water. In dark, the spiro isomer will gradually relax back to the merocyanine isomer which is thermodynamically more stable.

Host-guest chemistry can be used to modulate the relative stability between the spiro and merocyanine isomers of spiropyran compounds in water. The Klajn group and the Mukeherjee group[4] reported that positively-charged coordinated cages can stabilize the merocyanine isomer in water under room light. Crown ether,[5] sulfonatocalixarene,[6] and cucurbit[n]urils[7] show binding preference to the positively charged region of the merocyanine isomer and thus stabilize this isomer. Cyclodextrins[8] and octa-acid capsules[9] prefer the neutral spiro isomer. Photoswitchability between the spiro and merocyanine isomers have been studied inside these hosts. However, the conversion kinetics between spiro and merocyanine isomers in water has not been studied in details. In addition, the colour change of the process has not been used for the purpose of detection.

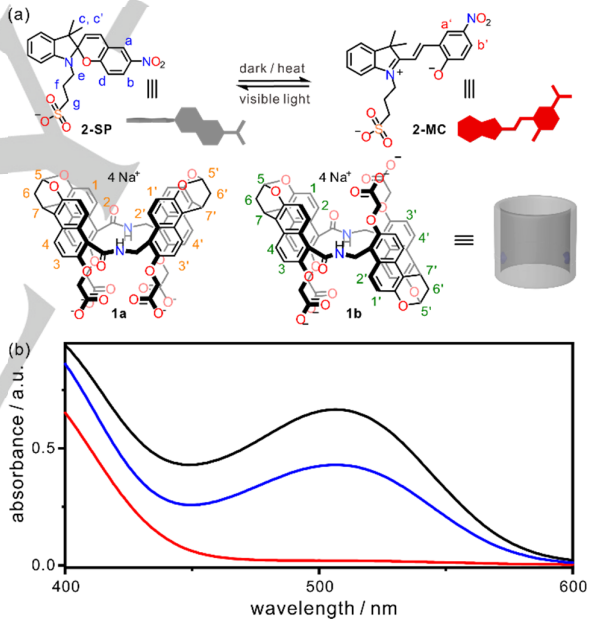

Figure 1. (a) Chemical structures and cartoons of the two isomers **2-SP** and **2-MC** of spiropyran **2** and the syn- (**1a**) and anti-configured (**1b**) isomers of amide naphthotubes **1**; (b) UV-Vis absorption spectra of spiropyran **2** in dark (black line), under room light in our lab (blue line) or after irradiation undervisible light for 10 s with a SupFire L6 torch (10 W, luminous flux =950 lm) (red line).

Over the past years, we have reported several macrocyclic hosts with functional groups inside their deep cavities.[10] In particular, water-soluble amide naphthotubes (**1a** and **1b**, Figure 1a) are able to strongly and selectively bind functional organic molecules in water through hydrogen bonding and the hydrophobic effect.[11,12] We are wondering whether naphthotubes **1a** and **1b** can be used to modulate the thermodynamic equilibrium and interconversion kinetics between the spiro and merocyanine isomers. Herein, we reported our findings: the anti-configurational isomer **1b** can stabilize **2-SP** in water, change its equilibrium with **2-MC**, and inhibit its conversion kinetics to **2-MC** in dark.

Moreover, an indicator displacement assay was constructed to visibly detect the toxic paraoxon using the selective binding of amide naphthotube **1b**.

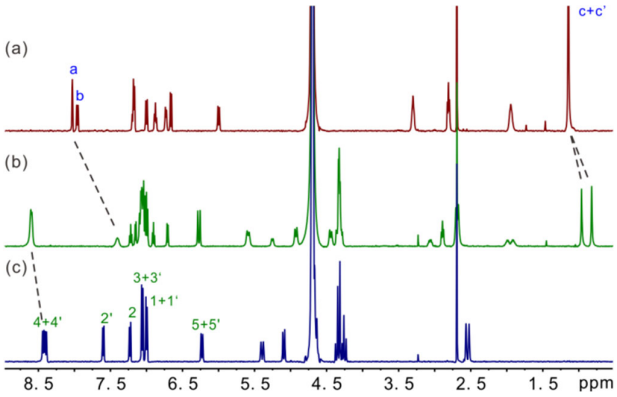

Figure 2. ^{1}H NMR spectra (500 MHz, D_2O, 0.5 mM, 298 K) of (a) **2-SP**, (c) **1b**, and (b) their equimolar mixture.

The nitro-spiropyran **2** is water-soluble and predominately exists as the **2-MC** isomer in water in dark.[3] However, under room light in our lab, **2-MC** will partly convert to **2-SP**. The ratio between **2-MC** and **2-SP** at the steady state is ca. 2:1 after exposing the solution for 24 hours under the room light in our lab, as determined from the ^{1}H NMR spectrum (Figure S1). After irradiating the solution with visible light with a SupFire L6 torch (10 W, luminous flux= 950 lm) for 10 seconds, **2-MC** (0.5 mM, 0.5 mL) can be completely converted to **2-SP**. While the solution of **2** was kept in dark for overnight, **2-MC** was found to be the sole product. These processes can also be monitored by UV-Vis spectroscopy (Figure 1b), in which **2-MC** has strong absorption of the visible light while **2-SP** is not. These results lay the basis for the studies on the binding properties of amide naphthotubes **1a** and **1b** to **2-SP** and **2-MC** separately.

Naphthotube **1a** is able to bind both **2-MC** and **2-SP** (Figures S2-S3). The Job's plot and molar ratio plot support a 1:1 binding stoichiometry (Figure S4). The binding constants were determined to be 890 M^{-1} for **2-SP** (Figures S5-S6) and 740 M^{-1} for **2-MC** (Figures S7-S8). This indicates that naphthotube **1a** is only a modest binder for the spiropyran compound; in addition, **1a** cannot differentiate the two isomers. In contrast, naphthotube **1b** selectively binds the **2-SP** isomer over the **2-MC** isomer. In an equimolar mixture of **1b** and **2-SP**, large chemical shift changes were observed for both the signals of the host and the guest (Figure 2). The binding constant between **1b** and **2-SP** was determined to be 5.3×10^4 M^{-1} in D_2O by ^{1}H NMR titrations (Figures S9-S11). However, the binding of **1b** to the **2-MC** isomer is much weaker, as indicated by the very small chemical shift changes of the signals upon adding the guest into the host (Figure S12). The binding constant between **2-MC** and **1b** was determined to be 330 M^{-1} (Figures S13-S14). The weaker binding of **1b** to the **2-MC** isomer may be due to the charge repulsion between the phenolate of the guest and the carboxylate groups of the host. Naphthotube **1b** has a clear binding preference to **2-SP** over **2-MC**, and thus can be further used to modulate the kinetics and thermodynamics of the spiropyran **2**. Therefore, the following discussion will be focused on the complexes between **1b** and **2**.

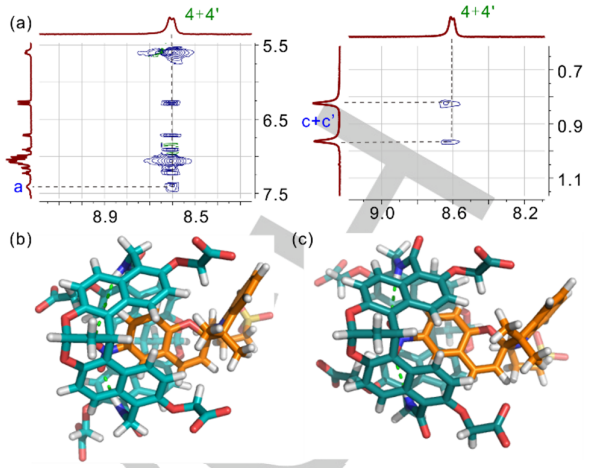

Figure 3. (a) Partial ^{1}H,^{1}H-ROESY NMR spectra (500 MHz, D_2O, 1 mM, 298 K) of **2-SP@1b**; (b, c) two possible orientational isomers of **2-SP@1b** as calculated by DFT (wB97XD) with the SMD solution model in water at 298 K.

Due to the low symmetry of **2-SP** and **1b**, there are four orientational isomers for their complex. ^{1}H,^{1}H-COSY and ^{1}H,^{1}H-ROESY NMR spectra were obtained to reveal the preferred binding orientation (Figures S15-S16). As shown in Figure 3a, nuclear Overhauser effect (NOE) signals were detected between protons 4+4' of **1b** and protons *a* and *c+c'* of the guest. This suggests the methyl groups of **2-SP** are close to protons 4/4', excluding two possible isomers (Figure S17). But further differentiation by ^{1}H NMR is not possible. Nevertheless, computational results (Figures 3b and S18) show the orientational isomer in Figure 3b is more stable than the other possible isomer in Figure 3c by 2.9 kcal/mol. The nitrophenyl group was included in the cavity of naphthotube **1b** (Figure 3b). Hydrogen bonds between the nitro group of the guest and the amide groups of the host and the hydrophobic effect should be the driving force for the binding. A similar binding geometry has been observed for **1b** with nitrobenzene as the guest.[11h]

How would naphthotube **1b** influence the thermodynamic equilibrium and kinetic conversion between **2-MC** and **2-SP**? Several NMR experiments have been performed to reveal the thermodynamic consequence (Figure S19). In dark, **2-MC** is more stable than **2-SP** in water and thus predominantly exists. When adding one equiv. of **1b** into the solution of **2-MC** and keeping the solution in dark, equilibrium was reached at 24 hours with the ratio between **2-SP** and **2-MC** was stabilized at 1:3 after 24 hours. This indicates that naphthotube can shift the equilibrium of **2-MC** and **2-SP**. Under room light in our lab, **2-MC** and **2-SP** exist in 2:1 ratio. When adding one equiv. of **1b** into this solution, the ratio between **2-MC** and **2-SP** was changed to 1:1. Obviously, the existence of the **2-SP** isomer was biased in the presence of **1b**. The change in ratio is a consequence of the binding. The resulting solution was kept in dark for 24 hours, and the ratio between **2-SP** and **2-MC** is 1:3 after reaching thermodynamic equilibrium. These results indicate the existence of **1b** can stabilize the closed-ring isomer **2-SP**, which should be caused by the preferred binding of **1b** to **2-SP** over **2-MC**.

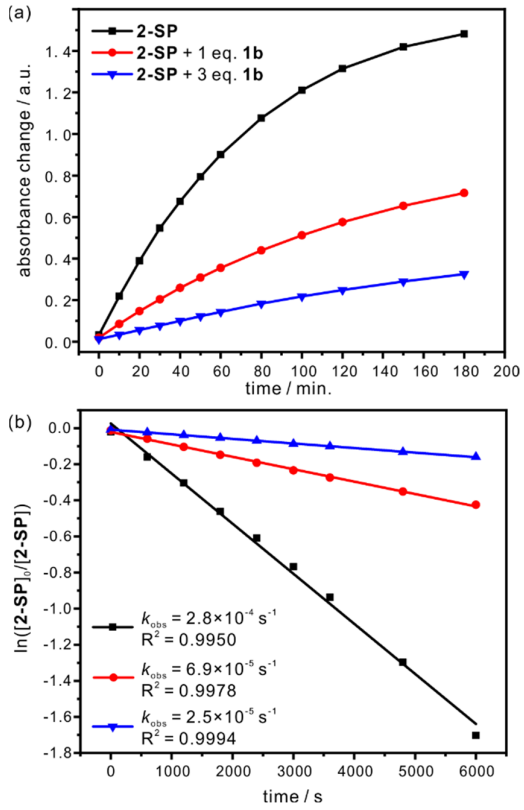

Figure 4. (a) Evolution of UV-Vis absorbance at 503 nm of spiropyran **2** (after irradiation under visible light for 10 s, 0.1 mM in pH = 7.2, 50 mM phosphate buffer) over time in the absence (black line) or the presence of one (0.1 mM, red line) or three (0.3 Mm, blue line) equiv. of **1b**; (b) Plots of ln([**2-SP**]$_0$/[**2-SP**]) versus reaction time t for the determination of the conversion kinetics according to a first-order rate law. (k_{obs}: observed rate constant)

The existence of naphthotube **1b** also slows down the kinetic conversion of **2-SP** to **2-MC** in dark. When irradiated at 10 W visible light for 10 seconds, **2-MC** in solution (0.1 mM, 2 mL) can be completely converted to **2-SP**. The resulting solution was kept in dark and the conversion of **2-SP** to **2-MC** was monitored by UV-Vis experiments.[13] As shown in Figure 4, the conversion of **2-SP** to **2-MC** follows first-order rate law and the rate constant k_1 was calculated to be 2.8×10^{-4} s^{-1} (Figures 4b and S20). The half-life is 6.9 hours. However, in the presence of one equiv. of **1b**, the rate constant and half-life were changed to 6.9×10^{-5} s^{-1} and 28 hours (Figures 4b and S21), respectively. With three equiv. of **1b**, the observed rate constant was changed to 2.5×10^{-5} s^{-1} (Figures 4b and S22). This shows that the naphthotube **1b** slows down the conversion kinetics of **2-SP** to **2-MC**, and **1b** is much more effective than that of cyclodextrins.[8a] The slowed kinetics is due to the binding between **2-SP** and **1b**. In the mixture of **2-SP** and **1b**, **2-SP** can only isomerize to **2-MC** when it is released from the cavity of **1b**. By assuming [**1b**] ≫ [**2-SP**] and **1b** does not bind **2-MC**, the relationship among observed rate constant (k_{obs}), the conversion rate constant (k_1) from **2-SP** to **2-MC**, and the binding constant (K_a) between **2-SP** and **1b**, and the concentration ([**1b**]$_0$) of **1b** can be simplified as follows (for details, see supporting information):[8a]

$$k_{obs} = \frac{k_1}{1 + K_a[\mathbf{1b}]_0}$$

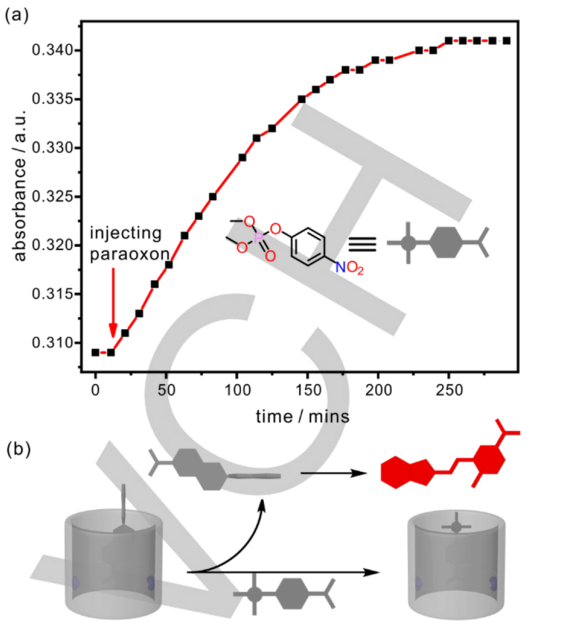

Figure 5. (a) Evolution of UV-Vis absorbance at 503 nm of **2-SP@1b** (0.1 mM. pH = 7.2, 50 mM phosphate buffer) after injecting two equiv. of paraoxon; (b) Cartoon representation of the principle of indicator displacement assay used for the naked-eye detection of paraoxon with **2-SP@1b**.

However, the current experimental condition does not strictly meet the condition of [**1b**] ≫ [**2-SP**] and that **1b** does not bind **2-MC**. Nevertheless, the calculated K_a (3.4×10^4 M^{-1}) according to the above equation by using the kinetic data with three equiv. of **1b** still qualitatively agrees with the binding constant (5.3×10^4 M^{-1}) as determined by NMR titrations.

When adding three equiv. of **1b**, the ratio between **2-SP** and **2-MC** changed to 4:1 in dark (Figure S22). Thus, the existence of **2-MC** was further inhibited; the solution changed from red colour to colourless. When a guest, which can be selectively complexed by **1b**, is added into the solution, **2-SP** would be displaced from the cavity of **1b** and further converted to **2-MC**. Consequently, the solution will become red. This colour change may be used for naked-eye detection of toxic chemicals. This design follows the principle of indicator displacement assay. [14]

As a demonstration, paraoxon (Figure 5a) was selected as a toxic compound. In our earlier work,[12] **1b** was shown to bind strongly to paraoxon in water (K_a = 2.8×10^4 M^{-1}); the toxicity of paraoxon to acetylcholinesterase was even inhibited by **1b**. **1b** is fluorescent and may work as a fluorescent sensor for paraoxon. However, paraoxon quenches the fluorescence of **1b**. A turn-on sensor is usually considered to be better than a turn-off sensor. Therefore, the fluorescent detection of paraoxon by **1b** is not ideal, leaving room for the development of a better sensor, for example, capable of naked-eye detection of paraoxon.

As shown in Figures 5a and S24, two equiv. of paraoxon was added into the pre-equilibrated equimolar mixture of **2** and **1b** (0.1 mM) in dark. The resulting solution was monitored at 503 nm by a UV-Vis spectrometer. The absorption at 503 nm was gradually increased, indicating that **2-SP** was displaced from the cavity of **1b** and gradually converted to **2-MC**. The principle of indicator displacement assay can be expressed in Figure 5b.

This design can be further used to prepare a test paper for naked-eye detection of paraoxon. The test papers were prepared by immersing the filter papers into the solution of **2-SP** (1.0 mM) and **1b** (3.0 mM) and then dried under air in dark (Figure 6a). No colour change was observed during the preparation of the test papers, showing that the complex **2-SP@1b** is stable in the solid state. When the test paper was moistened with the solution of paraoxon (5.0 mM), the paper becomes red after keeping it in dark for 1 hour (Figure 6b). In contrast, moistening the test paper with deionized water cause no obvious colour change. This demonstrates that the test paper containing the complex of **2-SP@1b** can be used for naked-eye detection of toxic compounds that can be selectively bound by **1b**.

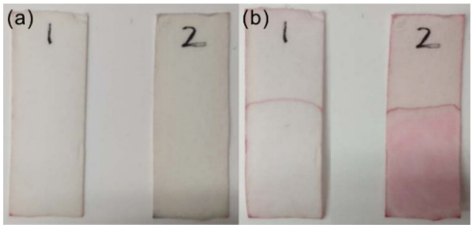

Figure 6. (a) Two test papers prepared by immersing them into the solution of **2-SP** (1.0 mM) and **1b** (3.0 mM) and then dried under air in dark; (b) the test papers that are moistened with deionized water (left) or 5.0 mM paraoxon solution (right) and then standing for 1 hour.

In summary, we report the anti-configured isomer of amide naphthotube is able to selectively bind the colourless, closed-ring spiro isomer of spiropyran over the red-coloured merocyanine isomer. Therefore, the amide naphthotube bias the equilibrium of the two isomers of spiropyran in dark and under room light, and inhibit the conversion kinetics of the spiro isomer to the merocyanine isomer. This selective binding of amide naphthotube causes a colour change of the solution of spiropyran, which was further used to prepare a test paper for naked-eye detection of toxic paraoxon. Considering the selective binding ability of amide naphthotubes,[11] this test paper may be used for the detection of other environmental contaminants, drug molecules, or toxic compounds.

Acknowledgements

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Keywords: spiropyran • molecular recognition • paraoxon • naphthotube

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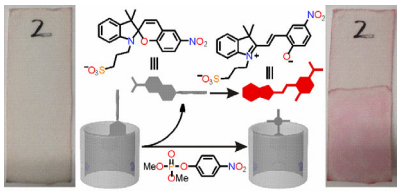

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