Kinetic characterization of spiropyrans in aqueous media†

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Received (in Cambridge, UK) 14th October 2008, Accepted 18th November 2008
First published as an Advance Article on the web 8th December 2008
DOI: 10.1039/b818050d

Detailed kinetic investigations of the most common photoswitchable spiropyran, 6-nitro-BIPS, reveal that hydrolytic decomposition of its merocyanine isomer limits its utility in aqueous buffer; however, simple replacement of the 6-nitro substituent with an 8-carboxylate yields a BIPS photoswitch that is potentially better suited for biological applications.

Spiropyrans are valuable photochromic compounds that are easily interconverted between colorless ring-closed spiropyran (sp) and colored ring-opened merocyanine (mc) isomers either by irradiation or heat (Fig. 1).[1] They have been intensively studied because of their utility in materials science.[1] Although biological applications are also desirable,[2] relatively little is known about the stability or kinetic properties of these dyes in water.[3] We have therefore examined the frequently used 6-nitro derivative of spiro-(2*H*-1-benzopyran-2,2′-indoline) (BIPS) in aqueous solution to determine the relative rates of thermal switching and decomposition.

Peptide **1a**, which contains the 6-nitro-BIPS chromophore and three lysine residues for solubility, was assembled by Fmoc solid phase peptide synthesis.[4] After HPLC purification, it was isolated in the closed spiropyran form, $\mathbf{1a}_{sp}$, which has an absorption maximum at 352 nm (Fig. 2). The open isomer, $\mathbf{1a}_{mc}$, was obtained by heating a millimolar solution of $\mathbf{1a}_{sp}$ in 0.2% TFA to 90 °C for one minute and then diluting the sample 100-fold in buffer. The merocyanine form of the dye has absorbance maxima at 373 and 520 nm (Fig. 2). As previously reported,[1] the dye isomers are readily interconverted by light of different wavelengths. If an aqueous solution of $\mathbf{1a}_{sp}$ is irradiated at 350 nm, as is typically done,[1] conversion to $\mathbf{1a}_{mc}$ is only about 10%. However, irradiation with UV light at 275 nm affords a 1 : 1 mixture of $\mathbf{1a}_{sp}$ and $\mathbf{1a}_{mc}$ within minutes, and irradiation of this mixture with visible light (λ = 520 nm) shifts the equilibrium back to the closed form $\mathbf{1a}_{sp}$. A *ca.* 1 : 1 mixture of $\mathbf{1a}_{sp}$ and $\mathbf{1a}_{mc}$ is also established thermally in buffer over several hours. Under these conditions, however, decomposition of the merocyanine isomer to give Fischer's base **2** and 4-nitro-salicylaldehyde **3a** competes with formation of the closed spiropyran (Fig. 1), as established by LC-MS and absorption spectroscopy. Hydrolysis is apparently initiated by conjugate addition of water to the ene-iminium cation of $\mathbf{1a}_{mc}$, followed by a retro-aldol reaction.

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† Electronic supplementary information (ESI) available: Experimental section and data fitting. See DOI: 10.1039/b818050d

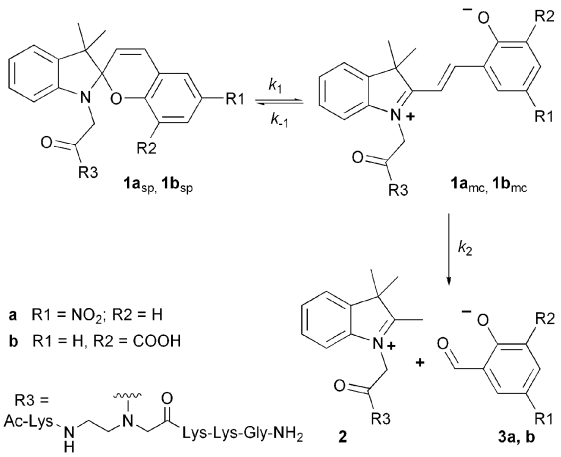

Fig. 1 Isomeric forms of the spiropyran moiety embedded in peptides **1a** and **1b**. In aqueous solution the merocyanine form of the chromophore undergoes hydrolysis to give Fischer's base **2** and salicylaldehyde **3**.

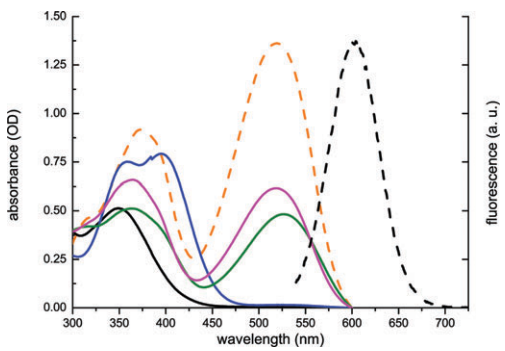

Fig. 2 Spectroscopic characterization of **1a**. Absorption spectra: $\mathbf{1a}_{sp}$ (—) and $\mathbf{1a}_{mc}$ (- - -); equilibrium mixture achieved by irradiation of **1a** at 275 nm (—); thermal equilibrium established upon heating **1a** for 5 min at 60 °C (—); hydrolysis products obtained by heating the sample at 60 °C for 90 min (—). Fluorescence emission of $\mathbf{1a}_{mc}$ (520 nm excitation, ---). In each case, the concentration of peptide **1** was 50 μM in 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0.

This system was characterized kinetically over a range of pH values, starting from either $\mathbf{1a}_{sp}$ or $\mathbf{1a}_{mc}$, by monitoring the formation or disappearance of the merocyanine isomer at 520 nm (Fig. 3). The data were analyzed by fitting the absorbance changes to the mechanistic model shown in Fig. 1 using the program DynaFit.[5] The rate constants for thermal ring opening and ring closure are the same within error over the pH range 5.0–8.0, and only about two times faster than the pseudo-first order rate constant for hydrolysis of $\mathbf{1a}_{mc}$ (Table 1). The fact that hydrolysis of the merocyanine ($\tau_{1/2} \approx 4$ h) occurs on roughly the same time scale as thermal

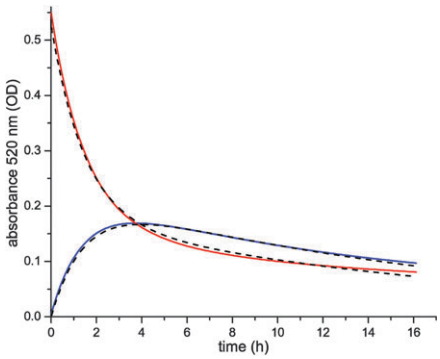

Fig. 3 Typical time course of the thermal equilibration/degradation of $\mathbf{1a}_{sp}$ (—) and $\mathbf{1a}_{mc}$ (—). Reactions were carried out with 20 μM peptide in 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0, 25.0 °C and monitored spectroscopically at 520 nm. The kinetic parameters k_1, k_{-1} and k_2 (Table 1) were obtained by fitting the data (---) to the model shown in Fig. 1.

Table 1 Kinetic parameters for interconversion and degradation[a]

Peptide	pH	k_1/s^{-1}	k_{-1}/s^{-1}	k_2/s^{-1}	$K = k_1/k_{-1}$
1a	0.1% TFA	8.0×10^{-5}	$<10^{-7}$	6.0×10^{-7}	$\gg 1$
	5.0	7.0×10^{-5}	7.0×10^{-5}	2.0×10^{-5}	1
	6.0	1.1×10^{-4}	1.1×10^{-4}	2.0×10^{-5}	1
	7.0	8.0×10^{-5}	9.0×10^{-5}	4.0×10^{-5}	0.9
	8.0	1.0×10^{-4}	2.6×10^{-4}	6.0×10^{-5}	0.4
1b	0.1% TFA	1.6×10^{-2}	n.d.	$<10^{-7}$	
	5.0	1.4×10^{-2}	n.d.	5.5×10^{-6}	
	6.0	1.0×10^{-2}	n.d.	5.0×10^{-5}	>100
	7.0	1.0×10^{-2}	n.d.	1.2×10^{-4}	
	8.0	1.4×10^{-2}	n.d.	1.8×10^{-4}	

[a] Reactions with peptides **1a** (20 μM) and **1b** (25 μM) were carried out in 0.1% TFA–water or in 10 mM sodium phosphate buffer, 100 mM NaCl, pH 5.0–8.0. Kinetic data were obtained from absorbance time traces at 520 nm and 25.0 °C for **1a** and 440 nm and 29.0 °C for **1b**. Estimated errors for all kinetic values are ±20%.

equilibration of the open and closed forms of the dye ($\tau_{1/2} \approx 2$ h) seriously limits the utility of 6-nitro-BIPS as a photoswitch in aqueous solution. Although the spiropyran form of the dye is stable, decomposition begins as soon as $\mathbf{1a}_{mc}$ is produced, either photochemically or thermally, thus reducing the number of times that $\mathbf{1a}_{sp}$ and $\mathbf{1a}_{mc}$ can be productively interconverted.

To obtain dye molecules with more favorable properties for biological applications, we prepared and isolated differently substituted spiropyran derivatives by aldehyde exchange from commercially available aldehydes. For example, peptide **1b**, containing an 8-carboxylic acid rather than a 6-nitro substituent, was generated by heating millimolar aqueous solutions of **1a** in the presence of a 10-fold excess of 3-formylsalicylic acid. In contrast to **1a**, which is isolated as the spiropyran $\mathbf{1a}_{sp}$, peptide **1b** is obtained as the merocyanine isomer $\mathbf{1b}_{mc}$. In aqueous buffer, the thermal equilibrium between $\mathbf{1b}_{sp}$ and $\mathbf{1b}_{mc}$ lies completely on the side of the open merocyanine as a consequence of a 125-fold faster rate of ring opening compared to **1a** (Table 1). The merocyanine can be quantitatively converted to pure $\mathbf{1b}_{sp}$ by irradiation with blue light (440 nm),

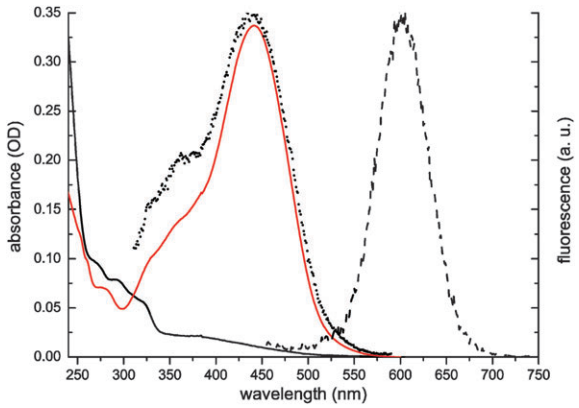

Fig. 4 Absorption spectra of $\mathbf{1b}_{sp}$ (—) and $\mathbf{1b}_{mc}$ (—) and fluorescence emission and excitation spectra of $\mathbf{1b}_{mc}$ (440 nm excitation, ···; 600 nm emission, ---). The spectra were measured in 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0 with 25 μM peptide.

but thermal reconversion to $\mathbf{1b}_{mc}$ occurs within minutes. Although $\mathbf{1b}_{mc}$ decomposes at roughly the same rate as $\mathbf{1a}_{mc}$, the time scale of hydrolysis ($\tau_{1/2} \approx 4$ h) is much longer than thermal ring opening ($\tau_{1/2} \approx 1$ min) or photochemical ring closure ($\tau_{1/2} \approx 1$ min), allowing peptide **1b** to be switched many times between pure $\mathbf{1b}_{sp}$ and pure $\mathbf{1b}_{mc}$ with only minor degradation. The large Stokes shift (160 nm) and the absence of distinct features above 280 nm in the absorption spectra of $\mathbf{1b}_{sp}$ (Fig. 4) render this new photoswitch suitable for applications involving Förster resonance energy transfer.

In conclusion, relatively rapid hydrolysis of the merocyanine form of 6-nitro-BIPS competes with thermal interconversion of the open and closed forms of the dye under physiological conditions, significantly limiting the utility of this photochromic compound in water. Replacement of the 6-nitro substituent with an 8-carboxy group enhances the rate of ring opening by two orders of magnitude, making 8-carboxy-BIPS an attractive alternative to 6-nitro-BIPS as a chemical switch in biological systems.

This work was generously supported by the Deutschen Akademie der Naturforscher Leopoldina (Bundesministerium für Bildung und Forschung BMBF-LPD 9901/8-158) and the ETH Zürich.

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