Basic amino acid induced isomerization of a spiropyran: towards visual recognition of basic amino acids in water

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Basic amino acids (BAA) induced 1-(β-carboxyethyl)-3,3-dimethyl-6′-nitrospiro(indoline-2,2′[2*H*-1]benzopyran) (SP) isomerization to its open form, *i.e.* merocyanine (MC), and the formation of its complex with BAA in water was reported for the first time, which may provide a potential application for *in situ* recognition of BAA.

Spiropyrans are well-known photochromic compounds, *i.e.* undergoing reversible structural isomerization between a colorless spiro form and a colored open form by UV light and *vice versa* by visible light or heat. The open form is not thermally stable, and the decay rate decreases with increasing solvent polarity. As early as 1965, Phillips *et al.* pointed out the chelating capability of merocyanine.[1] Since then, tremendous attention has been focused on metal ions and their effects on the photochemical and photophysical properties of merocyanine.[2–5] One distinct property is cation induced isomerization for some spiropyran derivatives, which has potential for metal ion recognition.[6]

The interactions between spiropyrans and amino acids is a very sensitive and interesting field. Only a few papers have been published so far.[7,8] A premise is that before the interaction occurs the merocyanine form needs to be present, which means that the SP solution is irradiated by UV light first,[9,10] otherwise the merocyanine would be thermally stable at room temperature. Inouye *et al.* reported the selective coloration of spiropyridopyrans with guanosine derivatives based on a three hydrogen bond complementarity between the open form of spiropyran and guanine.[11,12] Recently, Yang *et al.* presented a new strategy for the recognition of cysteine and homocysteine based on the fact that metal ions can bind both an amino acid and the open form of spiropyran.[13]

Though Inouye and Yang have offered solutions, a drawback towards practical implementation of amino acid recognition still exists: all the proposed systems were operated in organic solvents or mixtures of water with organic solvent, which are quite different from the live environment. This report offers a method for basic amino acid (BAA) induced spiropyran isomerization and formation of a complex between the open form and BAA in pure water.

After the aqueous solution of mixed 1-(β-carboxyethyl)-3,3-dimethyl-6′-nitrospiro(indoline-2,2′[2*H*-1]benzopyran) (SP) and histidine (His) was kept in the dark for 1 h at room temperature, the colorless solution became red (Fig. 1). Reversibility between the red and colorless solution was observed. When the red solution was exposed to white light, the red color disappeared, and when it was incubated in the dark again, it reverted to red. This cycle can be repeated many times. Obviously, this cycle is related to the ring-opening and ring-closing processes of SP. The possibility of thermochromism of SP in the dark was excluded by a control experiment. In the absence of His, no red color change was observed no matter how long it was kept in the dark. In addition, zinc acetate or ethylenediamine was added to the water solution of SP in order to increase its solubility. After they were kept in the dark for 24 h, no color change and no absorption band in the visible region were observed. Thus, the possibility that water may induce reverse photochromism of SP was excluded.[14] These results indicated His was responsible for the SP coloration, and the red color species in solution should be a complex between the open form of SP (MC) and His, *i.e.* MC–His (Scheme 1).

SP displays no absorption bands in the visible region, however, upon formation of MC–His, a broad irregular band centered at λ = 505 nm was observed. Time-dependent absorption intensity centered at λ = 505 nm is displayed in Fig. 2a. The recognition process proceeded to completion in about 1–6 h, which was dependent on the His concentration. An obvious feature was the decreasing baseline with increasing incubation time. It is understandable considering the Mie scattering effect of the SP suspension,[15] and with the gradual formation of zwitterionic MC–His, the Mie scattering effect

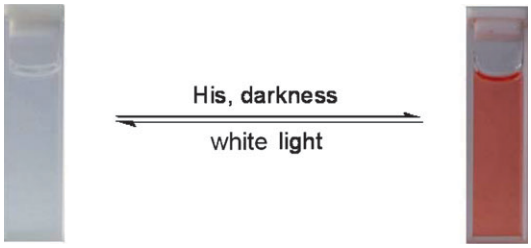

Fig. 1 Photograph of the color changes in the mixture of SP and His in water by alternate exposure to darkness and white light.

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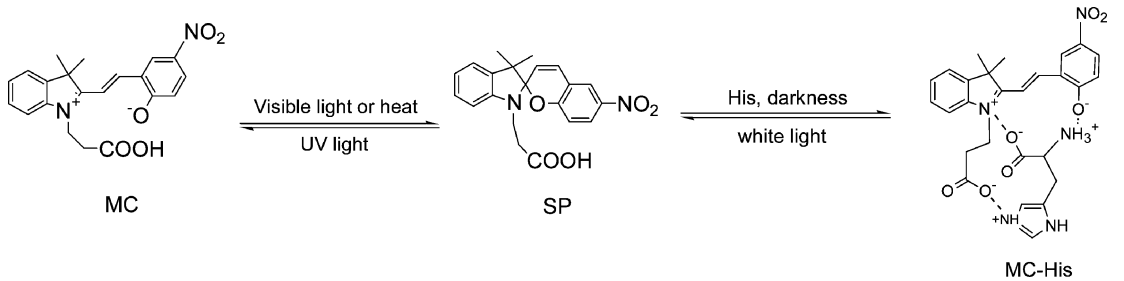


Scheme 1 The structure of SP, its isomerization upon irradiation, and the proposed structure of the binary complex between MC and His.

was gradually suppressed. The inset in Fig. 2a shows the reversibility with alternate exposure to darkness and white light in which the decrease and recovery of the 505 nm absorption band were monitored.

MC–His in water is fluorescent, displaying an emission band centered at $\lambda = 620$ nm. In addition, its excitation spectrum shows a good mirror image of its fluorescence spectrum (Fig. 2b). The peak centered at $\lambda = 380$ nm is ascribed to the closed SP. The lifetime of the excited singlet state of MC–His was also investigated. It followed a monoexponential decay, with a lifetime of 503 ps. This is different from the results reported in the literature, which showed biexponential decay, with two shorter lived species (100 and 250 ps). The complexion with His may restrict the torsional dynamics of MC, resulting in a longer lifetime.[10]

The complex was thermally stable in water. When irradiated with white light, it decayed back to SP and His. This process obeyed first order kinetics, with a rate constant $k = 6.7 \times 10^{-4}$ s^{-1} at room temperature (Fig. 2c). Two steps could be included in the process; the first was the very fast decomposition of the complex and then the slow ring closure of MC, which is the kinetic decisive step.

For a reference study, UV irradiation of the water solution of SP was conducted for 5 minutes. Due to the quite low solubility of SP in water, it aggregated fast, which resulted in a high baseline, and only a shoulder for MC was obtained. The

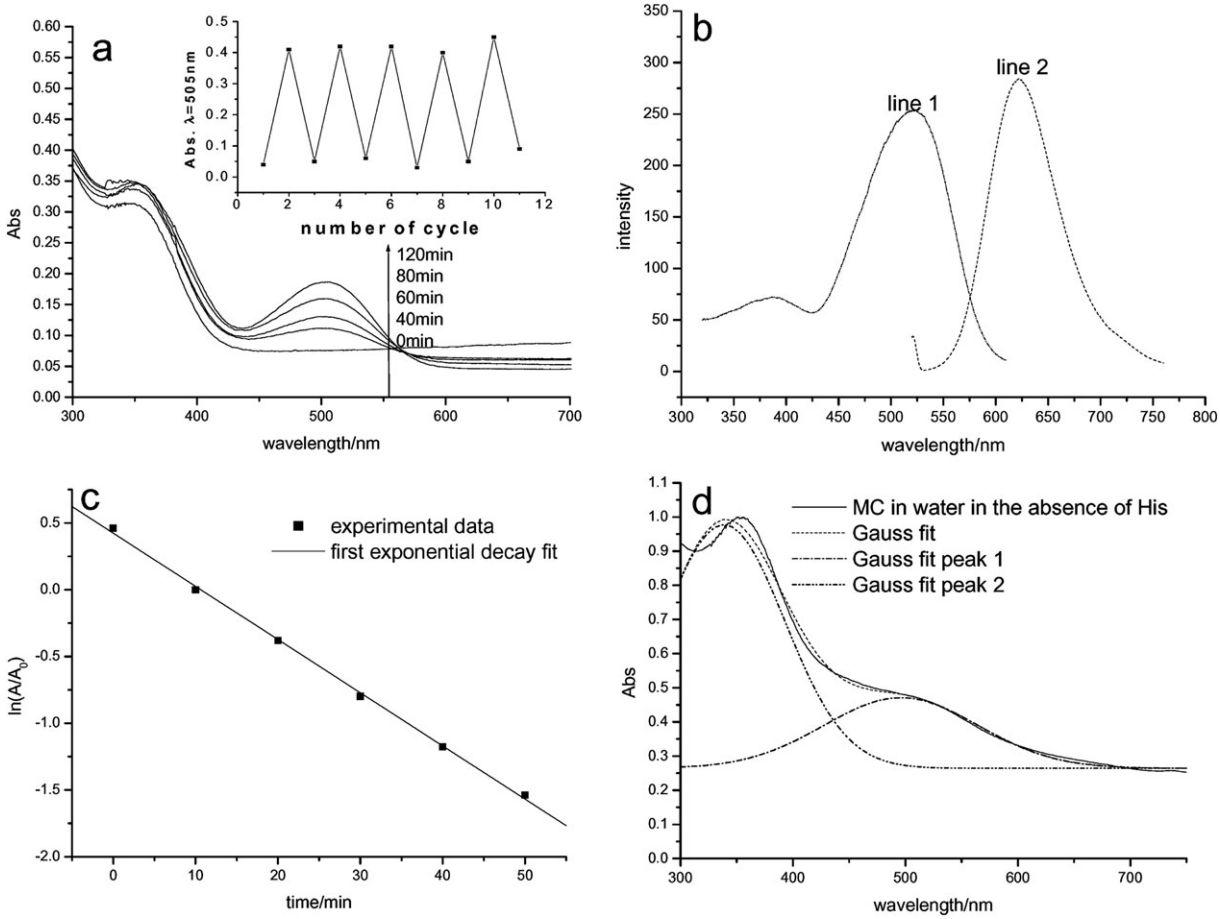
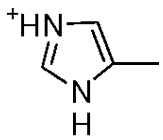


Fig. 2 (a) Time dependent absorption intensity of MC–His kept in the dark; inset shows the reversibility with alternate exposure to darkness and white light; (b) excitation (line 1) and emission (line 2) spectra of MC–His; (c) first order decay of MC–His irradiated with white light at room temperature; and (d) Gauss fit of MC absorption spectra in water in the absence of His.

spectrum was fitted to two Gaussian peaks centered at λ = 350 nm and 505 nm, which are the maximum absorption wavelengths for SP and MC, respectively (Fig. 2d). This confirmed our conclusion that the new red solution is related to MC and binding His had little effect on the energy of its first excited state, which is in agreement with the literature.[16] The emission spectra of MC and its decay in this study were not determined because of the very low concentration of MC.

Similar results were obtained for the other two BAA, lysine (Lys) and arginine (Arg). No spectral differences were observed, which is consistent with above discussion that amino acids have little effect on the maximum absorption wavelength of MC. The kinetics, however, distinguished the three complexes from each other under the same experimental conditions (temperature, concentration, *etc.*) as that for His (Fig. 3). Considering that the BAA has little effect on the molar absorption coefficients of the three complexes,[16] the kinetic order is His > Lys > Arg, which is opposite to their isoelectric points (Ip).

Based on the pK_a values of the different groups in SP and His, one can discuss the formation mechanism of the complex MC–His. The pK_a value for the carboxyethyl in SP is 3.5,[17] while the pK_a values for the –COOH, $-NH_3^+$, and **1** in His is 1.82, 9.17, and 6.00, respectively.



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Thus, when SP and His are both present in water, the predominant species is $-COO^-$, $-NH_3^+$, and **1**. The anionic carboxyethyl in SP cost one ammonium (**1**) through electrostatic effects, and the remaining cationic ammonium ($-NH_3^+$) induces the ring-opening of SP to produce the anionic phenolic group, since its pK_a is 2.25,[17] which resulted in the formation of the second point of electrostatic interaction. The $-COO^-$ in His formed another ion pair with the just-formed $C{=}N^+$ in MC. This fits to the other two BAA (Lys and Arg, Scheme 1). According to this, the different kinetics of MC with the three

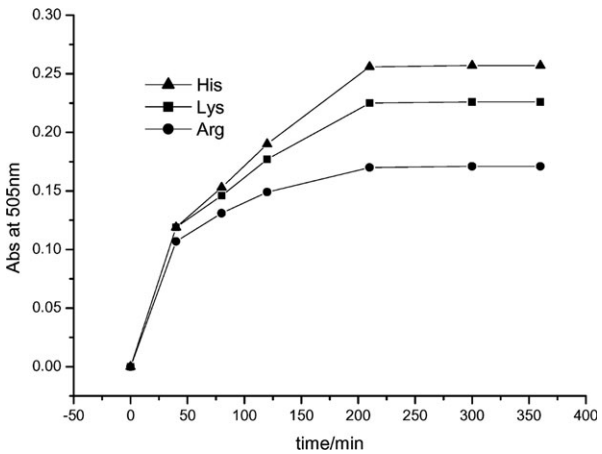

Fig. 3 Comparison of the kinetics for the formation of complexes of SP with the three BAA in the dark.

Table 1 The maximum absorption wavelengths (λ_{max}) and first order thermal decay rates (k) in the dark of MC–His and MC (in parentheses) in organic solvents after 5 s UV light irradiation

Solvents	λ_{max}/nm	$k/10^2$ s^{-1}
THF	577 (575)	5.18 (5.33)
DMF	559 (560)	0.738 (1.05)
MeCN	568 (569)	0.510 (1.19)
DMSO	577 (575)	0.158 (0.489)
MeOH	531 (532)	0.0390 (0.0542)

BAA can be understandable. Specifically, the lower the Ip, the faster the kinetics.

The influence of solvents on SP in the absence and presence of His was examined in organic solvents: tetrahydrofuran (THF), acetonitrile (MeCN), methanol (MeOH), *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). Negligible absorption intensity in the presence of His was observed in these organic solvents, indicating the unique effect of water on BAA induced SP isomerization.

Table 1 displays the thermal decay rates of the complex (His–MC) and MC (in parentheses) in these organic solvents after 5 s UV light irradiation. Obviously, the presence of His stabilized MC to some extent due to the formation of the complex, though the complex is not thermally stable in these organic solvents.[16] What's more, His was more effective at stabilizing MC in polar solvents than in less polar solvents. In less polar THF, the stability of the MC–His and MC are almost the same. With increasing solvent polarity, however, the decay rate of MC–His decreased gradually. The high polarity of water made MC–His very stable, *i.e.* it did not isomerize to SP unless it was exposed to white light. Thus, it is the high polarity of water that is responsible for the unique phenomenon of BAA induced SP isomerization.

In summary, by allowing a mixture of SP and His in water to stand in the dark for one hour, a visual color change from colorless to red spontaneously occurred. Two major facts are noteworthy and are addressed in this report (1) the system was operated in pure water, and the study on the photochemical and photophysical properties of the complex between MC and His has been reported for the first time; (2) it provides a simple way for potential *in situ* recognition just through a visual color change after keeping the mixture in the dark. A further study on the synthesis of a water soluble spiropyran is under way with the aim to find highly sensitive amino acid sensors.

Experimental

SP was synthesized according to the literature.[18] Ultrapure water was produced using a Milli-Q apparatus with a resistivity of 18.2 MΩ cm (Millipore). Amino acids, zinc acetate and ethylenediamine were of analytical reagent grade.

The concentrations of SP and BAA were 3.0×10^{-5} mol L^{-1} and 1.0×10^{-4} mol L^{-1}, respectively. All the experiments were carried out at room temperature (298 K). The UV-visible absorption spectra were measured using a Perkin Elmer Lambda 35 UV/Vis spectrophotometer with the solution in a quartz cuvette with a 1 cm path length. The steady-state fluorescence was carried out in a Hitachi F-4500 Fluorospectrometer. The time-resolved fluorescence was

measured using an Edinburgh Analytical Instrument FLS920 time-resolved spectrofluorometer, using the time-correlated single photon counting (TCSPC) method. A 500 W high-pressure mercury lamp was applied during UV irradiation.

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