Photochromic and Fluorescence Properties of a Hemiindigo in the Presence of Bovine Serum Albumin

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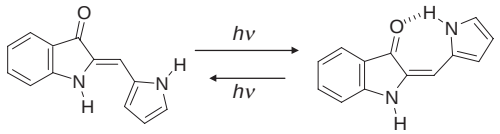
The hemiindigo derivative having a pyrrole ring exhibited photochromic and thermochromic properties in the presence of bovine serum albumin (BSA) in phosphate buffer solution.

We have previously reported that the hemiindigo derivative having a pyrrole ring (**1**) exhibited the photochromic properties in organic solvent.[1,2] *E*-**1** forms intramolecular hydrogen bonding in non-protic solvents. However, protic solvents may interact with **1** by forming intermolecular hydrogen bonding. Actually, *Z*-**1** is more stable by 0.85 kcal mol^{-1} than *E*-**1** in methanol due to the formation of intermolecular hydrogen bonding between *Z*-**1** and methanol. But *E*-**1** is more stable by 1.4 kcal mol^{-1} than *Z*-**1** in acetonitrile.[1] These results suggest that *E*-**1** is more hydrophobic than *Z*-**1**, and therefore, the hydrophobic site of protein such as bovine serum albumin (BSA) may give an interesting environment for photochemical reaction of **1**.



Z-**1** *E*-**1**

In the absence of BSA:	Non-fluorescent	Non-fluorescent
In the presence of BSA:	Non-fluorescent	Highly fluorescent

BSA is a well known host of organic molecules in aqueous solution.[3–5] For example, the photoisomerization of bilirubin, which is the degradation product of the heme, is affected by the presence of albumins.[6]

Figure 1 shows the change in the absorption spectrum of *E*-**1** (5×10^{-6} M in phosphate buffer; pH = 7.4; Wako 166-17445) by the addition of BSA (Sigma 7030). *E*-**1** exhibited the absorption spectrum with the maximum wavelength of 524 and 531 nm in the absence of BSA and in the presence of BSA (3×10^{-5} M), respectively in phosphate buffer solution (Figure 1a). No fluorescence emission of *E*-**1** was observed in the absence of BSA. However, the fluorescence spectrum of *E*-**1** was observed on addition of BSA and the intensity increased with increasing the concentration of BSA as shown in Figure 1b. The maximum wavelength of the fluorescence spectrum of *E*-**1** (5×10^{-6} M) was 558 nm and its fluorescence lifetime in the presence of BSA (3×10^{-5} M) was 4.2 ns. The absorption spectrum of *Z*-**1** (5×10^{-6} M) was not affected by the addition of BSA and *Z*-**1** did not exhibit a fluorescence emission in phosphate buffer solution even in the presence of BSA. These results indicated that *E*-**1** was bound to a hydrophobic site of BSA, but *Z*-**1** did not interact with BSA.

There might be some degree of aggregation of *E*-**1** in aqueous solution, which might cause *E*-**1** to have a low molar absorption coefficient and a low fluorescence quantum yield. Thus, it is possible to assume that the addition of BSA might simply facilitate deaggregation. However, the addition of surfactants such as sodium dodecyl sulfate (SDS) could solve *E*-**1** and *Z*-**1** in water by forming micelle and the addition of SDS did not enhance the fluorescence emission of *E*-**1**. This result also suggests that the fluorescence enhancement is due to the binding of *E*-**1** to BSA.

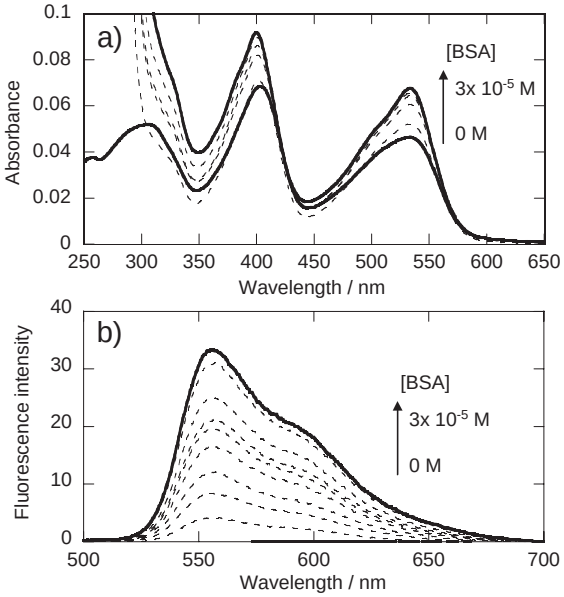


Figure 1. The change of (a) absorption and (b) fluorescence spectra of *E*-**1** (5×10^{-6} M) by the addition of BSA at the concentration from 0 to 3×10^{-5} M in phosphate buffer solution.

The change of the fluorescence intensity of *E*-**1** upon addition of BSA is consistent with the change of the absorbance upon addition of BSA as shown in Figure 2, indicating that the increase in the fluorescence intensity is resulted from the binding of *E*-**1** to BSA. The association constant $K = [E\text{-}\mathbf{1}\cdot\text{BSA}]/([E\text{-}\mathbf{1}][\text{BSA}])$ was calculated to be $(5.4 \pm 0.9) \times 10^4$ M^{-1} by least squares curve fitting.

The fluorescence lifetime (Quantum yield; Φ_f) of *E*-**1** is 210 ps (6×10^{-3}), < 200 ps (2×10^{-3}), < 200 ps (2×10^{-3}) in benzene, acetonitrile, and methanol, respectively.[1] The fluorescence lifetime of *E*-**1** increases with decreasing temperature from 210 ps at 295 K to 2.9 ns at 185 K in toluene.[1] No fluorescence spectrum of *E*-**1** was observed in phosphate buffer solution as well as in water. Very interestingly, *E*-**1** gave a fluorescence emission in the presence of BSA with a lifetime of 4.2 ns as mentioned above. The large enhancement of fluorescence intensity of *E*-**1** in the presence of BSA in aqueous solution indicates that a hydrophobic site of BSA plays an important role in affecting

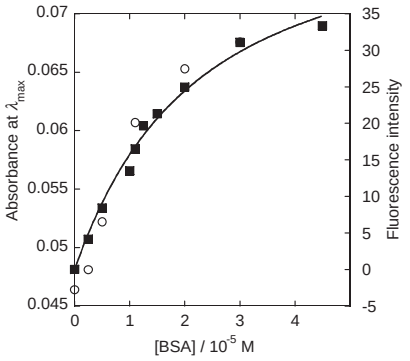


Figure 2. The change of the absorbance (open circle) and the fluorescence intensity (square) of *E*-**1** (5×10^{-6} M) by the addition of BSA in phosphate buffer. The solid line represents the fitting curve for estimating the binding constant of *E*-**1** to BSA.

the fluorescence property of *E*-**1**. In a hydrophobic site of BSA, the rotational relaxation process of *E*-**1** may be suppressed to increase the fluorescence intensity.[7] The quantum yield of *E*-to-*Z* isomerization of **1** was less than 0.01. Thus, intermolecular hydrogen bonding or other interactions in the hydrophobic site may decrease the non-radiative rate constant from the singlet excited state of *E*-**1**.

Compound **1** underwent *Z*-to-*E* isomerization photochemically and *E*-to-*Z* isomerization thermally in water, and therefore, **1** exhibited interesting photochromic property accompanied by the change of the fluorescence intensity. Figure 3a shows the change of the absorption spectrum of compound **1** by the isomerization in the presence of BSA (1×10^{-5} M) in phosphate buffer solution. Just after the sample preparation, *E*-**1** exhibited fluorescence with the intensity of 10 upon irradiation at 400 nm. Then, the maximum of the absorption spectrum shifted to the shorter wavelength region with the concomitant decrease in the fluorescence intensity with the timescale of minutes, and the rate constant was 2.5×10^{-4} s^{-1} at 295 K. The observation was explained by the thermal *E*-to-*Z* isomerization to give an equilibrium mixture. Upon irradiation at 366-nm light, *Z*-**1** isomerized to the *E* isomer to give a photostationary mixture and the fluorescence intensity upon irradiation at 400 nm became 8 in one cycle. The repetition characteristic of the fluorescence intensity is shown in Figure 3b.

On the basis of these results, compound **1** isomerizes mutually between a highly fluorescent *E*-isomer and a non-fluorescent *Z*-isomer photochemically and thermally. The system is very interesting from the view point of not only a hybrid photochromic system based on the host–guest interaction, but also fluorescent sensor or color indicator for revealing function of proteins. Further investigation to reveal the mechanism of the fluorescence enhancement in the presence of BSA will lead us to develop the newly fluorescent molecules having intramolecular hydrogen bonding.[1,8]

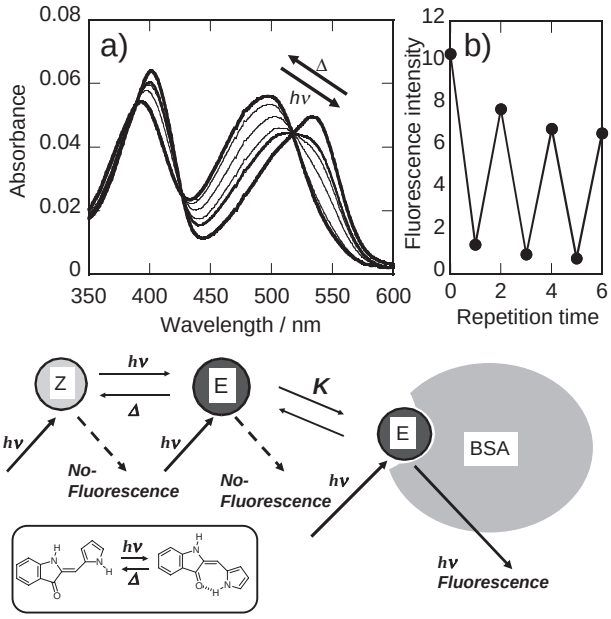


Figure 3. (a) Change of the absorption spectrum of compound **1** by the photochemical and thermal isomerization. (b) Change of the fluorescence intensity at 558 nm on irradiation at 400 nm.

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