Characteristic Fluorescence Behavior of Dialkynylpyrene Derivatives in Hydrophobic Cavity of Protein

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Water-soluble fluorescent molecules consisting of dialkynylpyrene skeleton with an oxyethylene unit were designed and synthesized for interacting with hydrophobic cavities of proteins. In the presence of serum albumin, the fluorescent spectra of dialkynylpyrene derivatives were remarkably changed. These behavior can be explained by monomer–excimer emission switching and twisted intramolecular charge-transfer mechanism.

Fluorescent molecules are essential for detecting biomolecules and analyzing their functionalities in biochemistry and chemical biology.[1] Among them, pyrene derivatives have been extensively used as fluorescent molecular probes for various proteins due to their inherent photophysical characteristics.[2] In the fluorescence spectra of pyrene derivatives, vibronic bands originating from monomer emission and excimer emission depend on the concentration of the pyrene solution.[3] Monomer to excimer emission switching induced by the assembly of pyrene derivatives can be used for ratiometric fluorescence analysis as a highly sensitive assay.[4] Also, pyrene derivatives attached to a donor such as an amino group formed corresponding exciplexes via intramolecular charge transfer (ICT).[5] In particular, the twisted intramolecular charge transfer (TICT) reflects structural information between donors and acceptors.[6] However, pyrene has serious drawbacks; it has a relatively short absorption wavelength and undergoes significant quenching of its fluorescence in the presence of oxygen. Recently, it was reported that the introduction of alkynyl groups into the pyrene skeleton induced a red shift in the absorption maxima, as well as retaining strong fluorescence intensity even in the presence of oxygen.[7] Taking these aspects into account, we expected that alkynylpyrenes bound to hydrophobic cavities of protein might show intriguing fluorescence properties, which promise to be highly potential probes for various proteins.

In this paper, we report the characteristic fluorescence of alkynylpyrenes in the presence of proteins. Recently, **1** has been synthesized (Chart 1) and it was found that the introduction of alkynyl groups into pyrene nuclei induced a red shift in the absorption maxima and an increase of quantum yield compared with the parent pyrene as mentioned above.[7b] The aniline-linked dialkynylpyrene **2** was newly prepared for this study (Chart 1). Since **2** possess an amino group as a donor at the para position of the benzene ring in **1**, a donor–acceptor ICT will appear in **2**, in which the pyrene moiety works as an acceptor. Furthermore, ICT emission will be observed from **2** in the protein cavity via a controlled TICT pathway. Bovine serum albumin (BSA) is well-known to have several hydrophobic binding sites for guest molecules, so that its interaction with various organic molecules has been explored in detail.[8–11] Therefore, BSA is mainly used as a model protein indicating hydrophobic interaction with dialkynylpyrenes.

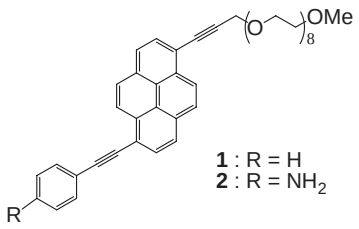

Chart 1.

The two dialkynylpyrenes **1** and **2** designed as water-soluble pyrene derivatives were synthesized by Sonogashira coupling reactions[5c] in a stepwise manner via a monosubstituted pyrene derivative attached to an octa(ethylene glycol), followed by coupling with ethynylbenzene and 4-ethynylaniline, respectively.

To investigate the binding ability of **1** to the hydrophobic cavity of BSA, a fluorescence titration experiment was performed in 10 mM HEPES buffer (pH 7.4) at 25 °C. In the fluorescence spectrum of **1**, both monomer ($\lambda_{max} = 410$ and 433 nm) and excimer ($\lambda_{max} = 528$ nm) emissions were observed at a concentration of 4.7 μM. When BSA was added to **1**, the fluorescence spectrum changed remarkably (Figure 1). As the BSA concentration was increased, the emission intensity of the monomer band increased at the cost of the excimer band at 528 nm. The emission maximum in the monomer band was shifted from 410 to 415 nm. In circular dichroism (CD) measurements of **1** with BSA, induced CD bands were observed in the absorptive region of the pyrene moiety (Figure S1).[12] These bands were attributed to binding of the pyrene within the chiral environment of the BSA cavities. The UV spectra of **1** also changed upon addition of BSA passing through an isosbestic point (Figure S2),[12] assuming that pyrene and BSA might form a 1:1 complex because of the presence of a large excess of BSA. Analysis of the BSA-binding profile by a nonlinear least-squares method

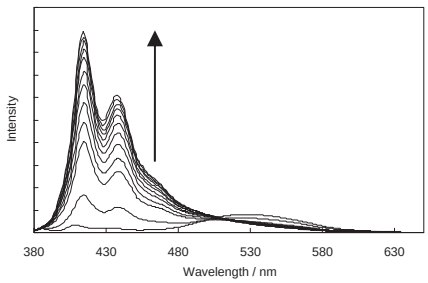

Figure 1. Fluorescence spectral changes of **1** (4.7 μM) upon the addition of BSA (0–45 μM); $\lambda_{ex} = 341$ nm.

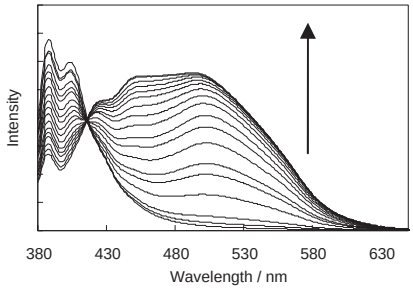

Figure 2. Fluorescence spectral changes of **2** (4.7 μM) upon the addition of BSA (0–44 μM); $\lambda_{ex} = 363$ nm.

gave an apparent association constant of $6.2 \times 10^5\ M^{-1}$. These findings obtained from the CD and UV–vis analyses also confirm that the excimer formation of the pyrene moiety is hindered in the protein environment upon the addition of BSA. Thus, the monomer–excimer emission for **1** can be controlled by complexation with BSA.

With respect to the other derivative **2** including an aniline moiety, the emission bands in aqueous buffer solution (pH 7.4) appeared at 388 and 404 nm; **2** exhibited only monomer emission. The polar amino group on the benzene of **2** contributes to the water solubility of **2** and perhaps leads the suppression of its excimer formation in the buffer solution. Upon the addition of BSA to **2**, the broad emission band of **2** at around 502 nm significantly increased with an isoemissive point at 417 nm, while the monomer bands decreased (Figure 2). The broad band newly appearing at 502 nm in the titration spectra differed by 26 nm from that observed in the case of **1**. This difference implies that the band at 502 nm can not be attributed to excimer emission. The increase in the emission at 502 nm can be rationalized by TICT mechanism.[5,6] In the TICT of **2**, a twisted angle between the aniline and pyrene planes should result from the regulated rotation around the acetylene linkage by the encapsulation of **2** into the BSA cavity. This supposition is consistent with the circular dichroism spectra of **2** with BSA because an exciton coupling was observed from the pyrene absorptive region in the CD spectra of **2** unlike in those of **1** (Figure S1).[12] The exciton coupling of **2** could be attributed to the hetero combination of the pyrene and aniline chromophores under the chiral environment of the BSA cavity. Interestingly, a small difference in functional group, i.e. the presence or absence of NH_2, resulted in the pyrene molecules having totally different photochemical characteristics in terms of interaction with the protein.

To evaluate the selectivity of **1** and **2** for the binding to various proteins, fluorescence spectra were measured in the presence of BSA, lactalbumin, γ-globulin, chymotrypsin, ribonuclease, and lysozyme. Figure 3 displays the fluorescence response of **1** and **2** at 415 nm (monomer band for **1**) and at 502 nm (TICT emission band for **2**) in the presence of the proteins. In both cases, the largest fluorescence changes were observed upon the addition of BSA. On the other hand, the other proteins hardly gave significant enhancements. This finding suggests that the binding of **1** and **2** with the proteins may be driven by hydrophobic interaction on the site having the high surface hydrophobicity[13] because the order of enhancement in fluorescence is independent of the pI values for the proteins.

In summary, the two dialkynylpyrene derivatives used in this study have shown specificity for BSA. In the binding of **1** to BSA, the excimer to monomer emission switching was observed, and CD bands were newly induced from the pyrene moiety of **1**. Introduction of a polar amino group into the benzene ring of **1** led **2** to the TICT emission and the exciton coupling at the pyrene absorptive region. The photophysical properties of the dialkynylpyrenes were found to be influenced by the steric effect in the microenvironment arisen from the complexation with BSA. The selective response of the dialkynylpyrenes to BSA encourages us to develop a new class of fluorescent probes directed toward various proteins because very simple functional modification of the pyrene nuclei causes the fluorescent profiles to be diverse.

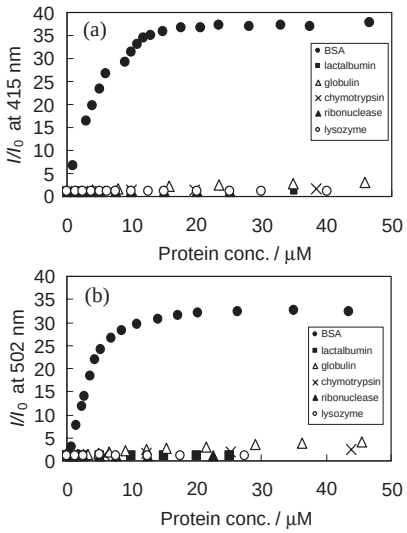

Figure 3. Fluorescence response of **l** (a) at 415 nm and **2** (b) at 502 nm (4.7 μM) upon addition of a series of proteins.

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