A fluorescent photochromic compound for labeling biomolecules†

Nobuaki Soh,[a] Kenji Yoshida,[a] Hizuru Nakajima,[a] Koji Nakano,[a] Toshihiko Imato,*[a] Tuyoshi Fukaminato[b] and Masahiro Irie*[bc]

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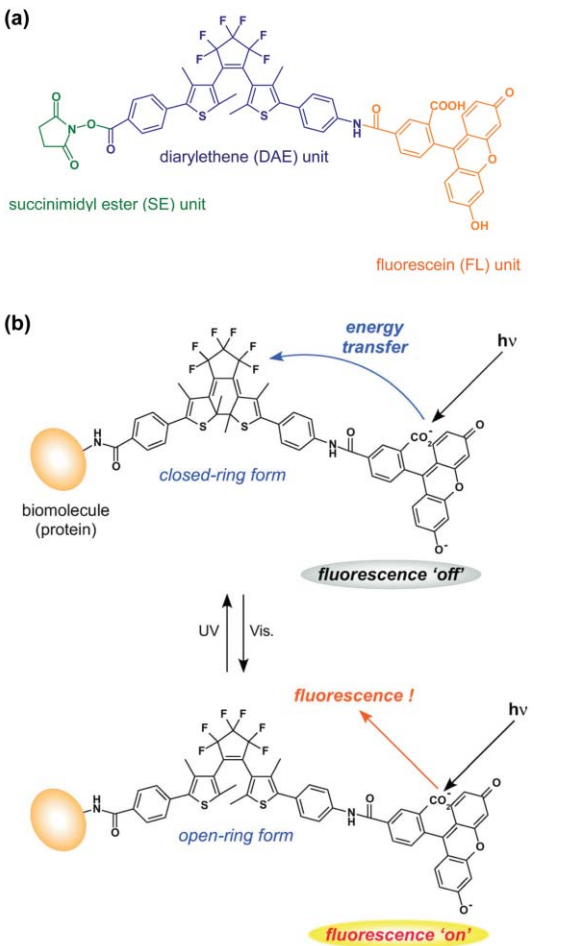
A fluorescent photochromic compound, composed of diarylethene, fluorescein and succinimidyl ester units, was developed for the controllable fluorescent labeling of biomolecules based on a small molecule.

Photochromism is a reversible transformation of chemical species induced by photoreaction between two forms with different absorption spectra.[1] Photochromic compounds have attracted much interest because of their potential applications to photonic devices such as optical memories and display devices.[2] On the other hand, biological applications of them have not yet been extensively explored although a few preceding works have been reported.[3]

Recently, a photochromic fluorescent protein, Dronpa was isolated by engineering a novel coral protein.[4] By tagging the photochromic protein, a regulated fast nucleocytoplasmic shuttling of mitogen-activated protein kinase (extracellular signal regulated kinase; ERK) was successfully observed. Generally, fluorescent proteins are able to be genetically fused with host proteins to yield fluorescent chimeras *in situ*[5] and a labeling of such fluorescent proteins is thus useful to monitor the behavior of a target protein (protein of interest) using fluorescent microscopy with high resolution. However, the large size of fluorescent proteins (*i.e.* 27 kDa for GFP) can perturb the inherent function and distribution of the target protein.[6] Therefore, the development of new alternative labeling methods for proteins based on smaller fluorescent molecules is important.[7] Especially, a novel small photocontrollable fluorescent labeling molecule would be a powerful tool to elucidate the unexplored regulation of fast protein dynamics in more detail.

Here, we report on a photochromic compound for fluorescent labeling of proteins. The compound, named DAE-FL-SE, is composed of a photochromic diarylethene (DAE) unit, a fluorescent fluorescein (FL) unit, and an amine-reactive succinimidyl ester (SE) unit (Scheme 1(a)). Diarylethene derivatives are known to have excellent thermal stability of two isomers (open- and closed-ring isomers) and fatigue resistance among several photochromic molecules.[8] Photoswitching of fluorescence in diarylethene derivatives linked to an appropriate fluorophore has been demonstrated.[9] Such photoswitching of fluorescence is generally based on the intramolecular energy transfer from the fluorophore to the diarylethene derivative. In DAE-FL-SE, the fluorescence would be efficiently quenched along with the photocyclization, since the fluorescent spectrum of the FL unit and the absorption spectrum of the closed-ring form of the DAE unit are well overlapped. Thus, DAE-FL-SE, in which a SE unit is introduced into the DAE-FL for effective binding with proteins, is expected to function as a novel labeling reagent, which can control the "off/on" state of fluorescence in the protein of interest (Scheme 1(b)). In this report, we selected ERK protein and



Scheme 1 (a) Chemical structure of DAE-FL-SE. (b) Photoswitching of fluorescence in DAE-FL labeled biomolecule based on the photochromic reaction.

[a]*Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744, Moto-oka, Nishi-ku, Fukuoka, 819-0395, Japan. E-mail: imato@cstf.kyushu-u.ac.jp; Fax: +81-92-802-2889; Tel: +81-92-802-2889;*
[b]*Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, 744, Moto-oka, Nishi-ku, Fukuoka, 819-0395, Japan. Fax: +81-92-802-2873; Tel: +81-92-802-2873*
[c]*Department of Chemistry, Rikkyo University, 3-34-1, Nishi-Ikebukuro, Toshima-ku, Tokyo, 171-8501, Japan. E-mail: iriem@rikkyo.ac.jp; Fax: +81-3-3985-2397; Tel: +81-3-3985-2397*
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investigated the binding ability of DAE-FL-SE with the target protein and the photoswitching of fluorescence in the DAE-FL-labeled protein.

DAE-FL-SE was synthesized by coupling a boronate diarylethene with an iodo-phenylated fluorescein derivative and subsequent esterification using *N*-hydroxysuccinimide (synthetic procedures are described in ESI†). The binding ability of DAE-FL-SE toward the target protein was initially investigated. Fig. 1 shows the result of SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) for ERK samples, which were prepared by incubation of ERK with DAE-FL-SE and subsequent purification by ultrafiltration (see ESI†). As can be seen from the gel stained by CBB (Coomassie Brilliant Blue) shown in Fig. 1(a), a protein band with a little higher molecular weight than control ERK (lane 7) was observed at the position of ~80 kDa in lanes 2–4, corresponding to the concentration of samples. Simultaneously, the above-mentioned bands at ~80 kDa in lanes 2–4 emitted strong fluorescence upon irradiation with 488 nm light using a molecular imager system, as shown in Fig. 1(b). The results clearly demonstrated that DAE-FL-SE combined with ERK and produced the expected DAE-FL-labeled ERK successfully.

Fig. 2 shows the absorption and fluorescence spectral changes of DAE-FL-labeled ERK upon irradiation with UV (365 nm) and visible ($\lambda > 550$ nm) light. In Fig. 2(a), the absorption band at 505 nm is characteristic for the 6-carboxyfluorescein unit. Upon irradiation with UV light, a new absorption band appeared at 580 nm, which was bleached after irradiation with visible light. This absorption band was attributed to the closed-ring isomer of the diarylethene unit. The photochromic behaviour was observed reversibly many times. A characteristic fluorescence band was observed at 520 nm in the open-ring isomer (Fig. 2(b)). Upon irradiation with UV light, the fluorescence intensity decreased gradually. Upon irradiation with visible light, the fluorescence intensity returned fully to the initial intensity.

Because it is difficult to quantitatively evaluate the fluorescence photoswitching performance of protein-labeled DAE-FL, that of a model compound (DAE-FL-CO_2Et: **16**) (see ESI†) was investigated quantitatively. Reversible fluorescence switching was observed along with photochromic reaction. Upon UV (365 nm) light irradiation, a new absorption band appeared at 580 nm and the fluorescence intensity decreased at that time. By irradiating with visible ($\lambda > 550$ nm) light, both absorption and fluorescence spectra were returned to their original state. The fluorescence intensity at the photostationary state was 39% of the original intensity of the open-ring isomer. A conversion ratio from the open- to closed-ring isomer of **16** upon UV light irradiation was estimated from ^{1}H NMR spectral measurement. Before UV light irradiation, multiple ^{1}H NMR signals corresponding to two methyl groups on the thiophene rings were observed at around 2.38 and 2.13 ppm. After UV light irradiation, these signals were

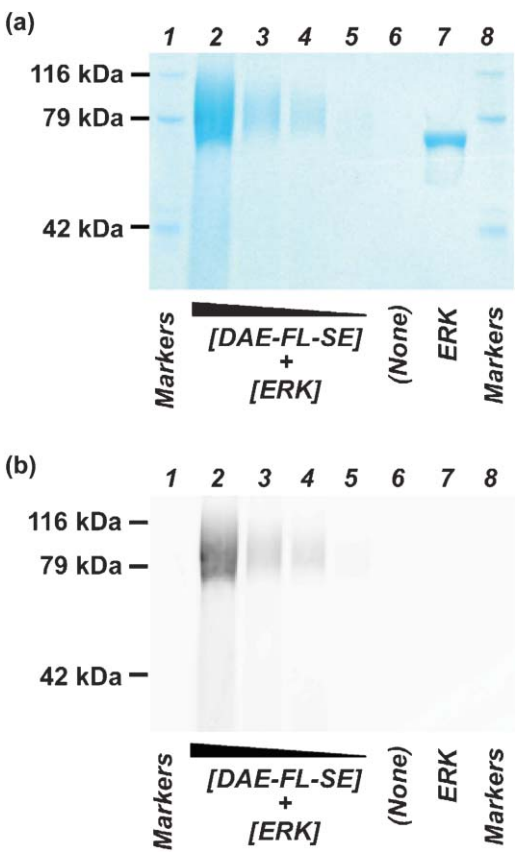

Fig. 1 SDS-PAGE of ERK samples which were reacted with DAE-FL-SE and purified by ultrafiltration. (a) Proteins in the gel were stained with CBB. (b) Fluorescent bands in unstained gel were visualized by a molecular imager system.

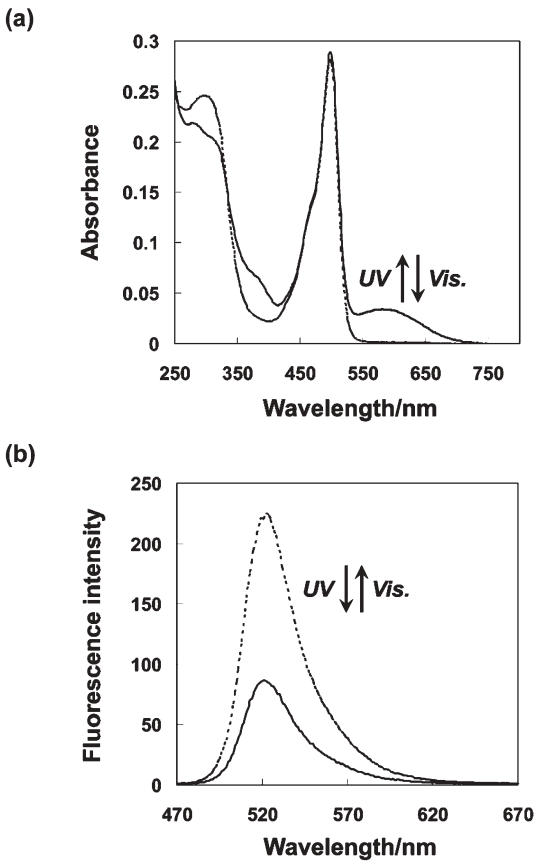

Fig. 2 (a) Absorption and (b) fluorescence spectral changes of DAE-FL labeled ERK protein in PBS buffer–EtOH (7 : 3) upon irradiation with UV (365 nm) and visible ($\lambda > 550$ nm) light.

suppressed and new two signals appeared at around 2.30 and 2.04 ppm. The spectral changes in the NMR measurement were completely reversible and the original spectrum was recovered by irradiating with visible light. On the basis of the integrated intensities of the methyl signals, the content of the closed-ring isomer in the photostationary state upon UV light irradiation was estimated to be 60%. The value of the conversion is in good agreement with the quenching ratio of fluorescence intensity at the photostationary state. This indicates that the closed-ring isomer is non-fluorescent and the quenching ratio of fluorescence intensity corresponds to the amount of the closed-ring isomer. It seems that the results can be also adapted to the protein-labeled DAE-FL molecule. Therefore, 62% of open-ring isomer of DAE-FL labeled with ERK protein converted to the closed-ring isomer along with the photocyclization reaction. The fluorescence quantum yields Φ of **16** in ethanol before and after UV light irradiation were 0.71 and 0.14 (at photostationary state), respectively (fluorescein was used as the standard, $\Phi = 0.97$). These results indicate that the fluorescent switching of the DAE-FL-labeled protein can be accomplished by irradiation of externally-regulated UV/visible light. Thus, DAE-FL-SE is promising for the controlled labeling of target proteins which exist in specific areas in single cells such as photochromic Dronpa. Furthermore, DAE-FL-SE is also applicable to various biomolecules containing amino groups.

In conclusion, we have synthesized photochromic DAE-FL-SE composed of diarylethene, fluorescein and succinimidyl ester units and demonstrated that the novel compound is applicable to the controllable fluorescent labeling of biomolecules based on small molecules. It is noteworthy that the SE unit in the DAE-FL-SE can be easily converted to other functional groups such as an NTA-Ni^{2+} unit, which recognizes a repeat of a histidine sequence (His-tag) with a high selectively. Current efforts are directed toward applying DAE-FL-SE and relevant fluorescent photochromic compounds for studying dynamics of biomolecules in living systems.

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