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Photochromic DNA having fluorescent protein-inspired nucleosides

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ABSTRACT

Molecular switches controlled by light stimuli can be applicable to the variety of the biological application. In this study, skeletal structures of a chromophore of fluorescent protein were applied as aglycones of newly designed photochromic nucleosides, "Fluorescent protein-inspired nuceloside: FIN". Phosphoramidite units of the photochromic nucleosides having imidazolinone derivatives with benzylidene or 3-pyridilidene groups were successfully synthesized for FIN-containing ODNs. Thermodynamic studies of the FIN-containing ODNs revealed that photo-irradiation with specific wavelength induced stability change of the duplexes.

Introduction

In recent year, molecular switches controlled by various stimuli have been used in very different applications with great success [1]. Among the stimuli, light stimuli have some advantages 1) selected wavelength can be used for stimulation, 2) stimulation–period and –region can be liberally altered, 3) contamination of residues derived from the stimuli to the reaction system can be circumvented [2–4]. Therefore light irradiation is preferably used for the control of bio-systems as an external stimulus [5]. To realize the control of the bio-systems, a number of photochromic groups, which reversibly alter the steric structures upon light irradiation with specific wavelength, have been artificially incorporated to biomolecules. Photo-control of DNA hybridization can be used as a robust tool to regulate and elucidate DNA-involving biological processes. In previous reports, azobenzene [6,7], stilbene [8,9], and derivatives [10] are incorporated into oligonucleotides to control its duplex-forming activities by light illumination.

A chromophore of blue fluorescein protein (BFP) [11], which is constituted by a 5-phenylidene-4H-imidazolin-4-one derivative as a skeletal structure, is also one of a good candidate as a photochromic group. From detailed studies on photochromic properties of fluorescent protein chromophores, it has been revealed that vinyl group of the chromophores are efficiently isomerized and ratio of E- and Z- isomers are changeable by light irradiation with certain wavelength [12,13]. Obtained E- and Z- isomers are both thermodynamically stable under physiological conditions. Furthermore, photo-irradiation wavelength for E-Z isomerization can be tunable by systematic substitution on the phenyl and imidazolinone groups [14,15]. Considering potential benefits of photochromic properties of the FP chromophores, development of nucleotide derivatives [16] with FP-chromophores would be beneficial for photo-control of DNA hybridization.

In this study, we developed new photoresponsive nucleosides that are inspired from the FP chromophores, called "fluorescent protein-inspired nucleoside (FIN)" (Fig. 1) and evaluated regulatory effect of DNA duplex-forming ability.

Synthesis and photochromic properties of GFP-chromophore analogues

Voliani *et al.* reported that photo-chromic properties of GFP chromophore and T66F GFP chromophore analogue [12]. In that report, *cis-trans* photo-switching kinetics were depend on illumination intensity and the extinction coefficient of cis and trans isomers.

To explore photo-chromic properties of GFP chromophore analogues, an imidazolinone derivative with a pyridine ring was synthesized as shown in Scheme 1. Condensation of 3-pyridinecarboxa aldehyde with imidazolinone (**1**) [17] produced fluorescent protein-chromophore analogues (**2**) in modest yields. Z-isomers were thought to be more stable than E-isomers from the experimental [18–20] and theoretical [21] results. Next E-Z photo-isomerization of **2** was examined according to previous procedures [18]. Irradiation wavelength around 350 nm which include the absorption maximum derived from HOMO->LUMO transition of each Z-isomer (**2**) was used for isomerization from Z-isomer to E-isomer. As is the case with the T66F GFP chromophore analogue, absorption intensity of low-energy bands of **2** was decreased and

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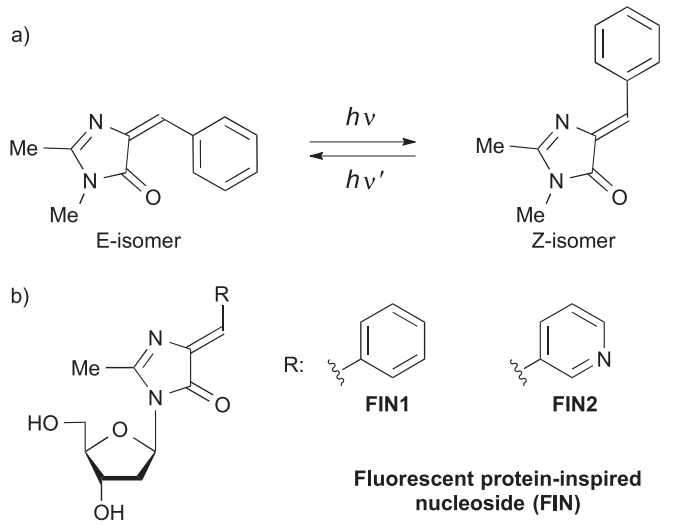
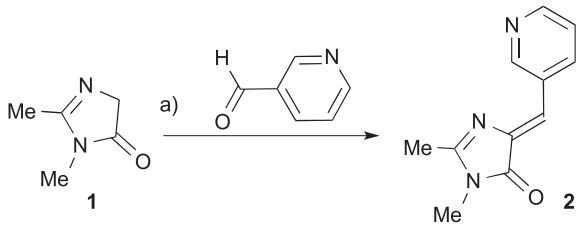


Fig. 1. (a) Photo-isomerization of a chromophore of blue fluorescent-protein (BFP). (b) Chemical structures of Fluorescent protein-inspired nucreosides (FIN).



Scheme 1. 1.1 equiv 3-pyridinecarboxa aldehyde, piperidine, rt, ethanol,1 h. 41%.

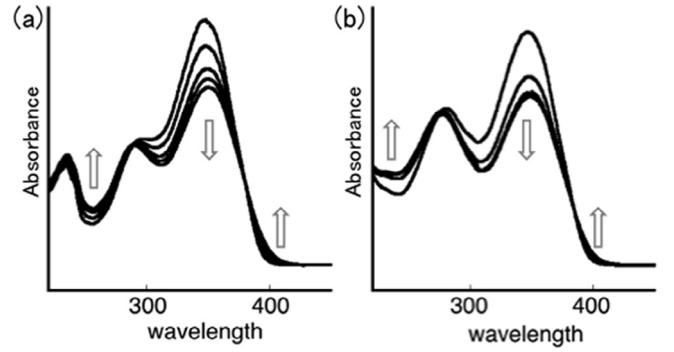
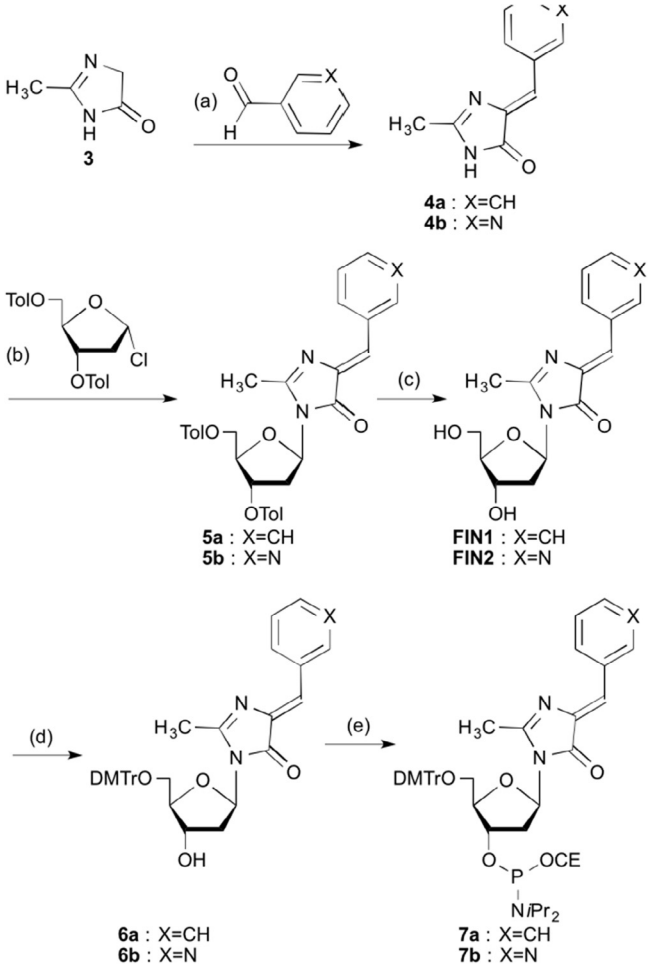


Fig. 2. Absorption spectra of 60 μM of (a) T66F GFP chromophore analogue and (b) **2** with increasing photo-irradiation time (0–10 min). Excitation wavelengths were (a) 350 nm and (b) 355 nm. Arrows indicate changes upon irradiation.

that of high-energy band was increased with two isosbestic points. (Fig. 2) From the reversed-phase HPLC analysis (Fig. S1), we estimated that the ratio of Z-isomer to E-isomer of **2** under photostationary state was 36:64. Under UV irradiation around 410–420 nm which excited mostly E-isomer of **2**, photostationary state of E-Z mixtures were changed and ratio of Z-isomers were increased (78:22, Z-isomer: E-isomer). Hence, GFP-chromophore analogues obtained have properties of reversible conformation-change by alternative photo-irradiation and two isomers are both thermodynamically stable under physiological conditions.

Synthesis of FIN containing ODNs

In order to introduce the GFP-chromophore analogues into DNA duplexes, we prepared the photochromic nucleosides **FIN1** and **FIN2** and their phosphoramidite derivetives (Scheme 2). Aldol



Scheme 2. (a) 1.0 equiv aldehyde, piperidine-ethanol (1:7, v/v), rt, 5 h. 67%(**4a**) and 19%(**4b**). (b) 1.1 equiv NaH, 1.1 equiv chlorosugar, acetonitrile, rt, 30 min. 59%(**5a**) and 43%(**5b**). (c) NaOEt, CH_3OH, rt, 1 h. 87%(**FIN1**) and 24%(**FIN2**). (d) 1.2 equiv DMTrCl, pyridine, rt, 2 h. 57%(**6a**) and 23%(**6b**). (e) 2 equiv cyanoethyl-*N*,*N*-diisopropylphosphrochloridite, 5 equiv *N*,*N*-diisopropylethylamine, rt, 1 h. 38% (**7a**) and 31%(**7b**).

condensation of **3** with corresponding aldehydes gave Z-isomers of **4a** and **4b** as dominant products. E-Z configuration of **4a** and **4b** were confirmed by coupling between C6-H proton and the C5 = O carbonyl carbon (J_{C5H6} = 4.7 Hz for **4a** and 5.3 Hz for **4b**); small coupling constants that were assigned Z-isomers were obtained for both cases. Hoffer's chlorosugar [22] was used as a glycosyl donor for stereoselective glycosylation and mono-isomeres were obtained at this stage. Following dimethoxytritylation and phosphitylation, phosphoramidite units (**7a**, **b**) were obtained. From NOESY spectra of **FIN1** and **6b**, the stereochemistry of obtained nucleosides were confirmed to be the β-isomers. Then the phosphoramidite units were used for the synthesis of FIN-containing ODNs (**ODN1** and **ODN2**) with ultramild DNA synthesis reagents [23]. After purification by reversed-phase HPLC, mass of the ODNs were confirmed by high-resolution MASS. (ESI-TOF-MS *m*/*z*: $[M-4H]^{4-}$ calcd for **ODN1** 947.43, found 947.49; $[M-4H]^{4-}$ calcd for **ODN2** 947.68, found 947.63).

Photo-isomerization of FIN-containing ODN was evaluated using **ODN1** by reversed-phase HPLC. Before photo-irradiation, **ODN1** overwhelmingly contained (more than 90%) a Z-isomer of **FIN 1**, confirmed by UV absorption spectroscopy. Fig. 3 shows reversed-HPLC profiles of **ODN1** after photo-isomerization reactions and time course of photo-isomerization of **ODN1**. **ODN1** (20 μM) were isomerized by photo-irradiation in phosphate buffer