Photophysical studies on a photoactive yellow protein fluorophore analog with the 4-Hydroxy group replaced by 4-Dimethylamino group

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ABSTRACT

Although several fluorescent analogs of the PYP Fluorophore have been studied so far, the focus has been overwhelmingly on the effect of the electron-withdrawing carbonyl end substituent of the fluorophore. Here, we have introduced for the first time, a PYP Fluorophore analog named pDMACT where the electron-donating phenolate has been replaced instead, by another electron-donating substituent: the N, N-dimethylamino group. Spectral properties of pDMACT were found to be extremely sensitive to the polarity of the solvents, as measured by parameters like $E_T(30)$, $F(e,n)$ or p^*. In fact, for an increase of $E_T(30)$ from 31.1 (in n-heptane) to 45.6 (in acetonitrile), pDMACT registers a fluorescence Stokes shift of 75 nm, as against ~70 nm reported with the well known polarity probe PRODAN for a comparable increase in $E_T(30)$ (Ref. [25]). The high sensitivity of pDMACT is attributed to a strong push-pull charge-separated structure which is further enhanced in the excited state. This enables the molecule to also serve as a probe for ultrafast solvation dynamics. The neutral character of pDMACT further ensures that it can be employed as a fluorescent probe for highly non-polar domains which are inaccessible to the original, anionic PYP fluorophore.

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1. Introduction

The Photoactive Yellow Protein (PYP) is a small, water soluble protein that is responsible for the negative phototactility of the host organism—halophilic purple bacteria [1–3]. At the heart of its photodynamics is the PYP fluorophore, which is the phenolate form of a trans- 4-hydroxy cinnamoyl moiety bound to the Cys69 residue of the protein backbone by a thioester bond, as shown in Fig. 1a. In order to elucidate the photophysics of the PYP Fluorophore, extensive spectroscopic studies have been carried out on many compounds regarded as its analogs [4–20]. These have been augmented by computational studies on the electronic states and relaxation pathways of the PYP fluorophore and its analogs [21–24]. Most of these analogs are essentially derivatives of the phenolate form of trans- 4-hydroxy cinnamic acid, where the carbonyl end substituent X (as in Fig. 1b) has been varied to produce carboxylates, esters, thioesters, ketones, amides, etc

[13–16]. It has been demonstrated that the electron-withdrawing capacity of the X-group is crucial in determining the course of excited state dynamics. When X is relatively electron-withdrawing (like —SR or —OMe), fluorescence decay becomes faster, and intramolecular charge-transfer becomes the main radiationless relaxation process rather than photoisomerization. But when it is weakly electron-withdrawing (like —O$^-$ or —NH$_2$), photoisomerization occurs [16].

In other words, the photophysics of these PYP Fluorophore analogs is governed by a combination of the electron-withdrawing capacity of the carbonyl end substituent and the electron-donating capacity of the 4-phenolate group. This implies that replacing the 4-phenolate group by other, potentially electron-donating groups may also significantly affect the photophysics, an aspect that has not been hitherto explored. Keeping this in mind, we have introduced in this work a new type of analog, where, instead of the carbonyl end substituent, the 4-phenolate group has been replaced by the dimethylamino(Me$_2$N—) group. The resulting compound, the thioester of p-Dimethylaminocinnamic acid (pDMACT, Fig. 1c), essentially combines the efficient electron donation of the Me$_2$N— with the electron acceptance of the thiophenolate group
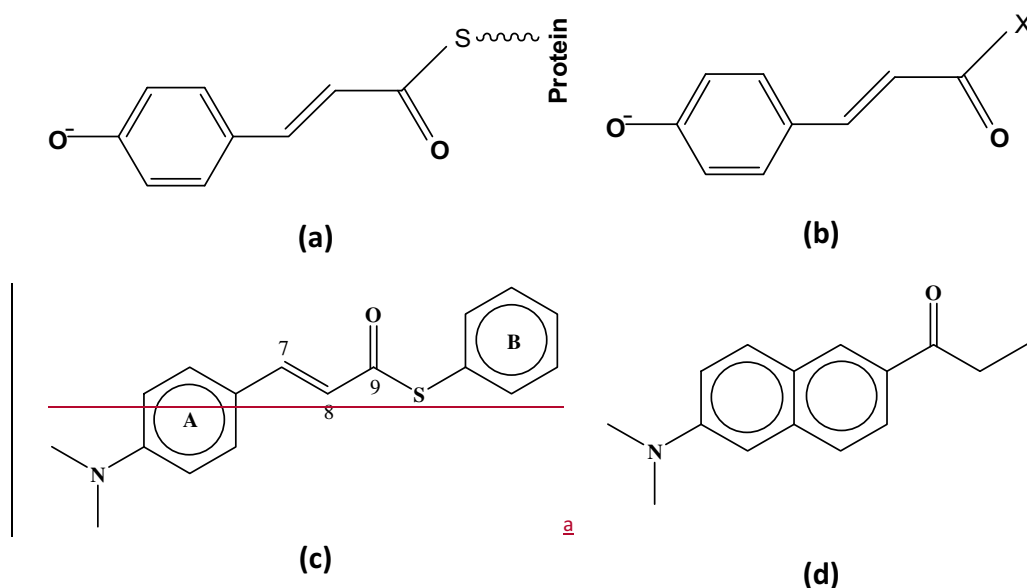
Fig. 1. Structures of (a) the PYP Fluorophore bound to the protein; (b) PYP Fluorophoreanalogswith different substituents on the carbonyl end (*see text*); (c) PYP Fluorophorep DMACT; (d) polarity probe PRODAN.

on the carbonyl end to produce a strong donor-acceptor couple that facilitates intramolecular charge-transfer. In fact, pDMACT is structurally quite similar to the well-known fluorescent polarity probe PRODAN (Fig. 1d), which has a push-pull charge-transfer character with the Me_2N- group responsible for electron donation [25].

In a previous report, it was noted that replacing the 4-phenolate group by the 4-dimethylamino group rendered the absorption peak of the fluorophore less sensitive to changes like thioester- ification, pH variations, or binding to the PYP apoprotein [26]. This was attributed to a decrease in fluorophore-apoprotein interac- tions in case of the substituted derivative, which probably explains why PYP analogs devoid of the 4-phenolate group do not exist in nature. Nevertheless, in order to get a deeper understanding of the nature of these interactions, it is imperative to probe how molecular properties of the fluorophores like electronic distribu- tion and excited state relaxation pathways are affected by the introduction of ring-substituents, and how these properties respond to the choice of local host environment. Systematic photophysical studies performed in a range of solvents can elucidate these issues and lead to further insight into the fluorescence of the PYP Fluorophore and its analogs. Moreover, this will help in the development of newer fluorescent derivatives adaptable to a wider range of environments. For example, in the present work, replacing the anionic phenolate with the neutral Me_2N- group rendered the pDMACT fluorophore soluble in non- polar alkanes, something which was impossible for the original PYP Fluorophore. Thus, unlike the latter, pDMACT serves as an excellent fluorescent probe molecule for exploring relatively less polar domains in a biological environment which are inaccessible to the original PYP Fluorophore.

2. Experimental

2.1. Materials

p-hydroxycinnamic acid was purchased from Alfa-Aesar (98%). Other chemicals used for synthesis of pDMACT were of synthesis grade. Solvents used for spectroscopy were purchased from Merck, India and were of either UV or HPLC grade. They were freshly distilled before conducting spectroscopic measurement.

2.2. Synthesis of pDMACT

The synthesis is described elsewhere in details [27]. Briefly, to a solution of *p*-hydroxycinnamic acid in anhydrous dichloromethane (DCM), excess oxalyl chloride and DMF were added dropwise at 0 °C. After gas evolution, the reaction mixture was stirred for 30 min at 0 °C and then 1.5 hr at room temperature. The solvent was evaporated to give yellow solid material that was dissolved in anhydrous DCM. Triethylamine (TEA) and then thiophenol were added at 0 °C to the reaction mixture and stirred for 16 hr.The resulting reaction mixture was successively washed with 2 M HCl, H_2O and brine and dried with Na_2SO_4. Then it was concentrated under reduced pressure to give the crude product, which was subjected to silica gel chromatography (2% EtOAc/pet ether) to afford the compound pDMACT (yield∼ 10%) as a yellow powder; mp 166–167 0 °C; IR (KBr, cm^{-1}) ν_{max}: 1649, 1581, 1373, 1184, 1024; ^1H NMR (400 MHz, $CDCl_3$): δ 3.043 (6H, s, N(CH_3)$_2$), 6.587 (1H, d, J = 15.6 Hz, C^8-H), 6.673 (2H, d, J = 8J = 8.8 Hz, Ar-H), 7.410–7.428 (3H, m, Ar-H), 7.447 (2H, d,J = 8.8 Hz,Ar-H), 7.484–7.508 (2H, m, Ar- H), 7.628 (1H, d,J = 15.6 Hz, C^8-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 40.26 (N(CH_3)$_2$), 111.95, 118.83, 121.74, 128.53, 129.21, 129.26, 130.59, 134.88, 142.56, 152.26, 187.81 (C^9); Anal. Calcd for $C_{17}H_{17}NOS$; C 72.05%, H 6.05%, N 4.94%; Found C 72.14%, H 6.12%, 4.86%. The NMR spectra are given in Fig. SF1 and SF2 of the Supporting information section.

2.3. Spectroscopy

Absorption and fluorescence spectra were measured in a HITACHI UV spectrophotometer (U-3501) and Perkin Elmer LS55B fluorimeter, respectively. Emission quantum yields were calculated using Coumarin 153 in ethanol as the standard for reference. For fluorescence dynamics studies in the nanosecond time-scales, a picosecond time correlated single photon counting (TCSPC) system of JobinYvon Horiba was used, employing a picosecond diode laser operating with λ_{ex} = 375 nm and pulse- width of ∼70 ps. For fluorescence dynamics studies in the <100 ps time-scale, femtosecond frequency upconversion technique was adopted. Here, the output of a femtosecond pulsed oscillator from a mode-locked Ti:sapphire laser (MaiTai-HP), centered at 750 nm and with a repetition rate of 80 MHz, was used as the gate pulse for