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Photoantenna in two cryptochrome–photolyase proteins from *O. tauri*: Presence, nature and ultrafast photoinduced dynamics

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ABSTRACT

Cryptochromes and photolyases are homologous flavoproteins either involved in photosensory functions or in the photorepair of UV-damaged DNA. We report on the presence, nature and ultrafast photoinduced dynamics of a photoantenna in two recently discovered cryptochrome/photolyase proteins (OtCPF1, a (6-4) photolyase, and OtCPF2, a cryptochrome-DASH), coming from the green alga *Ostreococcus tauri*. Whereas OtCPF1 does not show any apparent photoantenna after purification, OtCPF2 is found to bind the folate MTHF. Resonance energy transfer, from MTHF to the flavin cofactor (FAD) of OtCPF2, was studied by femtosecond transient absorption spectroscopy. It takes place in 15 ps when FAD is oxidized and 100 ps when it is fully reduced. The intrinsic photophysics of MTHF in acidic solution was studied for comparison. Two decays in the sub-100 ps regime were tentatively attributed to the presence of two conformers or to a quenching mechanism involving large-amplitude motions of the 1,2,3,4-tetrahydropyraxine ring. On the other hand, the nature of the missing photoantenna of OtCFP1 was questioned by means of sequence alignment and homology modeling. We propose that the deazaflavin 8-HDF could be the photoantenna of OtCFP1 *in vivo*.

1. Introduction

Cryptochromes and photolyases form a family (called CPF) of homologous flavoproteins involved in different light-activated biological functions [1]. Photolyases are able to photorepair UV-damaged DNA without nucleotide excision: CPD photolyases split cyclobutane pyrimidine dimers [1–3], and (6-4) photolyases repair the pyrimidine (6-4) pyrimidone photoproducts [1]. Cryptochromes are signaling photoreceptors [1,4–8], but cryptochromes-DASH are believed to exhibit specialized single-stranded photolyase activity [9,10]. All CPF proteins non-covalently bind flavin adenine dinucleotide (FAD, Fig. 1, left) as the main photoactive cofactor. They generally bind a secondary cofactor, a photoantenna that absorbs light with large extinction coefficient and improves the efficiency of the protein through resonant energy transfer to FAD. The two main photoantennae found in CPF proteins are 5,10-methenyl-tetrahydrofolate (MTHF, Fig. 1, bottom right) and 8-hydroxy-7,8-didesmethyl-5-deazariboflavin (8-HDF, Fig. 1, top right, also known as F_0) [1,11]. MTHF was found in several CPD photolyases and cryptochromes-DASH, and 8-HDF in various CPD photolyases [12–15]. On the other hand (6-4) photolyases are consistently purified without photoantenna [16–20]. If a photoantenna is bound to (6-4) photolyases *in vivo* its nature is not yet known.

In this paper we report on the presence, nature and ultrafast photoinduced dynamics of a photoantenna in two recently discovered CPF proteins (OtCPF1 and OtCPF2) coming from the green alga *Ostreococcus tauri* [21]. OtCPF1 groups with the (6-4) photolyase and animal cryptochrome sub-family, and OtCPF2 is a cryptochrome-DASH. OtCPF1 is purified (after heterologous expression in *Escherichia coli*) without any photoantenna [19,22], while OtCPF2 is found to bind the photoantenna MTHF [22].

By using femtosecond broadband transient absorption spectroscopy, we studied the photoinduced resonance energy transfer between MTHF and FAD, within OtCPF2. Energy transfer from MTHF to FAD has already been reported for several CPF proteins [23–25]. We focus on comparing the energy transfer rates from MTHF to two different redox states of FAD: FAD_{ox}, the neutral oxidized form, and $FADH^-$, the anionic fully reduced form. Such a comparison was previously made by Saxena et al. [25] in the case of

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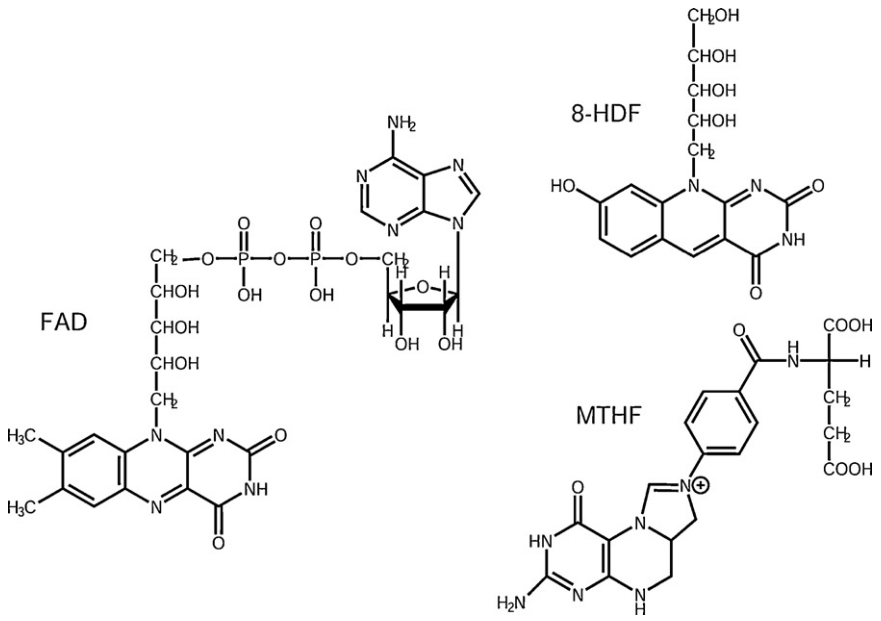

Fig. 1. Chemical structures of FAD (left), 8-HDF (top right) and MTHF (bottom right).

the cryptochrome-DASH of *Vibrio cholerae*, and lead the authors to propose a local change of the protein structure when the flavin is reduced from FAD_{ox} to $FADH^-$. We here reexamine this issue for OtCPF2 and confront it to structural information obtained by homology modeling.

For comparison we also studied the intrinsic photophysics of MTHF in aqueous solution, by femtosecond broadband transient absorption spectroscopy. Only one time-resolved fluorescence study on MTHF in water was previously reported [24]. The authors concluded to a fluorescence lifetime shorter than 30 ps, limited by the temporal resolution of their apparatus. They also showed that the excited-state lifetime of MTHF increases with the viscosity of the solvent.

Regarding the fact that OtCPF1 is purified without photoantenna, we used sequence alignment with other CPF proteins, as well as homology modeling, in order to propose a candidate photoantenna and give reasons for which the protein is purified without it.

2. Materials and methods

2.1. Sample preparation

OtCPF2 was overexpressed in *E. coli* as a GST (glutathione S-transferase) fusion protein. The expression and purification protocols have already been described elsewhere [22]. Briefly, purification was made on a Glutathione Sepharose 4B resin (Amersham Biosciences) which binds the GST tag. The protein was released by adding a GSH (reduced L-glutathione) rich elution buffer (Tris–HCl at pH 8.0 100 mmol L^{-1}, NaCl 100 mmol L^{-1}, GSH 20 mmol L^{-1}). The purified proteins were concentrated by means of Microcon centrifugable membrane filters (10 kDa cutoff) in order to obtain a final volume of 50 μL and a maximal absorbance (over 1 mm optical path) of 0.42 at 386 nm. After addition of glycerol (10%, v/v), they were stored at −80 °C.

MTHF (Schircks Laboratories) solution was dissolved in acidic (HCl) aqueous solution at pH 2.0, in order to prevent the hydrolysis of MTHF into 10-formyl-tetrahydrofolate (10-FTHF) [26]. The concentration of MTHF for transient absorption experiments was 170 μmol L^{-1}, corresponding to an absorbance over 1 mm optical path of 0.75 at the absorption maximum (352 nm).

2.2. Steady-state spectroscopy

UV–vis absorption spectra were recorded with double-beam UV spectrophotometers: UV-mc^2 (Safas). Fluorescence spectra were measured with a fully corrected Fluoromax-3 (Horiba Jobin-Yvon) spectrofluorimeter. The cell was thermostated at 5 °C by a temperature-controlled bath (Minichiller Inox, Huber).

2.3. Time-resolved absorption spectroscopy

Broadband (340–750 nm) femtosecond transient absorption spectra were recorded by the pump-probe with white-light continuum technique, as described elsewhere [27]. The laser source is a commercial amplified Ti:Sapphire laser system (Tsunami and Spitfire, Spectra Physics) delivering 50-fs pulses at 775 nm, at 1 kHz repetition rate. The 388-nm pump beam (55 fs, 0.13 μJ per pulse) was generated by focusing the 775 nm beam on a BBO crystal. The continuum probe beam was generated by focusing a few μJ per pulse of the 775-nm beam on a moving CaF_2 plate. The probe beam was then split into a sample beam and a reference beam. The pump and probe beams were focused on a diameter of about 80 μm onto the sample cell and crossed at an angle of *ca.* 5°. The probe beam was delayed with respect to the pump beam by a motorized optical delay line. The cuvettes were thermostated at 5 °C to prevent protein degradation, and continuously moved up and down in order to avoid photolysis. The probe beams (reference and sample) were dispersed in a spectrograph (Acton SP306i) and the spectra were recorded at 333 Hz on a CCD camera (Roper Scientific, Spec-10 100B, 100 × 1340 pixels). The linear polarizations of the pump and probe beams were set at the magic angle (54.7°).

The data were globally fitted to a sum of exponential functions and/or step function, after dimensional reduction and noise filtering by singular value decomposition (see technical details in Ref. [27]). Briefly, the fit functions were convoluted by a Gaussian function (100–130 fs FWHM) representing the instrument response function. In the first stage of analysis, the presence of a cross-phase modulation artifact during pump-probe overlap was empirically taken into account by adding the sum of the same Gaussian