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Detailed insight into the ultrafast photoconversion of the cyanobacteriochrome Slr1393 from *Synechocystis* sp.

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ABSTRACT

The initial light-induced processes of the photochromic, phycocyanobilin-binding GAF domain of Slr1393 from *Synechocystis* sp. PCC6803 have been studied by ultrafast transient absorption spectroscopy. We use lifetime density analysis as a model-independent method for the evolution of the experimental data, which gives a comprehensive overview of the excitation wavelength dependence of the photoconversion kinetics. The method is particularly suitable for this highly complex and not purely exponential kinetics. In contrast to previously studied cyanobacteriochromes (CBCRs), here both the red- and the green-absorbing forms show significantly slower reaction dynamics, which proceed also via ground state intermediates. The photoconversion of the green-absorbing form is faster than that of the red state, which allowed a clear detection of the primary photoproduct Lumi-G. Strong coherent oscillations of the recorded transient absorption due to wavepacket motion on the excited state potential energy surface were observed and analyzed for both (red and green) forms of Slr1393g3. The vibrational modes responsible for the coherent oscillations could play a role in the dynamics of the initially heterogeneous excited state (ES) population and direct the system towards the minima on the potential energy surface that determine the ES decay pathway. Furthermore, the coherent oscillations appear to be a common feature of bilin-based photoreceptors and thus deserve further attention. The investigated CBCR exhibits an extraordinary high level of heterogeneity due to the remarkable flexibility of the phycocyanobilin and the protein binding pocket. These properties should allow spectrally tuned response to the light stimuli and thus have significant biological implications.

1. Introduction

Cyanobacteriochromes (CBCRs) constitute a subfamily of bilin-binding photoreceptors [1,2]. Their photochemistry is based on a covalently bound phycocyanobilin (PCB) chromophore that in some CBCRs is modified into PCB derivatives, e.g., phycoviolobilin or phycourobilin [3,4]. CBCRs show structural and functional similarities to canonical phytochromes in undergoing light-driven photochromic conversions. Furthermore, as in canonical phytochromes, the chromophore is bound to the protein via a thioether linkage to a conserved cysteine within a GAF domain. Whereas, however, canonical phytochromes exhibit a highly conserved PAS–GAF–PHY protein domain arrangement with all three structural elements essential to maintain the spectral and photochemical properties [5], CBCRs are most often composed of tandem arrays of GAF domains, and even CBCRs with a sole GAF domain have been reported [6]. Functional similarities were also identified in the signaling function of these photoreceptors, such that two-component systems (histidine kinases communicating with a response regulator unit) or cyclic-di-GMP regulating enzyme activities (GGDEF- and EAL domains) are present in both CBCRs and canonical (cyano)bacterial phytochromes [7].

CBCRs are gaining increasing scientific attention, as some of their properties extend far beyond those reported for the canonical phytochromes. Most stunning is the finding that even isolated GAF domains of CBCRs show absorbance properties in virtually any region of the visible and even the near ultraviolet spectral range [3]. Besides the unexpected wide absorbance range, also the chemical reactivity of CBCRs, i.e., the abovementioned capability to modify the chemical structure of

Abbreviations: CBCR, cyanobacteriochrome; EADS, evolution-associated difference spectra; ESA, excited state absorption; GAF, cGMP-specific phosphodiesterases, Adenylyl cyclases and FhlA (protein domain); GGDEF/EAL, protein domains with phosphodiesterase activity, coined according to protein sequence signatures; GSB, ground state bleach; GSI, ground state intermediate; IRF, instrument response function; LDA, lifetime density analysis; LDM, lifetimes density map; NOPA, non-collinear optical parametric amplifier; PAS, Per Arnt Sim (protein domain); PCB, phycocyanobilin; PES, potential energy surface; P_G, green-absorbing state of GAF3 from Slr1393; P_R, red-absorbing state of GAF3 from Slr1393; PHY, phytochrome-specific protein domain; QE, quantum efficiency; SE, stimulated emission; SI, supplementary information; TA, transient absorption

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the initially present PCB into phycoviolobilin, phycourobilin, or even into rubinoid compounds calls for further studies to understand the protein-driven (photo)chemistry. As even CBCR-GAF domains separated from the full-length protein maintain the chromophore-lyase function, this protein family is not only challenging in unraveling its properties, but also offers broad biotechnological applications [8,9].

Among the CBCRs reported so far, red–green switching proteins represent the largest group. In those CBCRs the red absorbing parental state (P_R, λ_{max} = 640–650 nm) carries the chromophore in its 5-, 10-, and 15-*Z* isomeric state that upon light absorption is converted into its 15-*E* isomer (P_G), causing a shift to a wavelength range around 540 nm (Fig. 1). Prototypal for this group of CBCRs are AnPixJg2 (from *Anabaena* (*Nostoc*) sp. PCC 7120) and Slr1393g3 (from *Synechocystis* sp. PCC6803) [9–11]. Whereas in AnPixJ it is the second GAF domain (AnPixJg2) that carries the chromophore, in Slr1393 the third GAF domain (Slr1393g3) – out of a tandem array of three GAF domains – has been identified with chromophore-binding capacity. This latter protein is composed of three GAF domains, followed in C-terminal direction by a PAS domain and a histidine kinase function.

Both GAF domains, AnPixJg2 and Slr1393g3, have been studied for their conversion dynamics in the (sub)-μs-to-ms time range and structural data have been reported for both proteins, demonstrating their conformational and functional similarity [10,11]. However, no information is available on the ultrafast, initial photoprocesses for either of these two proteins. The early photochemical reactions have been followed for a number of other CBCRs [15–23]. Recently, the primary photoreactions from the parental state ($^{15Z}P_R \rightarrow {}^{15E}P_G$) and also from the photoproduct state ($^{15Z}P_G \rightarrow {}^{15Z}P_R$) in this class of red–green absorbing CBCR-GAF domains have been studied in great detail for NpR6012g4 and NpF2164g6 [15–18]. According to these studies, the forward $^{15Z}P_R \rightarrow {}^{15E}P_G$ reaction of these proteins exhibits an unusually high quantum efficiency (QE) of ~30–40% compared to canonical phytochromes that typically show a QE of ~10–18% [24–28]). Formation of the primary photoproduct (Lumi-R_r) proceeds from two excited state (ES) intermediates with lifetimes of ~50–60 ps and ~300–320 ps [15,18]. A third ES intermediate (ESI) (~5–6 ps lifetime) was interpreted as not being involved in Lumi-R_r formation. Further pump–dump–probe experiments on the $^{15Z}P_R \rightarrow {}^{15E}P_G$ reaction in Np6012g4 and NpF2164g6 indicated formation of short-lived (10 and 2 ps lifetimes, correspondingly) ground state intermediates (GSI). In the case of Np6012g4 those GSI contribute significantly to the formation of the primary photoproduct Lumi-R_r [16], while in the case of NpF2164g6 such contribution was not observed most likely due to very short lifetime of the GSI [18]. The reverse dynamics of Np6012g4 and NpF2164g6 ($^{15Z}P_G \rightarrow {}^{15Z}P_R$) is considerably faster than the forward one with primary photoproduct (Lumi-G_{O1}) formation on the picosecond timescale (~1–5 ps), which evidently allows evolution of Lumi-G_{O1} into Lumi-G_{O2} on the 100 ps timescale [17,18]. The QE of the reverse reaction appears to be even greater with values in the range of 40–50% and does not involve a GSI pathway [18]. The ground state population of the abovementioned red → green CBCRs [15–18] as well as of other CBCRs (green → red, violet → orange, blue → green) [19–22] often involves a significant level of inhomogeneity, which renders the investigation of the underlying reactions difficult.

Considering the already available structural data and the kinetic analysis in the μs-to-ms time range for Slr1393g3 [11], we hereby report our studies for the ultrafast processes in the sub-ps-to-ns time range for both the red- and the green absorbing states of this protein, and thus, combined with the μs-to-ms results [11], provide a complete picture of the light-induced interconversion of the green and the red forms.

2. Materials and methods

2.1. Sample preparation and characterization

Slr1393g3 from *Synechocystis* PCC6803 has been cloned and expressed as an independent protein [9,11,29]. The recombinant protein was furnished with an N-terminal His_6 tag. Chromophore incorporation was accomplished in vivo during expression, making use of a two plasmid approach as recently described [11]. Slr1393g3 obtained by this

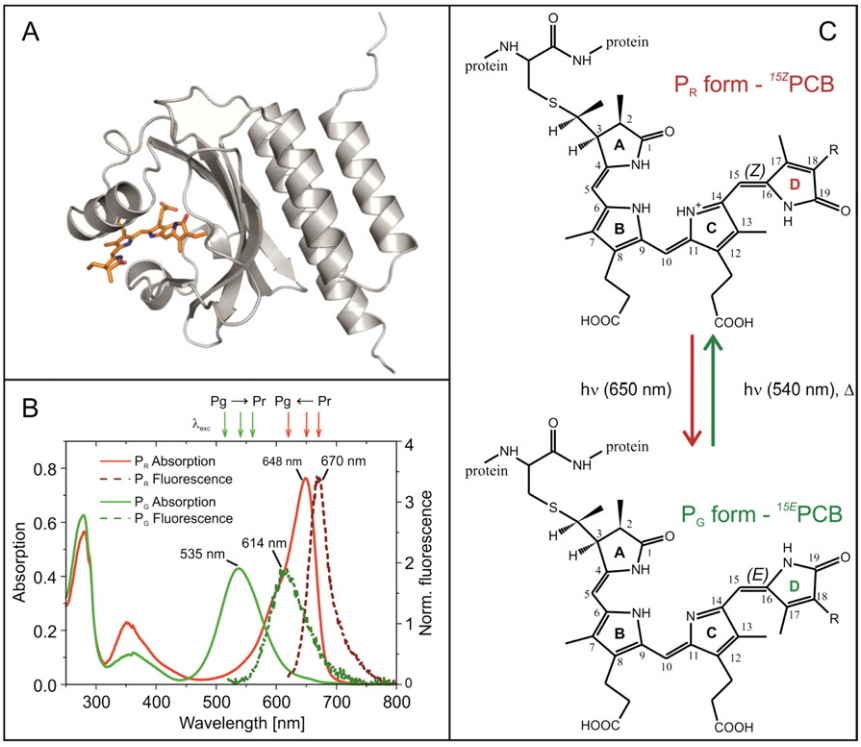

Fig. 1. A) Model structure of the GAF3 domain of Slr1393 protein from *Synechocystis* sp. PCC6803 constructed from the structure of AnPixJg2 (*Anabaena* sp. PCC7120, PDB ID: 3W2Z) [12] as a template and using Modeller [13]. B) Stationary absorption and fluorescence spectra of the $^{15E}P_G$ and the $^{15Z}P_R$ forms of Slr1393g3 CBCR. C) Model structures of the phycocyanobilin chromophore in the $^{15E}P_G$ and the $^{15Z}P_R$ forms as proposed in [14].