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Light-induced oligomerization of cryptochrome4 from European robin

Shigeki ARAI[1*], Rumi SHIMIZU[1], Motoyasu ADACHI[1],
Hirokazu MASAI[2], Koji KIMURA[3], Mitsuhiro HIRAI[4]

[1]National Institutes for Quantum Science and Technology, 4-9-1 Anagwa, Inage, Chiba-city, Chiba 263-8555, Japan, [2]Department of Materials and Chemistry, National Institute of Advanced Industrial Science and Technology (AIST), 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan. [3]Graduate School of Engineering Global College, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, Aichi 466-8555, Japan, [4]Graduate School of Science and Technology, Gunma University, 4-2 Aramaki, Maebashi, Gunma 371-8510, Japan

1 Introduction

Cryptochromes (CRYs), highly conserved blue light absorbing flavoproteins containing a flavin adenine dinucleotide (FAD), in retinal cells of some animal species could act as a quantum sensor to detect the angle of the geomagnetic field [1–3]. The long-lived spin-correlated radical pair (RP) generated in CRY by blue light illumination could detect the angle of the magnetic field [4]. As described in the Photon Factory activity report (PF-report) previously, the small-angle X-ray scattering (SAXS) analysis in the q-range of 0.06–0.85 Å^{-1} revealed that the blue light illumination causes not only the RP formation but also the light-induced structural ordering of erCRY4 in the monomer-scale. Moreover, we are currently working on multifaceted structural analysis, including SAXS measurements in the lower q-range and electron magnetic resonance (EPR), because blue light illumination is known to affect the association of erCRY4 [5]. In order to clarify the effect of blue light illumination on the structure of erCRY4 in the oligomer-scale, we conducted SAXS analysis in the q-range of 0.006–0.195 Å^{-1} and the size exclusion chromatography (SEC) analysis of erCRY4 before and after blue light illumination.

2 Experiment

The construction of erCRY4 using the *E.coli* expression system was performed by almost same procedure as previously reported [4]. Before SAXS measurements, SEC analysis was performed to confirm the effect of blue light illumination on erCRY4 oligomerization. In brief, to obtain the erCRY4 sample in the ground state before blue light illumination, a tube containing erCRY4 solution was surrounded by an aluminium foil and set in the incubator (4 ˚C) for 24 hours. For the ground state measurement with SEC, the SEC column (Superose 6 increase 10/300gl, Cytiva) was surrounded by an aluminium foil to avoid the light illumination, and a sample solution containing erCRY4 (65 μg), 20 mM Tris-HCl buffer (pH 8), 150 mM NaCl and 10 mM 3-mercapto-1,2-propanediol was injected into the SEC column. For the excited state measurement after blue light illumination with SEC, an aluminum foil surrounding the SEC column was removed, and the same amount of erCRY4 as used for the ground state measurement was injected into the SEC column. Flow rate was 0.5 mL/min.

SAXS measurements were conducted at the BL-10C. Approximately a 40 μL sample solution containing 2.7 mg/mL erCRY4 was used for SAXS measurements. The wavelength of the incident X-ray beam was 1.55 Å. The sample-to-detector distance was 3 m. Other experimental conditions for SAXS measurements were same as described in the previous PF-Report. In brief, for the ground state measurement, windows of a sample cell were covered by aluminum foils in shielding ultraviolet light ~ visible light. For the excited state measurement, aluminum foils on windows of a sample cell were removed, and the blue LED light (wavelength: 454nm) irradiated to the erCRY4 solution for 10 min. SAXS data were collected using a PILATUS3 2M detector (Detectris) at 20 ˚C. X-ray exposure time to collect one SAXS curve was 1 min.

3 Results and Discussion

The SEC analysis revealed that erCRY4 (molecular weight : 66.0 kDa) in both the ground state (dark condition) and the excited state (under blue light) eluted earlier than the standard sample, bovine serum albumin (66.5 kDa) (Fig. 1a), suggesting that erCRY4 self-associated to form dimer or larger. In addition, erCRY4 in the excited state eluted earlier than that in the ground state. This result indicates that the molecular size of erCRY4 oligomer increased with the excitation by blue light illumination.

To clarify the structural features of erCRY4 in the ground state and the excited state, we measured the scattering curves in the q-range of 0.006–0.195 Å^{-1} by SAXS, which reflects structural information with a real spatial resolution of d = 32–1047 Å (Fig. 1b). Buffer scattering was subtracted from each curve to yield the sample scattering curves $I(q)$s using the program *SAngler* [6]. Here, q denotes the magnitude of the scattering vector defined by the following equation:

$$q = 2\pi/d = (4\pi/\lambda)\sin(\theta/2)$$

d, θ and λ are the real-space resolution, the scattering angle and the wavelength, respectively. The average radius of gyration R_g of erCRY4 estimated from the Guinier region ($q \cdot R_g < 1.3$) was increased from 179 Å to 223 Å with blue light illumination.

Moreover, the distance distribution functions $P(r)$s were obtained by Fourier transform of $I(q)$s in the q-range of 0.06–0.20 Å^{-1} using following equation:

$$P(r) = \frac{2}{\pi}\int_0^\infty rqI(q)\sin(rq)dq$$

r is the real space distance. The obtained $P(r)$s showed that the peak position corresponding to the center of gravity of the intramolecular vector distribution and the maximum particle diameter (D_{max}) determined from the position of $P(r)$ = 0 were both larger in the excited state than in the ground state (Fig. 1c). These results suggest that the self-association of erCRY4 is promoted by blue light irradiation.

To estimate the shape of the erCRY4 oligomer more specifically, we constructed bead models reflecting the external shapes of the erCRY4 oligomers from the SAXS data using the program *Dammin* [7] (Fig. 1d, e). The obtained models exhibited that amorphous-like but flat oligomers could be formed in both the ground state and the excited state. However, the bead model of the erCRY4 oligomer was slightly expanded by blue light illumination (Fig. 1d, e). Taking together the results obtained so far, it was clarified that blue light illumination to erCRY4 induces not only RP formation and the structural change in the monomer-scale but also the structural change in the oligomer-scale. We are planning to clarify whether erCRY4 exhibits such molecular behavior even in the molecular crowding condition as *in vivo*.

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* arai.shigeki@qst.go.jp

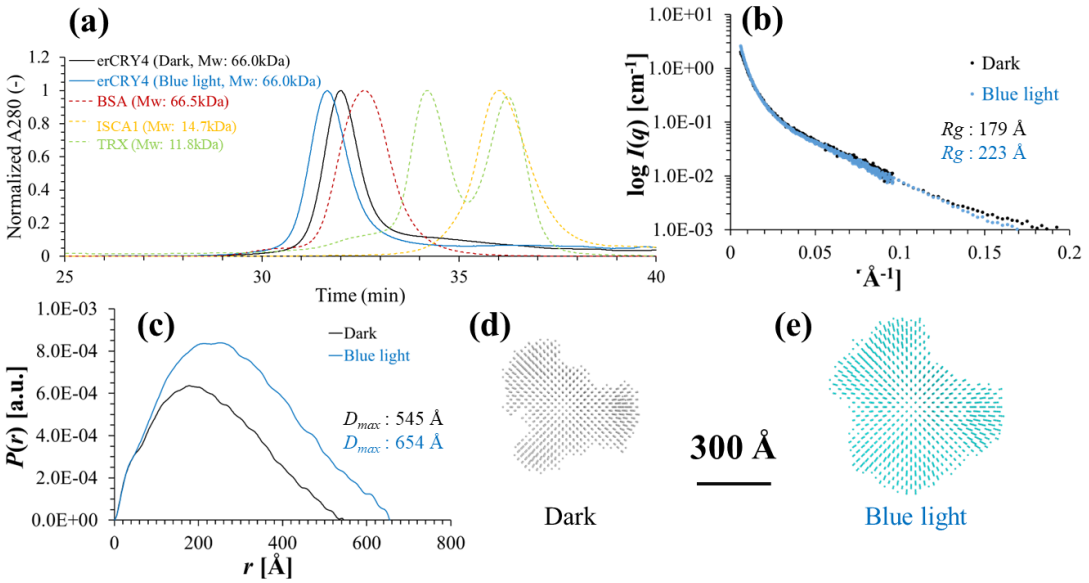

Fig. 1. Structural features of erCRY4 oligomers. (a) Elution curves observed by SEC. Black and blue curves indicate the elution of erCRY4 in the ground state (in the dark condition) and the excited state (under blue light illumination), respectively. Dotted curves show the elution curves of bovine serum albumin (BSA), ISCA1, and thioredoxin (TRX) as standard samples. (b) and (c) show $I(q)$s and $P(r)$s obtained by SAXS measurements of erCRY4. (d) and (e) show bead models of erCRY4 oligomers in the ground state (d) and in the excited state (e). These models were estimated from SAXS data shown in (c) with the *Dammin* program.