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Time-Resolved Tracking of Interprotein Signal Transduction: *Synechocystis* PixD–PixE Complex as a Sensor of Light Intensity

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Supporting Information

ABSTRACT: PixD (Slr1694) is a blue light receptor that contains a BLUF (blue light sensors using a flavin chromophore) domain. A protein–protein interaction between PixD and a response regulator PixE (Slr1693) is essential to achieve light signal transduction for phototaxis of the species. Although the initial photochemical reaction of PixD, the red shift of the flavin absorption spectrum, has been investigated, the subsequent reaction dynamics remain largely unresolved. Only the disassembly of the $PixD_{10}$–$PixE_5$ dark complex has been characterized by static size exclusion chromatography. In this report, interprotein reaction dynamics were examined using time-resolved transient grating spectroscopy. The dissociation process was clearly observed as the light-induced diffusion coefficient change in the time domain, and the kinetics was determined. More strikingly, disassembly was found to take place only after photoactivation of two PixD subunits in the complex. This result suggests that the biological response of PixD does not follow a linear correlation with the light intensity but appears to be light-intensity-dependent.

Although characterization of the dynamics of intermolecular interactions is important in chemistry, there is a paucity of time-resolved studies of interprotein signaling processes, as it is usually difficult to measure the kinetics in real time. In this Communication, we investigate interprotein interaction dynamics of the blue light photoreceptor PixD (Slr1694) with its response regulator PixE (Slr1693) using time-resolved diffusion detection based on the pulsed laser-induced transient grating (TG) technique.

The photochemistry of blue light receptors has recently attracted considerable attention.[1] PixD is a cyanobacterial blue light receptor from the mesophile *Synechocystis* sp. PCC6803.[2,3] PixD is composed of a typical blue light sensing domain, the BLUF (blue light sensors using a flavin adenine dinucleotide) domain, and two additional α-helices. The representative photochemistry of PixD is a rearrangement of a hydrogen-bonding network in the vicinity of the flavin, which is characterized by a ~10 nm red shift of the absorption spectrum.[4−6] Ultrafast visible and infrared transient absorption studies of PixD demonstrated a proton-coupled electron transfer between the Tyr residue and the chromophore within 100 ps after photoexcitation, resulting in the generation of the signaling state.[7,8] Since there is no signal output domain in PixD, light signal transduction is probably mediated by a change in protein–protein interactions. Related to the interprotein interaction, PixD has a unique oligomeric structure (Figure 1). Here, the protein decamer consists of an asymmetric unit with two pentameric rings in a crystal.[9] It was previously reported that PixD in the dark state forms a dimer in solution, and in the presence of the cyanobacterial two-component response regulator PixE, the PixD dimer associates with PixE to form a hetero-oligomeric complex, the $PixD_{10}$–$PixE_5$ complex.[10,11] Upon blue light irradiation, the complexes disassemble into 5 PixD dimers and 5 PixE monomers. This light-induced change in the PixD–PixE interaction was suggested to be a crucial part of the early signal transduction process.

Recently, we investigated the light-induced reaction dynamics of PixD by the TG method and size exclusion chromatography (SEC).[12] We found that PixD is in equilibrium between the dimer and decamer forms in the dark, even in the absence of PixE. When the solution was illuminated with blue light, a conformational change associated with a volume contraction occurred with a time constant of 45 ms. Upon strong light irradiation, a significant diffusion coefficient (D) increase of the PixD decamer was observed with a time constant of 350 ms. These observations were interpreted by dissociation to the dimer upon photoexcitation. Thus, we are now in a position to study a more functionally important reaction: the effect of PixE on the reaction of PixD using the $PixD_{10}$–$PixE_5$ complex.

The experimental methods and analyses are described in the Supporting Information (SI). In a short time range, a weak rise component was observed in several tens of milliseconds and is attributed to the volume contraction process, indicating a light-induced conformational change (SI-2). After this dynamics, the TG signal showed rise–decay features. Figure 1 depicts the TG signal of the $PixD_{10}$–$PixE_5$ complex at the grating wavenumber of $q^2 = 5.6 \times 10^{10}$ m^{-2} and a laser power of 4.3 mJ/cm^2. For

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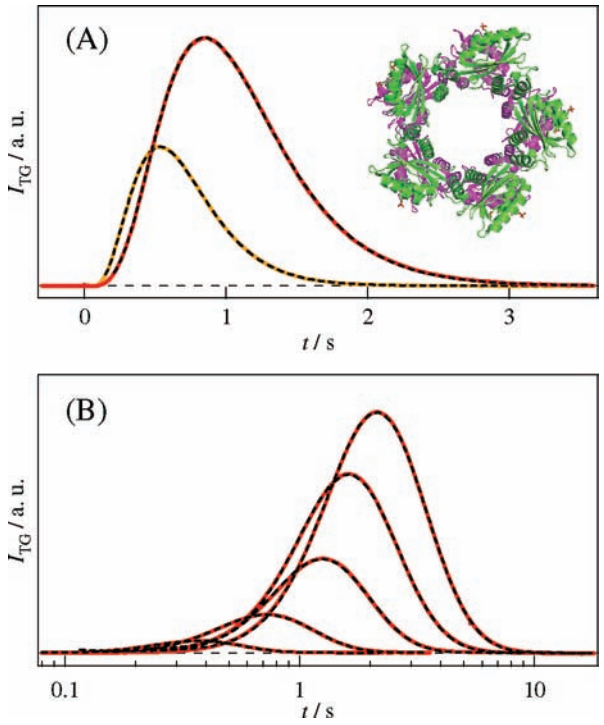

Figure 1. (A) Typical TG signal after the photoexcitation of the PixD−PixE complex at $q^2 = 5.6 \times 10^{10}$ m^{-2} and a laser power of 4.3 mJ/cm^2 (red) with that of PixD (yellow) under the same experimental conditions. The inset is a crystal structure of PixD decamer.[9] Two pentameric rings are shown in green and magenta. (B) q^2 dependence of the TG signal of the PixD−PixE complex (red lines). The q^2 values were 21-, 7.6-, 4.7-, 3.0-, and 2.0×10^{10} m^{-2}, from left to right. The signals were normalized by the thermal grating intensity. The best-fitted curves using the equations of SI-1 are shown by the black dashed line on the observed data.

comparison, the TG signal of PixD without PixE measured under the same experimental condition is also shown. These signals are qualitatively very similar to each other.

Since the rise and decay components depended on q^2 value, these phases represented the molecular diffusion processes. By comparing the sign of the refractive index change (SI), we determined that the rate constants of the rise and decay phases represent the diffusion of the product and the reactant, respectively. For analyzing the signal, we first determined the diffusion coefficient of the product (D_P) and the reactant (D_R) by fitting the diffusion signal in the long time region ($t > 1$ s) using a biexponential function (eq S-2). The D_P and D_R values were determined to be 7.5- and 2.5×10^{-11} m^2/s, respectively. This result indicates that a photoproduct diffuses faster than the reactant; that is, the photoproduct should have a smaller molecular size than the reactant. Compared with the D values associated with the photoreaction of PixD alone, D_R of the PixD−PixE complex was smaller than that of PixD (3.7×10^{-11} m^2/s). The smaller value of D_R is quantitatively consistent with the previous finding that PixD oligomerizes in the presence of PixE to form a hetero-oligomeric protein complex composed of 10 PixD and 5 PixE molecules,[10] whereas the reactive PixD forms a decamer. (According to the Stokes−Einstein relationship, the D value is proportional to the reciprocal value of the cube root of molecular weight. The ratio of D of $PixD_{10}$−$PixE_5$ to that of $PixD_{10}$ is close to that expected from their molecular size.) It is interesting to note that the D_P value (7.5×10^{-11} m^2/s) is close to that of PixD dimer, which was reported previously.[12] Hence, it is reasonable to assign a photoproduct from the $PixD_{10}$−$PixE_5$ complex to the dimer of PixD. Furthermore, since the molecular mass of the monomer of PixE (43 kDa) is close to that of PixD dimer (36 kDa), we consider that another photoproduct is a PixE monomer; hence, the rise component represents a superposition of two diffusing species, the PixD dimer and the PixE monomer. This assignment, which clearly indicates the disassembly of the $PixD_{10}$−$PixE_5$ complex into the PixD dimer and PixE monomer upon blue light irradiation, is fully consistent with the previous observation obtained using static SEC.[10]

The time-resolved diffusion detection method directly provides the PixD−PixE interprotein interaction change in the time domain. To determine the kinetics of the dissociation reaction, the signals at different q^2 values were measured (Figure 1B). The peak intensity was weak on a fast time scale and increased with increasing the observation time by decreasing q^2. This feature is typical of a time-dependent D by a reaction that can be fitted using the model

$$R \xrightarrow{h\nu} I \xrightarrow{k} P$$

where R is the $PixD_{10}$−$PixE_5$ complex, I is an intermediate, P is the photoproduct which includes the dissociated PixD dimer and PixE monomer, and k is the rate constant of the D change, i.e., the dissociation rate. The signals at various q^2 values were fitted by the above model (eq S-3), and D_I and the time constant k^{-1} were determined to be 2.5×10^{-11} m^2/s and 500 ms, respectively. This time constant was similar to but slightly longer than that of PixD (350 ms).[12]

It would be interesting to determine whether the light-induced dissociation of the $PixD_{10}$−$PixE_5$ complex is caused by the multiple-excitation of the monomer subunits of PixD. For examining this point, we measured the TG signal at various excitation light intensities. The amplitude of the reactant (δn_R), which is proportional to the number of $PixD_{10}$−$PixE_5$ complexes undergoing dissociation, is plotted against the excitation laser intensity in Figure 2. For obtaining the number

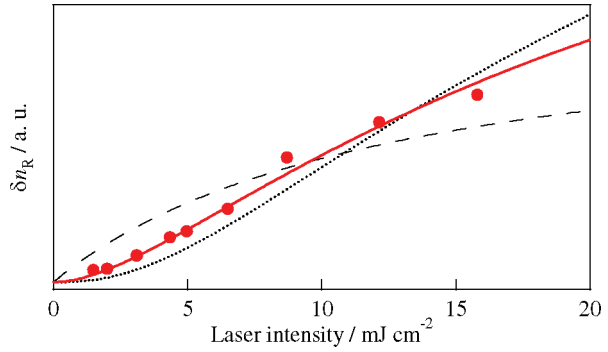

Figure 2. Light intensity dependence of the number of reactive PixD−PixE complexes (δn_R, closed red circles). Best-fit curves by P_1, P_2, and P_3 are shown by the black dashed line, red solid line, and black dotted line, respectively.

of excited monomer units for this reaction, the observed light intensity dependence was fitted by the number of complexes containing one red-shifted species from the probability of the Poisson distribution at one (P_1), two (P_2), or three (P_3) excited subunits (SI-3). As shown in Figure 2, the probable disassembled complex calculated from P_1 increases linearly at

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a weak light intensity and then is saturated in a stronger light intensity region. Also P_3 should increase with the third power of laser intensity and is not saturated in the simulated intensity range examined. Conversely, the dissociation calculated from P_2 initially increases by the second order of the light intensity and is then gradually saturated. The best-fit curves of P_1 and P_3 did not reproduce the observed light intensity dependency of δn_R at all; however, the simulated curve of P_2 provided a good fit to the data. This result clearly indicates that only a decamer containing two red-shifted monomers is responsible for the dissociation reaction.

To further support the above consideration, we performed an experiment with a weak laser intensity and a high repetition rate (data in SI-4). The signal intensity gradually increased as the number of excitation pulses increased. This increase is explained as follows. Just following the excitation by a weak light, the excitation of one PixD subunit in the complex should be dominant. Hence, the signal intensity is weak because the number of dissociated complexes should be stochastically low. However, by the next pulse, the other monomer unit could be excited, and this complex is disrupted to show the diffusion signal. Therefore, this successive irradiation effect on the signal intensity represents clear evidence for the above mechanism.

The present observations suggested that the dissociation is triggered by a conformation strain due to a conformation change; however, this strain of a single unit is not large enough to trigger dissociation, and the strain of two subunits is essential. Prior to completing this research, we hypothesized that the presence of PixE may change interprotein interactions and photoexcitation of one PixD may be sufficient to facilitate dissociation of the $PixD_{10}$–$PixE_5$ complex, because the light sensitivity should be much higher for this complex and it may be favorable for the light sensing function. However, we found that the dissociation schemes of PixD in the presence and absence of PixE are similar, both of which require the photochemical reaction of two PixD subunits. Hence, the strain caused by the excitation of one monomer unit is not large enough to facilitate dissociation of the PixD–PixE complex. So what is the effect of PixE on the PixD reaction? There may be two apparent effects. First, without PixE, PixD is in equilibrium between the dimer and decamer forms and the dimer form is dominant. On the other hand, in the presence of PixE, the decamer form ($PixD_{10}$–$PixE_5$ complex) becomes dominant. Therefore, PixE stabilizes the decamer form of PixD. Second, the dissociation rate of the oligomer was found to slightly decrease; dissociation of the PixD decamer is 350 ms and that of PixD–PixE complex is 500 ms (Figure 3). This reduction of the rate may be related to the stabilization of the parent $PixD_{10}$–$PixE_5$ complex.

How is this effect related to biological function? The average solar irradiance at the surface of the earth in the visible wavelength region during the lifetime is ~100 mJ/cm^2, which is sufficient to excite two PixD subunits in the $PixD_{10}$–$PixE_5$ complex, i.e., higher than that used in our TG experiments.[12] Therefore, the photoactivation of multiple subunits in the $PixD_{10}$–$PixE_5$ complex is possible in nature, and this photoactivation induces the dissociation reaction that controls the subsequent light signal transduction for biological function. This probability is low for weak light intensity but increases as the light intensity increases. Hence, the organism may be using this photoreceptor as a light intensity sensor. This light intensity sensing is reasonable biologically, since the cyanobacteria can use blue light as the energy source.

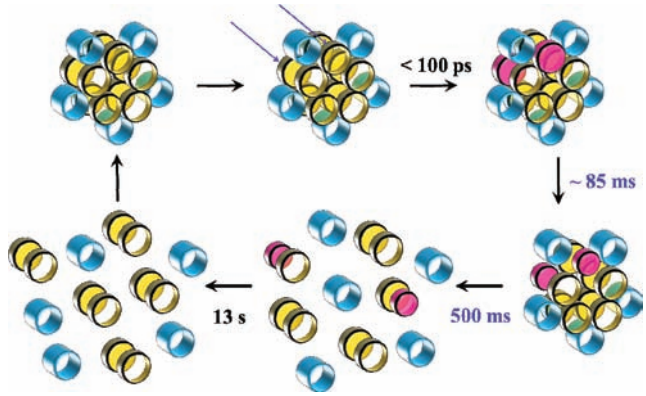

Figure 3. Photoreaction scheme of PixD–PixE complex.

■ ASSOCIATED CONTENT

Supporting Information

Material and methods; dynamics of conformational change in a fast time scale; analysis by Poisson distribution; and further support for the dissociation by excitation of two monomer units. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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