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Exploring excited state properties of BLUF domain of AppA by photothermal methods

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Introduction:

AppA is a novel blue-light receptor that regulates photosynthesis gene expression in response to blue light in the purple bacterium *Rhodobacter sphaeroides* [1,2]. AppA interacts with a transcriptional repressor PpsR to form a stable AppA-$(PpsR)_2$ complex in the dark or lowlight conditions [1,2]. Blue light disrupts the binding of AppA to PpsR and thus restoring the PpsR binding activity to the promoter region of photosynthesis genes [1,2]. Although AppA mediated signal transduction is rather well understood, its initial photochemistry and signal transduction pathway still remain to be solved. Since the time-resolved transient grating (TG) technique has been well established to detect kinetics and conformational change or aggregation process which may occur far away from chromophore, we investigated AppA (BLUF domain of AppA, $AppA_{5\text{-}125}$) photoreaction after the blue light irradiation by the time-resolved TG method. We have also used the transient lens (TrL) method for quantitative measurements of the enthalpy change of the final product.

Experimental:

The principle of TG and TrL techniques are described elsewhere [3-5]. Our excitation wavelength in both TG and TrL experiments was 465 nm (XeCl excimer laser pumped dye laser). The probe wavelength for the TG measurement was 780 nm (from diode laser) and the probe wavelength for the TrL measurement was 633 nm (from He-Ne laser). BLUF domain of AppA, $AppA_{5\text{-}125}$ was cloned, and expressed from *Escherichia coli* [6].

Results and Discussions:

The TG signal of AppA upon the photoexcitation rose quickly with the time response of our system (~20 ns) and there appeared a weak slow rising component with a time constant of ~3.4 μs, which was attributed to the decay of the excited triplet state. After this component, the thermal grating signal and finally a rise-decay component representing protein diffusion process were observed. From the rate constants of the rise and decay of the latest signal, the diffusion coefficient of AppA and the product can be determined. Interestingly, we found that this component was concentration dependent in all q^2 condition (Fig.1). In going from low q^2 to high q^2 condition, the signal approaches to a single exponential like feature. We interpreted this concentration dependent feature in terms of the aggregation formation in the excited state of this protein. The observed TG signal was fitted well by an aggregation model. The observed aggregation rate constant (k_{obs}) increases linearly as the concentration increases. This dependence suggests that the dimerization reaction takes place in the photoreaction of AppA. From the slope of the plot of k_{obs} against the concentration, we got second order rate constant with a value $\sim 2.5\times10^5$ $M^{-1}sec^{-1}$. This value is lower than the diffusion controlled rate

observed for bimolecular association reaction.

Furthermore, we have measured the enthalpy change associated with the formation of the first intermediate (which is formed in several hundreds of picosecond time-scale after the blue light irradiation [2]) by the TG method. The ΔH value of this intermediate was ~85 (±12) KJ/mole at 25^0C. This ΔH value decreased with decreasing the temperature and this temperature dependence indicates nonzero heat capacity change between the ground state and the intermediate state (ΔC_p). Since the heat capacity is an indicator of the solvent accessible surface area, this positive ΔC_p value may indicate that the hydrophobic surface area is exposed in this intermediate.

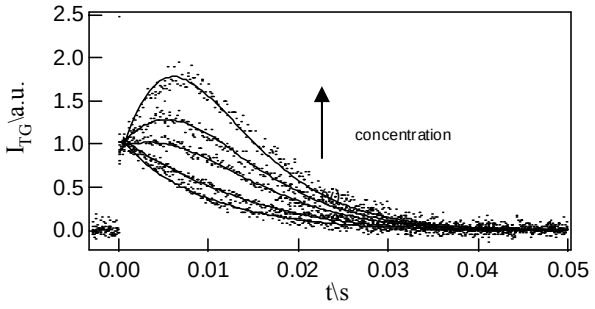

Fig.1: Concentration dependence of the TG signals of AppA at $q^2=1.3\times10^{12}$ m^{-2}.

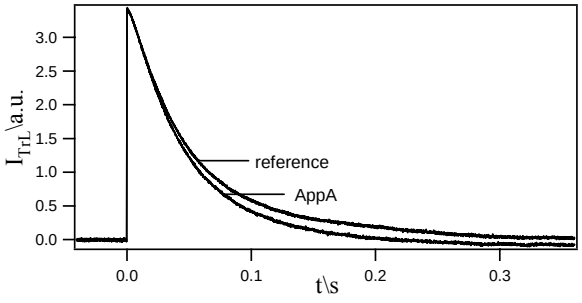

Fig.2: Typical TrL signals of calorimetric reference sample and AppA at 298K.

We have also measured the enthalpy change associated with the dimerization reaction by the TrL method (Fig.2). Analysis of the TrL signal indicates that this dimer formation is accompanied by negative ΔH. That means that dimerization reaction is accompanied by heat release rather than heat absorption. Thus we conclude that the dimerization reaction of AppA stabilize the product energetically. We hope that these thermodynamic parameters may give new insight to the photocycle of the BLUF domain of AppA.

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