Time-resolved detection of photoinduced heating and volume changes during protein reactions

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Background – The photothermal methods detect heating of various materials after illumination. The heating is caused by the nonradiative transition from the excited states, and it is general phenomena particularly in condensed phase. We have been using the photothermal techniques, in particular, the transient grating (TG) and transient lens (TrL) methods, to understand chemical reactions of biomolecules in solution. Since these methods detect the refractive index change upon photoexcitation, a variety of properties, e.g., thermodynamical properties and diffusion, can be detected. There are many advantages in these methods. One of the merits is very high time-resolution. Changes in the thermodynamics properties can be monitored in time domain. For example, although the thermodynamics have been playing very important roles in revealing the nature of molecules, the thermodynamic properties are not easily accessible for transient species. The TG and TrL methods remove this limitation. Here we will report on the thermodynamical studies of short-lived intermediate species of a biological protein, phototropins.

Phototropins (phots) are blue-light sensors, and the photoreactions have been widely investigated. In particular, the reaction of the LOV2 domain with the linker region from phot of *Arabidopsis thaliana* have been extensively studied by a variety of methods, and all results indicated that the linker helix is eventually unfolded upon the photoexcitation of the LOV2 domain. Using the TG method, we have determined the reaction scheme and the kinetics of this protein (Fig.1) [1]. Although this is a very prominent reaction, the driving force of the unfolding of the linker has not been well elucidated. On this aspect, we proposed that structural fluctuations might be essential for this reaction. For proving the importance of the structural fluctuation during the reaction, we tried to measure the compressibility of the intermediate species by using the TG and TrL methods with a special designed high pressure optical cell. From the pressure dependence data, the compressibility changes for the short lived intermediate and the final product formation were determined. The compressibility change will be discussed in terms of the cavities inside the protein.

Methods – The protein Arabidopsis phot1-LOV2-linker was dissolved in buffer solution. The purified protein was concentrated by ultrafiltration and then used for the measurements.

The pressure resistance of a high pressure cell we used was up to 500 MPa. High pressure was generated by pumping water with a compact hand pump. The sample temperature was controlled by circulating temperature-controlled water through a hole drilled within the cell. The sample solution was encapsulated in an inner cell and incorporated into the high-pressure apparatus. The pressure was reset to 0.1 MPa after every compression (high pressure experiment) to check the recovery of the signal. The signals were confirmed to be completely reversible.

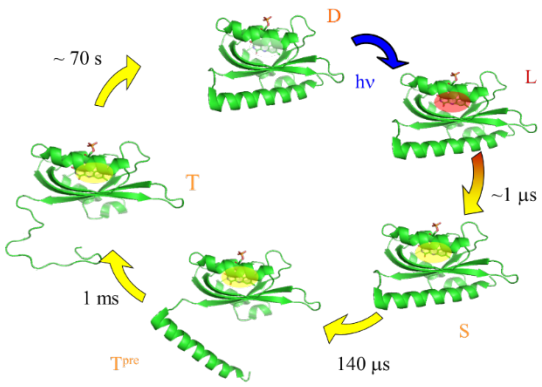

Fig. 1. Reaction scheme of a blue light sensor protein, phot1LOV2-linker determined by the TG method. D: dark state, L: excited triplet state, S: initial intermediate, T^{pre}: precursor state of the T state, T: final photoproduct state.

For the TG and TrL measurements, a XeCl excimer laser-pumped dye laser beam (λ = 462 nm) was used for the excitation laser. The signal was detected by a photomultiplier tube. The repetition rate of excitation was usually 0.015 Hz and the laser power for excitation was usually set to be weak enough to not excite the photoexcited protein twice by the laser pulse. A CW diode laser (835 nm) was used as a probe light source. The grating wavenumber, q value, at each experimental setup was determined from the decay rate of the thermal grating signal of the calorimetric reference.

Results and discussion – The compressibility change can be determined simply by measuring the volume changes after photoexcitation at various pressures, if the reaction yield does not depend on pressure. However, since proteins are generally flexible, the protein reaction yield could be pressure dependent. If this is a case, the measurement of the compressibility change is difficult. Hence, we first investigated a pressure dependent reaction yield using the pressure dependence of the transient absorption method of the phot1LOV2-linker. The pressure effects on the UV-Vis absorption spectra in the dark state were found to be negligible after the correction of the increase in the density of the solution. This fact ensures that the conformation, at least around the chromophore, does not change with pressure over the range of 0.1 - 400 MPa. Next, the pressure dependence of the reaction yield of the S state formation was measured. The time profiles of the absorption changes after the photoexcitation of the phot1LOV2-linker at various pressures was slightly dependent on the pressure. However, the change was minor, so that the pressure effect can be corrected.

The time-evolution of the TG signal after photoexcitation of the phot1LOV2-linker at 0.1 MPa consists of five components: the adduct formation process (occurs over a few μs), the thermal grating component (decay rate constant $D_{th}q^2$ (D_{th}: thermal diffusivity)), a volume contraction process associated with the transition from the S state to the T state with a time constant of ~1 ms, and a peak of the molecular diffusion signal, which represents the diffusion of the T and D species. By taking the sum of these contributions, the signal was expressed well by an equation of;

$$I_{TG}(t) = \alpha\{\delta n_{th}\exp(-D_{th}q^2t) + \delta n_P\exp(-D_Pq^2t) - \delta n_R\exp(-D_Rq^2t)\}^2 \qquad \text{Eqn. 1}$$

where α is a constant, subscripts of th, P, and R represent the thermal component, product, and reactant, respectively, δn_i is the peak-to-null refractive index difference in sinusoidal modulation at $t = 0$, and q is the grating wavenumber. For measuring the volume change associated with the unfolding process of the linker (S → T process), we used the TrL methods.

The isothermal compressibility (κ_T) reflects the volume fluctuation. We succeeded in detecting a transient enhancement of the isothermal compressibility (i.e., fluctuation) in the S state, and also in the T state compared with the D state at ambient pressure. The observed enhancement of the compressibility

relative to the dark state are shown in Fig.2. Using this value, the observed enhancement of the structural fluctuation in the S state is an increase of ~9.3% from the D state. This increase of 9.3% in the compressibility does not come from the whole protein, but is likely to be localized probably around an important region involved in the subsequent reaction. Hence, we consider that this is a relatively large enhancement and that this enhancement of the fluctuation can trigger the unfolding of the linker-helix. The extent of the enhancement of the compressibility in this LOV domain sample is surprisingly similar to that of the BLUF domain protein [2]. The similar extent of the increase of the fluctuation suggests that the dissociation of the LOV domain and the linker domain is also controlled by the fluctuation of the LOV domain.

An important contribution to the compressibility could be cavities inside the protein. A detailed description that explains the enhanced compressibility is obtained from the crystal structure of the phot1LOV2-linker for dark and light states. Using the PDB data, which correspond to the dark and light structures, we calculated their cavities by the 3D program with a water probe radius of 1.5 Å. We found that the cavity volume of the light state was larger than that of the dark state. This change may be sufficient to explain the observed compressibility change.

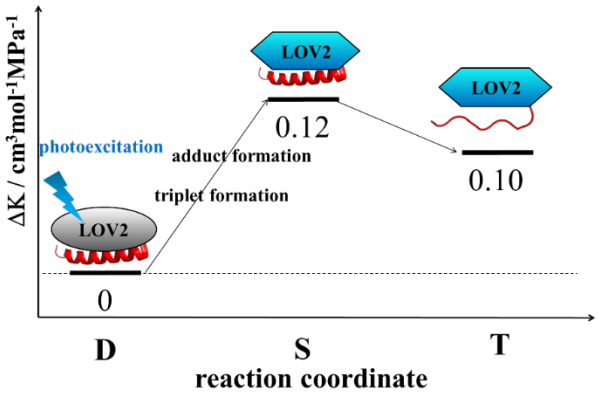

Fig. 2. Compressibility changes along the reaction coordinate of phot1LOV2-linker.

References

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