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Conformational change of photoactive yellow protein studied by the time-resolved thermodynamics

Partha Hazra[1], Yasushi Imamoto[1], Miki Harigai[1], Mikio Kataoka[2] and Masahide Terazima[1]
Kyoto University[1], Nara Institute of Technology[2]

Introduction: Conformational changes play a central role in the molecular mechanism of many photoreceptor proteins and presently it is a burgeoning interest to researchers how this photoreceptor proteins transfer signal to its downstream proteins. Among the various photoreceptor proteins, PYP has become a model system for the study of signal transduction mechanism in photoreceptor proteins due to its excellent photochemical stability. Its photocycle has been characterized by various experimental techniques [1]. In brief, upon photoexcitation of the chromophore, the ground state species (pG) is converted into a spectrally red-shifted intermediate (pR_1) within 2 ns. Subsequently, pR_1 is converted to pR_2 without any spectral change. The pR_2 species decays on the submillisecond timescale to a blue-shifted intermediate (pB'), which is converted to pB, finally returns to pG on a timescale of seconds. This pB state is considered to relay the signal to downstream proteins. Recently, heat capacity changes (ΔC_p) of pR and pB state were measured by the transient grating (TG) and transient lens (TrL) techniques [2]. The measured ΔC_p associated with the pB state formation is 2.7 kJ/mol K and the unfolding of the N-terminal helices was believed to be responsible for the observed high positive ΔC_p [2]. Very recently, conformational changes of this pB intermediate of PYP were studied from a viewpoint of the diffusion coefficient (D) change of several N truncated intermediates, which lacked the N-terminal 6, 15 and 23 amino acid residues (T6, T15 and T23, respectively) [3]. This diffusion study also confirms the involvement of N-terminal helices during the pB formation. In this study, we measured ΔC_p, enthalpy change (ΔH), and the thermal expansion volume ($V\Delta\alpha_{th}$) of pR and pB for several N-truncated PYPs; T23, which lacks two α-helices in N-terminal region, and T15, which lacks one α-helix by the TG and TrL methods.

Experimental: The principle of the TG and TrL techniques were described elsewhere [2,3]. Our excitation wavelength in both TG and TrL experiments was 465 nm (XeCl excimer laser pumped dye laser). The probe wavelength for the measurement was 633 nm (from He-Ne laser).

Results and Discussions: Fig. 1(a) represents the TG signal on a time scale of ~30 μs. The signal rises quickly after photoexcitation with the instrument response of our system and then it shows a weak rising component that represents pR_1 to pR_2 transition. After that, the signal decays to certain intensity with the time constant $D_{th}q^2$. This thermal grating signal arises due to the heat releasing process accompanied by the pG→pR_2 transition. Then signal shows a rising component that reflects the pR_2→pB kinetics. Comparing the thermal grating signal intensity of T23 with that of a calorimetric reference sample (BCP) at various temperatures, we calculated ΔH_{pR} using the quantum yield of the reaction Φ=0.35 (it was verified by transient absorption (TA) study that Φ is the same as that of the wild type). The plot of ΔH_{pR} against temperature is shown in the inset of Fig. 1(a). The value was almost temperature independent. This result indicates that C_p of pR_2 is the same as that of pG of T23 and it means that the solvation structure of pR_2 does not change so much from pG. The high enthalpy in the pR_2 state is due to the strained protein conformation

in this state. Furthermore, we also calculated $\Delta\alpha_{th}$ in going from pG to pR_2 from the temperature dependence TG signal and we found that $\Delta\alpha_{th}$= 0.3 cm^3/mol.K, which is significantly smaller than that of wild type ($\Delta\alpha_{th}$= 0.6 cm^3/mol.K) [1(f)]. It suggests that the conformational flexibility of pR_2 in T23 is very similar to that of pG but it is quite less than that of pR_2 of wild type.

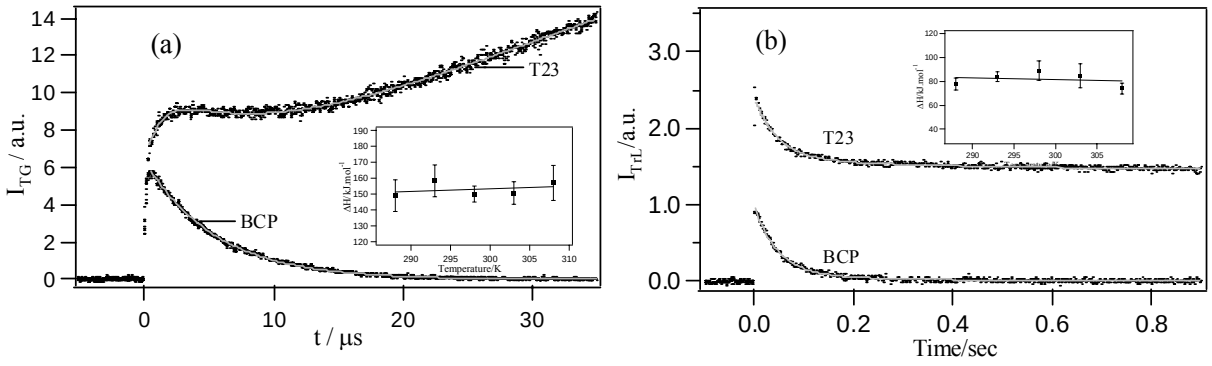

Fig.1 (a) TG signals of T23 and BCP in a time range of thermal grating decay at 298 K. Plot of ΔH_{pR} against temperature is shown in the inset. (b) TrL signals of T23 and BCP at 298 K. ΔH_{pB} against temperature is shown in the inset.

We used the TrL method for the determination of ΔH for the formation of pB. Fig. 1(b) is typical TrL signals of T23 and BCP at 298 K. Thermal energy released from the photoexcited state to the pB formation can be detected as a decaying component of the TrL signal. Similar to TG signal analysis, the relative thermal energy was compared with that of BCP and we determined the enthalpy change (ΔH_{pB}) of pB formation to be 85 (± 10) kJ/mol at 293 K. This value is close to that of wild type (75 (± 12) kJ/mol at 301 K) [3]. Moreover, unlike wild type, we observed that ΔH_{pB} is practically remains constant over the wide range of temperature. This means that pG→ pB in T23 is accompanied by almost zero ΔC_p. The measured ΔC_p associated with the pB state formation of wild type PYP was 2.7 kJ/mol [3] and this large ΔC_p was interpreted in terms of exposure of non-polar amino acid residues to the aqueous phase [3]. In the N-terminal truncated T23, all N-terminal helices are absent. Moreover, we observed almost zero ΔC_p in going from pR_2→pB. Thus our results may be direct proof that the N-terminal helices are mainly responsible for the observed high ΔC_p in case of wild type PYP and these N-terminal helices play a major role for the signaling state formation of PYP. Furthermore, we also calculated $\Delta\alpha_{th}$ for the pR_2 and pB from the temperature dependent TG signals and the measured value is 0.47 cm^3/mol.K. This positive value of $\Delta\alpha_{th}$ in going from pR_2 to pB state indicates that pB state is less compact than the pR_2 due to the conformational change in the pB state.

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