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Protein dynamics detected by the time-resolved transient grating technique*

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Abstract: Dynamics of two proteins [myoglobin and photoactive yellow protein (PYP)] initiated by photoexcitation were studied by the time-resolved transient grating (TG) and photoacoustic methods. For both proteins, the temporal profiles of the TG signals showed new features that have not been detected before by the transient absorption method. These dynamics were attributed to the volume change, which is caused by the protein dynamics apart from the chromophores.

INTRODUCTION

Dynamics of proteins have been attracting many scientists in many fields and have been one of the most important research areas. For detecting the protein dynamics in time domain, there are two important factors we should consider; how to initiate the dynamics and how to detect it. Protein dynamics in some biological systems can be initiated by light irradiation of the chromophores, and such systems are suitable for studies with a fast time resolution. Here we chose myoglobin (Mb) triggered by photodissociation reaction of the ligand and photoactive yellow protein (PYP) triggered by the photoisomerization reaction as target systems for the study of the protein dynamics. As for the detection, many spectroscopic techniques, such as transient absorption (TA), infrared absorption, Raman scattering, NMR, etc., have been applied to the studies of the protein structures and the dynamics. Among them, TA is a powerful detection method for monitoring the time development in a fast time scale (>a few 10 fs) and indeed has been widely used so far. However, by this technique, only the structural changes close to the chromophore can be detected, and the dynamics cannot be related to the energetics of the system. In this respect, dynamics of the volume change (ΔV), which reflects the dynamics of the whole protein part, and the energy change (ΔH) are complementary and good properties we should measure. However, it has been impossible to trace the temporal change of ΔH and ΔV by traditional techniques.

Only one technique for measuring ΔH and ΔV of irreversible reactions developed so far was the photoacoustic (PA) method [1]. The observed PA signal consists of the thermal as well as the volume contributions simultaneously. A difficulty of the application of this method is that these contributions have to be separated using some assumptions, which have never been tested rigorously. Another difficulty of the PA method is that the time window is rather limited (from 10 ns to a few µs). Recently, we have reported that these difficulties can be overcome using the transient grating (TG) technique [2]. The time window of the TG method is quite wide. We have also demonstrated that ΔV and ΔH can be separately measured without any assumption [2]. Here, we applied this TG method to studies of the structural and energy changes of Mb after photodissociation of carboxymyoglobin (MbCO) and the photocyclic reaction of PYP from 10 ns to milliseconds continuously.

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EXPERIMENTAL

The details of the TG and PA setup have been described previously [2]. For the Mb study, the second harmonic of a Nd:YAG laser (Spectra-Physics-CGR-170) was used for the photoexcitation. For the PYP study, the third harmonic of a Nd:YAG laser pumped dye laser (Lumomics, HyperDye 300; 465 nm) were used for the excitation. The pulse width is 10 ns. The laser light was split by a beam splitter and crossed inside a quartz sample cell (2-mm path length). The created interference pattern (transient grating) was probed by a diode laser (840 nm) or a He-Ne laser (633 nm) as a Bragg diffracted signal (TG signal). The TG signal was detected by a photomultiplier (Hamamatsu R928), averaged by a digital oscilloscope, and transferred to a computer for averaging and analysis.

RESULTS AND DISCUSSION

Myoglobin

Myoglobin has been used as a model system for experimental and theoretical studies of a kinetics-structural relation. A heme is embedded within the protein, and a small ligand such as carbon monoxide (CO) is reversibly bound to the sixth coordination site, on the distal side, of the heme. By the photoexcitation of the heme, the CO–metal bond is photodissociated with a quantum yield of almost unity, and the protein structure is forced to change. This photoreaction can be used, therefore, to trigger a perturbation to the protein [3].

Figure 1a depicts the TG signal after the photoexcitation of horse MbCO in buffer solution at 10 °C. The TG signal rises initially very fast within laser pulse width, and a decay-grow-decay (10 μs) feature was observed. The signal decays further to the baseline by the molecular diffusion process in a ms time scale. These rich features are sharply contrasting to the TA signal, which is almost constant in this time range (Fig. 1b) and decays to the baseline with 2–3 ms by the recombination reaction.

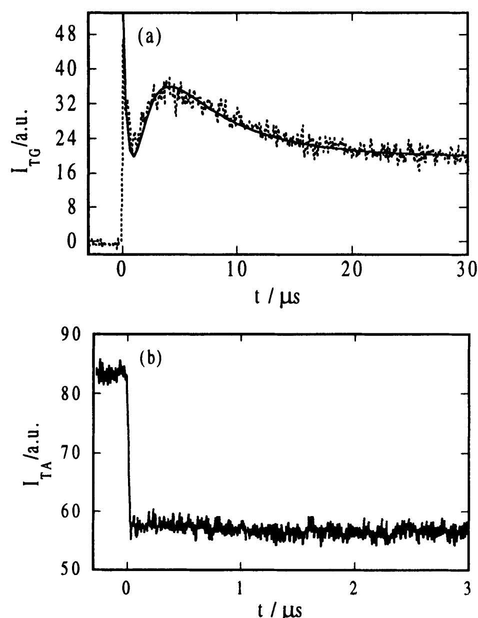

Fig. 1 (a) TG signal (dotted line) after the photoexcitation of horse MbCO at 10 °C and (b) TA signal under the same condition. The best fitted line of the TG signal using the δn_{th}, δn_{pop}, and δn_{vol} terms is shown by the solid line in (a).

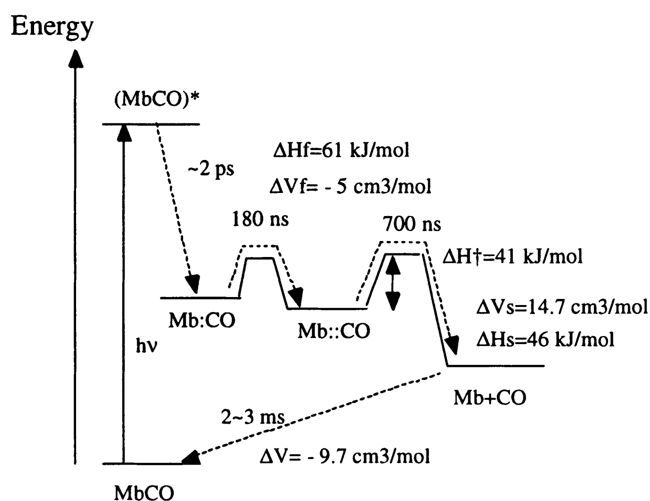

Fig. 2 Schematic representation of energy and volume changes of photodissociation reaction of MbCO.

The TG signal intensity (I_{TG}) is related to the refractive index change (δn) and the absorption change (δk) by the excitation. Under a weak diffraction limit, it is expressed by

$$I_{TG} = A(\delta n)^2 + B(\delta n)^2$$

where A and B are constants. There are several origins of δn; thermal effect (thermal gating; δn_{th}), absorption change (population grating; δn_{pop}), and volume change (volume grating; δn_{vol}). Considering a fact that the thermal grating signal decays with a rate of $D_{th}q^2$ (D_{th}; thermal diffusivity, q; grating wavenumber) [2], which is about 10 µs under this condition, we can attribute the 10 µs decay component of Fig. 1a to the thermal grating signal. From the intensity, we can determine the energy of the final dissociated state (Mb + CO).

In particular, the decay-rise component (Fig. 1a) is a new and interesting observation. This dynamic has never been observed by the TA detection. The origin of this component was attributed to the heating effect and the volume effect of Mb. We separated these components by a TG setup with a high wavenumber [4]. The principle of the separation was described elsewhere in detail [4].

From these measurements and analysis, the protein dynamics of Mb can be described as follows (Fig. 2). We found that initially, within ~10 ns after the photodissociation, a small volume contraction takes place (–5 cm^3/mol), and an energy of 61 kJ/mol is stored in the system. This energy is smaller than that expected from the Fe–CO bond energy. This fact indicates a rather large protein structural relaxation in this fast time scale. Subsequently, a structural change, volume expansion of 14.7 cm^3/mol, occurs with a lifetime of 700 ns at 20 °C. This indicates the presence of the second intermediate state, from where the ligand cannot return back to the heme, and the 700 ns process is attributed to the protein structural change induced by the escaping of CO from the protein to the outer solvent phase. We are now further investigating the origin of the volume change by using a site mutation technique.

Photoactive yellow protein (PYP)

The second example of the TG application is the dynamics of PYP. Interests in the photophysical and photochemical processes of PYP are rapidly increasing recently because of the structural simplicity and resemblance of the reaction to that of Rhodopsin [5,6]. Upon excitation of the chromophore, the ground-state PYP (pG) is converted into a red-shifted intermediate (pR) and subsequently another blue-

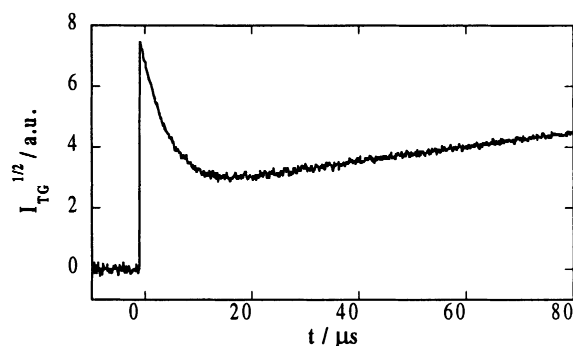

Fig. 3 The TG signal after photoexcitation of PYP at 20 °C.

shifted intermediate (pB), which returns to pG in a sub-s time scale. This cyclic reaction is triggered by the *trans*→*cis* isomerization of the chromophore (p-coumaric acid).

The thermal grating signal of the PYP solution after excitation at 465 nm rises within the excitation pulse and decays with $D_{th}q^2$ (Fig. 3). The intensity of this component represents the thermal energy released by the first photocycle step $pG^* \rightarrow pR$. The background signal beneath the thermal grating is the species grating signal, which consists of δn_{pop} and δn_{vol} [7]. Hence, this signal intensity represents the absorption spectrum change as well as the volume change during this process. Comparing the intensity of the thermal grating with that of a reference sample, we determine $\Phi\Delta H = 57 \pm 7$ kJ/mol. The quantum yield of the reaction was measured as $\Phi = 0.35$ [6] and hence, we determine ΔH to be 160 ± 20 kJ/mol. The enthalpy difference indicates that pR has a strained structure and stores a large energy in the protein part [8]. The volume contraction of $\Delta V = -7 \pm 2$ cm^3/mol is obtained at 20 °C.

When the temperature of the sample decreases, the thermal grating intensity decreases, because of the temperature dependence of the refractive index (dn/dT). More importantly, the background signal intensity, which is the sum of δn_{pop} and δn_{vol} also decreases with temperature. It is important to note that the temperature dependence of the TA signal intensity is minor in this temperature range. Hence, the temperature dependence of the species grating signal should come from that of the volume change. In other words, the volume change of PYP depends on the temperature.

It is surprising that the volume contraction becomes larger with the decrease of the temperature. The volume contraction around 0 °C becomes about twice as large as that at room temperature. We consider that the volume change may come from the change of the protein structure of PYP. Many biological systems such as PYP are not rigid, but there are many local free-energy minima (substates) along the reaction coordinate. These substates and associated thermal fluctuation of the protein structure are essential for the biological function. The observed temperature-dependent volume change may reflect the structural flexibility of the PYP protein structure.

A structural change that cannot be detected by the TA method was also observed for a mutant of PYP. At present, we think that the dynamics represents a movement of an amino acid that locates apart from the chromophore, and it will provide unique information on the structural change of PYP. The results and interpretation will be reported elsewhere.

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