Development and Application of a Femtosecond Time-Resolved Mid-Infrared Transient Absorption Spectrometer

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Synopsis

The design and performance of a femtosecond time-resolved mid-infrared transient absorption spectrometer is illustrated. The spectrometer was developed from a set of commercially available Ti: sapphire femtosecond lasers utilizing difference frequency mixing technique. With this spectrometer, the transient absorption in the mid-infrared region from 800 to 2800 cm\(^{-1}\) was detected with a sensitivity of \(1 \times 10^{-4}\) for difference absorbance. With a sub-picosecond visible or ultraviolet pump, a time-resolution of 250 fs has been obtained. A study on the binding and dissociation processes of carbon monoxide in myoglobin is presented as an example of its applications.

1. Introduction

In the past decades, lasers with ultrashort pulses have been widely used in chemistry, physics, biology, and the ultrafast time-resolved spectroscopy has become an important means in scientific research. However, relatively few works have been carried out in the mid-infrared (mid-IR) region, due partially to the technical difficulties in obtaining ultrashort IR laser pulses. Nevertheless, ultrafast time-resolved study in the mid-IR region (3–10 \(\mu\)m) is very necessary, since the characteristic absorptions of the functional groups of molecules in this region provide unique information of the reaction dynamics, which is difficult to obtain by UV-visible spectroscopy\(^{1,2}\). A typical example is given with biological samples. Some simple molecules such as carbon monoxide (CO) and nitric oxide (NO), which play important roles in biological and physiological processes, can be detected in the IR region, but not in the visible and near ultraviolet region. For these reasons, the ultrafast time-resolved IR (TRIR) spectroscopy is considered as an indispensable tool for scientific researchers and continuous efforts have been made to develop ultrashort laser pulses in the mid-IR region. In 1989 Hochstrasser and co-workers using an up-conversion of the CW IR probe by a sub-picosecond laser pulses achieved a time resolution of about 300 fs\(^{3}\). In 1991, Dyer and co-workers studied ligand transfer dynamics in cytochrome \(c\) oxidase using a visible-pump mid-IR probe spectrometer with a time resolution of 2 ps\(^{4}\). In 1994, Anfinrud et al. studied protein dynamics with a 35 ps visible-pump 0.2 ps mid-IR probe spectrometer\(^{5-7}\). These studies demonstrated the usefulness of these ultrafast mid-IR spectrometers for the studies of molecular, biological, and semiconductor systems. However, though these picosecond systems had an advantage of spectrally narrow propping or pumping, the time-resolutions of most of these systems were limited to picoseconds, which is not high enough for some ultrafast processes. The dye lasers used in these systems had drawbacks such as instability and difficulty in maintenance. The emergence of commercial femtosecond mode-locked Ti: sapphire (Ti:S) lasers provided laser sources with improved performance and speeded up the development of TRIR spectroscopy. With advances in the technology of nonlinear optical crystals, sub-picosecond IR laser pulses became facile\(^8\). Correspondingly, the time-resolution of TRIR spectroscopy advanced into sub-picosecond timescale.
Stimulated by the prolificacy of its visible counterpart and prompted by the pioneering works, the application of sub-picosecond mid-IR laser pulses becomes more and more attractive. In 1994, Hamm et al. illustrated the mid-infrared sub-picosecond transient absorption spectrometer with a time-resolution of 400 fs. In 1996, Akhremtcheyev et al. developed a mid-IR spectrometer based on all-solid-state laser with a time-resolution of 100 fs. Methods to improve the signal-to-noise ratio were proposed as well.

In this paper, we report a sub-picosecond visible-pump mid-IR probe spectrometer, developed in our laboratory utilizing a set of commercially available Ti:sapphire femtosecond lasers. Using this spectrometer, transient absorption in spectral region from 800 to 2800 cm$^{-1}$ is detected with reliable accuracy (absorbance change $<1 \times 10^{-4}$) and high time-resolution ($<250$ fs). As an application, a study of the dynamics of carbon monoxide ligand dissociation from myoglobin is demonstrated.

2. Apparatus

Figure 1 shows a schematic illustration of the whole setup of the spectrometer. A mode-locked Ti:sapphire oscillator (Tsunami 3960, Spectra-Physics) pumped by a CW diode laser (Millennia, Spectra-Physics) gave pulses of 9 nJ/pulse at 800 nm with a repetition rate of 80 MHz and a pulse width of 80 fs (FWHM). A regenerative amplifier (Spitfire, Spectra-Physics) pumped by an intra-cavity doubled Nd:YLF laser (Merlin, Spectra-Physics) amplified the laser energy to 0.9 mJ/pulse at a repetition rate of 1 kHz. The amplified pulse had a pulse width of about 120 fs. An OPA (OPA-800, Spectra-Physics) pumped by a fraction (about 0.42 mW) of the amplified pulses was used to produce signal and idler beams in the region of 1.14~1.6 μm and 1.98~1.6 μm, respectively. The total power of signal and idler beams reaches 70 mW. The signal and idler beams were mixed collinearly in a 2 mm thick type I AgGaS$_2$ crystal to generate IR pulse from 800 to 2800 cm$^{-1}$.

The generated mid-IR beam was then split into two beams by a 50% transmittance aluminum beam splitter on a CaF$_2$ substrate. One beam was used as a probe and focused to a rotating sample cell by a CaF$_2$ lens with a focus length of 15 cm. The other, as a reference, detoured the sample cell by another pathway. The second harmonic of the 800 nm was generated with a 1 mm thick β-barium borate (BBO) crystal and used as a pump beam. The pump beam, chopped by a chopper at 500 Hz, passed an optical delay line which changed the time difference between the pump and the IR probe pulses. In the path of the pump beam, a half-wave plate was inserted to rotate the polarization to any orientation with respect to the polarization of the IR probe beam. At last, the pump beam was focused to the sample cell by a lens with a focus length of 20 cm and overlapped the probe IR beam. The size of the focus of the IR beam on the sample cell was 100 μm while that of the pump beam was about 150 μm. The focus length is 15 cm for the probe and 20 cm for the pump beam.

The sample cell consisted of two pieces of 1 mm CaF$_2$ plates and a Teflon spacer in between. By using different spacers with thickness from 0.1 mm to 2 mm, the thickness of the sample cell varied to meet the desired absorbance. To avoid photodegradation, the sample cell was rotated at a speed of 3 cycles/second which kept providing fresh sample for every pulse.

The transmitted IR probe beam was then focused to the entrance slit of a monochromator together with the reference beam that was vertically shifted with respect to the probe beam. Finally the two beams were separated by a half-height mirror in the monochromator and sent out through two output slits. Two mercury cadmium telluride (MCT) detectors (KLD-2-J1-3/11, Kolmar Technologies), attached to the output slits, detected the IR beams. The detectors had active size of 2 × 2 mm and built-in amplifiers. The response of the detectors was sampled by two boxcar integrators (SR250, Stanford Research) whose output were digitized by a computer-controlled data acquisition system.

The chopper in the pump path worked at a frequency equal to the half repetition rate of the pump, so every other pump pulse was blocked. Thus, every two consecutive responses of the detectors consisted of one with pump ($I_{on}$) and one without pump ($I_{off}$). Then the absorbance change, $\Delta A$, induced by photoexcitation was obtained by calculating the difference between the two consecutive ratios of the probe response, $I_{pr}$, to the reference response, $I_{ref}$: $\Delta A = \log \left( \frac{I_{pr}}{I_{off}} \right) \cdot \frac{I_{ref}}{I_{ref}}$. In this way, the
noise coming from shot-to-shot fluctuation was mostly removed by using the reference and the effect of the long-term fluctuations in pulse energies was also minimized. In order to increase the S/N ratio, the alignment of the detectors and the configuration of the boxcar integrators were carefully adjusted. A sensitivity better than $1 \times 10^{-4}$ was achieved in an accumulation time of two seconds (a thousand pulses for each case, with and without pump).

The duration of mid-IR pulses is estimated to be about 200 fs. The cross-correlation time between the idler (1.97 μm) and the pump (400 nm) was about 165 fs (FWHM), suggesting that the time resolution of the instrument at the mid-IR region was better than 250 fs. To verify this time-resolution, the sample cell was replaced by a silicon wafer. It has been known that on visible photo-excitation, hot electrons are generated in the conducting band of silicon in less than 0.4 ps\(^2\). In Fig. 2, the result probed at 1960.6 cm\(^{-1}\) is illustrated. From deconvolution, taking a Gaussian with a FWHM of 250 fs as the instrument response function, a rising time of 0.3 ps was obtained, which is consistent with the reported time for photo-generation of electrons in silicon\(^{12}\). The duration of the mid-IR pulses is estimated to be about 200 fs. The bandwidth of the mid-IR pulses was about 150 cm\(^{-1}\), which produced a time-bandwidth product ($t_{\text{pulse}} A f_{\text{pulse}}$) of 0.9, about twice of the value for a Gaussian-shaped transform-limited pulse. In experiments, the width of the output slit of the monochromator was set at 0.3 mm, corresponding to a spectral resolution of 3.6 cm\(^{-1}\). Spectra were obtained by scanning the wavelength with the monochromator at fixed time delays.

Anisotropy measurement was made by rotating the polarization of the pump beam with a half-wave plate. Anisotropy decays were reconstructed from the decay curves measured with polarization parallel or perpendicular to the probe beam. To increase S/N ratio, about ten scans with alternating polarizations of the pump were measured.

### 3. Applications

The ultrafast time-resolved mid-IR spectrometer facilitates the studies on the vibrational and structural relaxation processes in molecular systems\(^{13,14}\), as well as the dynamics of carriers in semiconductors\(^{15,16}\). One significant application is the investigation of protein dynamics in biological systems. Here we present an application of our ultrafast time-resolved mid-IR spectrometer to a study of the carbon monoxide (CO) dissociation dynamics in myoglobin.

As a transporter and reservoir of small molecules such as O\(_2\), CO or NO, myoglobin repeatedly binds and releases these ligands\(^{17}\). The binding site of myoglobin is an iron-containing porphyrin group, so-called heme. Mechanistic studies would be suggestive for understanding the functional, structural and spectroscopic properties of other heme-containing proteins. Furthermore, the investigation on these biologically important processes is useful for revealing the general principles that regulate the proteins binding with the ligands, and further understanding how proteins discriminate toxicants from other molecules.

Though the X-ray analysis is a powerful method to reveal protein structure, no pathway has been found for small molecules to reach the iron atom from the outside of the heme protein pocket\(^{18,19}\). This indicates that structural changes must happen during the reaction to provide a pathway for the ligand\(^{20,21}\). To detect structural changes, the time-resolved IR spectroscopy is a useful method.

Myoglobin has been intensively studied for many years by different spectroscopic methods. Here we present the data of the CO recombination, particularly focusing on the determination of the attachment geometry of the bound CO. The experiment was performed on the horse myoglobin (Mb) purchased from Sigma in D\(_2\)O/glycerol solution (1:1, volume ratio). To prepare CO bound sample, Mb solution (~1 mM) was deaerated and reduced by an excessive amount of sodium dithionite. CO-bound Mb was prepared by introducing gaseous CO into the reduced sample. The sample cell (0.2 mm thick) was filled with the sample solution in a glove box under nitrogen atmosphere. The absorbance of the sample in the cell was ~0.6 at 400 nm. To avoid photodegradation the sample cell was spun and energy of the pump pulses was kept below 0.4 μJ/pulse, which corresponds to an excitation of less than 25% of the molecules. The steady-state absorption spectra of the sample before and after experiment were compared and no difference was observed. The temperature of the sample during experiment was kept at 5°C by a flow of cooled nitrogen gas.

![Fig. 2](image-url) Transient infrared absorption of silicon wafer probed at 1960 cm\(^{-1}\). The open squares are experimental data. The smooth curve is the fitting result of the instrument response function convoluted with a 0.3 ps exponential rise and two exponential decays.
In Fig. 3 the transient absorption spectrum of Mb-CO sample measured at 6.2 ps time delay after photoexcitation is presented. It shows a single peak at 1944 cm$^{-1}$ with a spectral width of 12.5 cm$^{-1}$, which clearly belongs to the bleach spectrum of the C-O stretching vibration of the bound CO molecules. No difference was observed in transient absorption spectra at longer delay times up to 600 ps.

In the upper panel of Fig. 4, the transient absorption decay curves at the peak wavelength of the bleach spectrum are shown for two polarizations, parallel and perpendicular. The appearance of the bleach spectrum is associated with CO dissociation. No dynamics is observed (Fig. 4), which is in agreement with the previous reports. It was shown that about 4% of dissociated CO recombine geminately with a time of $\approx 4$ ms. The rest of CO molecules escape from the heme pocket and recombine in bimolecular reaction with the rate of $17 \mu$M s$^{-1}$.

The geometry of the CO ligand attached to the heme can be obtained from the anisotropy value of the CO bleach signal (see Fig. 4 (lower panel)), $r(t)$, which is defined by

$$r(t) = \frac{\langle A_\parallel(t) - A_\perp(t) \rangle}{\langle A_\parallel(t) + 2A_\perp(t) \rangle},$$

where $A_\parallel$ and $A_\perp$ are the photo-induced bleaching measured under parallel and perpendicular excitation conditions. The transition moment for the absorption at 400 nm is directed in the porphyrin plane, while the transition moment of the CO vibrational transition is directed along the C-O line. The value of the initial anisotropy can be expected to be $-0.2$ for the perpendicular orientation of the two transition moments. The anisotropy value obtained for Mb-CO ($\lambda_{\text{max}} = 1944.5$ cm$^{-1}$) is $-0.184 \pm 0.003$. The correction for the finite bleaching of the Mb-CO gives the value of $-0.197 \pm 0.005$. As the heme has a pseudo $D_{2h}$ symmetry, it behaves as a circular or elliptical absorber to the excitation. In an approximation of circular absorber, the anisotropy value can be expressed by an equation,

$$r(\phi) = 0.1 (3 \cos^2 \phi - 2),$$

where $\phi$ is the angle between the porphyrin plane and the C-O line of CO molecule. From the corrected anisotropy value, by using the above equation, the angle $\phi$ was calculated to be $84 \pm 6$ degrees. Thus, it is shown that CO is attached almost perpendicular to the heme plane, with a deviation from the normal of about 6 degrees. A 6 degrees deviation can be considered as the lowest limit for this angle, as no distribution of the bound angles was considered. An alternative explanation of the anisotropy value larger than $-0.2$ could be made by considering a static or dynamic distribution of the binding angles (inhomogeneity). Considering the average bind angle to be zero, a Gaussian distribution with a width of 6.5 degrees will result in an anisotropy value of $-0.197$. However, no orientational relaxation of the bound CO was observed for Mb in mid-IR pump/mid-IR probe experiments, which suggests that the distribution of the bound angles is rather narrow, or that the motion of the bound CO is very restricted.

A deviation of $<10$ degrees was reported in the previous time-resolved IR experiment. There is no consistency in the X-ray structural data. Though a 19 degree deviation angle was obtained with a spatial resolution of 1.9 Å, recently the angle was measured to be 7.4 degrees with 1.15 Å resolution. Our result is in a good agreement with the latest X-ray analytical data.
4. Conclusions

In this paper, we reported a femtosecond time-resolved visible pump/mid-IR probe spectrometer. A sensitivity of absorbance change better than $1 \times 10^{-4}$ in 2 seconds of data accumulation was obtained. As an example of its application, a study on the CO dissociation and rebinding processes with myoglobin was demonstrated. By the anisotropy measurement, the bound angle of the CO in Mb-CO protein was determined to be less than 6 degrees from the normal to the heme plane.

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References