# Investigation of Chlorophyll Protein 43 and 47 Denaturation by Terahertz Time-Domain Spectroscopy *

CHEN Hua(陈华)[1,2], QU Yuan-Gang(曲元刚)[3], PENG Wei-Xian(彭慰先)[1], KUANG Ting-Yun(匡廷云)[3], LI Liang-Bi(李良壁)[3], WANG Li(汪力)[2**]

[1] *College of Physics, Jilin University, Changchun 130023*

[2] *Beijing National Laboratory for Condensed Matter Physics, Institute of Physics, Chinese Academy of Sciences, PO Box 603, Beijing 100080*

[3] *Institute of Botany, Chinese Academy of Sciences, Beijing 100080*

(Received 31 October 2006)

*We demonstrate the applications of terahertz time-domain spectroscopy to distinguish conformation changes of the chlorophyll proteins CP43 and CP47 induced by the treatment of guanidine hydrochloride, light irradiation and heating. It is indicated that THz transmission spectroscopy can be used for monitoring protein denaturation and associated conformation change processes in a feasible and effective way.*

*PACS: 87.15.He, 42.25.Bs, 87.14.Ee*

The use of terahertz time-domain spectroscopy (THz-TDS) for studies of conformational flexibility and conformational change in biomolecules has attracted great interest in recent years.[1−3] Protein structure dynamics is a fundamental process for biological systems to function properly in nature, such as in enzymatic reaction, sensory transduction and photosynthetic processes. It is now known that for a given molecular configuration and conformation, there are some unique collective vibrational modes associated with large scale structural motions in the far infrared or THz range.[1] When the collective vibrational modes coexist with electric charge oscillations, the THz absorption spectroscopy can be used to study the processes. The THz-TDS technique has been successfully performed to identify different molecular species, molecular structure change by mutation, and different conformations of a given biomolecule.[2,3] It is well known that the secondary structure and/or the tertiary structure of protein is influenced by the denaturation by different mechanisms, which results in changes of the low-frequency vibrational modes involving rigidly bound subgroups which are connected by the weaker bonded or non-bonded interactions. THz-TDS offers direct insight into the configurational and conformational dynamics of proteins under investigation. In this Letter, we show the capability of the THz-TDS technique in sensing protein denaturation of CP43 and CP47 caused by interaction with GuHCL, light irradiation and temperature rising, and prove the applicability of THz-TDS in distinguishing different confirmation and conformation states of a given protein.

The chlorophyll binding proteins CP43 and CP47, also called the internal antennae, are important components in photosystem Ⅱ (PSⅡ), a photosynthetic pigment-protein complex. They are encoded by the psbC and psbB genes, respectively, in the chloroplast genome of higher plants, green alga and in the genomic DNA of cyanobacteria.[4,5] Both CP43 and CP47 have six transmembrane $\alpha$-helices, which are separated by five extrinsic loop domains.[5] The large loop domain of CP43 contains two long and three short helices. In the case of CP47, the lumenal domain contains two long and four short helices and three $\beta$ sheets.[6] The more detailed structure characteristics of the extramembrane domains of CP43 and CP47 still remains to be studied. The protein CP43 and CP47 contain both chlorophyll *a* (Chl *a*) and $\beta$-Carotene ($\beta$-Car), but no chlorophyll *b* (Chl *b*).[4,5] The newest revealed PSⅡ crystal structure shows that there are 14 Chl *a* and 4 $\beta$-Car molecules in CP43 and 16 Chl *a* and 2 $\beta$-Car molecules in CP47.[6] Both CP43 and CP47 can accept excitation energy that is harvested by the light-harvesting complex Ⅱ (LHC Ⅱ) and then transfer it directly to the PSⅡ reaction center (RC).[4] In our experiment, the CP43 and CP47 were abstracted from spinach leaves using the method described by Kuwabara and Murata.[7] The purified CP43 or CP47 was suspended in 20 mM Bis-Tris and 0.05% DM (pH 6.0). The final concentration of CP43 and CP47 in the buffer liquid was 3 $\mu$g Chl *a* per ml. The denatured samples were obtained after CP43 and CP47 were incubated in the buffer with various concentrations of GuHCl in the dark at room temperature for 6 h. Before the THz-TDS measurement, the protein colloidal solutions were first mixed with polyethylene (PE) powder, which is nearly transparent in the THz waveband and is a suitable filling material for spectroscopic applications in this spectral region. For each

* Supported by the National Natural Science Foundation of China under Grant No 10390160.

** To whom correspondence should be addressed. Email: wangli@aphy.iphy.ac.cn

©2007 Chinese Physical Society and IOP Publishing Ltd

measurement, 50 $\mu$l protein colloidal solution was put into 880 mg PE powder. The mixture was carefully stirred till uniformly mixed and then was held in a sample cell with high density polyethylene windows. The sample filled the inner space of the cell with 10 mm diameter and 0.5 mm thickness. An electric blower running at room temperature was used to vaporize the moisture in the mixture. All the THz-TDS measurements were conducted in a vacuum chamber to avoid the interference of water vapour and to ensure the sample was in dry condition.

A standard THz-TDS configuration was used in our study.[8,9] In brief, the THz radiation is generated from a piece of $\langle 100 \rangle$-oriented InAs wafer, which was excited by optical pulses from a mode-locked Ti-sapphire laser ($\lambda = 800$ nm, $\tau_{\mathrm{FWHM}} = 100$ fs). The repetition rate of the laser is 82 MHz. The THz radiation from the InAs is collimated and focused onto the sample by means of off-axis parabolic mirrors. Using another pair of parabolic mirrors, the transmitted THz pulses through the sample were collected, collimated and focused onto the sensor, a piece of electric-optic (EO) crystal ZnTe. The probe beam, derived from the same optical source by a beam splitter is employed to gate the THz temporal waveform. As the THz pulses change the probe beam's polarization via the EO effect in ZnTe wafer, the two cross-polarized components in the probe beam will change their intensity ratio and then reach two photodiodes, respectively. The differential current proportional to the amplitude of the THz electric field from the diodes is recorded by a lock-in amplifier. The acquired sample and reference waveforms were converted to their Fourier transforms $E_s(\omega)$ and $E_r(\omega)$, respectively, and the transmission spectra were obtained as the ratio of the their Fourier spectra, $E_s(\omega)/E_r(\omega)$. The complex system response simply gets rid of this way. Different from the traditional FTIR measurement, probable multi-reflection pulses can be well resolved temporally in THz-TDS measurement and therefore, the spectral interference resulted from the etalon effect could be easily ruled out by truncation of the reflected pulses.

Figure 1 shows the Fourier transform spectra of the THz temporal waveforms after passing the GuHCl-denatured CP43 and CP47 as well as the intact ones. The data were normalized to the peak values of the reference spectra. It is clear that the denaturation by GuHCl treatment causes distinct spectral responses of CP43 and CP47 in THz region, which could be attributed to protein conformation changes. The absorptance of samples can be expressed as

$$E_s(\omega)/E_r(\omega) = T(n)\exp(-\alpha d + in\omega d/c),$$

where $T(\omega)$ is a factor related to the reflection at the sample/PE window interfaces, $d$ is the sample thickness, and $c$ is the light velocity in vacuum. The absorptions $\alpha d$ of the samples are plotted in Fig. 2. The GuHCl induced denaturation shows quite different effects on CP 43 and CP47. In comparison to CP43, denatured CP47 shows an additional featured absorption peaked around 1.6 THz, demonstrating that the tertiary structure of CP47 is more sensitive to the GuHCl treatment. The reason may be due to the fact that there are more lipids in CP43 than in CP47 while GuHCl interacts mainly with polar groups in proteins by hydrogen bonds.[7]

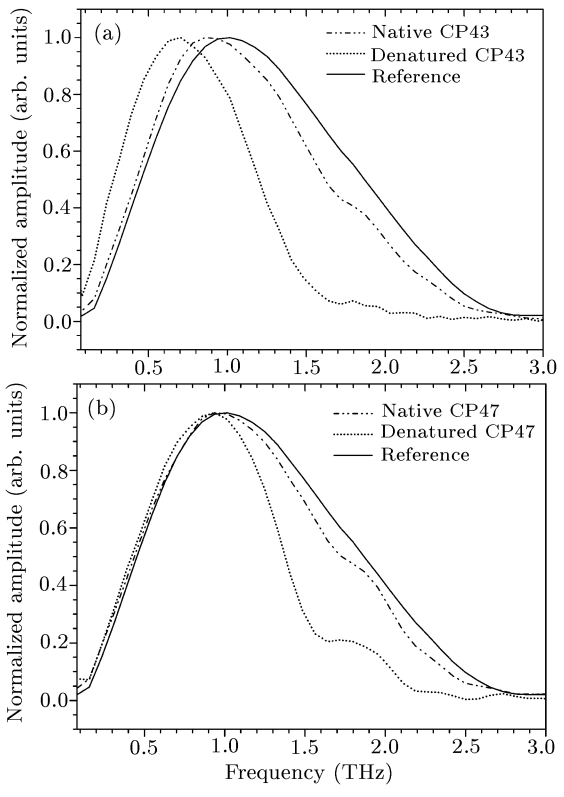


**Fig. 1.** Fourier transform spectra of the THz temporal waveforms from (a) CP43 and (b) CP47 with and without the treatment by GuHCl.

In the study on the irradiation effects, a halogen lamp was used to illuminate the samples through an infrared band-reject filter preventing temperature rise from heating, and the light intensity (PAR)[10] at the surface of the sample was about 1000 $\mu$mol m$^{-2}$s$^{-1}$. The samples were exposed in light for different times and then took the THz-TDS measurement for each case. The light intensity was kept to be constant in all measurements. The Fourier transform spectra of the THz temporal waveforms passing CP43 and CP47 after the irradiation are shown in Fig. 3. The responses to the irradiation of the CP43 and CP47 samples are quite diverse. For CP43, the transmission tends to increase with the light exposure time at beginning. It reaches a saturation level after the exposure lasts for 10 min and longer. The spectral response of CP47 shows a totally different behaviour. After an initial

red-shift, the transmission decreases and is saturated finally at a lower level. It has been reported that the irradiation on CP43 or CP47 can induce a transient conformational change of the membrane chromophoric site and gives rise to the spectral intermediates of protein.[11] These changes will subsequently affect the extradiscal surface of CP43 or CP47, which triggers the further conformation change of the proteins. The both CP43 and CP47, containing about 15 chlorophyll and 2-4 $\beta$-carotenes, have six transmembrane helical domains with the C- and N-terminal end exposed at the stromal surface. When illuminated by light, the pigments $\beta$-Car and Chl $a$ are excited and will start an energy transfer process. The probably different transferring efficiency in CP43 and CP47 could make the light induced conformation dissimilar. The frequency dependent absorptions of the samples after irradiation for 5 min are also shown in Fig. 2.

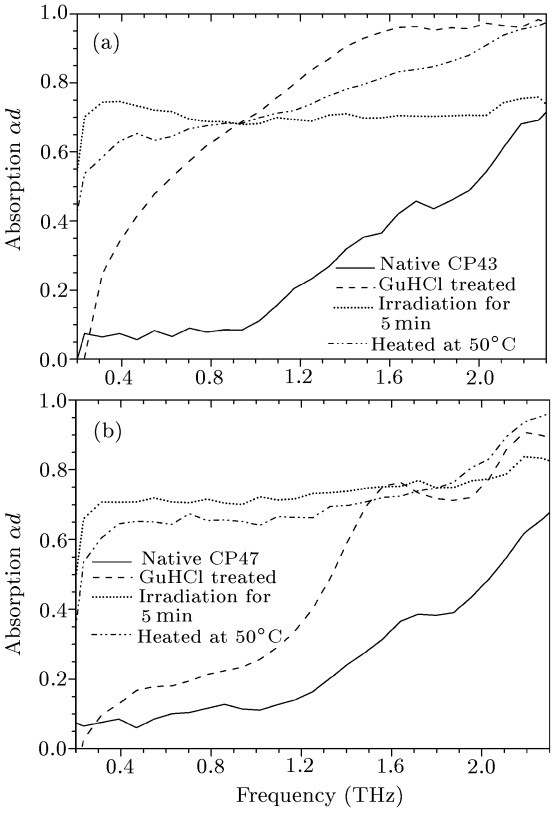


**Fig. 2**. THz absorption spectra of CP43 and CP47 under different conditions.

Heat denaturation induced conformation changes of CP43 and CP47 was also observed in THz spectra at 50 and 70°C. The samples were prepared at room temperature, and heat treatment was performed by placing the protein samples in a water bath at preset temperatures for 5 min. Figure 4 displays the Fourier transformed spectra of the waveforms taken at 50 and 70°C, where the data are normalized to the

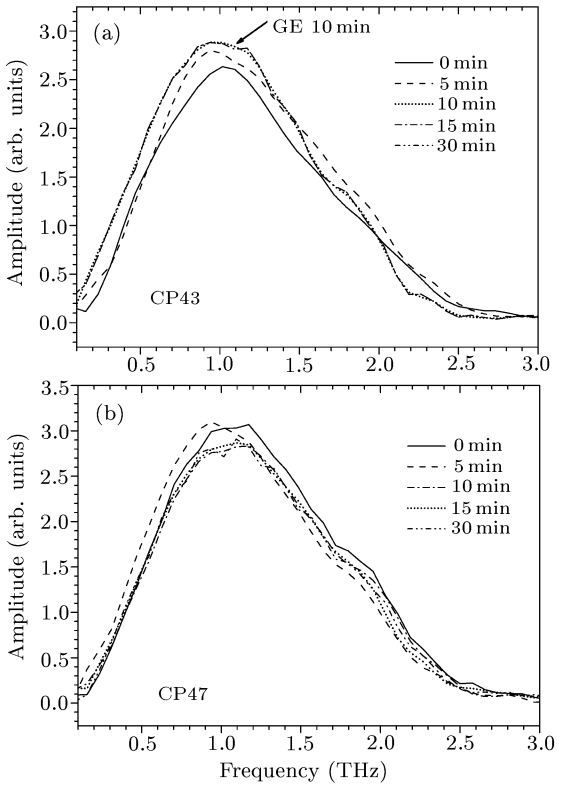


**Fig. 3**. Fourier transform spectra of the THz temporal waveforms from CP43 and CP47 prior to and after light exposure for 5, 10, 15, and 30 min.

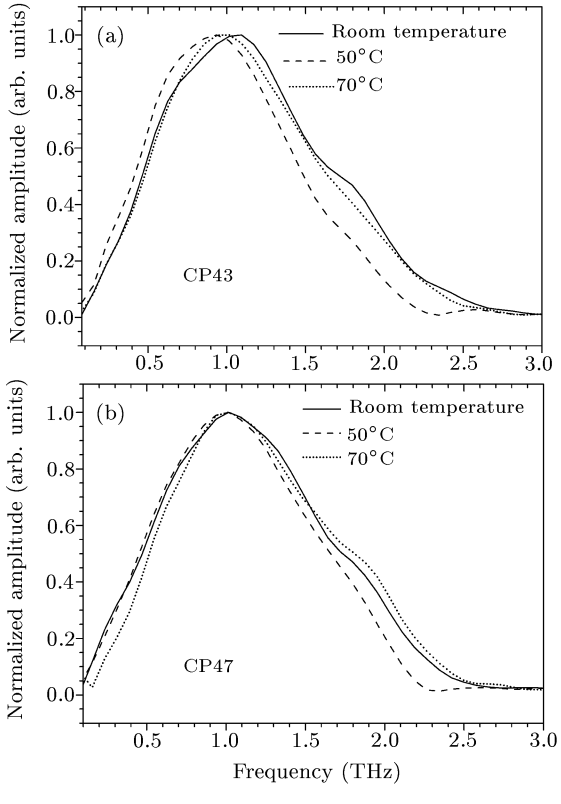


**Fig. 4.** Fourier transform spectra of the THz temporal waveforms from CP43 and CP47 at room temperatures 50°C and 70°C.

peak value of data at room temperature. The spectrum from the CP43 shifts to low frequency in 50°C, and resumes the profile at room temperature more or less when temperature reaches 70°C. On the contrary, the spectrum from the CP47 at 50°C has a moderate red-shift, and moves to higher frequency at 70°C. Figure 2 shows the absorption spectra of the two proteins at 50°C. Research on the heat denaturation process in the photosynthetic system has revealed the changes in the structural organization of proteins and cofactors in electron transfer.[12,13] The secondary structural reorganization induced the conformation state changes of the CP43 and CP47 are well detected here by the THz-TDS technique.

In summary, it have been demonstrated that the THz spectroscopy may be used to examine protein denaturation induced by interaction with reactive chemicals, light irradiation and temperature rise. The results reported here show that under suitable conditions, the sensitivity and reproducibility of the currently available THz-TDS technique is feasible in discrimination of the conformation changes in the secondary and tertiary structures of proteins, structure-sensitive energy and electron transfer in a bio-system, and other molecule reactions.

# References


[1] Markelz A G, Whitmire S, Hillebrecht J and Birge R 2002 *Phys. Med. Biol.* **47** 3797

[2] Whitmire S E, Wolpert D, Markelz A G, Hillebrecht J R, Galan J and Birge R R 2003 *Biophys. J.* **85** 1269

[3] Globus T R, Woodlard D L, Samules A C, Gelmount B L, Hesler J, Crowe T W and Bykhovskaia M 2002 *J. Appl. Phys.* **91** 6105

[4] Bricker T M 1990 *Photosynth. Res.* **24** 1

[5] Bricker T M and Frankel L K 2002 *Photosynth. Res.* **72** 131

[6] Ferreira K N, Iverson T M, Maghlaoui K, Barber J and Iwata S 2004 *Science* **303** 1831

[7] Kuwabara T and Murata N 1982 *Plant. Cell. Physicol.* **23** 533

[8] Yang Y P, Xu X L, Yan W and Wang L 2005 *Chin. Phys. Lett.* **8** 2123

[9] Yang Y P, Yan W, Xu X L, Shi Y L and Wang L 2005 *Chin. Phys. Lett.* **22** 1401

[10] Velitchkova M and Popova A 2005 *Bioelectrochem.* **67** 81

[11] Pellicone C, Nullans G and Virmaux N 1985 *Febs. Lett.* **181** 179

[12] Amane Y, Ashino Y and Koike H 1988 *Photosynth. Res.* **57** 51

[13] Thompson L K, Sturrevent J M and Brudvig G W 1986 *Biochemistry* **25** 6161