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LETTER

Application of terahertz time-domain spectroscopy in intracellular metabolite detection

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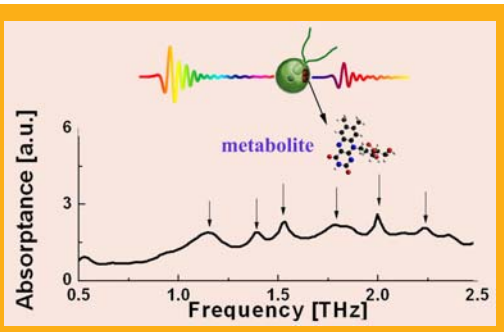
Using terahertz time-domain spectroscopy (THz-TDS), we have investigated the THz spectra of astaxanthin and riboflavin and the spectra of two kinds of cell, haenatcoccus plusivalis and bacillus subtilis, which could produce astaxanthin and riboflavin, respectively, during their metabolite process. Riboflavin was found to be much more absorptive to THz radiation and have richer spectral characteristics than astaxanthin. As an intracellular metabolite, riboflavin could be distinguished from the cells by using THz-TDS. The technique has potential applications in high-throughput screening of industrial strains.



THz rays can penetrate into the cell and distinguish some metabolites inside by the spectrum changing, which could serve as a potential tool in high-throughput strain screening

1. Introduction

Terahertz (THz) radiation, ranging from 0.3–10 THz, was traditionally termed the submillimeter-wave band. The basic electromagnetic properties make this kind of radiation a valuable tool in biological and life science [1–6]. First, the photon energy of THz radiation is very low (~1–40 meV) and therefore damage to issues or cells is nonionizing and limited to thermal effects. Secondly, energies of about 10^{-21} J are consistent with discrete molecular vibrational, torsional and librational modes in liquids and solids [7–10]. There have been many reports on the applications of THz technology in the biological and chemical fields, such as protein-structure recognition [11], label-free DNA sequencing [12] and cell-change sensing [13]. THz spectroscopy provides a new way for sensing metabolite in the cells, which can be used in high-throughput strain screening, one of the key problems in metabolic engineering [14].

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In this letter, we report results obtained from THz time-domain spectroscopy (THz-TDS) investigation of two different intracellular metabolites, riboflavin and astaxanthin, in the 0.5–2.5 THz region. In the experiment, riboflavin showed very strong absorption of THz rays with clear discrete absorption lines, and was able to be distinguished from the cells. The technique could be used to increase the throughput in high-yielding strain screening.

2. Experimental

Riboflavin, also known as vitamin B_2, is one of the most widely used medicines to keep human beings and animals healthy. It plays a key role in energy metabolism, and in metabolism of fat, carbohydrates, and proteins. Astaxanthin, a red carotenoid pigment, is a powerful biological antioxidant. It has been shown that astaxanthin has many potential applications in human health, the aging process and cancer curing. For comparison, pure astananthin and riboflavin and cells that can produce astananthin and riboflavin inside, respectively, during the metabolite process are both prepared. The haenatcoccus plusivalis cells are compared with riboflavin, and bacillus subtilis with astaxanthin. The pure astananthin and riboflavin are both acquired from Sigma Co Ltd. The haenatcoccus plusivalis cells are cultured for two different periods, six days and twelve days, respectively, with different metabolite amounts. The bacillus subtilis cells were harvested from two different strains that have been gene-engineered and cultured for eighteen hours. Within these two strains, one is high yielding while the other is not.

All the samples were first dried and then mixed with polyethylene powder, which was proven to be transparent in the terahertz range at a mass ratio of 1 : 1, and finally pressed to a thin circular tablet applying a pressure of about 5 tons. The diameter of the tablet is 12 mm and the thickness is 1 mm.

A standard 8-f THz-TDS system [15] was used in the measurement. The samples were placed at the focus of the THz beam. The THz transmission spectrum modified by the samples was measured. The system was placed in a chamber filled with N_2 to avoid the interference of water vapor in the air. The humidity in the chamber was about 4%.

3. Results and discussion

Figure 1a shows the time-domain waveforms of the THz electric field. The reference signal (black trace) is the measured THz signal without the sample. The transmitted signal of axtanxathin is shown as the red line. The measured signal with the sample is delayed because of the difference between the index of the reference tablet and the sample tablet. Figure 1b shows the transmission spectra of the measured signals. The time-domain waveforms and spectra for riboflavin are shown in Figure 2. From these curves, it can be observed that the waveforms, as well as the spectra, are transformed by the samples not only in amplitude but also in shape, and thus the absorption information of the substance can be extracted.

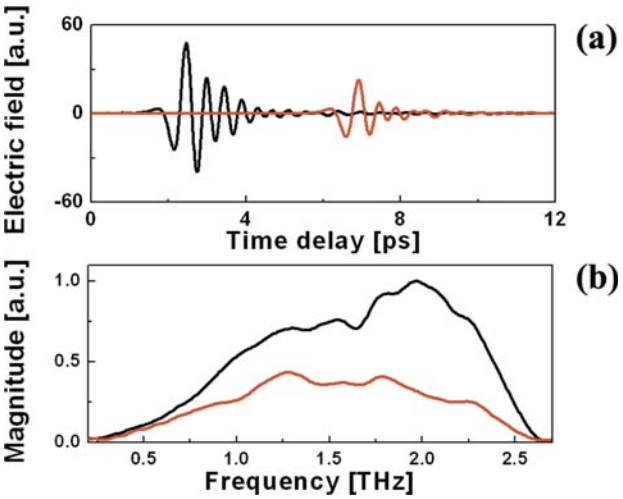

Figure 1 (online color at: www.biophotonics-journal.org) Time-domain waveforms of astaxanthin and its corresponding spectrum. (**a**) Time-domain signal of reference (black trace) and the astaxanthin sample (red trace). (**b**) Spectra of the signals.

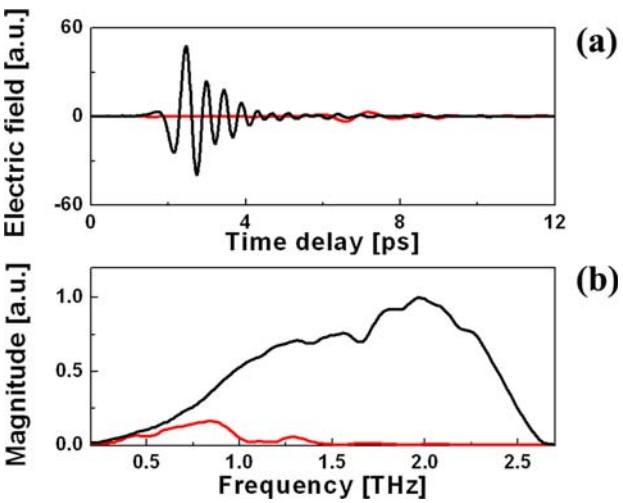

Figure 2 (online color at: www.biophotonics-journal.org) Time-domain waveforms of riboflavin and its corresponding spectrum. (**a**) Time-domain signals of reference (black trace) and the astaxanthin sample (red trace). (**b**) Spectra of the signals.

By ignoring the reflectance of the sample, the absorbance can be expressed approximately as followed [6]:

$$\alpha = -\log\left(I_s/I_r\right)$$

where I_r is the intensity of the reference signal and I_s that of the sample signal.

Using this formula, we calculated the absorbance spectra of riboflavin and anstanxathin from the data shown in Figures 1 and 2. The results are plotted in Figures 3a and 4a, respectively. The absorption peaks are marked with black arrows. It can be easily seen that the absorbance spectra for these two samples are totally different: the absorptance of riboflavin is much higher than that of anstanxathin, and the discrete absorption lines of riboflavin are more noticeable and narrower, while those of astaxanthin are flat and wide. The absorption peaks are considered to be due to the hydrogen bonds of the molecules, which are very significant in the crystallization process. The THz resonant modes, corresponding to out-of-plane vibrations or torsions, are regarded as collective modes involving a very large number of atoms, and these modes are very sensitive to the hydrogen bonds of the molecules [16–19]. The hydrogen bonds in riboflavin molecules are more complicated than those in astaxanthin molecules, which lead to the stronger and more complicated THz responses in riboflavin. For metabolite detection, using THz-TDS, the discrete strong absorption lines will be very useful in characterizing the substance and very helpful in strain screening.

Figure 3b and c show the absorbance spectra of haenatcoccus pluvialis cells cultured for different time, while Figure 4b and c are for bacillus subtilis cells gene-engineered in different ways. Figure 3b is the absorbtance of haenatcoccus pluvialis strains cultured for six days with very little axtaxanthin inside. There is not enough spectra information in Figure 3a and c to distinguish the metabolite, astaxanthin, from the absorbance of the haenatcoccus pluvial cells. This is because the absorption of metabolite is too small compared with that of haenatcoccus cells and the absorption information of as-

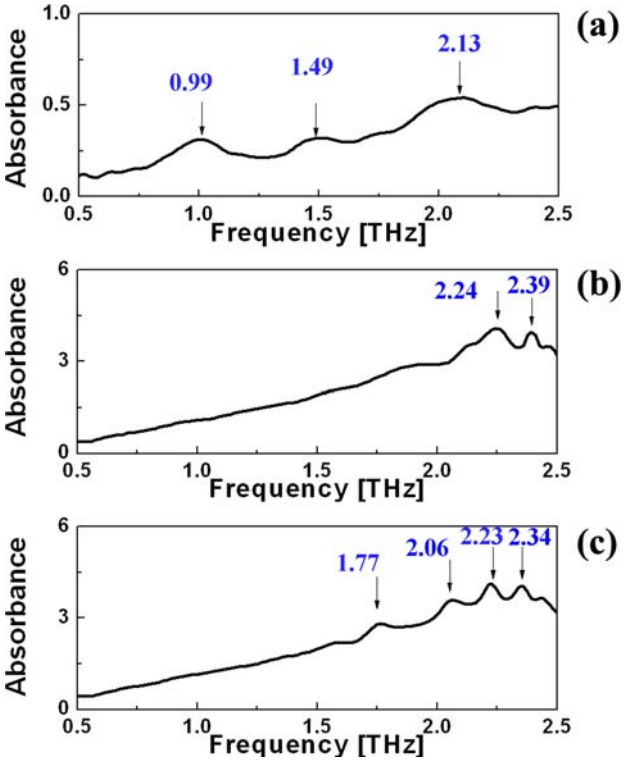

Figure 3 (online color at: www.biophotonics-journal.org) The THz absorbance of the astaxanthin tablet and that of haenatcoccus pluvialis cultured for alternative periods of time. (**a**) The absorbance of astaxanthin; (**b**) The absorbance of haenatcoccus pluvialis strain cultured for 6 days with very little astaxanthin inside the cells. (**c**) The absorbance of haenatcoccus plusivalis cells cultured for 12 days with a high content of astaxanthin.

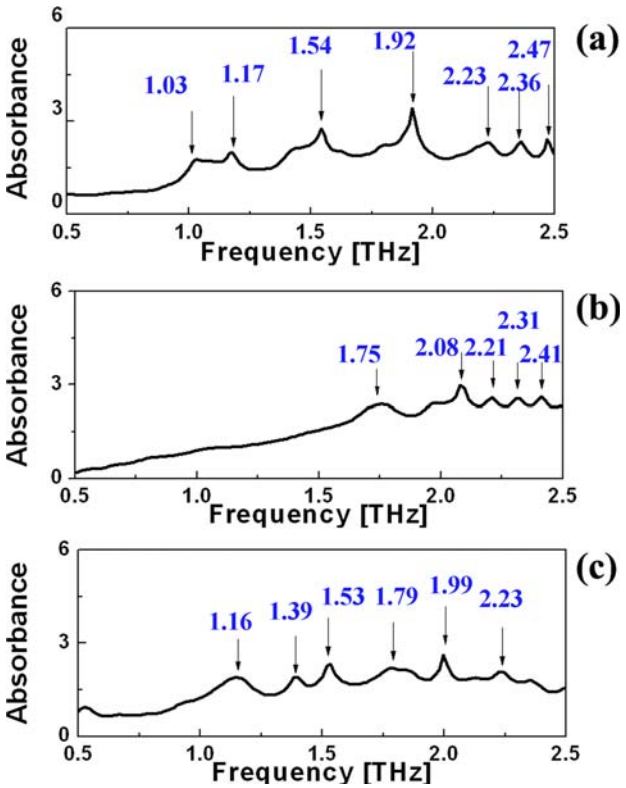

Figure 4 (online color at: www.biophotonics-journal.org) The THz absorbance of riboflavin tablet and that of bacillus subtilis cultured for alternative periods of time. (**a**) The absorbance of riboflavin; (**b**) The absorbance of bacillus subtilis strains with very poor riboflavin productivity; (**c**) The absorbance of bacillus subtilis strains with high riboflavin productivity.

taxanthin is covered up by the absorption of the cell itself. Besides, it can be seen that the line shapes in Figure 3b and c, corresponding to differently cultured haenatcoccus pluvialis cells, are similar, except with some tiny bulges that may have resulted from the cells' unavoidable change during their growth. This indicates that the absorption in Figure 3c is mainly resulted from the biological components of the cells, e.g. the organelles, not the metabolite.

On the other hand, riboflavin is very absorptive to THz radiation and makes it possible for the absorption information of riboflavin to be distinguished from the absorbance spectra of bacillus subtilis cells. A comparison of Figure 4b and c shows that the line shape has changed significantly. The difference between these two strains is mainly the amount of the metabolite, riboflavin. Therefore, the additional absorption peaks are mostly due to the absorption of riboflavin. Although the absorption peaks are not the same as riboflavin because of the hybridization with the absorbance spectrum of the cells, we could also determine the corresponding relation between the metabolite and the pure riboflavin by analyzing the line shape of Figure 4c. In Figure 4c, the absorption peaks are at 1.16, 1.39, 1.53, 1.79, 1.99 and 2.23 THz. By comparing Figure 4a and c, we can find the following: The peak at 1.16 THz is very wide, which corresponds to the 1.02 THz and 1.17 THz peaks of riboflavin; The 1.53-THz peak corresponds to 1.54 THz; The 1.99 THz peak comes from the hybridization of 1.92 THz of riboflavin and 2.08 THz of the cells; and the 2.23 THz peak is the same as for riboflavin. However, the high-frequency modes cannot be distinguished because the spectrum is very flat under the absorption mixing of the metabolite and the cells. The 1.79 THz line in Figure 4c is the absorbance of the cell and can be found in Figure 4b as well. Although there still exist some unexplainable lines in Figure 4c, like the one at 1.39 THz, it is enough to confirm the existence of the metabolite, i.e. riboflavin, in the cells.

Generally, it is very difficult to quantitatively analyze the intracellular metabolite using THz-TDS because there are too many things to be taken into account and the absorbance spectrum usually exhibits high hybridity. However, under some special conditions, the case is different. For bacillus subtilis cells, on the one hand, the metabolite, riboflavin is very absorptive in the THz band and there are many strong absorption peaks corresponding to different vibrational modes; on the other hand, the absorption of the cells is relatively small and very flat over the range of 0.2–2.5 THz. This results in a high contrast ratio in the spectra for the intracellular metabolite and the cells. This is why riboflavin could be distinguished from the cells while anstanxathin could not.

4. Conclusion

In this letter, we studied two metabolites, riboflavin and anstanxathin, and succeeded in distinguishing riboflavin from the cells. Though the spectral result is not perfect, it still makes the intracellular metabolite distinguishable to a certain extent. By comparing the absorbance spectra of pure riboflavin, cells with little metabolite and cells with high content of metabolite, the use of THz spectral technology to detect the intracellular metabolite is proved to be feasible. This technology enables label-free investigation of cells, providing information unavailable by other conventional methods. This could have great potential to remove one of the key bottlenecks in high-throughput screening of high-producing change strains to cells.

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