Invited Paper

Study of gene transcription and bio-molecular information by terahertz waves

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Abstract: Terahertz (THz) technologies have shown great potentials for biomedical applications such as THz radiation effects on cells and spectroscopy of water studied in terahertz range. Water's strong absorption to THz wave restricts the acquisition of high frequency information, promoting the development of THz attenuated total reflection system (THz-ATR). Besides, the advances in terahertz applications have stimulated renewed interest regarding the THz bio-safety, which results in development related to biological effects studies of terahertz radiation.

Keywords: Terahertz spectroscopy, Protein, Hydrating water

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1. Introduction

As a booming technology, terahertz (THz) method is drawing more attention in diverse aspects. The THz wave lies between infrared waves and microwaves, with characteristic frequency of 0.1-10 *THz*. Owing to its high time-resolved feature and high signal-to-noise ratio, THz spectrum provides a new insight into the study of structural and conformational changes within biomolecules [1]. Its low energy photon and polarity sensitivity provide new opportunities to more comprehensively understanding on how biological macromolecules function and play their critical roles in sustaining life [2]. Along with the development of recent decades, terahertz time-domain spectroscopy (THz-TDS) has already been widely applied in biological field, such as pharmaceutical measurement [3, 4] cancer diagnosis [5, 6] and medical imaging [7-9]. It has been shown that THz spectroscopy is particularly adept at characterizing the features of biomolecules in solid state [10, 11], including chiral amino acids and racemic compounds [12, 13]. Apart from them, THz spectroscopy has been applied to study α-helical polypeptides, providing intermolecular information on protein structure and dynamics [14]. Recent work has shown that THz is a promising way in future research into insulin fibrillation and the detection of amyloid aggregation [15]. Some applications, like the body scanner which is employed at airports, and THz imaging used in diagnostic methods will result in exposure of general public to THz radiation, which has prompted an increased interest in biological effects with THz wave. Although many researches have proven that THz radiation show little genotoxicity to cells, THz electromagnetic fields may influence gene expression and DNA replication. In this review, we summarize the study of protein and hydrating water by THz spectroscopy, the bottleneck in the measurement of aqueous samples and the approach to overcome it, and the researches performed to investigate the possible biological effects.

2. Terahertz spectroscopy of protein and hydrating water

Terahertz studies of macromolecules can date back to the 1990s, including amino acid, proteins and DNAs. Low frequency vibrational modes were found related to the conformational information about biomolecules [16]. Under physiological conditions, proteins perform the function under the participations of surrounding water molecules. Generally, the surrounding water molecules around proteins is divided into three categories: (1) the bulk water molecules surrounding proteins at a distance out of van der Waals contact, (2) hydrating water molecules at the surface of proteins with direct interactions, (3) water molecules that individually hydrogen-bounded to polar proteins in cavities inside biomolecules [17]. Hydrating water is the major form of protein-bound water that contributes to many molecular properties such as protein folding, solubility, drug docking, and oligomer formation. The interactions of proteins with water are important to their structural, dynamical and functional properties [18], which are believed to be responsible for peculiar bio-protective mechanism such as governing protein glass transition [19]. Thus, a three-component model is widely used to describe the absorption features of protein solutions [20].

Although some spectroscopic methods can provide insight into single molecule dynamics, THz spectroscopy allows the detection of changes in the fast collective coupled protein-hydrogen bond network dynamics. With such experimental method, the correlation between protein function and solvent dynamics can be revealed. Two key concepts are used to describe the THz experimental results of biomolecules. One is the ‘terahertz defect’, which means the decreasing trend of THz absorption coefficient with increasing protein concentration [21]. This is because protein absorbs much less THz wave than water. The other is ‘terahertz excess’, which shows that the hydration shell forms in the protein–water mixture, causing an increase in the absorption coefficient, and resulting in a nonlinear behavior. For example, Born et al. [22] explored the hydration shell in ubiquitin solutions with terahertz pulsed spectroscopy. A hydration shell with the thickness of at least 18Å was discovered which exceeded the static hydration layer typically observed with scattering measurements. In the case of antifreeze proteins, the alteration of the water network dynamics caused by extended dynamical hydration shell has been found [23, 24].

3. Water's strong absorption to THz and THz system geometry

There are two challenges restricting the terahertz (THz) transmission intensity and dynamic spectral range of THz-TDS in study of aqueous samples [25-27]. One is the performance of THz-TDS system, including the output power of THz pulse, the dynamic spectral range of reference and the signal to noise ratio (SNR). The other is the strong background absorption of water solvent. As a highly structured polar solvent, water has broad and intensive absorption in THz region, thus intrinsic THz features and discrepancy of aqueous samples are buried deeply in water background, making the spectral THz-TDS extremely difficult to understand.

As an alternative, the THz-TDS system in reflection geometry has been utilized to investigate aqueous samples, avoiding high power loss during the penetration of water layer [28]. However, a restrict requirement should be met to minimize the phase change: the surface of reflection mirror is placed on the same horizontal plane as the samples. An appropriate technique to circumvent this problem is THz attenuated total reflection spectroscopy (THz-ATR) [29]. Using this approach, an evanescent wave interacts with the strongly absorbing sample [30]. This principle allows for spectroscopic measurements of highly absorbing substances in the THz frequency range [31-33]. Naito et al. [34] built a THz-ATR system to measure the solution of P-NIPAAm. The results are in good consistence with those obtained from THz-TDS in transmission geometry. Ogawa et al. [35] applied the THz-ATR to investigate the hydration state of an intact HeLa cell monolayer. Combined with the extended theory of Onsager, a quarter of HeLa intracellular water molecules were hydrogen-bonded to biomolecules, exhibiting slower relaxation dynamics than bulk water. By using THz-ATR, the THz pulse intensity and dynamic frequency range are improved.

4. Biological effect studies of terahertz radiation

Terahertz (THz) radiation is one type of non-ionizing electromagnetic wave due to its low photon energy, which could not cause direct ionization damage to water and biomolecules with frequencies ranging from 0.1 to 10 *THz*. However, non-thermal effects of non-ionizing radiation were observed in some researches on radiofrequency electromagnetic fields (RF-EMF) [36]. Therefore, many researchers have focused on THz biological effects, though only a few studies have been conducted to determine whether THz radiation is safe for human.

Actually, most of pioneering contributions on biological effects of THz wave belong to an international project, the "THz-BRIDGE" project [37], which aimed at examining the mechanisms governing interactions with cells to assess the possible genotoxicity of THz radiation. According to their findings, it is obvious that THz radiation can cause thermal effects due to strong absorption of THz wave by water in biological materials, while some studies of the THz-BRIDGE project reported potential genotoxic and epigenetic effects on human lymphocytes [38]. It is known that the frequency range of THz wave corresponds to rotational spectra of molecules, as well as oscillations of biologically important collective modes of biomolecules. Besides, the energy scale of THz radiation is within the range of hydrogen bonds, van der Waals forces and charge-transfer reactions that determine the higher-order structures of the most important biomacromolecules [22]. Therefore, THz radiation may influence the responsible function of biomolecules in various cellular processes, through causing low-frequency intramolecular vibrations in biomolecules [39]. Actually, about 40 years ago, Fröhlich already suggested that non-thermal impact of THz radiation may be caused by coherent excitation through nonlinear resonance mechanism [40]. Armed with Fröhlich's initial postulates, a mathematical model of DNA breathing mode have been proposed, which predicted that THz radiation may couple to the breathing mode of biomolecules, through which THz electromagnetic fields can influence gene expression and DNA replication [41].

Recently, some investigations on non-thermal effects of THz radiation were conducted at strictly controlled thermal conditions to minimize thermal effects. It is found that extended exposure of mouse mesenchymal stem cells to the broadband THz radiation centered on ~10 *THz* results in specific changes in transcription of certain genes. Besides, the THz radiation tends to accelerate stem cells differentiation toward adipose phenotype [42]. Similar effects were detected in follow-on studies which reported that the effect of gene expression by THz radiation not only depends on irradiation parameters like the duration and type of THz source, but also relates to the degree of stem cell differentiation [43-44]. Experiments on artificial human skin tissues also demonstrated that intense picosecond-duration THz pulse radiation affected expression levels of numerous genes associated with some skin diseases. Besides, exposure to intense THz pulses may cause DNA damage and lead to an increase of several proteins responsible for cell-cycle regulation and tumor suppression [45-46]. In agreement with these data, changes in the gene transcription of human embryonic stem cells have also been documented after exposed to the narrow-band 2.3 *THz* radiation [47].

5. Conclusion

Researches into biological applications have been in rapid growth over the past decade. Many interesting studies have demonstrated the high potential of terahertz radiation and spectroscopy to probe subtle changes in both molecular level, such as protein and amino acids, and macroscopic level, such as cell and tissue. Using THz-ATR system, high frequency information of aqueous samples is available for further study on hydration process. Besides, since THz radiation possesses the potential to be capable of influencing cellular gene expression, a detailed investigation on its effects could be of great practical necessity and importance [48], which would be a revolutionary technology in the fields of medicine and biotech industry.

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