Comparing Terahertz transmission response on pH-dependent Apomyoglobin proteins dynamics with Circular Dichroism

Junyi Qiu[*†], Bin Yang[‡], Oleksandr Sushko[*], Richard W. Pikersgill[†] and Robert S. Donnan[*]

[*]School of Electronic Engineering and Computer Science
Queen Mary University of London, Mile End Road, London E1 4NS, United Kingdom
Email: r.donnan@qmul.ac.uk
† School of Biological and Chemical Science
Queen Mary University of London, Mile End Road, London E1 4NS, United Kingdom
Email: r.w.pikersgill@qmul.ac.uk
[‡]Engineering, Sports & Sciences Academic Group
University of Bolton, Deane Road, Bolton, BL3 5AB, United Kingdom

***Abstract* — Terahertz time domain spectroscopy (THz-TDS) was used to study the transmission responses of pH-dependent apomyoglobin (ApoMb) dissolved solutions at 0.2 – 2.2 THz, the THz-TDS technique was also benchmarked against circular dichroism (CD) by studying pH-related folding states changes of ApoMb protein. Results revealed that differences of pH-dependent ApoMb /water dynamics can be detected directly by the THz refractive index spectrum, and these differences are further proved to be caused by the major effect of protonation of water and possibly water response leaded by protein conformation change.**

***Index Terms* — Terahertz, Time domain, Circular dichroism, refractive index, protein dynamics**

I. Introduction

During the last 20 years, increasing attention has been focused on the dynamic aspects of protein structure and function, in which play an important role in protein folding. Due to their transient nature, however, states in the protein-folding process often cannot be observed directly on the existing technologies including x-ray crystallography [1], and their properties are only inferred from indirect measurements, like UV circlular dichroism spectroscopy (UV-CD), SAXS and Fourier transform infrared spectroscopy (FTIR) [2]. Recent advances in terahertz (THz) technology have made the application of THz waves ranging from 0.1 -10 THz (~ 3.3-333 cm^{-1}) attractive in the fields of chemicals and biomolecules studies: THz waves are sensitive to water absorption makes it possible to investigate water-biomolecules dynamics [3], additionally, the sensitive amplitude and nonlinear vibrations of proteins detected in the THz frequency range that are considered to be associated with protein function and protein folding [4]. THz time domain spectroscopy (TDS) is also capable of obtaining the refractive index in addition to the absorption coefficient, in order to deliver corresponding features directly base on measurements. The protein used in this work, apoMyoglobin (ApoMb), is produced from myoglobin (Mb) by removal of the heme group [5]. As the remove of heme group provide ApoMb more flexibility in conformation changes [6], which is a good candidate on studies of protein folding. In this work, we engage to take ApoMb as templates to investigate how the hydration dynamics of solvated protein responds upon different folding states based on the obtained THz refractive index and absorption information, by combining THz-TDS spectroscopy with circular dichroism (CD).

II. Materials and Methods

A. Materials

1 gram Mb from equine skeletal muscle was purchased from Sigma Aldrich (Protein product number: M0630). The Mb was first purified by gel chromatography (*HiLoad 26/60 Superdex75*, GE Healthcare) using FPLC (Fast Protein Liquid Chromatography) with 10mM phosphate buffer at pH 6.5, the purity was checked by SDS-PAGE followed by Coomassie Brilliant Blue staining. Purified protein was dialyzed extensively against de-ionized water at 4°C, and concentrated protein to 20mg/ml. ApoMb was obtained from the above purified Mb by heme group remove step using 2-butanone extraction method [7].

Different pH condition solutions were reached by adding small amount of hydrogen chloride (pH 5 to pH 2) or sodium hydroxide (for pH 7); pH 6 was in its native protein solution. Reference buffers for each pH condition were prepared with same amount of hydrogen chloride/ sodium hydroxide in volume. All chemicals added amount was less than 5µl for not disturbing the solution concentration.

B. Far-UV circular dichroism (CD)

Absorbance scans were carried out from 190 to 260 nm at 0.5 nm intervals at a set temperature of 25°C. For each sample scan, three trials were taken and then averaged. An absorbance scan of the buffer was also taken and used as a baseline to deduct the absorbance scan of samples.

C. Terahertz Time Domain Spectroscopy (THz- TDS)

The THz-TDS system used has its THz pulses generated and detected using 100 fs light pulses from a mode-locked Ti: sapphire laser at a wavelength of 800 nm and a repetition rate of 80 MHz [8]. The measurement was performed at a controlled temperature 25±0.1°C and with a nitrogen purge that reduced the relative humidity (≤ 0.3%) caused by water vapor. A bruker liquid cell (A145) was utilized as a sample holder for the protein solutions. The cell consists of two poly-4-methyl pentene-1 (TPX) plates and a 100µm polytetrafluoroethylene (PTFE) spacer. A 50µl of solutions were injected into the cell with pipette before each measurement, and a wash and pump-empty procedure was applied after each measurement to ensure thickness of the samples is constant during the experiments.

III. RESULTS AND DISCUSSION

A. Estimation of ApoMb secondary structure from CD spectra

Fig. 1 shows the ApoMb absorption spectra monitored between pH 7.0 and 2.0 at wavelength 190-260 nm by CD. Both pH 6 and 7 spectra reserve two negative bands of similar magnitude at 222 and 208 nm, and a positive band at~ 190 nm, aligning well with the all alpha-helical native state of ApoMb protein; pH 2 and 3 has a negative band of great magnitude at around 200 nm, which are supposed to be in its disordered state while pH 4 and 5 tend to achieve equilibrium intermediate states separately.

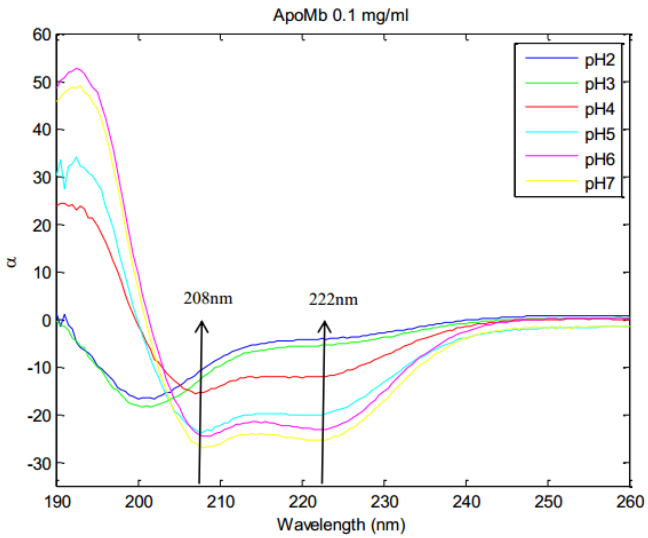

Fig. 1. CD spectra of ApoMb as a function of acidic conditions (pH 2 to pH 7) in 190nm to 260nm wavelength range. Two lines were added at 208nm and 222nm positions to indicate the absorption changes due to pH decrease.

As marked in the figure, pH deceases from pH 7 to pH 2 results in an increased absorption in 208nm, which are dominated by the π^{o}- π^{*} electronic transitions of the protein backbone and indicate the unfolding of alpha helical contents, the dynamic absorption shift from pH5 to pH4 shows a big change of the equilibrium protein state; an smooth continuous decreased at 222nm absorption by pH deceases from pH 7 to 2, is related to the strong hydrogen-bonding environment of alpha helices and are dominated by the n- π^{*} transitions.

B. THz spectra of pH dependent ApoMb

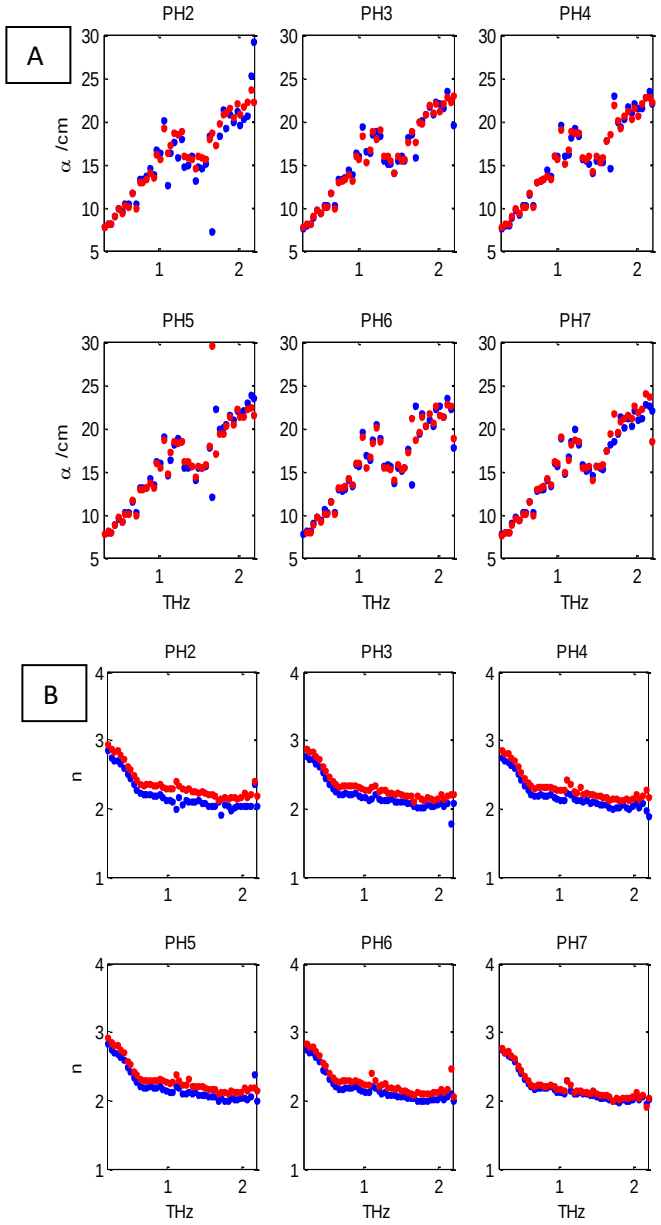

Fig. 2. THz transmission spectra of two ApoMb concentration, 20mg/ml (Red line) and 10mg/ml (Blue line), under different pH conditions from pH 2 to pH 7 in 0.2-2.2 THz frequency range. A shows absorption coefficient and B presents refractive index.

By comparing two ApoMb concentration spectra (20mg/ml and 10mg/ml) under different pH conditions in Fig.2, the refractive indices of two different concentration ApoMb solutions gradually distinct with an acidic increase in pH

conditions (from pH 7 to pH 2) while no clear trend was observed in absorption coefficient spectra. During the acidic denaturation process from pH 6 to pH 2, the chemical environment of protein is altered by hydrogen protons which lead to exposure of the protein's near-surface hydrophobic side chains to the water. Protein conformation and stability are supposed to be affected more by this alteration in lower pH condition and higher protein concentration, which align well with the observed spectra trend; in pH 7 condition, the added hydroxide compound is contributed in the stablization of protein structure while no significant change was observed in spectra. The spectra shift in refractive index is possible caused by chemical environment changes and protein conformation change leading altered response from water.

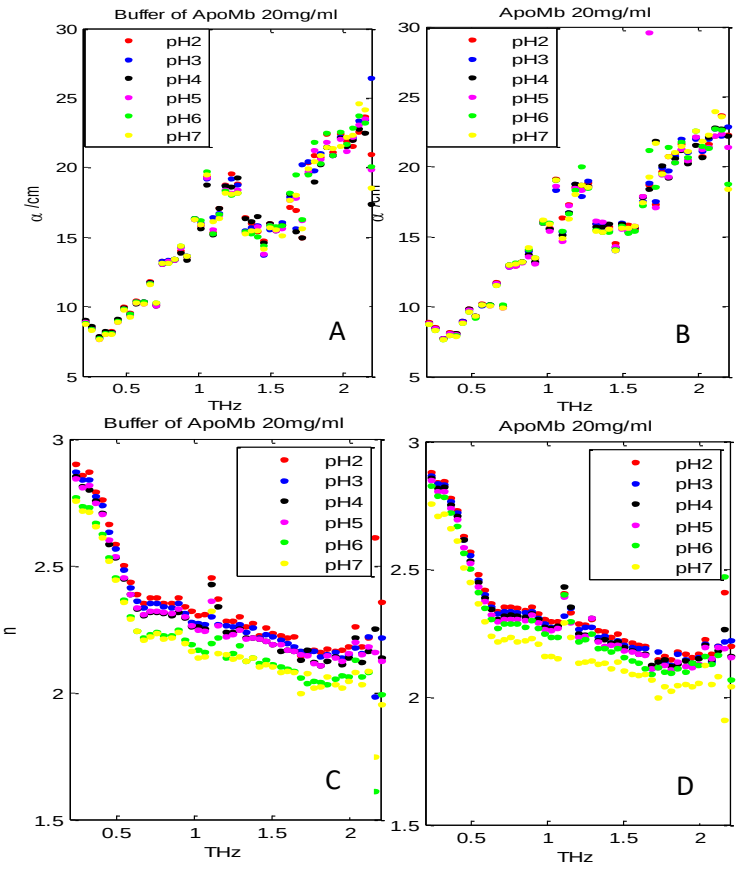

Fig. 3. THz transmission spectra of pH-dependent ApoMb protein solutions and their relaive buffers as a function of acidic conditions (pH 2 to pH 7) measured in 0.2-2.2 THz frequency range on 20mg/ml protein concentration. A and B shows absorption coefficient of buffers and proteins, respectively. C and D presents refractive index spectra.

In Fig. 3, we compared both absorption coefficient and refractive index spectra of pH-dependent ApoMb protein under 20mg/ml in 0.2- 2.2 THz range. As a general trend, a gradually increased refractive index is found with an acidic increase in pH conditions on 20mg/ml protein concentration (fig. 3D), Similarly, this trend is clearly observed in different buffer conditions for 20mg/ml ApoMb solution in fig. 3C. This suggests that the change in ApoMb protein-water dynamics is also contributed by the fact that the protonation of water leading to different dynamic response can be detected in refractive index spectra, which cannot be detected with CD and THz absorption spectra.

IV. Conclusion

Instead of performing the relationship between protein dynamics and THz absorption coefficient which was investigated and accepted by the majority of THz society, we have experimentally shown that pH-dependent ApoMb/ water dynamics differences can be detected directly by refractive index spectrum, and even more sensitive than absorption information given. These differences are further proved causing by the major effect of protonation of water, and possible contribution from changes in the conformation of the protein leading altered response from water.

Meanwhile, we have also indicated that THz refractive index spectra is not sensitive enough in detecting small water-protein dynamic change nor secondary structure contents comparing with CD spectroscopy, as the spectra information of small dynamic change has been largely covered by strong water absorption. However, THz techniques are capable of delivering unique dynamic response data for protein analysis, which cannot be achieved by other approaches. These corresponding features obtained potentially can be combined to other techniques of protein chemistry.

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