Terahertz Time-Domain Spectroscopy of Peptides in Solution

R. Li[a], T. Ding[b], T. Huber[b], R.J. Falconer[b], A.P.J. Middelberg[b,c], L.F. Gladden[a], and J.A. Zeitler[a]
[a] Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB2 3RA, UK
[b] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia
[c] School of Chemical Engineering, The University of Queensland, Brisbane, Australia

***Abstract*—The terahertz absorption spectra of different peptides in buffer solutions were measured in the frozen state (80 K) with different peptide concentrations. The observed absorption peaks, confirmed by independent measurement at the Australian synchrotron, are believed to directly relate to the formation of KF crystals upon freezing. This process is significantly suppressed by including peptide in the buffer.**

I. Introduction and Background

Computational studies predict that the collective vibrational modes of biomolecules fall in the terahertz frequency range[1,2]. With the advent of terahertz spectroscopy, extensive experimental activity is investigating the dynamics and conformational changes of proteins and peptides[3-6]. Initially, the strong absorption of bulk water and the limited power of laboratory terahertz radiation confined measurement to proteins prepared in compressed pellets[5] or hydrated thin films[6]. In order to study protein and peptide dynamics in an aqueous environment, two approaches have been attempted recently: freezing the sample solution to reduce the absorption of bulk water[4] or introducing a more powerful but narrow bandwidth terahertz sources such as systems based on p-Ge lasers (2.1 ~ 2.7 THz)[3].

In this work we use a traditional terahertz time-domain spectroscopy (THz-TDS) setup equipped with a GaAs emitter (0.2-4.0 THz) to study the dynamics of peptides in buffer solutions. In addition, we compare the THz-TDS results to independent measurements performed on the far-infrared beamline at the Australian Synchrotron. The buffer solution used in this study is 0.1 M potassium fluoride (KF) with 1 mM potassium phosphate (KH_2PO_4) solution (*pH*=7). The peptide system in this study consists of three chemically similar peptides dissolved in buffer solution. AK17 is a seventeen amino acid alanine-rich peptide containing three lysine residues. AK9P differs from AK17 with one proline substitution at the 9th position. AK10G differs from AK17 with one glycine substitution at the 10th position. Although chemically similar, these three peptides vary considerably in their secondary structure (Table 1 helicity measurement) when dissolved in buffer, indicating substantial differences in their energy landscape. AK17 is alpha helical with the highest measured helicity; AK9P is a purely random coil; AK10G has an intermediate measured value of helicity. In contrast to most of the earlier studies[3,4] the peptide solution is kept at low concentrations (below 50mM) to ensure that all the peptides are dynamically independent from each other. The aim of this study is to investigate the underlying physics of this biochemical system using THz-TDS, and to compare the applicability of this experimental approach to experiments performed using high power central facilities for the investigation of the dynamics of biomolecules.

Peptides	Amino Acid Sequence	Helicity (Chakrabartty et al. 1994)	Helicity Measured at 0°C
AK17	AC-AKAAAAKAAAAKAAAAK-NH2	67.70%	72.40%
AK10G	AC-AKAAAAKAAGAKAAAAK-NH2	25.60%	29.50%
AK9P	AC-AKAAAAKAPAAKAAAAK-NH2	0	0

Table 1 Helicities of three chemically similar peptides

II. Results

All three peptides were measured at three concentrations (12.5 mM, 25 mM, 50 mM) at temperatures varying from 260 K to 80 K in 20 K increments. Room temperature measurements were acquired before and after freezing the samples to check the reproducibility of the system. The absorption coefficients were calculated by measuring pure buffer solution as a reference. The results of the THz-TDS measurements of all three peptides at 80 K are shown in Fig. 1. The terahertz spectra of AK17 are compared with the synchrotron measurements.

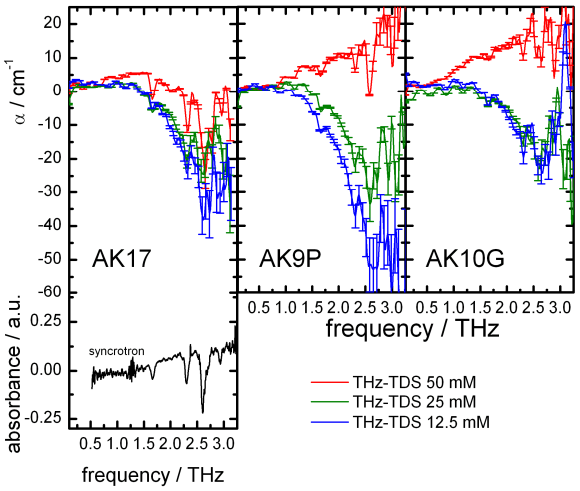

Fig. 1: Terahertz spectra of all three peptides at 80 K and the corresponding synchrotron measurement for AK17.

Both THz-TDS and synchrotron measurements showed terahertz absorption peaks at 1.66, 2.32 and 2.64 THz in the case of AK17. The intensities of the inverse absorption peaks varied monotonically with peptide concentration. This result implies that the observed terahertz features must be strongly influenced by the existence of peptide. However, the THz-TDS experiments on AK9P and AK10G both exhibit the same inverse absorption peaks as observed in AK17. Therefore, the existence of terahertz inverse absorption peaks cannot be directly attributed to the secondary structure of the peptide. Furthermore, the temperature dependence of the inverse absorption peaks was also investigated. The terahertz spectra of AK17 (50 mM concentration) are shown as an example in Fig. 2: the peaks around 1.7 THz and 2.3 THz shift to higher frequencies with decreasing temperature. Similar temperature dependencies were also observed for the other two peptides at different concentrations. This strong effect is indicative of

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phonons in crystals.

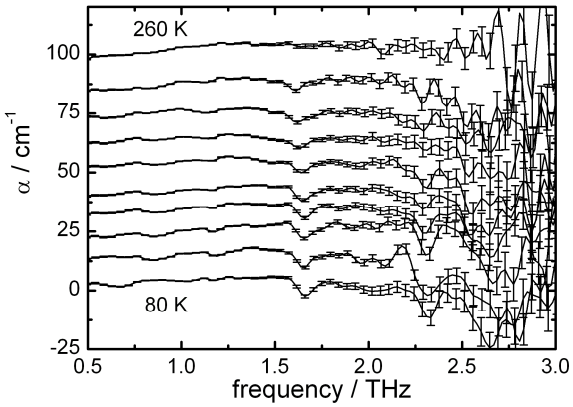

Fig. 2: Temperature dependent terahertz spectra of AK17 at a concentration of 50 mM.

The terahertz absorption spectra of peptides and buffer solutions were also calculated by measuring the empty cell as the reference. By highlighting parts of the spectra as shown in Fig. 3 (still taking AK17 50 mM as the example), the spectral features emerging from the absorption baseline of buffer solution spectrum were assigned to the inverse absorption peaks shown in Fig. 1 and 2. Accordingly, all the terahertz absorption features observed in our experiments are characteristics of the buffer solution used, which mainly contains KF ions.

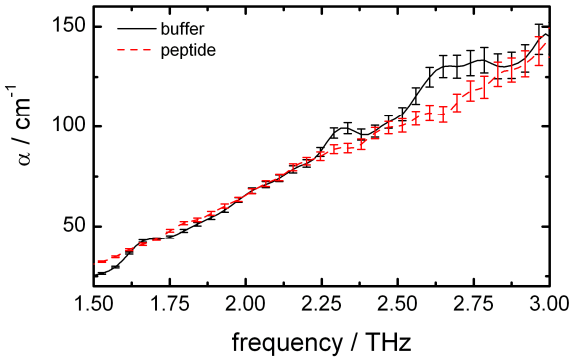

Fig. 3: Terahertz spectra of buffer and AK17 peptide solution (recorded at 80 K) within the frequency range where inverse absorption peaks were observed.

Recent far-infrared experiments done at the Australian Synchrotron suggest the absence of these features in samples of pure water or KF free buffer solution. All three absorption peaks identified in our experiments were found to be consistent with the absorption peaks of KF dihydrate ($KF{\cdot}2H_2O$) measured by Chantry *et al.* in 1981[7]. From our results we conclude that we observe the formation of $KF{\cdot}2H_2O$ crystals upon freezing samples of KF buffer solution. In the presence of peptide, the crystallization is inhibited. This effect is observed for all three peptides investigated in this study. Future works need to address the exact mechanism of the interaction between peptide, KF and the water molecules.

Apart from features within a spectrum, the baseline difference between terahertz spectra also provides important additional information. As shown in Fig. 1, the overall absorption of all three peptide solutions increased as the concentration increased. This is in contrast with the observation at room temperature that the overall absorption is reduced by adding peptide, due to peptide being much less absorbing than aqueous solution. According to Fig. 1, the peptide buffer solution is higher absorbing than the corresponding buffer solution at 80 K. This effect could be due to the disrupted crystallization of $KF{\cdot}2H_2O$ in the buffer solution caused by doping with peptide. Especially in the case of AK9P and AK10G, a dramatic jump in the baseline absorptions was observed as the peptide concentration increased from 25 mM to 50 mM. This phenomenon might be related to the existence of a critical concentration, beyond which a large scale phase transition is accomplished. One hypothesis is that, about this concentration, the peptides can no longer be considered independent from each other within the buffer solution.

III. CONCLUSIONS

We report the formation of strong spectral features of terahertz frequencies in an aqueous KF buffer solution upon freezing. The spectral features are attributed to the formation of $KF.2H_2O$ upon freezing, which can be suppressed by the existence of peptide. This suppression effect is not significantly influenced by peptide secondary structure. Good agreement was found between synchrotron and THz-TDS measurements.

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