

# Low frequency modes of biomolecules in the hydrated states

Ohki Kambara<sup>1</sup>, Norihisa Hiromoto<sup>2,3</sup>

<sup>1</sup>Research Institute of Electronics, Shizuoka University, Hamamatsu, 432-8011, Japan

<sup>2</sup>Graduate School of Engineering, Shizuoka University, Hamamatsu, 432-8011, Japan

<sup>3</sup>Graduate School of Science and Technology, Shizuoka University, Hamamatsu, 432-8011, Japan

**Abstract**—Hydration process of few organic molecules is examined in the low-frequency region under  $100\text{ cm}^{-1}$ . THz spectra of both hydrated states and anhydrous states in the solid crystal samples are measured by the THz-TDS instrument and their vibrational modes are analyzed by periodic boundary condition implemented DFT calculation. By comparing with single molecule calculation, peak assignment of their peaks was performed and the effects of each water molecules which bind to the anhydrous molecules are discussed.

## I. INTRODUCTION

LIVING things cannot live without water. When major biomolecules like proteins and DNAs play important roles in the vital activities, several water molecules always bind to these molecules. Understanding how these water molecules affect to the low-frequency vibrational modes of the anhydrous molecules will throw light on the structure-function relationship problems of biomolecules. In this paper, low-frequency spectra of both hydrated states and anhydrous states of small organic molecules including ampicillin and L-asparagine are investigated by experimentally and theoretically. THz spectra of the solid samples in these states under  $100\text{ cm}^{-1}$  are measured by a self-build THz-TDS instrument [1] under the nitrogen-gas atmosphere with the number of scans of 4 times. The pellet used for measurement was made by grinding these samples up by a mortar and mixing with a polyethylene powder. To assign these low-frequency bands, both positions and intensities of each vibrational mode are compared with the theoretically obtained values from density theory calculation (DFT) by CRYSTAL package [2], where periodic boundary condition (PBC) is implemented. Without this fatal condition for solid-state calculation, that is, by calculating single molecule, intermolecular interaction will be ignored and frequency calculation results are only of gas-phase [3,4]. The initial structures for the optimization calculations were taken from the X-ray crystallographic structure and all the cell parameters were kept fixed and followed by frequency calculation step. The crystal systems for anhydrous/hydrated L-asparagine are  $P21$  and  $P2_12_12_1$ , respectively. In addition, hydration process were tried to be monitored by incubating the anhydrous samples at various humidity level, which are prepared by saturated solution of various salts including potassium chloride or sodium chloride etc. Detailed analysis of DFT calculation was also performed to further study.

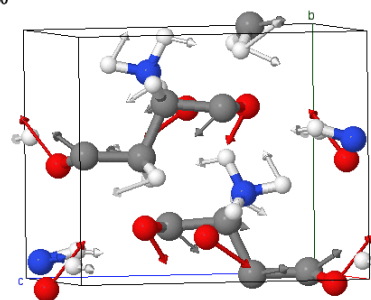
## II. RESULTS

One of the amino acid, L-asparagine and its monohydrate sample are adopted as an example. Optimized structure of anhydrous and monohydrate L-asparagine in the solid state are shown in Fig. 1. Number of the molecule in the single unit cell

for anhydrate sample is two and that for hydrate sample is four. Resulting number of normal modes of these samples also different and they were 102 and 234, respectively. These numbers contain three harmonic modes. Among them, there were three and nine IR active bands for anhydrate and mono hydrated L-asparagine under  $100\text{ cm}^{-1}$ , which is our measuring range, respectively. The representative IR active vibrational bands existed in the similar frequency were selected and depicted in Fig. 1. Both two bands look rotational bands. These

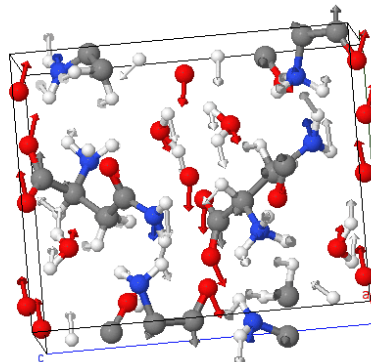
(A)

```
P 1 [P 1] #1
a=5.062Å
b=6.700Å
c=8.054Å
α=90.000°
β=91.706°
γ=90.000°
```



(B)

```
P 1 [P 1] #1
a=5.593Å
b=9.827Å
c=11.808Å
α=90.000°
β=90.000°
γ=90.000°
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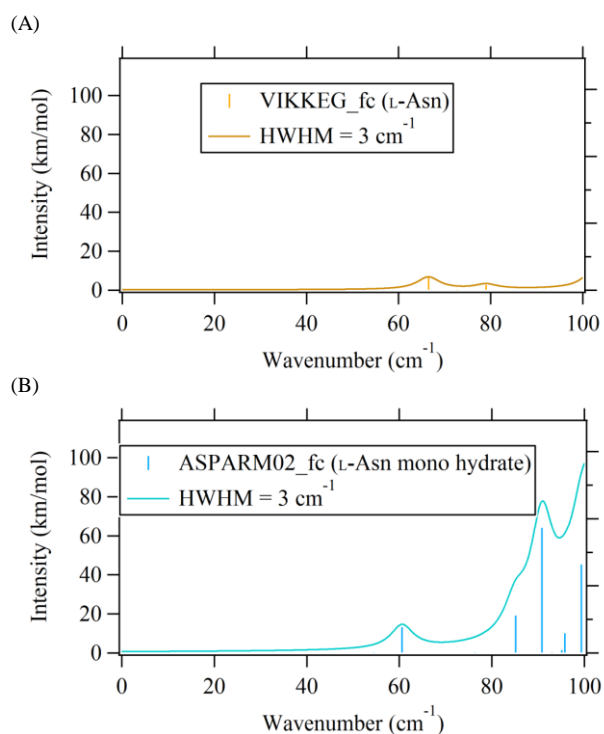


**Fig. 1.** Theoretically obtained optimized structures and these representative bands of (A) anhydrous and (B) monohydrate L-asparagine in the low-frequency vibrational regions under  $100\text{ cm}^{-1}$  calculated by CRYSTAL package.

vibrational modes were also observed by single molecule calculation, suggesting that these representative bands mainly derived from intermolecular vibrations.

On the other hands, THz spectra for these two samples were

measured by THz-TDS instrument. Anhydrous L-asparagine shows one representative band at  $62.7\text{ cm}^{-1}$ , and that of monohydrate sample shows sharper band at  $54.5\text{ cm}^{-1}$  and strong broad band at around  $80\text{ cm}^{-1}$ . The difference of two spectra is directly derived from the change of molecular structure and crystal system, which are results of the binding of the single water molecule. DFT calculation clearly reproduced these experimentally obtained spectra. Low-frequency modes of anhydrous/monohydrate state of L-asparagine calculated at B3LYP/6-31G(d,p) level are shown in Fig. 2. In this figure, pseudo spectra were drawn with each peak position and these intensities with HWHM =  $3\text{ cm}^{-1}$ . Though high frequency shifts from thermal effects were uniformly observed for both samples [5], whole spectral contour with single representative bands and strong broad band at high frequency region are well reproduced by DFT calculation. These modes consist of various collective modes with/without single water molecule.



**Fig. 2.** Vibrational bands of (A) anhydrous and (B) monohydrate L-asparagine calculated by CRYSTAL. The number of IR active bands in these figures are (A) three and (B) nine, respectively. Pseudo spectra were drawn with each peak position and these intensities with HWHM =  $3\text{ cm}^{-1}$ .

Finally, the hydrating experiment of anhydrous l-asparagine was performed to observe the hydration process of this small organic molecule via low-frequency spectra. In this experiment, the simple decreasing of the representative band of anhydrous sample and increasing of the band at  $54.5\text{ cm}^{-1}$  was observed with increasing the hydration level. This result indicates that the hydration process for this molecule has no intermediate structural phase. This result is interesting in the following aspect. The binding of the water molecule must involve drastic structural change for these small molecules because their crystal systems before/after hydration are thoroughly different and they

are monoclinic lattice and orthorhombic lattice, respectively. Furthermore, single molecular calculation without PBC is also performed and compared with “CRYSTAL” calculation results. By this comparison, the origin of each spectral band is revealed. Combining these types of analysis, hydration process of small organic molecule was elucidated from the aspect of low-frequency vibrational modes.

### III. SUMMARY

By comparing the experimental and theoretically calculated values, low-frequency vibrational modes of hydrated and anhydrous states of few molecules was revealed and discussed. Binding of even single small water molecule were revealed to change crystal structure of organic molecule and as a result, affected to the low-frequency vibrational modes of these samples.

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