# Hydration Profiles of Aromatic Amino Acids: Conformations and Vibrations of *L*-Phenylalanine-$(H_2O)_n$ Clusters

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## Abstract

IR-UV double resonance spectroscopy and *ab initio* calculations were employed to investigate the structures and vibrations of the aromatic amino acid, *L*-Phenylalanine-$(H_2O)_n$ clusters formed in a supersonic free jet. Our results indicate that up to three water molecules are preferentially bound to both the carbonyl oxygen and the carboxyl hydrogen of *L*-Phenylalanine (*L*-Phe) in a bridged hydrogen-bonded conformation. As the number of water molecules is increased, the bridge becomes longer. Two isomers are found for *L*-Phe-$(H_2O)_1$, and both of them form a cyclic hydrogen-bond between the carboxyl group and the water molecule. In *L*-Phe-$(H_2O)_2$, only one isomer was identified, in which two water molecules form extended cyclic hydrogen-bond with the carboxyl group. In the calculated structure of *L*-Phe-$(H_2O)_3$ the bridge of water molecules becomes larger and exhibits an extended hydrogen-bond to the π-system. Finally, in isolated *L*-Phe, the D conformer was found to be the most stable conformer by the experiment and by the *ab initio* calculation.

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## 1. Introduction

The three-dimensional structures of proteins and their dynamics are to a large extent governed by the conformational profiles of amino acids constituting them. In particular, competition between intra- and inter-molecular hydrogen-bonded interactions plays a vital role in determining the structure of these proteins in solution. Since amino acids exist as zwitter-ions in solution, it is interesting to investigate their hydrated structures [1-3]. The conformational landscape of isolated *L*-Phenylalanine(*L*-Phe) has been examined in detail previously [4-7]. The electronic spectrum of jet-cooled *L*-Phe was first reported by Levy and coworkers [4], and the IR spectra of various plausible conformers were investigated by Simons and coworkers [5, 6]. In an earlier study, we showed that the $S_1$ lifetime is considerably different for different conformers, which was described by the conformer dependent $^1\pi\pi^*$-$^1n\pi^*$ interaction of *L*-Phe[7].

The electronic spectrum of jet-cooled *L*-Phe-$(H_2O)_n$ was first reported by Kim and coworkers [8]. They observed mass-selected 1+1' resonant two photon ionization (R2PI) spectra of *L*-Phe-$(H_2O)_n$. Based on density functional theory (DFT) calculations, they suggested a cyclic H-bonding structure for *L*-Phe-$(H_2O)_1$. However, there has been no experimental evidence to confirm this structure of *L*-Phe-$(H_2O)_1$ as well as that of larger clusters. Since isolated amino acids in the gas phase are in the non-ionic state, it is particularly interesting to examine on how an incremental addition of water molecules leads to the formation and stabilization of the zwitter-ionic form.

In the present work, we therefore measured the LIF/R2PI and IR vibrational spectra of jet cooled *L*-Phe-$(H_2O)_n$. Kim and coworkers observed three bands, A', D' and α' of the *L*-Phe-$(H_2O)_n$ clusters [8] in the region of the $S_1 \leftarrow S_0$ transition of the monomer, which were assigned to *L*-Phe-$(H_2O)_1$ and a higher size cluster, $n \geq 2$. We observed the IR spectrum by monitoring each band. The cluster size as well as the corresponding structure was determined for each band by comparing the observed IR spectra with the frequencies evaluated for structures obtained from *ab initio* calculations. It was found that water molecules exclusively form the H-bond to the open conformers, i.e., the non-intramolecular hydrogen-bonded (NIMHB) conformers. The most stable forms of *L*-Phe-$(H_2O)_1$, *L*-Phe-$(H_2O)_2$ and *L*-Phe-$(H_2O)_3$ were found to have the structure in which water molecule(s) is bound to the carboxyl group to form a cyclic H-bonded system.

## 2. Methods

### 2-1. Experimental

The experiments have been performed in Hiroshima and Frankfurt. While the depletion spectra presented and discussed in the following are from the fluorescence depletion experiment performed in Hiroshima, the ion depletion spectra have been recorded (IR/R2PI) in Frankfurt. Since both types of spectra were in essence the same, except for a better S/N ratio for the fluorescence depletion spectra, we only display and discuss the latter. The detail of the experimental setups has been described in the previous papers [7, 9-12]. Here, the Hiroshima setup is described in greater detail.

For generating jet-cooled amino acids, we used a home-made heated pulsed nozzle. We attached a sample housing on the head of a commercial pulsed nozzle (General valve series 9). The housing is made of poly-imido resin and the sample was heated to about 100 $^{o}$C by a spiral heater. The mixture of the water vapor with He carrier gas passed through the sample housing and was expanded into vacuum through a 1 mm orifice nozzle. A tunable pulsed UV laser light crossed the supersonic free jet at 20 mm downstream of the nozzle. The emission from molecules and clusters was collected by a series of lenses and detected by a photomultiplier tube after passing through a band pass filter. We measured IR spectra of each species by IR-UV double resonance spectroscopy with the fluorescence detection, called Fluorescence detected IR(FDIR) spectroscopy [9, 10]. In this measurement, a tunable IR laser light was introduced into the vacuum chamber 50 ns prior to the UV laser pulse. The two laser beams were coaxially focused on the supersonic jet. The UV laser frequency was fixed to the band origin of each species, and the IR laser frequency was scanned while monitoring the fluorescence signal. The depletion of the ground state population induced by the IR absorption was recorded as the fluorescence-dip spectrum. The wavelength of the dye laser output was calibrated by the opt-galvanic method. The observed IR spectra were compared with those calculated for the *ab initio* optimized structures.

The Frankfurt experimental setup was described in detail elsewhere [11, 12]. It is very similar to the Hiroshima apparatus, except that a time of flight mass spectrometer was utilized to analyze the ions. Mainly ions of the monomer and of the 1:1 cluster showed up when ionizing the expansion by one color-R2PI in the region of the vibronic origin of the $S_1 \leftarrow S_0$ transition. An R2PI spectrum is the yield of an ion as function of the frequency of the ionizing laser. The IR OPO laser was superposed to the cluster beam in an antiparallel way. Its pulse arrived 200 ns before the UV ionizing laser pulse. The depletion of an ion signal by tuning the IR laser represented an IR/R2PI depletion spectrum.

### 2-2 Computational

The geometry optimizations and the evaluation of the vibrational frequencies was carried out by using density functional theory (DFT) calculation with B3LYP/6-31+G* level of theory [12, 13]. Further geometry optimizations and vibrational frequency evaluations were carried out by using the 6-31+G* and aug-cc-pVDZ basis sets at the second order Møller-Plesset (MP2) level of theory [14]. The BSSE corrections were carried out using the counterpoise method of Boys and Bernardi [15]. The zero point vibrational energy (ZPVE) corrections were computed from frequencies evaluated at the MP2/6-31+G* and MP2/aug-cc-pVDZ levels of theory. All the calculations were performed by using GAUSSIAN 03 [16].

## 3. Results and discussion

### 3-1 Electronic spectrum

Fig. 1 shows R2PI spectra of jet-cooled *L*-Phe measured under different expansion conditions. In order to facilitate comparison, we adopted labeling for the conformers proposed by Simons and coworkers for bare *L*-Phe [5, 6], and those proposed by Kim and coworkers for *L*-Phe-$(H_2O)_n$ [8]. In the R2PI spectra depicted in Fig. 1, the spectrum in (a) was recorded with the jet seeded with Xe at 2 bar. The monomer is sampled from an early part of the gas pulse, where cooling is not optimal. Xe was chosen because it provides a better cooling power than He. Conformers A, B, C, D and X appear strongly, while conformer E is missing. It should be pointed out that the intensities assigned to conformers D and X are nearly the same. According to the assignment of Simons and coworkers, band X is the origin of the most stable conformer. In Fig 1(b) we increased the time delay between the gas pulse and the laser pulse thus probing the coldest part of the gas pulse. A clear decrease of band X is observed relative to band D. This decrease is more pronounced in Fig. 1(c), where the stagnation pressure of Xe was raised to 2.5 bar. As seen in Figs. 1(b) and (c), the bands A, B and C are heavily reduced relative to the band D, which is now the most intense. It is also seen that under these ultracold conditions the band D' appears, which was tentatively assigned to the 1:1 cluster between conformer D and water by Kim et al. [8]. Obviously, residual water in the gas line forms clusters under the very cold conditions. For comparison we show in Fig. 1(d) the spectrum measured with an expansion of He at 3 bar. Here all isomers reported by Levy and Simons are observed, the strongest ones being A, D, X and E. In Fig. 1(e) the expansion gas is now Ar at a pressure of 2 bar. Again, due to the better cooling conditions with Ar, conformer E no longer appears similar to the spectrum in Fig. 1(a). A better cooling is achieved by increasing the Ar pressure to 3 bar (Fig. 1(f)). This time, the spectrum is similar to that in Fig. 1(c) and the intensity of conformer X is substantially depleted. Thus ample evidence is given that conformer D is the energetically lowest isomer and that conformer X is less stable. Table 1 lists the zero point vibrational energies (ZPVE) levels of conformers A, B, C, D, X and E calculated at the MP2/6-31+G* level. Conformers B and X are classified to the IMHB closed conformer and A, C and D to the NIMHB open conformer [7]. In contrast to the work of Simons and co-workers, our ZPVE corrected relative energies well reproduce the experimentally found relative stabilities.

Fig. 2 shows the LIF spectra of jet-cooled *L*-Phe measured under different water vapor pressure in the He expansion. In Fig. 2(a), without the addition of water vapor, the six conformers of isolated *L*-Phe can be identified. As was described in our previous paper [7], the band assigned to conformer X shows a much weaker intensity than the band in the R2PI spectrum in Fig. 1. The addition of water vapor leads to a weakening of the bands associated with *L*-Phe. Especially as seen in Fig. 2(b), the band intensities due to conformers A, D and E become much weaker, and bands A' and D' newly appear at 37520 and 37582 $cm^{-1}$, respectively. Thus, it is concluded that water molecules exclusively form H-bonding to the open-type conformers A, D and E. There is no sign of an appearance of third cluster with water. This conformer-selective H-bond formation was suggested [8], and is also found in another aromatic acid, such as 3-Indole-propionic acid [17]. At higher partial pressure of water, another band (band $\alpha$') appears at 37494 $cm^{-1}$, as shown in Fig. 2(c). This band was assigned to *L*-Phe-$(H_2O)_2$ by Kim and coworkers, though its peak is observed only in the spectrum sampled at the mass of [*L*-Phe]$^+$ and [*L*-Phe-$(H_2O)_1$]$^+$, due to the fragmentation after the ionization. In the following section, we will discuss the structure of *L*-Phe-$(H_2O)_n$ based on the results of IR spectroscopic measurements and *ab initio* calculations.

## 3-2 IR spectra and the cluster structures

We measured IR spectra by fixing UV frequencies to bands A', D' and $\alpha$', which are shown in Figs. 3-5. The observed IR frequencies and corresponding calculated B3LYP and

MP2 frequencies are listed in Table 2. In Fig. 3(a) is shown the FDIR spectrum of band A', which was assigned to *L*-Phe-$(H_2O)_1$ [8]. In the IR spectrum, there are two intense bands at 3246 and 3506 $cm^{-1}$, and three sharp bands at 3040, 3421 and 3724 $cm^{-1}$. Though it is *apriori* not clear whether *L*-Phe retains a similar conformation under the formation of the H-bonding with water, it is reasonable to assume that the isomer due to band A' may have the *L*-Phe conformation similar to that of conformer A of bare *L*-Phe [8]. This is because the intensity of band A' increased parallel to the decrease of band A, and the small red-shift in the electronic transition of band A' with respect to band A, that is 7 $cm^{-1}$, is also observed in other monohydrated aromatic acids [17-19]. Thus, we calculated the structures of *L*-Phe-$(H_2O)_1$ with *L*-Phe forming conformer A. The optimized structures are shown in Figs. 3(b)-(d). For the calculation, we considered three H-bonding sites; the carboxyl group, the amino group and the phenyl group. Fig. 3(b) shows the most stable isomer (Aw1-I), where the water forms a cyclic H-bond with the carboxyl group. In isomer Aw1-II (Fig. 3(c)), one of OH of the water is H-bonded with nitrogen of the amino group. The energy of isomer Aw1-II is 11.8 kJ/mol higher than Aw1-I. In isomer Aw1-III (Fig. 3(d)), which is 29.6 kJ/mol less stable than Aw1-I, water is H-bonded to the phenyl group as a H-bond donor, while the amino hydrogen is bound to the oxygen atom of water. By comparing the calculated spectra with the observed one, it is clear that the calculated IR spectrum of isomer Aw1-I reproduces very well the observed one with respect to the intensities and the band positions, compared to the other two calculated spectra. On the other hand, the relative intensities of the bands of Aw1-II do not agree with the observed ones of Fig. 3(a). Similarly, the calculated IR spectrum of the higher energy isomer, Aw1-III, is quite different from the observed one. Thus, we conclude that the structure of *L*-Phe-$(H_2O)_1$ of band A' is that of Aw1-I. The bands observed in the IR spectrum are assigned as follows; the intense and broad bands at 3246 and 3506 $cm^{-1}$ are the H-bonded carboxyl OH and the water donor OH stretch bands, respectively, within the cyclic H-bonding network. The sharp bands at 3421 and 3724 $cm^{-1}$ are assigned to the asymmetric $NH_2$ stretch of amino group and free OH stretch of water, respectively. The band at 3040 $cm^{-1}$ is the aromatic CH stretch vibration.

Fig. 4(a) shows the FDIR spectrum observed by fixing UV frequency to band D'. The spectrum is essentially very similar to that of band A'. That is, the intense bands at 3255 and 3513 $cm^{-1}$ and the sharp band at 3726 $cm^{-1}$ are very similar to those of A' in Fig. 3(a). Slight differences are that the band at 3513 $cm^{-1}$ is split into two, and the CH stretch bands of the aromatic group appear more intense than those of band A'. Kim and coworkers suggested that the isomer due to band D' may have conformer D of bare *L*-Phe, because the red-shift of band D' from band D in the electronic transition, that is 8.5 $cm^{-1}$, is close to that of band A' from band A. Thus, we calculated the structure of *L*-Phe-$(H_2O)_1$ with *L*-Phe forming conformer D, which are shown in Figs. 4(b)-(d). Similar to isomer Aw1-I, the isomer in which the water molecule forms cyclic H-bond with the carboxyl group (Dw1-I) is the most stable structure and the spectrum of Dw1-I well reproduces the observed one, except the band split at 3500 $cm^{-1}$. On the other hand, the agreement of the IR spectra of higher energy isomers, such as isomers Dw1-II and Dw1-III, are poor compared to that of isomer Dw1-I.

We also examined the possibility of conformer E for the band D'. This possibility comes from the observation that the decrease of the fluorescence intensity of band E is more drastic than that of D upon the addition of water vapor. Conformer E of bare *L*-Phe was first assigned to the IMHB form by Simon and coworkers [5, 6]. However, on the basis of the IR spectroscopic measurement in our previous paper we reported that this conformer does not form the intramolecular H-bond [7]. So, we assumed that the conformer E of bare *L*-Phe is the fourth stable structure of the open form, which is shown in Fig. 5(a) together with the observed and calculated IR spectra. The energy of conformer E of bare *L*-Phe is higher than that of conformer X by 6.8 and 11.47 kJ/mol at the B3LYP/6-31G* and MP2/6-311G** calculations, respectively [5]. For *L*-Phe-$(H_2O)_1$ with conformer E, we calculated only the isomer with a water molecule H-bonded to carboxyl group (Ew1-I), which is shown in Fig.

5(b). As is expected, the calculated IR spectrum of Ew1-I is very similar to that of conformer D of *L*-Phe-$(H_2O)_1$, (Dw1-I), and the spectrum reproduces well the observed one. Very surprisingly, the energy of Ew1-I is only 1.21 kJ/mol higher than Dw1-I at the B3LYP/6-31G* level calculation. Thus, it is very hard to determine definitely which isomer the band D' belongs to from the IR spectroscopic data. However, we can conclude that band D' is due to *L*-Phe-$H_2O$ having the cyclic H-bonding between carboxyl OH and water, like Dw1-I or Ew1-I. At this stage, we should point out that band E is very sensitive to the cooling condition, that is the conformer E no longer appears at lower temperature nozzle condition such as in the Ar expansion in the R2PI spectra. Thus, we would prefer the assignment of band D' to the Dw1-I isomer. The split at 3513 $cm^{-1}$ in the observed IR spectrum is probably due to the overlap of the $NH_2$ asymmetric stretch with the H-bonded OH stretch of water or may originate from a combination band. It is actually observed in the spectra calculated a the MP2 level for the Dw1-I isomer as depicted in Fig. 8.

Fig. 6(a) shows the IR spectrum obtained by fixing UV frequency to band $\alpha$'. In the spectrum, a very broad and strong band is seen at 3002 $cm^{-1}$. Several sharp bands are overlapped with this broad band, which are assigned to the aromatic CH stretch. Other intense peaks are seen at 3330, 3474, 3680 and 3717 $cm^{-1}$. It is prominent that the bandwidth becomes broader with lowering the IR frequency, indicating an increase of the rate of the intracluster vibrational energy redistribution with the red shift. The two bands at 3680 and 3717 $cm^{-1}$ are easily assigned to the OH stretch of water molecules free from the H-bonding. The appearance of the two free OH stretch bands indicates that this species contains two water molecules, that is band $\alpha$' is assigned to *L*-Phe-$(H_2O)_2$. Since band $\alpha$' is located on the lower frequency side of bands A and A' in the electronic spectrum (Fig. 2(c)), we assumed that in the cluster behind band $\alpha$' *L*-Phe has the conformation similar to those of bands A (*L*-Phe) and A' (*L*-Phe-$(H_2O)_1$). The calculated structures of *L*-Phe-$(H_2O)_2$ and its IR spectra are shown in Figs. 6(b)-(e). Similar to *L*-Phe-$(H_2O)_1$ , isomer Aw2-I, in which two waters form cyclic H-bond with carboxyl group, is most stable. In addition, a good agreement is seen between the calculated IR spectrum of Aw2-I and the observed one; only isomer Aw2-I shows a strong band at 3000 $cm^{-1}$, which is coincident with the observed band. Other higher energy isomers, such as the H-bonded form both to the carboxyl and amino groups of Fig. 6(c) or the H-bonded form to the phenyl ring of Fig. 6(d), do not reproduce the observed spectrum. Thus, we conclude that band $\alpha$' is due to the cyclic-form *L*-Phe-$(H_2O)_2$, and the bands in Fig. 6(a) are assigned as follows; the band at 3002 $cm^{-1}$ is the H-bonded carboxyl OH stretch. Those at 3330, and 3474 $cm^{-1}$ are the H-bonded OH stretches of two waters in the H-bond ring, and the bands at 3680 and 3717 $cm^{-1}$ are OH stretches free from the H-bond. The weak and broad bands at ~3200 $cm^{-1}$ are due to the overlapped transitions of the aromatic CH stretches. One noticeable point in the IR spectrum of *L*-Phe-$(H_2O)_2$ is that the red-shift of the carboxyl OH stretch with respect to bare *L*-Phe is as large as 580 $cm^{-1}$, which is more than twice the red-shift of the phenolic OH stretch of phenol-$(H_2O)_2$ compared to bare phenol [20], indicating that the carboxyl OH is considerably weakened under the hydration of two waters. This can be regarded as favorable for the proton transfer to take place in zwitter-ion formation.

Finally, we would like to discuss the relationship between the size-dependent growth of H-bonding network and the IR spectra of the OH stretching vibration. Fig.7 shows the observed IR spectra of species with A conformer of *L*-Phe-$(H_2O)_{0-2}$, and calculated IR spectra of *L*-Phe-$(H_2O)_{0-3}$ with MP2/6-31+G* levels of theory; Fig. 8 shows those of D conformer. As seen in the figures, the calculated *L*-Phe-$(H_2O)_3$ exhibits a cyclic H-bonded structure similar to that of *L*-Phe-$(H_2O)_2$. The incremental red-shift associated with carboxyl hydrogen becomes progressively smaller as the number of water molecules increases. It is very interesting to see that *L*-Phe(D)-$(H_2O)_3$ exhibits a $\pi$-H-bond in contrast to *L*-Phe(A)-$(H_2O)_3$. Consequently, *L*-Phe(D)-$(H_2O)_3$ is energetically more stabilized than *L*-Phe(A)-$(H_2O)_3$. The involvement of the $\pi$-system in stabilizing the binding of the water cluster to the amino acid is quite interesting given the observation that hydrogen bonds involving water molecules and aromatic residues have longer lifetimes [21, 22].

In conclusion, we investigated the structure of the *L*-Phe-$(H_2O)_{n\,=\,1\text{-}3}$ clusters by IR-UV double resonance spectroscopy and theoretical calculations. It was found that conformer D is the minimum energy conformer and that water molecules preferentially form H-bonding to the carboxyl group of the open conformers D and A of *L*-Phe. Thus, of the 6 isomers of *L*-Phe only two 1:1 clusters exist at ultralow temperature conditions while only 1 isomer of the 1:2 cluster is observed. Thus the conformational complexity of isolated *L*-Phe is strongly reduced upon the solvation. The OH stretch frequency of the carboxyl group is drastically reduced by forming cyclic H-bond with two water molecules, suggesting an addition of a few more water molecules may promote a proton transfer reaction resulting in zwitter-ion formation.

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**Figure caption**

**Fig. 1.** 1C-R2PI spectra of jet-cooled *L*- Phe in the region of the vibrational origin of the $S_1$-$S_0$ transition, recorded with the following expansion conditions.

a) Expansion with 2 bar Xe.

b) Expansion with 2 bar Xe and an increased time delay between the laser pulse and the gas pulse, sampling colder regions of the gas pulse.

c) Expansion with 2.5 bar Xe.

d) Expansion with 3 bar He.

e) Expansion with 2 bar Ar.

f) Expansion with 3 bar Ar.

**Fig. 2.** LIF spectrum of jet-cooled *L*-Phe and *L*-Phe-$(H_2O)_n$ obtained under different water vapor pressure condition; (a) without water vapor. (b) water vapor at 0 $^{o}$C. (c) water vapor at room temperature. He gas at a total pressure of 2.5 bar was used as a carrier gas.

**Fig. 3.** (a) Fluorescence detected IR (FDIR) spectrum of *L*-Phe-$(H_2O)_1$ obtained by fixing the UV frequency to band A'. (b)-(d) Structures and IR spectra (stick diagram) for *L*-Phe-$(H_2O)_1$ obtained by DFT calculation at the B3LYP/6-31G* level. The vibrational frequencies were multiplied by a factor of 0.97.

**Fig. 4.** (a) FDIR spectrum of *L*-Phe-$(H_2O)_1$ obtained by fixing the UV frequency to band D'. (b)-(d) Structures and IR spectra for *L*-Phe-$(H_2O)_1$ obtained by DFT calculation. The vibrational frequencies were multiplied by a factor of 0.97.

**Fig. 5.** (a) (left) Structure of conformer E of *L*-Phe. (right) FDIR spectrum of band E and calculated IR spectrum of conformer E. (b) (left) Structure of isomer Ew1-I of *L*-Phe-$(H_2O)_1$.

(right) Calculated IR spectrum of isomer Ew1-I. (c) FDIR spectrum of band D'. (d) (left) Structure of isomer Dw1-I of *L*-Phe-$(H_2O)_1$. (right) Calculated IR spectrum of isomer Dw1-I. The vibrational frequencies were multiplied by a factor of 0.97.

**Fig. 6.** (a) FDIR spectrum of *L*-Phe-$(H_2O)_2$ obtained by fixing the UV frequency to band $\alpha$'. (b)-(d) Structures and IR spectra for *L*-Phe-$(H_2O)_2$ obtained by DFT calculation at the B3LYP/6-31G* level. The vibrational frequencies were multiplied by a factor of 0.97.

**Fig. 7.** FDIR spectra of bare *L*-Phe(A) and *L*-Phe(A)-$(H_2O)_{1\text{-}2}$, and MP2/6-31+G* calculated IR spectra of bare *L*-Phe(A) and *L*-Phe(A)-$(H_2O)_{1\text{-}3}$ in the CH, $NH_2$, and OH region. The calculated values were scaled by a factor of 0.97.

**Fig. 8.** FDIR spectra of bare *L*-Phe(D) and *L*-Phe(D)-$(H_2O)_1$ , and MP2/6-31+G* calculated IR spectra of bare *L*-Phe(D) and *L*-Phe(D)-$(H_2O)_{1\text{-}3}$ in the CH, $NH_2$, and OH region. The calculated values were scaled by a factor of 0.97.

**Table 1** The $S_1 \leftarrow S_0$ transition energies and the MP2 calculated relative zero point vibrational energies of each conformer of *L*-Phe.

| Bands | D | X | A | C | B | E |
|---|---|---|---|---|---|---|
| $S_1 \leftarrow S_0$ [$cm^{-1}$] | 37590 | 37597 | 37528 | 37560 | 37548 | 37604 |
| $\Delta E$(ZPVE) [kJ/mol] | 0.00 | 1.22 | 3.60 | 3.62 | 4.03 | 7.26 |

**Table 2** Infrared frequencies ($cm^{-1}$) of *L*-Phe-$(H_2O)_n$ ($n$ =1,2) in the OH and $NH_2$ stretching region.

| | Obsd. | | DFT[a] | MP2[a] |
|---|---|---|---|---|
| *L*-Phe-$(H_2O)_1$ | Band A' | Assignment | Isomer Aw1-I | |
| | 3723.9 | (Free OH) | 3704.7 | 3749.9(3803.0) |
| | 3505.5 | (H-Bonded OH) | 3466.1 | 3545.2(3544.8) |
| | 3420.9 | ($NH_{2\ \mathrm{asym}}$) | 3454.2 | 3531.2(3511.5) |
| | □ | ($NH_{2\ \mathrm{sym}}$) | 3386.7 | 3437.7(3417.2) |
| | 3245.7 | (Carboxyl OH) | 3207.3 | 3282.3(3298.6) |
| | Band D' | Assignment | Isomer Dw1-I | |
| | 3726.2 | (Free OH) | 3705.7 | 3750.8(3802.9) |
| | 3513.0 | (H-Bonded OH) | 3471.7 | 3552.9(3553.2) |
| | 3402.6 | ($NH_{2\ \mathrm{asym}}$) | 3454.4 | 3524.2(3501.8) |
| | □ | ($NH_{2\ \mathrm{sym}}$) | 3379.6 | 3431.7(3409.0) |
| | 3254.5 | (Carboxyl OH) | 3214.5 | 3297.9(3316.9) |
| *L*-Phe-$(H_2O)_2$ | Band α' | Assignment | Isomer Aw2-I | |
| | 3716.8 | (W1 Free OH) | 3748.1 | 3754.5 |
| | 3680.0 | (W2 Free OH) | 3743.7 | 3745.2 |
| | 3473.5 | (W2 H-Bonded OH) | 3437.0 | 3509.2 |
| | 3401.1 | ($NH_{2\ \mathrm{asym}}$) | 3499.0 | 3528.4 |
| | 3330.1 | (W1 H-Bonded OH) | 3336.0 | 3428.4 |
| | □ | ($NH_{2\ \mathrm{sym}}$) | 3420.4 | 3435.3 |
| | 3002 | (Carboxyl OH) | 3039.7 | 3116.7 |
| | | Assignment | Isomer Dw2-I | |
| | | (W1 Free OH) | | 3761.4 |
| | | (W2 Free OH) | | 3744.4 |
| | | (W2 H-Bonded OH) | | 3531.0 |
| | | ($NH_{2\ \mathrm{asym}}$) | | 3524.2 |
| | | (W1 H-Bonded OH) | | 3446.1 |
| | | ($NH_{2\ \mathrm{sym}}$) | | 3430.9 |
| | | (Carboxyl OH) | | 3136.4 |

[a] Both the B3LYP/6-31+G* the MP2 values were scaled by a factor of 0.97. The calculated MP2 values enclosed in parentheses were obtained using the aug-cc-VDZ basis set and those outside were obtained using the 6-31+G* basis set.

Fig. 1 Ebata et al.

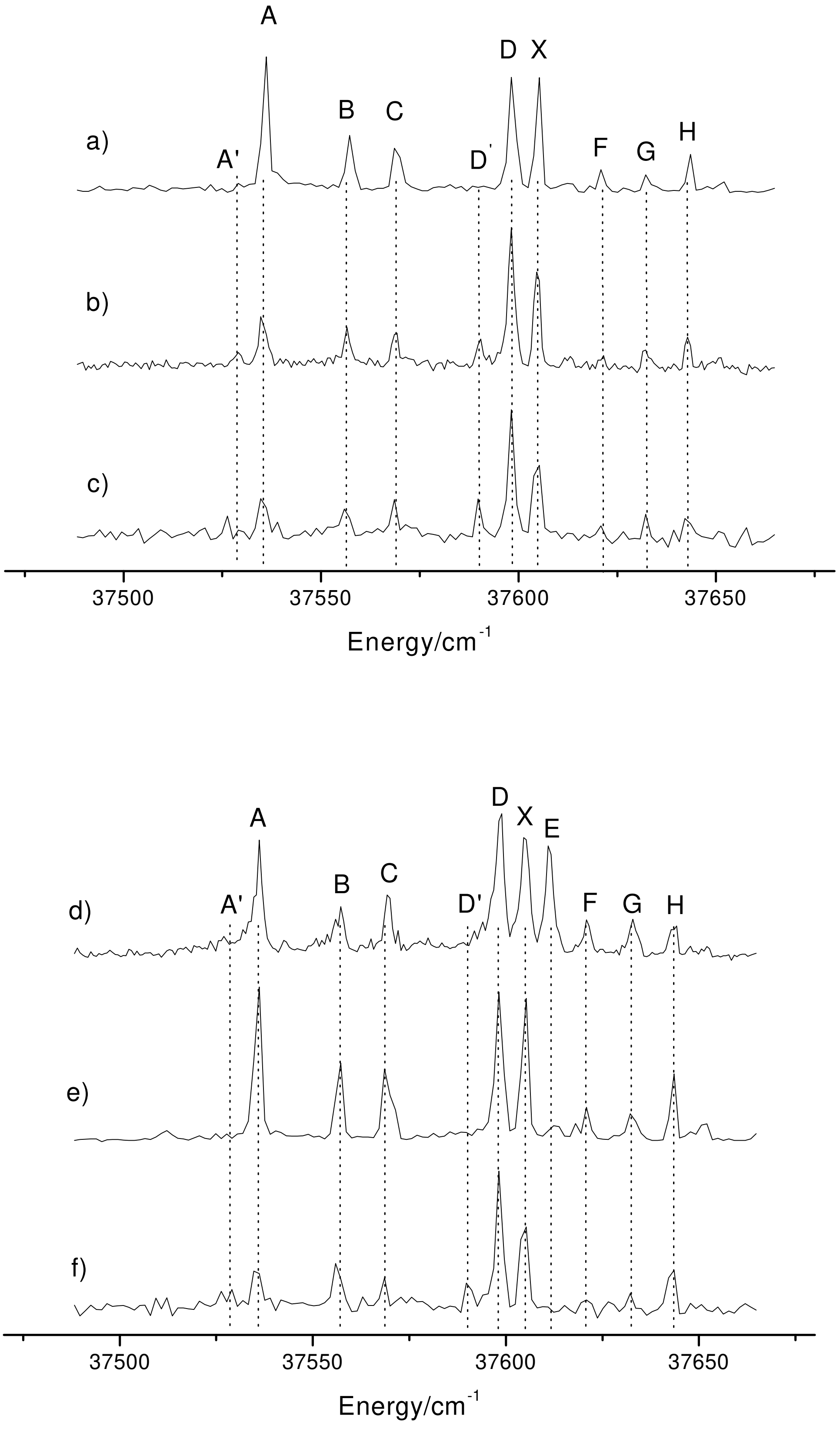

**Fig. 2 Ebata et al.**

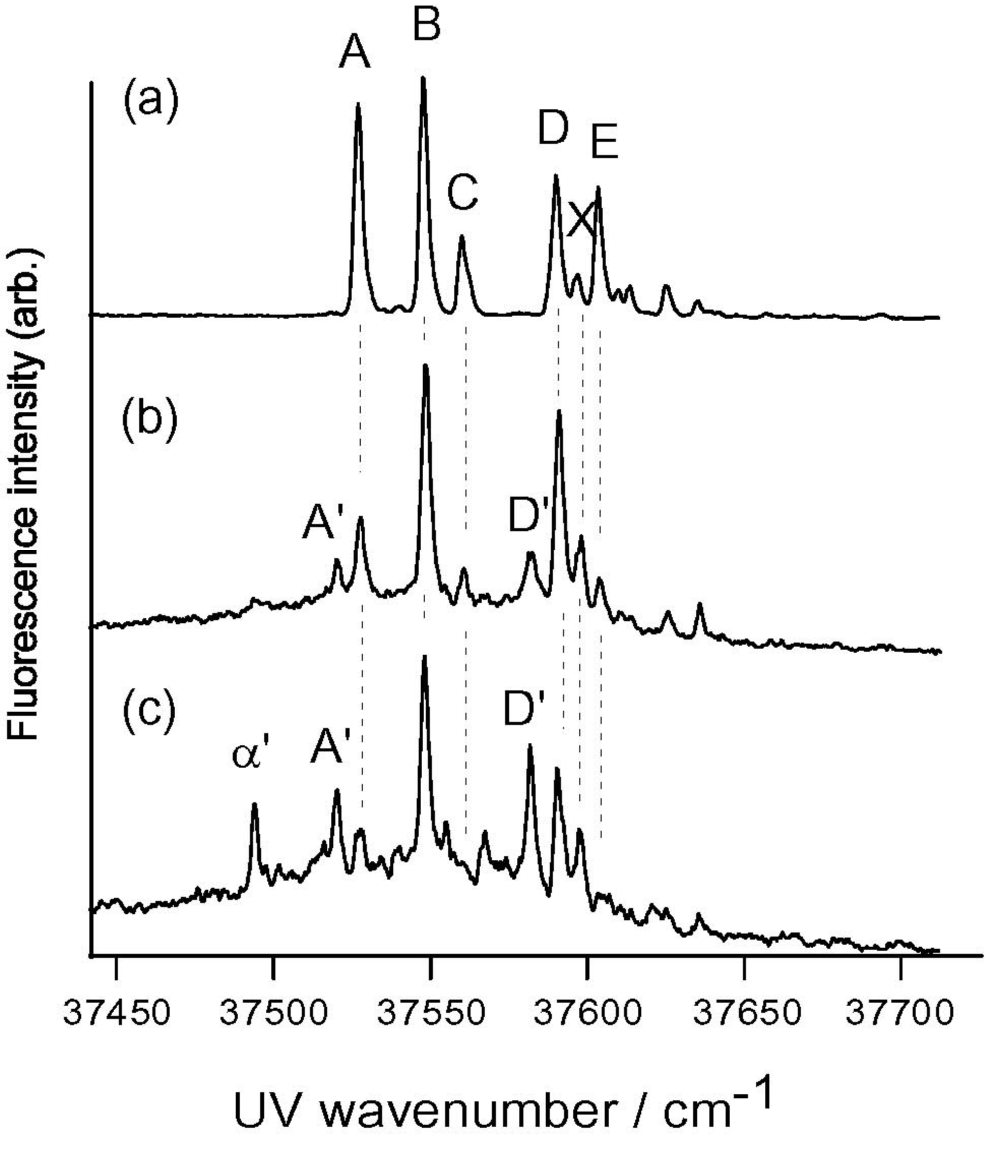

Fig. 3 Ebata et al.

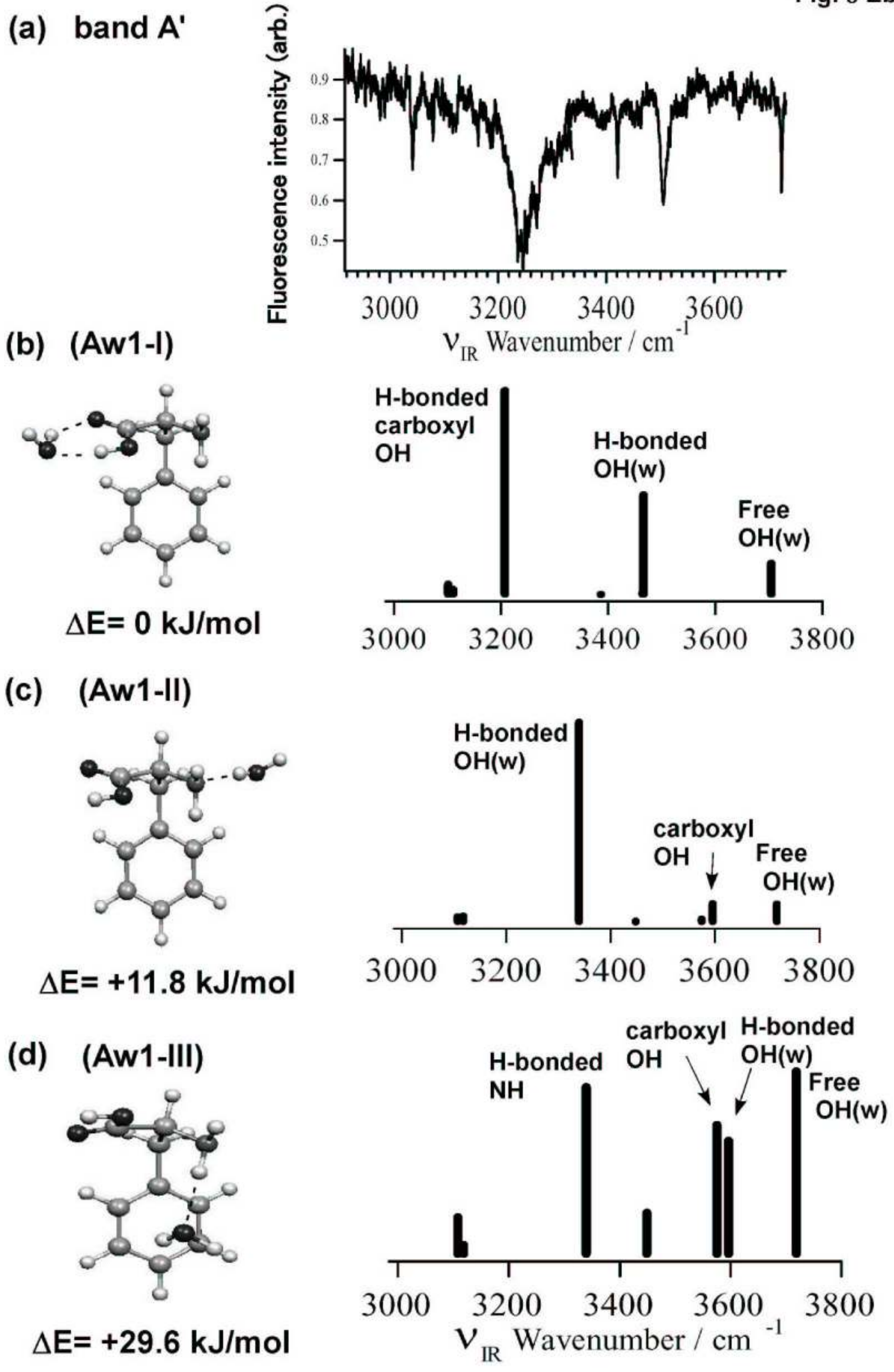

Fig.4 Ebata et al.

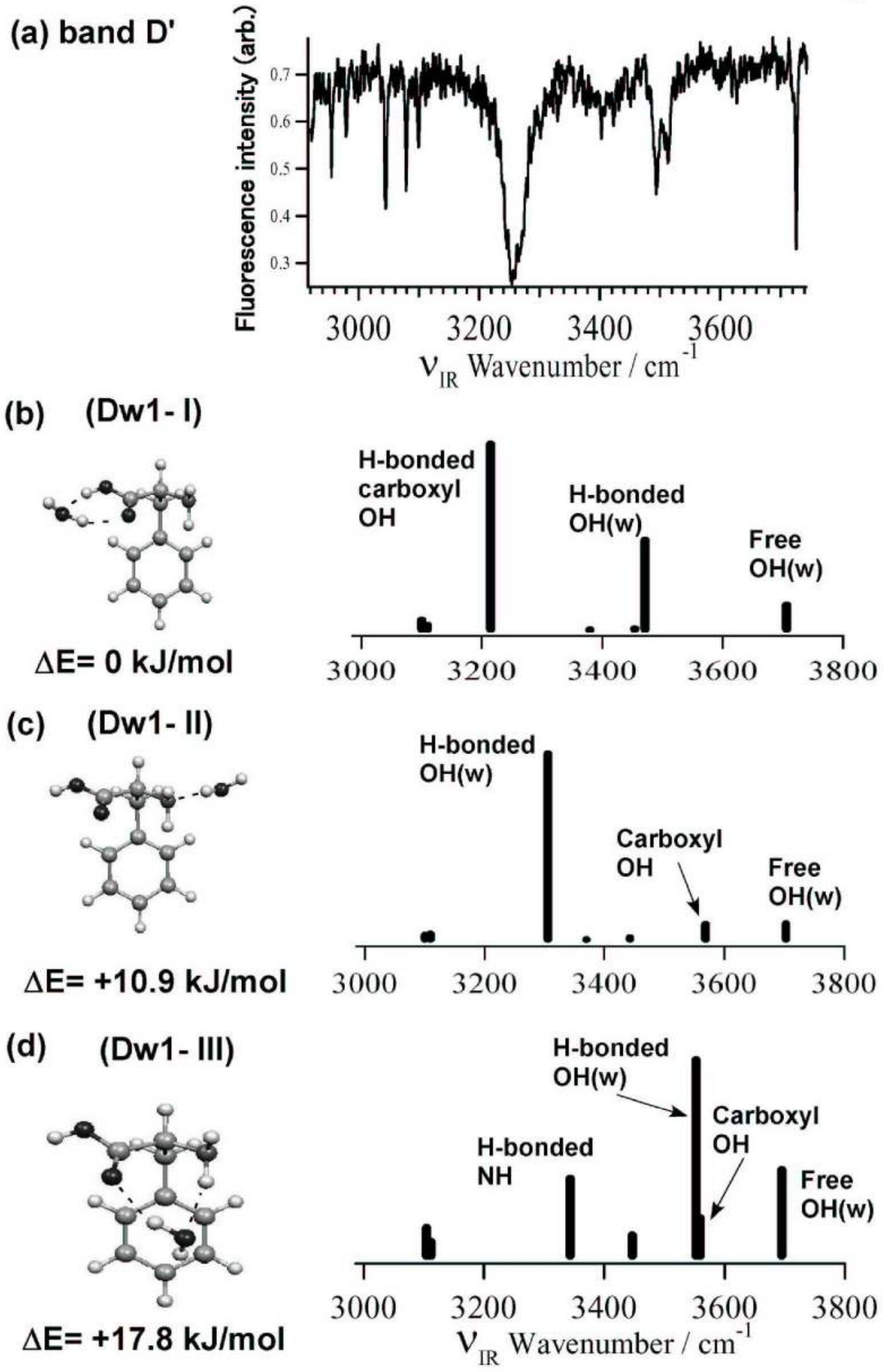

Fig. 5 Ebata et al.

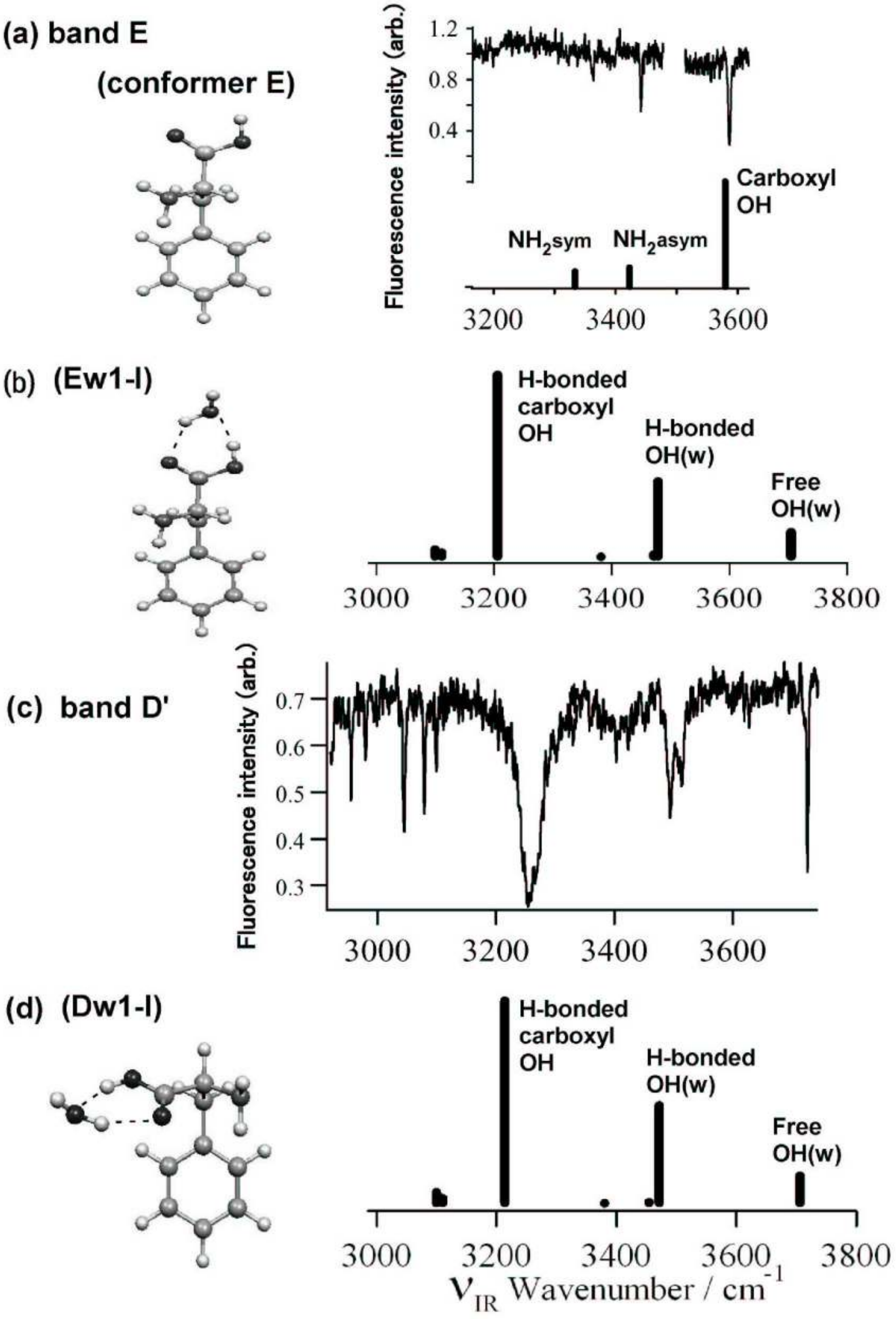

Fig.6 Ebata et al.

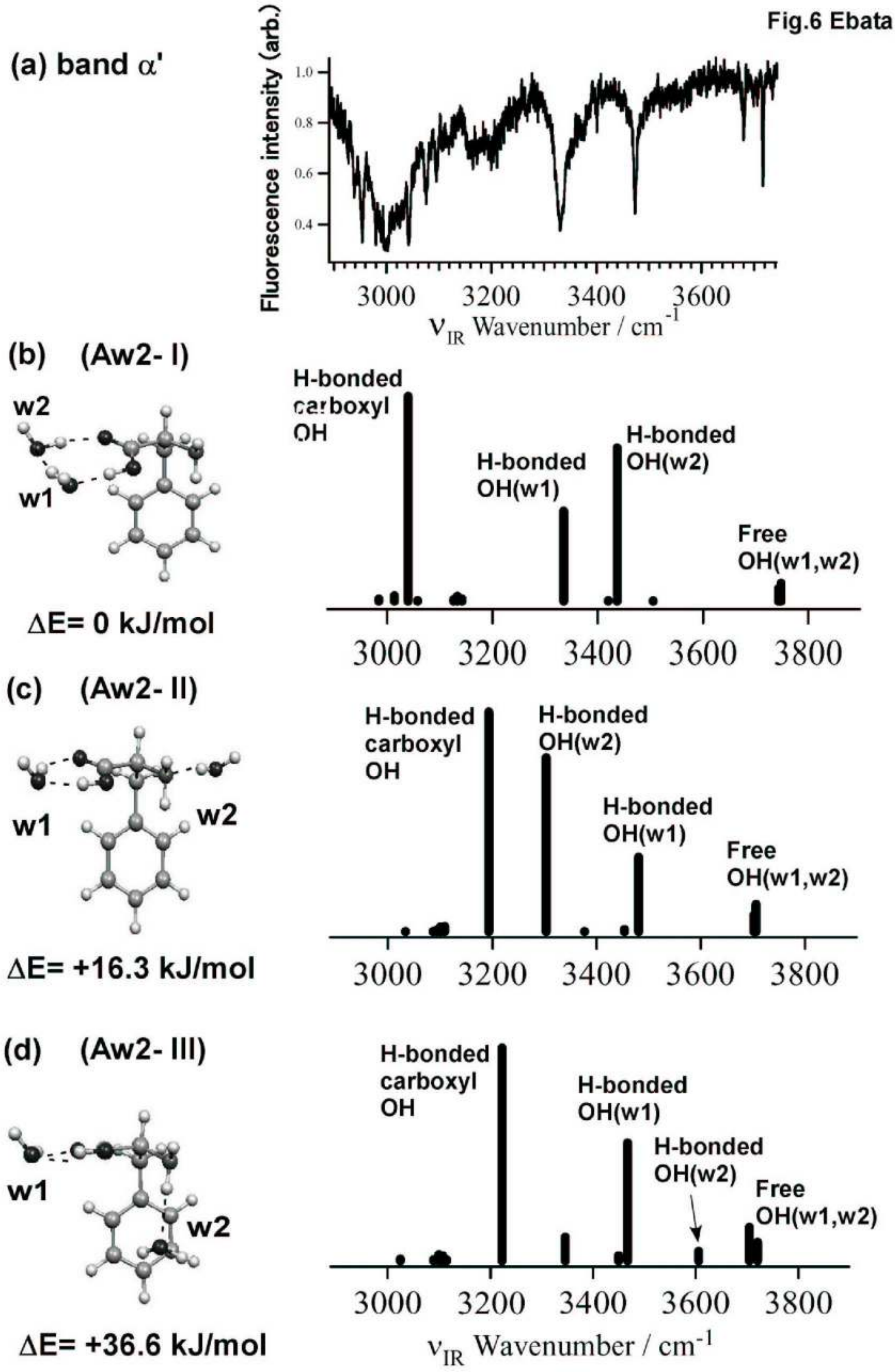

fig. 7 Ebata et al.

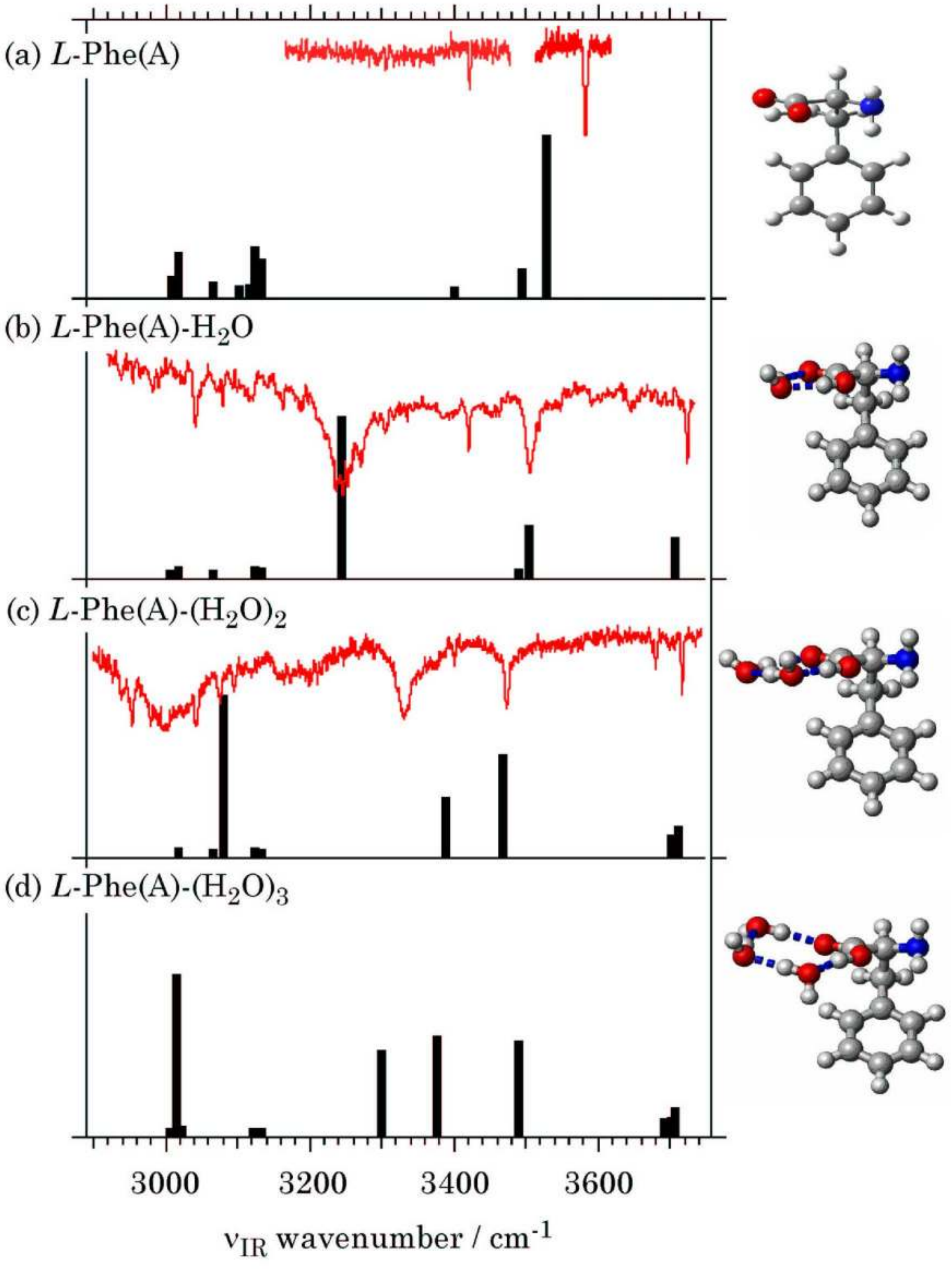

Fig. 8 Ebata et al.