Experimental and theoretical study of Aspartic acid

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Abstract: The aim of this research is to detect zwitterionic structure of the aspartic acid and confirm the experimental spectra with quantum chemical calculations. The experimental IR and Raman spectra of aspartic acid powder show no vibrational bands of OH and NH stretching in expected spectral region. We assume that zwitterionic structure of aspartic acid is responsible for lowering the frequencies of these vibrations. An extensive experimental and computational research supports this assumption. Our DFT calculation strongly suggests the need for the dielectric environment in order to stabilize the zwitterionic structure of a single molecule. The network of intermolecular hydrogen bonding between aspartic acid molecules provides this dielectric environment. The DFT quantum mechanical calculations corroborate this assumption by optimizing a four-member group of molecules, which also gives an explanation of broad IR spectrum lines.

INTRODUCTION

Aspartic acid is one of the twenty amino acids which together form the basic building blocks of proteins, enzymes and other body tissues. Proteolytic enzymes known as aspartic proteases each possess two aspartic acid residues at the active site. Aspartic acid provides the amino group in the urea cycle and in the biosynthesis of purine and synthesis of pyrimidine (Garett and Grisham, 2010). It is a precursor of the pyrimidine nucleotides and, in addition, is a key precursor for the synthesis of asparagine, methionine, lysine, threonine, and isoleucine (Garett and Grisham, 2010). It is known that these amino acids are participating in a variety of biochemical reactions, basic energy transfer and muscle activity and they are used for medical, cosmetic and industrial purposes (Zhu et al., 2011, Patil et al., 2018). It is also found that at higher concentrations aspartic acid affects the lifetime of the initial phase of CaCO₃ crystallization, the amorphous calcium carbonate, through the inhibition of crystal nucleation and growth (Tobler et al., 2014). The knowledge of the vibrational spectra of amino acids is very useful for lots of biochemical studies involving enzymes, proteins and their reactions (Navarrete, Hernández and Ramírez, 1994, Kumar, 2016, Numata et al., 2017). Several authors have demonstrated that the most stable structure for a free amino acid molecule is the nonzwitterionic form (NH₂-CHR-COOH) (Alam and Ahmad, 2012), but it is possible to find a local minimum for the zwitterionic form (NH₃+-CHR-CO₂-) and the calculated frequencies were compared to IR and Raman spectra (Navarrete, Hernández and Ramírez, 1994, Freire et al., 2017, Silva et al., 2015). The molecule structure in solid state has been determinate by x-ray diffraction techniques and it occurs in the crystal as a zwitterion (Derissen, Endeman and Peerdeman, 1968). Single molecule has the absolute minimum of energy in the nonzwitterionic structure but the zwitterionic structure is stabilized with other amino acid molecules in the solid state or with the solvent molecules in a solution (Navarrete, Hernández and Ramírez, 1994, Paxton and Harper, 2004, Nagy and Noszlá, 2000). That property is also observed with smaller amino acids such as alanine and glycine (Ilijima, Tanaka and Onuma, 1991, Alper, Dothe and Coker, 1991, Ilijima and Beaglev, 1991, Gontrani, Mennucci and Tomasi, 2000). Structure of the aspartic acid molecule in a solution also depends on the pH value of the solution (Paxton and Harper, 2004, Castro et al., 1995).

Our motivation to further contribute to this well explored subject is twofold. First, we have noticed there are no vibrational bands of OH and NH stretching in expected experimental spectral region. One possible explanation, as given in literature (Navarrete, Hernández and Ramírez,
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1994), is in vast number of intermolecular hydrogen bonds. However, to apply this explanation on bands at 2510, 2661 and 2729 cm$^{-1}$ the required frequency shift would be over 1000 cm$^{-1}$. The shift of such magnitude ascribed to hydrogen bonding is not commonly found. We argue that alongside hydrogen bonding there are quasi-free proton movements between two oxygen atoms and oxygen and nitrogen atoms. Second, our IR spectra of powder sample persistently showed broad bands, in spite of good spectrometer’s resolution of 1 cm$^{-1}$.

**MATERIALS AND METHODS**

The supplier of ≥ 98% L-aspartic acid was Sigma-Aldrich. To observe interactions experimentally, powder sample was characterized by attenuated total reflectance Fourier transform infrared spectroscopy (ATR FTIR), with Bruker Vertex 70 instrument. The absorbance data were collected between 400 and 4000 cm$^{-1}$ with spectral resolution of 1 cm$^{-1}$ and average of 32 scans. Raman spectra were recorded at room temperature with microtriple grating spectrometer Horiba Jobin Yvon model T64000. The spectra were excited by 514.5 nm line of the Coherent INNOVA-400 argon ion laser. Laser power at the sample was 7 mW.

Calculations

The quantum chemical calculations were performed with the Gaussian 09 package program at DFT–B3LYP level of theory (Frisch et al., 2009). The standard 6-311++G(d,p) basis set was used to carry out the calculation of molecular geometries, force fields, vibrational frequencies, as well as IR intensities and Raman activities.

**RESULTS AND DISCUSSION**

The molecule of aspartic acid is composed of 16 atoms and it does not have any molecule symmetry. It is well known from experimental research (Navarrete, Hernández and Ramírez, 1994) that aspartic acid (ASP) takes zwitterionic form. But DFT calculations of single free molecule fail to give zwitterionic form (Tobler et al., 2014).

Further calculations show that even a small group of molecules creates a necessary dielectric environment which makes all molecules stable in zwitterionic form. In other words, the dielectric environment is self-induced with sufficient dielectric constant. In Figure 2 an optimized geometry for a group of four molecules is shown in zwitterionic form without any specified environment. Stretching of OH and NH groups in our calculations of quadrimer structure have lowered significantly from the values calculated for one zwitterionic ASP molecule. For hydroxy groups surrounded with other molecules, the shifts are in the 600 – 1000 cm$^{-1}$ frequency range. The most IR intensive amino groups stretching have lowered from approximately 3400 cm$^{-1}$ to the 3000 and even to the 2700 cm$^{-1}$. One part of the lowered hydrogen bonding vibrational modes falls in the region of CH and CH$_2$ stretching. The other part of these modes is lowered even...
more, up to the 2500 cm\(^{-1}\). If the hydroxy or amino group is on the edge of the cluster, there is no shift, which can be explained by the lack of the influence of surrounding molecules.

Our biggest calculated cluster consists of four ASP molecules because of numerical limitations. In our powdered sample, we presume that clusters consist of larger number of ASP molecules. Because of that, number of "marginal" hydroxy and amino groups is small in comparison to the hydroxy and amino groups that are surrounded with other molecules. That is consistent with the experimental spectra of powdered samples, in which there are no OH and NH stretching bands at expected frequencies, around 3600 cm\(^{-1}\) and 3400 cm\(^{-1}\) respectively (Figure 3). But there exists a broad line around 3000 cm\(^{-1}\) consisting of CH stretching overlapping with lowered OH and NH stretching. Bands at 2510, 2661 and 2729 cm\(^{-1}\) (Figure 3) can be explained as OH stretching in intermolecular hydrogen bonding within the cluster (Stuart, 2004). Our calculations show these bands correspond to proton movement between O-O and O-N atoms (Figure 2).

![Figure 3. Infrared spectrum of aspartic acid in the 2400 – 4000 cm\(^{-1}\) region](image)

Bands at 2510, 2661 and 2729 cm\(^{-1}\) are interpreted as belonging to proton movements between two oxygen atoms or between oxygen and nitrogen atoms. Higher bands belong to CH and lowered OH stretching.

![Figure 4. Infrared spectrum of aspartic acid in the 400 – 2000 cm\(^{-1}\) region](image)

The main characteristic of our observed infrared spectrum is a composition of broad vibrational bands (Figure 3 and 4). Calculated IR spectrum of aspartic acid cluster also shows grouping of bands (Figure 5). Same vibrations in different molecules of the sample have slightly different frequencies. That is consistent with broader bands observed in experimental FTIR spectra.

Infrared and Raman spectra presented at Figures 4, 6 and 7 show vibrational bands assigned to NH\(^3\)+ group vibrations. Calculated and observed vibrational modes for zwitterionic form of aspartic acid molecule are presented in Table 1. Broad IR band at 1509 cm\(^{-1}\) belongs to NH\(^3\)+ asymmetrical bending. In the frequency range from 989 to 1247 cm\(^{-1}\), we observe, both in IR and Raman spectra, NH\(^3\)+ rocking combined with other modes. This is generally consistent with the results of past research although they have used different computational method (Navarrete, Hernández and Ramírez, 1994.)

![Figure 5. Calculated infrared spectrum of four members cluster presented in Fig. 2](image)

![Figure 6. Raman spectrum of aspartic acid in 2700 – 3600 cm\(^{-1}\) region](image)
Figure 7. Raman spectrum of aspartic acid in 400 – 1900 cm⁻¹ region

Table 1. Calculated and observed frequencies (cm⁻¹) for zwitterionic form of aspartic acid molecule

|   | IR    | Raman   | Calculated<sup>b</sup> | Vibrational mode                      |
|---|-------|---------|------------------------|---------------------------------------|
| 1 | 3722  | 3722    | 3722                   | OH str                                |
| 2 | 3507  | 3507    | 3507                   | NH str                                |
| 3 | 3353  | 3353    | 3353                   | NH<sub>3</sub> asym str               |
| 4 | 3069 w | 3069 w  | 3050                   | NH<sub>3</sub> sym str                |
| 5 | 3013 m | 3013 m  | 3013 m                | CH<sub>2</sub> asym str               |
| 6 | 2996 vs| 2996 vs | 2996 vs               | CH str                                |
| 7 | 2955 vs| 2955 vs | 2955 vs               | CH<sub>2</sub> sym str                |
| 8 | 1687 s | 1687 s  | 1687 s                | C=O str, NH<sub>3</sub> asym bend     |
| 9 | 1640 w | 1640 w  | 1640 w                | NH<sub>3</sub> asym bend, COO⁻ asym str|
|10 | 1618 w | 1618 w  | 1618 w                | COO⁻ asym str, NH<sub>3</sub> asym bend|
|11 | 1508 w | 1508 w  | 1508 w                | NH<sub>3</sub>⁺ asym bend             |
|12 | 1462 w | 1462 w  | 1462 w                | CH<sub>2</sub> sciss                   |
|13 | 1423 s | 1423 s  | 1423 s                | NH<sub>3</sub>⁺ sym bend              |
|14 | 1406   | 1406   | 1406                  | CO str, OH bend, CH<sub>2</sub> wag, CC str, CH bend |
|15 | 1396   | 1396   | 1396                  | CH bend                               |
|16 | 1330   | 1330   | 1330                  | CC str, COO⁻ sym str, NH<sub>3</sub> sym bend, CH bend |
|17 | 1330   | 1330   | 1330                  | CH<sub>2</sub> wagg, OH bend, CH bend  |
|18 | 1281   | 1281   | 1281                  | CH<sub>2</sub> twist, OH bend, CH band |
|19 | 1250   | 1250   | 1250                  | CH<sub>2</sub> twist, CH bend, NH<sub>3</sub> rock, COH bend |
|20 | 1183   | 1183   | 1183                  | OH bend, CH bend, NH<sub>3</sub> rock, CO str, CH<sub>2</sub> twist |
|21 | 1142   | 1142   | 1142                  | CH bend, NH<sub>3</sub> rock, CH<sub>2</sub> twist, OH bend |
|22 | 1103   | 1103   | 1103                  | NH<sub>3</sub> rock, CH bend, CH<sub>2</sub> twist |
|23 | 1049   | 1049   | 1049                  | CN str, CC str                        |
|24 | 964    | 964    | 964                   | CC str, NH<sub>3</sub> rock            |
|25 | 928    | 928    | 928                   | CC str, CCC bend                      |
|26 | 870    | 870    | 870                   | CC str, COH bend                      |
|27 | 843    | 843    | 843                   | CN str, CC str                        |
|28 | 765    | 765    | 765                   | OCO⁻ out of plane bending             |
|29 | 725    | 725    | 725                   | OCOH out of plane bending, COO⁻ in plane bend. |
|30 | 664    | 664    | 664                   | OCOH out of plane bending             |
|31 | 622    | 622    | 622                   | OCOH out of plane bending             |
|32 | 551    | 551    | 551                   | OCOH out of plane bending             |

<sup>a</sup> Observed in IR and Raman spectra of aspartic acid powdered sample. Abbreviations used: s, strong; m, moderate; w, weak; v, very; sh, shoulder

<sup>b</sup> The computed nonscaled values are calculated using B3LYP/6-311G++(d,p) method.
Table 2. The most relevant vibrational modes and frequencies (cm⁻¹) of group of four aspartic acid molecules

| Calculated | Observed | Vibrational mode |
|------------|----------|------------------|
| ASP quadrimer | ASP zwitterionic | IR | Raman | OH stretching |
| 3751, 3749, 3351, 2821 | 3722 | | | |
| 3527, 3481, 3456, 3447, 3336, 3290, 3254, 3031, 2988, 2966, 2762, 2714 | 3507, 3353, 3279 | | | NH stretching |
| 1650, 1623, 1590, 1553 | 1626 | 1509 s | 1508 w | NH₃⁺ asymmetric bending |
| 1512, 1484, 1478, 1443 | 1434 | 1419 s | 1423 s | NH₃⁺ symmetric bending |
| 1185, 1176, 1051, 893 | 1183 | 935 w | 939 s | OH in-plane bending OH out-of-plane bending |

The vibrational modes shown in Table 2 can explain broadening of observed infrared bands. As can be seen, the calculated frequencies of a four-member group can have a bandwidth of around 100 cm⁻¹. The broadening is mostly emphasized for NH₃⁺ asymmetric bending.

A strong Raman band at 939 cm⁻¹ is empirically assigned to OH out-of-plane bending (Zhu et al., 2011, Navarrete, Hernández and Ramírez, 1994, Castro et al., 1995). The calculation performed on single zwitterionic molecule shows very weak presence of this mode which is significantly entangled with skeletal vibrations, but shows strong presence of OH in-plane mode at 1183 cm⁻¹. However, the calculation performed on aspartic acid quadrimer shows both OH in-plane and OH out-of-plane bending as strong modes. The OH groups on cluster’s border show in-plane bending at higher frequencies (1185 and 1176 cm⁻¹ are very close to 1183 cm⁻¹ belonging to single zwitterionic molecule) while the OH group in the cluster’s interior clearly show out-of-plane bending at significantly lower frequencies which are in agreement with observed frequencies (see Table 2).

CONCLUSION

The main points of this work can be summarized as:

- The OH and NH stretching modes frequencies are significantly shifted to lower values within the powder of aspartic acid. Both experimental and calculated spectra prefer zwitterionic form of aspartic acid molecules.
- The DFT optimization of a single ASP zwitterionic molecule can be achieved only in dielectric environment with εr ≥ 3. The optimization of a group of aspartic acid molecules in zwitterionic form is possible due to the self-induced dielectric environment.
- The grouping of molecules is responsible for broadening of vibrational bands in infrared spectrum. This is confirmed by DFT calculations.
- The complexity of intermolecular hydrogen bonding is the main cause for the broadening of spectral lines and their significant shifting to lower values.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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Summary/Sažetak

Cilj našeg istraživanja je detekcija zwitterionske strukture asparaginske kiseline i potvrđivanje eksperimentalnih spektara s kvantno kemijskim proračunima. Eksperimentalni IR i Raman spektri asparaginske kiseline u prahu ne pokazuju OH i NH vibracijske vrpce u očekivanoj spektralnoj području. Pretpostavljamo da je zwitterionska struktura asparaginske kiseline odgovorna za snižavanje frekvencija tih vibracija. Opsežna eksperimentalna i računalna istraživanja podupiru tu pretpostavku. Izračun DFT-a snažno upućuje na potrebu za dielektričnom okolinom kako bi se stabilizirala zwitterionska struktura jedne molekule. Mreža intermolekularnih vodikovih veza između molekula asparaginske kiseline osigurava ovo dielektrično okruženje. DFT kvantno-mehanički izračuni potkrjepljuju ovu pretpostavku optimiranjem četveročlane skupine molekula, što također daje objašnjenje širokih linija IR spektra.