



Experimental and theoretical study of Aspartic acid

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Article info

Received: 26/03/2019

Accepted: 17/06/2019

Keywords:

Aspartic acid

FTIR

Raman

Zwitterion

Quantum chemistry

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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 non zwitterionic 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 Noszál, 2000). That property is also observed with smaller amino acids such as alanine and glycine (Iijima, Tanaka and Onuma, 1991, Alper, Dothe and Coker, 1991, Iijima and Beagle, 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,

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 $\geq 98\%$ 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).

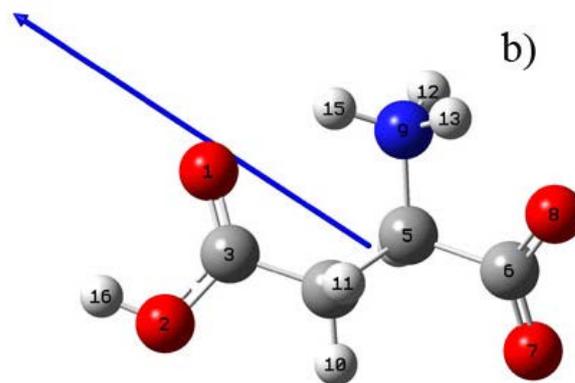
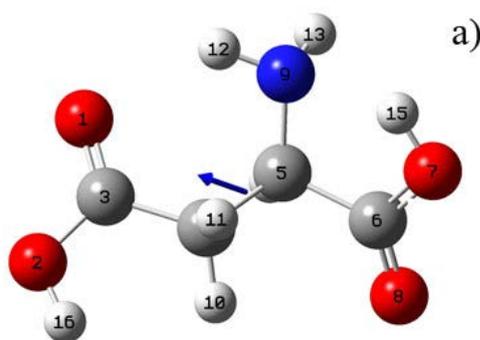


Figure 1. a) Single free molecule of aspartic acid. b) Zwitterionic structure of aspartic acid. The blue arrow line represents dipole moments

For a single free molecule ($\epsilon = 1$), the only way to obtain its zwitterionic structure is to place it in dielectric environment. Our first attempt was to calculate it in aqueous environment, by setting the relative dielectric constant to 82. Detailed inspection of relative dielectric constant set the low limit around $\epsilon = 3$ for stability of zwitterionic structure.

The dipole moments of these two forms significantly differ in magnitude but not in direction. Just for comparison, the dipole moment of single free molecule is 1.6 D and for zwitterionic conformation is around 7 D for $\epsilon = 3$ (Fig. 1).

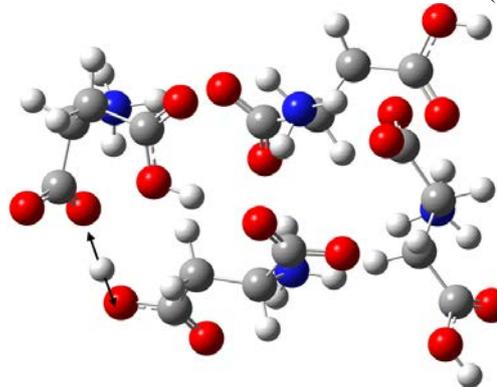


Figure 2. Optimized structure of four aspartic acid molecules in zwitterionic conformation. The arrows show proton movement in intermolecular hydrogen bonding corresponding to bands in the 2500 – 2700 cm^{-1} range

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).

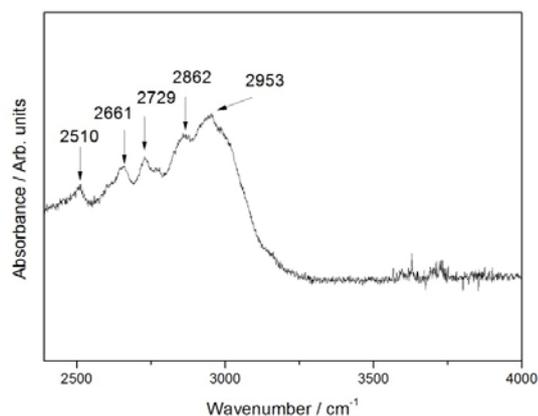


Figure 3. Infrared spectrum of aspartic acid in the $2400 - 4000\text{ cm}^{-1}$ region

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.

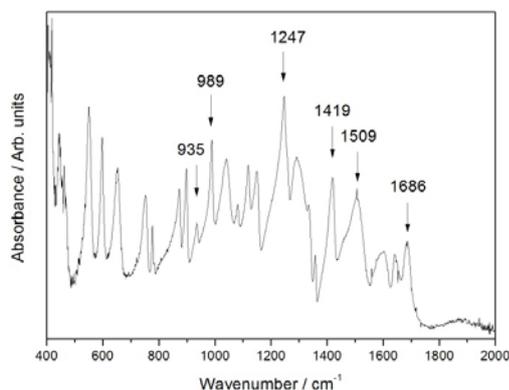


Figure 4. Infrared spectrum of aspartic acid in the $400 - 2000\text{ cm}^{-1}$ region

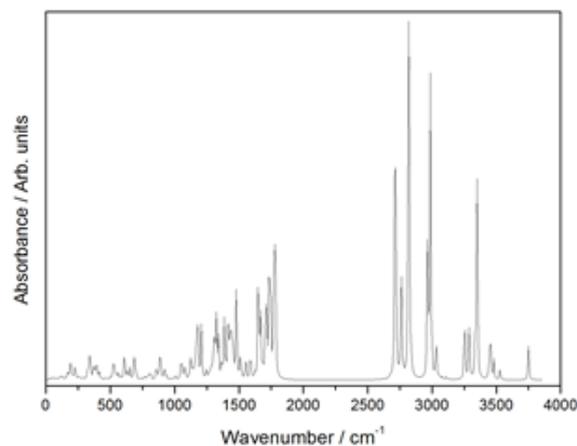


Figure 5. Calculated infrared spectrum of four members cluster presented in Fig. 2

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.)

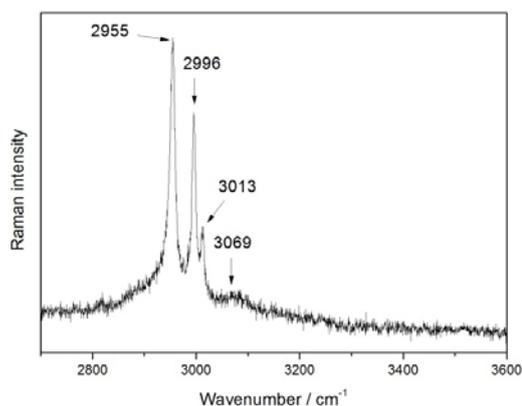


Figure 6. Raman spectrum of aspartic acid in the $2700 - 3600\text{ cm}^{-1}$ region

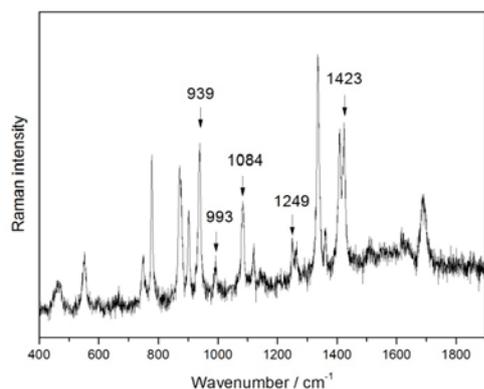


Figure 7. Raman spectrum of aspartic acid in 400 – 1900 cm^{-1} region

Table 1. Calculated and observed frequencies (cm^{-1}) for zwitterionic form of aspartic acid molecule

	Observed ^a		Calculated ^b	Vibrational mode
	IR	Raman		
1			3722	OH str
2			3507	NH str
3			3353	NH ₃ asym str
4		3069 w	3279	NH ₃ sym str
5	3018 w, sh	3013 m	3136	CH ₂ asym str
6	2985 w, sh	2996 vs	3127	CH str
7	2953, s	2955 vs	3037	CH ₂ sym str
8	1686 s	1687 s	1743	C=O str, NH ₃ asym bend
9	1641 m, broad	1640 w	1674	NH ₃ asym bend, COO ⁻ asym str
10		1618 w	1661	COO ⁻ asym str, NH ₃ asym bend
11	1507 s, broad	1508 w	1626	NH ₃ ⁺ asym bend
12	1459 w, sh	1462 w	1460	CH ₂ sciss
13	1419 s	1423 s	1434	NH ₃ ⁺ sym bend
14	1408 sh	1409 s	1406	CO str, OH bend, CH ₂ wag, CC str, CH bend
15	1358 m	1362 m	1396	CH bend
16	1334 m	1335 s	1350	CC str, COO ⁻ sym str, NH ₃ sym bend, CH bend
17	1291 broad		1330	CH ₂ wagg, OH bend, CH bend
18	1258 sh	1263 w	1281	CH ₂ twist, OH bend, CH band
19	1247 s	1249 m	1250	CH ₂ twist, CH bend, NH ₃ rock, COH bend
20	1149 m	1143 w	1183	OH bend, CH bend, NH ₃ rock, CO str, CH ₂ twist
21	1118 m	1121 m	1142	CH bend, NH ₃ rock, CH ₂ twist, OH bend
22	1081 w	1084 m	1103	NH ₃ rock, CH bend, CH ₂ twist
23	1042 m broad		1049	CN str, CC str
24	989 s	995 m	964	CC str, NH ₃ rock
25	935 w	939 s	928	CC str, CCC bend
26	897 m	903 m	870	CC str, COH bend
27	872 m	872 s	843	CN str, CC str
28	777 m	778 s	765	OCO ⁻ out of plane bending
29	752 m	752 m	725	OCOH out of plane bending, COO ⁻ in plane bend.
30	653 m	658 vw	664	OCOH out of plane bending
31	598 s	600 vw	622	OCOH out of plane bending
32	550 s	552 m	551	OCOH out of plane bending

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

^b 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	
3751, 3749, 3351, 2821	3722			OH stretching
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	NH_3^+ asymmetric bending
1512, 1484, 1478, 1443	1434	1419 s	1423 s	NH_3^+ 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 NH_3^+ 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 $\epsilon \geq 3$. 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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Acknowledgment

We thank prof. Vesna Volovšek, PhD and Assist. prof. Vilko Mandić, PhD from Faculty of Chemical Engineering and Technology, University of Zagreb, for help in obtaining experimental data of Raman and IR spectroscopy.

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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 očekivanom spektralnom 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 potkrepljuju ovu pretpostavku optimiranjem četveročlane skupine molekula, što također daje objašnjenje širokih linija IR spektra.