

## A DFT Study of the Intramolecular Hydrogen Bonding of Alanine and Its Effects on Ionization Energies

Gab-Yong Lee

Department of Life Chemistry, Catholic University of Daegu, Gyeongsan 712-702, Korea.

E-mail: gylee@cu.ac.kr

(Received September 3, 2015; Accepted October 11, 2015)

**Key words:** Alanine, Intramolecular hydrogen bond, Ionization energy, Electronic structure, *Ab initio* calculation

Alanine is the most significant amino acid detected in proteins, and it exists in the zwitterionic form in the solid phase. However, it has been determined through experimental studies<sup>1,2</sup> that only a neutral form of alanine exists in the gas phase. Computational studies have predicted 13 possible conformers for neutral alanine.<sup>3,4</sup>

Hydrogen bond in amino acids is highly important in decision the three-dimensional structure of proteins as well as for the thermodynamics of the biochemical molecules. It also plays an important role in protein folding and peptide assembly.<sup>5</sup> Experimental studies have shown that the ionization energy (IE) of a lone-pair orbital (n) in a molecule with intramolecular hydrogen bond is generally higher than that of a molecule with no such bond.<sup>6</sup> However, the effect of intramolecular hydrogen bonding on IEs is difficult to determine experimentally because of the fact that amino acids coexist as several conformers.<sup>7,8</sup>

The IE values of isolated amino acids have been obtained by gas-phase ultraviolet photoelectron (UP) spectroscopy experiments.<sup>7</sup> Tian *et al.*<sup>9</sup> assigned the UP spectrum of proline based on *ab initio* electron propagator theory.<sup>10</sup> However, to date, the UP spectrum of alanine<sup>11</sup> has not been theoretically analyzed. Here, we report the assignment of the UP spectrum of alanine, based on the electron propagator theory. Our aim was to optimize and estimate vertical ionization energy (IE<sub>v</sub>) values for a few stable alanine conformers, and to compare them with experimental values. We also examined the effect of intramolecular hydrogen bonding on the IEs of alanine.

Among the 13 possible conformers for the neutral alanine, the equilibrium geometries of the four most stable structures were taken from a previous study<sup>12</sup> as the beginning points for structural optimization.

All theoretical calculations on the structures were performed using the Gaussian 09 series of programs<sup>13</sup> using

Dunning's aug-cc-pVDZ basis set.<sup>14</sup> DFT calculations were carried out at the B3LYP, BLYP, and BP86 levels,<sup>15-17</sup> and *ab initio* calculations were carried out with the MP2 level of theory. Unrestricted open-shell methods were used to calculate the ionized conformers. Equilibrium geometries of each structure were fully optimized without any restriction on the symmetries. Vibration frequencies were also computed to confirm the characteristics of the critical point on the potential energy surface (PES). The stationary structures were identified by possessing no imaginary values of the harmonic frequency.

The IE<sub>v</sub> for each conformer can be evaluated by freezing the optimized geometries of the ground state and removing one electron from the HOMO. IE<sub>v</sub> is estimated as:

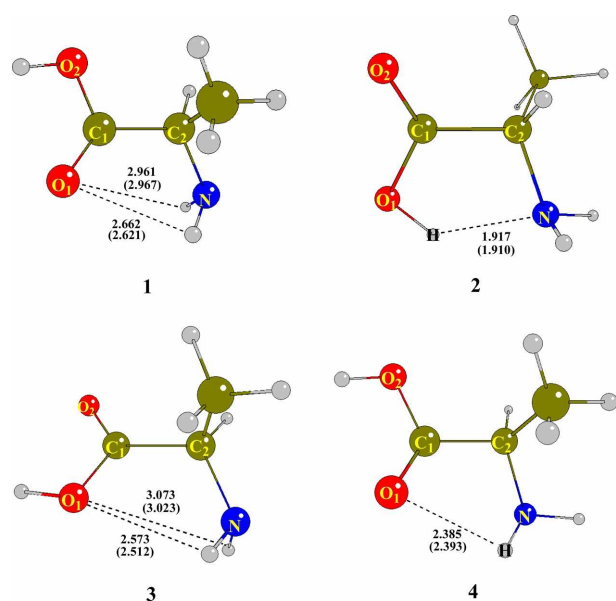
$$IE_v = E(A) - E^+(A)$$

where E(A) and E<sup>+</sup>(A) are the total electronic energies of the neutral and cationic conformers, respectively, at the neutral equilibrium structure.

Quasiparticle approximations in the electron propagator theory are convenient extensions of Koopmans' theorem for calculating electron binding energies.<sup>10</sup> These methods provide useful assignments of the photoelectron spectra of closed-shell molecules for IEs lower than 20–25 eV.<sup>18</sup> In this study, the IE<sub>v</sub> values were calculated in the partial third-order (P3) quasiparticle approximation and from Koopmans' theorem of the electron propagator theory.

The optimized geometrical structures at the B3LYP and MP2 levels for the four most stable conformers are shown in Fig. 1.

The optimized structural results at these levels of theory for the two lowest conformers of neutral alanine (structures 1 and 2 in Fig. 1) are in good agreement with the geometric parameters obtained from electron-diffraction experiments.<sup>11</sup> Conformer 1 has bifurcated hydrogen bonds from the NH<sub>2</sub>



**Figure 1.** B3LYP optimized geometries of the four lowest conformers of alanine. The distances are in Å and the values in parentheses are at the MP2 level.

group to the carbonyl O atom. Conformer 2 has a hydrogen bond from the OH group acting as a proton donor to the N lone pair acting as a proton acceptor. This structural tendency is analogous to experimental observations as seen in the microwave studies of the glycine structure.<sup>19–21</sup> Distance of intramolecular hydrogen bond for the conformer 2 is calculated to be 1.917 Å at the B3LYP level (1.910 Å at the MP2 level). Dihedral angle ( $\text{HO}_1\text{C}_1\text{N}$ ) is calculated to be  $-5.2^\circ$  at the B3LYP level. This result is due to a reduction in the repulsion between the  $\text{CH}_3$  group and the carbonyl oxygen atom.<sup>22</sup>

The absolute and relative energies of the four lowest conformers are listed in Table 1.

Table 1 shows that conformer 1 is the global minimum

structure of the potential energy at the B3LYP and MP2 levels, whereas conformer 2 has a global minimum structure at the BLYP and BP86 levels. The energy difference between the calculation methods is due to the correlation functionals. However the energy difference between conformers 1 and 2 was found to be very small ( $< 0.1$  kcal/mol at the B3LYP and MP2 levels, and  $< 0.2$  kcal/mol at the BLYP level). This implies that conformers 1 and 2 are in a nearly degenerate state. The degeneracy of these two conformers has been previously reported in different papers.<sup>3,12,22,23</sup>

Some IEs calculated for the four optimized conformers are compared with the experimental values<sup>7</sup> reported elsewhere in Table 2.

Alanine has lone-pair orbitals associated with both the N and O atoms. The N atom has a lone-pair orbital ( $n_N$ ), which is in the molecular plane. In the carboxyl group, one non-bonding O orbital ( $n_O$ ), is localized on the O atom of the carbonyl group and lies in the molecular plane, whereas the lone pair associated with the OH group is perpendicular to the molecular plane and interacts with the  $\pi$  orbitals of the carbonyl group. This orbital is labeled  $\pi_{OO}$  in amino acids. Mulliken population analyses of single positively charged amino acids show that the first ionized electron originates from the amino group of the amino acids.<sup>24</sup>

In this study, cations 1(+) and 4(+) corresponding to conformers 1 and 4, respectively, in Fig. 1 have not been found in their lowest energy form on the PES at the MP2 and BLYP levels, respectively. The optimization of ionized 4 converts it to 3(+) at the BLYP level by a facile rotation of the COOH group, and ionized 1 converts to structure 4(-) at the MP2 level by a similar internal rotation about the carbon-nitrogen single bond. Therefore, the direct IEs for conformers 1 and 4 were not obtained at the MP2 and BLYP levels, respectively. This tendency is similar to that of other reported glycine cations.<sup>25</sup>

**Table 1.** Total energies (in a.u.) of the four most stable conformers of neutral alanine and the corresponding cationic alanine at the various levels with aug-cc-pVDZ basis set

Conformer	B3LYP	BLYP	BP86	MP2
Neutral				
1	-323.801178(0.00)	-323.696413(0.00)	-323.803780(0.00)	-322.928393(0.00)
2	-323.801060(0.07)	-323.696674(-0.16)	-323.805505(-1.08)	-322.928323(0.04)
3	-323.799404(1.11)	-323.694808(1.01)	-323.801993(1.12)	-322.926664(1.08)
4	323.799258(1.21)	323.694442(1.24)	323.801868(1.20)	322.926567(1.15)
Cation				
1(-)	323.467411	323.375092	323.476294	
2(-)	323.465922	323.377268	323.478610	322.586295
3(-)	-323.477897	-323.388111	-323.489819	-322.589627
4(-)	323.474024		323.481287	322.595106

<sup>a</sup>The values in parentheses are relative energies in kcal/mol.

**Table 2.** Calculated vertical ionization energies (eV) at various levels for the four most stable conformers of alanine compared to experimental values

		B3LYP		BLYP		BP86		MP2	
		Koopmans	P3	Koopmans	P3	Koopmans	P3	Koopmans	P3
1	$n_N$	11.15(10.26)	9.75	11.20(10.40)	9.80	11.23(10.33)	9.83	11.23(10.33)	9.82
	$n_O$	12.54(11.54)	10.75	12.58(11.58)	10.80	12.58(11.57)	10.79	12.58(11.57)	10.79
	$\pi_{OO}$	13.24(12.18)	12.00	13.22(12.16)	11.97	13.22(12.16)	11.98	13.23(12.18)	12.00
2	$n_N$	11.45(10.54)	9.94	11.49(10.57)	9.97	11.45(10.53)	9.92	11.55(10.63)	10.01
	$n_O$	12.48(11.48)	11.11	12.48(11.48)	11.14	12.46(11.47)	11.17	12.46(11.47)	11.11
	$\pi_{OO}$	12.75(11.73)	11.26	12.73(11.71)	11.21	12.78(11.76)	12.22	12.73(11.71)	11.25
3	$n_N$	10.97(10.09)	9.52	11.05(10.17)	9.60	11.05(10.17)	9.60	11.04(10.16)	9.59
	$n_O$	13.02(11.97)	11.36	12.99(11.95)	11.27	13.02(11.98)	11.44	13.04(12.00)	11.51
	$\pi_{OO}$	13.17(12.12)	11.80	13.20(12.15)	11.88	13.17(12.11)	11.73	13.17(12.12)	11.69
4	$n_N$	10.88(10.01)	9.52	10.95(10.07)	9.58	10.94(10.07)	9.58	13.97(10.09)	9.96
	$n_O$	12.33(11.35)	10.68	12.35(11.36)	10.70	12.37(11.38)	10.72	12.35(11.37)	10.71
	$\pi_{OO}$	13.25(12.19)	11.98	13.22(12.16)	11.94	13.24(12.18)	11.96	13.22(12.16)	11.95
Direct <sup>b</sup>									
1		9.08		8.74		8.91		–	
2		9.11		8.69		8.90		9.31	
3		8.75		8.35		8.49		9.17	
4		8.85		–		8.72		9.02	
Exp. <sup>c</sup>									
	$n_N$	9.85							
	$n_O$	11.0							
	$\pi_{OO}$	12.1							

<sup>a</sup>The values in parentheses computed using Koopmans' theorem<sup>26</sup> and the 8% rule.<sup>27</sup>

<sup>b</sup>Calculated ionization energy from  $E(A) - E(A^+)$ .

<sup>c</sup>From ref. 8.

In Table 2, the P3 IEs in various DFT and MP2 levels are in accordance with the experimental results. Also, for all conformers, Koopmans values were found to be considerably overestimated, while direct IEs were underestimated. The average P3 value for the first IE of conformers 1 and 2 at the B3LYP level was calculated to be 9.845 eV, which is in excellent agreement with the experimental value (9.85 eV, with an error margin of 0.1%). The average value (10.93 eV) for the next IE of conformers 1 and 2 was only 0.07 eV less than that of the experimental value (11.0 eV). The average value (1.085 eV) of the difference between the first and the next orbital for conformers 1 and 2 is also similar to the experimental peak split (1.15 eV). This means that the peaks observed at 9.85 and 11.0 eV are due to the HOMO ( $n_N$ ) and the next HOMO ( $n_O$ ) of alanine, respectively.

The effect of intramolecular hydrogen bonding on the IE was further studied using P3 approximation by changing the value of the H-O<sub>1</sub>-C<sub>1</sub>-N dihedral angle of conformer 2, while the residual geometrical parameters were kept fixed. The change in the IE at different H-O<sub>1</sub>-C<sub>1</sub>-N dihedral angles at the B3LYP level, along with the computed hydrogen

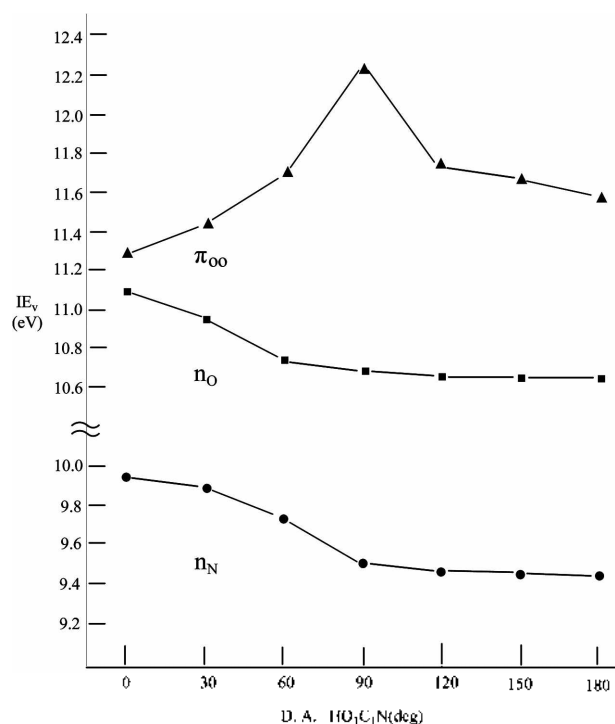
**Table 3.** Variation of vertical ionization energies (IE<sub>v</sub> in eV) and hydrogen bond distances (Å) with changing HO<sub>1</sub>C<sub>1</sub>N dihedral angles (D.A. in Degrees) for conformer 2 at the B3LYP level<sup>a</sup>

D. A.	$r(\text{H-N})^b$	IE		
		$n_N$	$n_O$	$\pi_{OO}$
-5.2(0)	1.917	9.94	11.11	11.26
24.8(30)	2.020	9.91	10.94	11.41
54.8(60)	2.370	9.74	10.74	11.69
84.8(90)	2.804	9.54	10.68	12.25
114.8(120)	3.198	9.44	10.65	11.75
144.8(150)	3.474	9.43	10.66	11.64
174.8(180)	3.591	9.44	10.68	11.59

<sup>a</sup>The values in parentheses are relative dihedral angles.

<sup>b</sup>Bond distances between H and N atom.

bond distances, is presented in Table 3 and is depicted in Fig. 2.



**Figure 2.** Variation of the vertical ionization energy with changing relative HO<sub>1</sub>C<sub>1</sub>N dihedral angles in conformer 2 at the B3LYP level.

Table 3 and Fig. 2 show that when the H atom in the carboxy group leaves the H-O<sub>1</sub>-C<sub>1</sub>-N plane, the IEs for  $n_N$  and  $n_O$  decrease, while the O-H...N hydrogen bond distance increases from 1.917 to 3.591 Å. The O-H...N hydrogen bond nearly disappears at the H-O<sub>1</sub>-C<sub>1</sub>-N relative dihedral angle of about 90°. Therefore, the intramolecular hydrogen bond shows a strong influence on the IE of alanine.

In conclusion, the ionization energies were calculated for the four lowest conformers of alanine. The P3 ionization energies in various DFT and MP2 levels show good agreement with the available experimental values. The average value (1.085 eV) of the difference between the first and next orbital for the two lowest conformers is in very close agreement with the experimental peak split (1.15 eV). Furthermore, the ionization energies decrease progressively with an increase in the H-O-C-N dihedral angles and the O-H...N hydrogen bond distances. Therefore, we concluded that the intramolecular hydrogen bond affects a strong influence on the ionization energy of alanine. The P3 theoretical method can also explain the experimental IE. Thus, the IEs for the other amino acids are expected to be predicted more reliably by using a P3 approximation of the electron propagator theory.

**Acknowledgment.** This work was supported by research grants from the Catholic University of Daegu.

## REFERENCES

- Iijima, K.; Beagley, B. *J. Mol. Struct.* **1991**, *248*, 133.
- Godfrey, P. D.; Firth, S.; Hatherley, L. D.; Brown, R. D.; Pierlot, A. P. *J. Am. Chem. Soc.* **1993**, *115*, 9687.
- Császár, A. G. *J. Phys. Chem.* **1996**, *100*, 3541.
- Godfrey, P. D.; Brown, R. D.; Rodgers, F. M. *J. Mol. Struct.* **1996**, *376*, 65.
- Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997.
- Tian, S. X. *J. Chem. Phys.* **2005**, *123*, 244310.
- Cannington, P. H.; Ham, N. S. *J. Electron Spectrosc. Relat. Phenom.* **1983**, *32*, 139.
- Zheng, Y.; Neville, J. J.; Brion, C. F. *Science* **1995**, *270*, 786.
- Tian, S. X.; Yang, J. *Angew. Chem. Int. Ed.* **2006**, *45*, 2069.
- Ortiz, J. V. *J. Chem. Phys.* **1996**, *104*, 7599.
- Blanco, S.; Lesarri, A.; López, J. C.; Alonso, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 11675.
- Gronert, S.; O'Hair, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 2071.
- Frisch, M. J.; et al. *Gaussian 09, Revision A.1*; Gaussian, Inc.: Wallingford, CT, 2009.
- Dunning, Jr. T. H. *J. Chem. Phys.* **1989**, *90*, 1007.
- Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
- (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372. (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
- Perdew, J. P.; Chevary, J. A.; Vosko, S. H.; Jackson, K. A.; Pederson, M. R.; Singh, D. J.; Fiolhais, C. *Phys. Rev. B* **1993**, *48*, 4978.
- Zakrzewski, V. G.; Ortiz, J. V.; Nichols, J. A.; Heryadi, D.; Yeager, D. L.; Golab, J. T. *Int. J. Quant. Chem.* **1996**, *60*, 29.
- Suenram, R. D.; Lovas, F. J. *J. Am. Chem. Soc.* **1980**, *102*, 7180.
- Brown, R. D.; Godfrey, P. D.; Storey, J. W.; Bassez, M.-P. *Chem. Commun.* **1978**, 547.
- Suenram, R. D.; Lovas, F. J. *J. Mol. Spectrosc.* **1978**, *72*, 372.
- Lee, G. Y. *Bull. Korean Chem. Soc.* **2012**, *33*, 1561.
- Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem. A* **1998**, *102*, 4623.
- Wright, L. R.; Borkman, R. F. *J. Am. Chem. Soc.* **1980**, *102*, 6207.
- Rodríguez-Santiago, L.; Sodupe, M.; Oliva, A.; Bertrán, J. *J. Phys. Chem. A* **2000**, *104*, 1256.
- Koopmans, T. *Physica* **1934**, *1*, 104.
- Brundle, C. R.; Robin, M. B.; Busch, H. *J. Chem. Phys.* **1970**, *53*, 2196.