

Notes

DFT Study of the Effects of Halogen Anions on the Stability of Alanine Zwitterion

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Alanine is the most abundant and important amino acid found in proteins, and is present on both the interior and exterior surface of proteins in contact with water.¹ The structure of alanine has been studied both experimentally and theoretically by many researchers.²⁻⁸ Using a high-pressure mass spectrometer (HPMS) and *ab initio* calculations, Wu *et al.*⁹ showed that certain organic ammonium ions are able to stabilize the zwitterionic structure of amino acids. Amino acids in aqueous solutions also exist in the zwitterionic form over a wide range of pH.^{10,11} However, they do not exist in the zwitterionic form in the gas phase.^{7,8}

Zwitterions play an important role in variety of biological reactions.¹² The electric fields of zwitterionic structures serve as the driving force that determines the activity of amino acids, peptides, and proteins.¹³ In particular, many drugs are present in their zwitterionic form and exhibit strong electrostatic interactions with protein receptors.¹⁴ Because of the instability of amino acid zwitterions in the gas phase, direct experimental evidence of their behavior is lacking; therefore, numerous attempts were made to stabilize gas-phase zwitterions.^{15,16} On the basis of *ab initio* calculations, Jensen *et al.*¹⁷ reported that two water molecules can stabilize the glycine zwitterion. Metalation^{18,19} and protonation²⁰ also stabilize the amino acid zwitterions. Kass²¹ stabilized the zwitterionic glycine using oxalic and malonic dianions, which cannot exist independently in the gas phase. He also examined the utility of self-stabilized anionic species to stabilize amino acid zwitterionic structures. On the basis of this finding, and as continuation of our studies on organic radicals^{22,23} and alanine radical cations,²⁴ we report the effects of halogen anions on the stability of the alanine zwitterion in this paper.

All theoretical calculations on structures considered in this study were carried out with the Gaussian 09 series of programs.²⁵ The relativistic effects play important roles in the Br and I elements, and accordingly their electrons near the nuclei are represented with the LanL2DZ basis set containing effective core potential (ECP). In accordance with ref 13, F and Cl elements were treated the same method as Br and I. DFT calculations were evaluated at the B3LYP level,²⁶ and *ab initio* calculations were performed using the MP2 level of theory. Equilibrium geometries of each structures were fully

optimized without any restriction on symmetries. Vibration frequencies were also calculated to confirm whether all the stationary points correspond to the true minima. The stationary structures were obtained by verifying if the harmonic frequencies for the local minimum were real. In this study, halogen anions (X = F⁻, Cl⁻, Br⁻, and I⁻) were used as the anionic stabilizer for zwitterionic alanine in the gas phase. Through interactions with binding sites of halogen anionic stabilizers (2X⁻), alanine zwitterions (AZW) can be stabilized and form AZW-2X⁻ complexes. The dissociation energy (DE) of the AZW-2X⁻ complexes were estimated using the following equation:²⁷

$$DE = E(\text{AZW}) + E(2\text{X}^-) - E(\text{AZW}-2\text{X}^-)$$

where the energies of the AZW and 2X⁻ species were obtained on optimizing the geometries of the AZW-2X⁻ complexes. The proton affinity (PA) of the halogen anionic stabilizer was calculated as the energy difference between

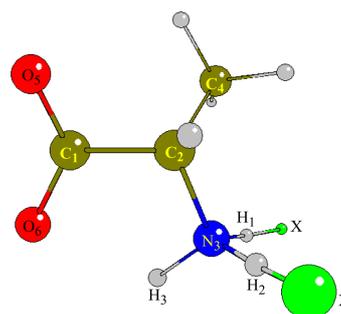


Figure 1. The structure of the AZW-2X⁻ complex (X = F, Cl, Br, and I).

Table 1. Dissociation energies (DE) of the AZW-2X⁻ complexes and proton affinities (PA) of the halogen anionic stabilizers at the B3LYP/LanL2DZ level (in kJ/mol)

Stabilizer (2X ⁻)	DE	PA
2F ⁻	617.0	1919.5
2Cl ⁻	356.6	1661.1
2Br ⁻	295.1	1593.1
2I ⁻	239.5	1537.7

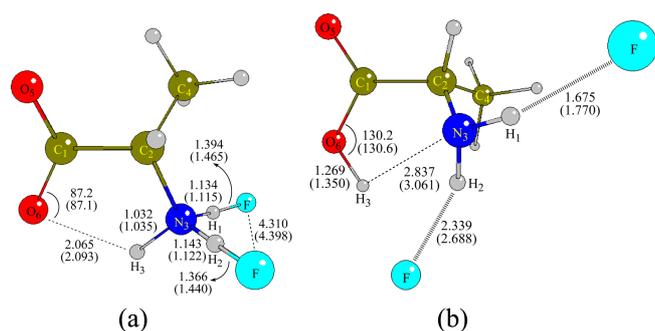


Figure 2. B3LYP-optimized geometries of the AZW-2F⁻ complex (a) and the neutral alanine complex with 2F⁻ (b). The distances are in Å and the bond angles are in degrees. The values in parentheses are at the MP2 level.

the halogen anionic stabilizer and its protonated form.

$$PA = E(2X^-) - E(2X^-H^+)$$

Figure 1 shows the structure of the AZW-2X⁻ complex adopted in this study.

The dissociation energies (DE) of the AZW-2X⁻ complexes and proton affinities (PA) of the halogen anionic stabilizers are given in Table 1.

As shown in Table 1, two of the same halogen anions can stabilize the alanine zwitterions. The dissociation energies increase from 239.5 kJ/mol (for 2I⁻) to 617.0 kJ/mol (for 2F⁻). This suggests that the dissociation energies of the AZW-2X⁻ complexes with halogen anions gradually increased with the decrease in the atomic size of the halogens. Thus F⁻ can better stabilize the alanine zwitterions as it has a large dissociation energy. This result indicates that the stabilization effect is considerably enhanced in the case of F⁻. To conclude, the halogen anions can be used to stabilize the unstable alanine zwitterions in the gas phase.

The optimized AZW-2F⁻ complex is shown in Figure 2(a). As can be seen, the geometric parameters at the MP2 and B3LYP levels of theory are close to each other.

On the other hand, the degree of stabilization is closely related to the proton affinities (PAs) of the anionic species. The PAs of the anions is a measure of their ability to accept

protons. Anions with larger PAs have stronger tendencies to remove protons of other molecules. Therefore, in the case of anions with very large PA, the protons gradually transfer from the alanine zwitterions to the anions, resulting in the formation of the protonated anionic species and deprotonated alanine. However, halogen anions used in this study do not show this tendency in the geometry optimization processes of the AZW-2X⁻ complexes (X = F, Cl, Br, and I). There is also a lower limit of proton affinities below which the anions fail to stabilize the amino acid zwitterions;¹³ In particular, anions with very low proton affinities fail to stabilize alanine zwitterions. This behavior is consistent with the intrinsic instabilities of the alanine zwitterions, which cannot exist independently in the gas phase.^{15,16} The four halogen anionic stabilizers used in this study have sufficiently large proton affinities that can stabilize the alanine zwitterions.

In the geometry optimization processes, the X-X distances in the AZW-2X⁻ complexes were found to be 4.310, 5.525, 5.970, and 6.528 Å at the B3LYP level, for 2F⁻, 2Cl⁻, 2Br⁻, and 2I⁻, respectively (Table 2). It can be deduced that larger atoms have a larger X-X distance. Accordingly, the I-I distance should be the largest in the AZW-2X⁻ complexes. Some of the optimized geometrical parameters of the AZW-2X⁻ complexes at the B3LYP level of theory are given in Table 2.

As shown in Table 2, when the proton affinities of the halogen anions have relatively smaller values, the three N-H bonds in the AZW-2X⁻ complexes are generally close to each other. It was also found that the X-H distances increase on going from the 2F⁻ to the 2I⁻ complex. That is, the X-H distances increase with a decrease in the PA of the halogen anionic stabilizer. Meanwhile, the O₆-H₃ distance decreases from 2.065 (in the 2F⁻ complex) to 1.779 Å (in the 2I⁻ complex); thus, the intramolecular hydrogen bond is strengthened.

To further investigate the effect of halogen anions on the stability of the alanine zwitterion, the neutral alanine structures (ANt-2X⁻, X = F, Cl, Br, and I) corresponding to AZW-2X⁻ were also optimized. The zwitterionic (AZW-2X⁻) and neutral (ANt-2X⁻) conformers (X = F, Cl, Br, and I) were found to coexist in the gas phase. Among the four

Table 2. Some optimized geometrical parameters of the AZW-2X⁻ and ANt-2X⁻ complexes at the B3LYP level. Distances are in Å and bond angles are in degrees

Parameters	AZW-2X ⁻				ANt-2X ⁻			
	X = F	Cl	Br	I	X = F	Cl	Br	I
r(X-H ₁)	1.394	2.130	2.399	2.735	1.675	2.540	2.943	3.179
r(X-H ₂)	1.366	2.104	2.359	2.653	2.339	2.736	2.968	3.077
r(N ₃ -H ₁)	1.134	1.062	1.052	1.044	1.061	1.031	1.025	1.028
r(N ₃ -H ₂)	1.143	1.062	1.053	1.045	1.019	1.024	1.022	1.026
r(N ₃ -H ₃)	1.032	1.044	1.050	1.059	2.837	2.792	2.778	1.587
r(O ₆ -H ₃)	2.065	1.897	1.842	1.779	1.269	1.030	1.015	1.067
r(C ₁ -O ₅)	1.294	1.284	1.281	1.277	1.277	1.256	1.252	1.249
r(C ₁ -O ₆)	1.297	1.298	1.299	1.301	1.327	1.363	1.368	1.361
r(X-X)	4.310	5.525	5.970	6.528	-	-	-	-
∠(C ₁ O ₆ H ₃)	87.2	89.7	90.3	91.0	130.2	119.3	118.8	99.9

Table 3. Total energies (in a.u.) of the ANt-2X⁻ and AZW-2X⁻ complexes. Values (in kJ/mol) in parentheses are relative energies

Stabilizer (X)	Total energy	
	ANt-2X ⁻	AZW-2X ⁻
F	-523.419192 (0.0)	-523.443117 (-62.8)
Cl	-353.697611 (0.0)	-353.717780 (-53.1)
Br	-350.157489 (0.0)	-350.176171 (-49.0)
I	-346.624272 (0.0)	-346.631808 (-19.7)

ANt-2X⁻ structures that were optimized in this study, the structure of ANt-2F⁻ is shown representatively in Figure 2(b). The F-H₁ and F-H₂ distances of the ANt-2F⁻ complex are much longer than those of the AZW-2F⁻ complex. In the case of the 2F⁻ complex, the energy of the zwitterionic conformer is lower than that of the neutral structure by 62.8 kJ/mol (see Table 3).

Some of the optimized geometrical parameters of the ANt-2X⁻ structures are inserted in Table 2.

Compared with the N₃-H₃ distances of the AZW-2X⁻ complexes, those of the ANt-2X⁻ structures are markedly longer, indicating that the strengths of the hydrogen bonds are weaker than those in the AZW-2X⁻ complexes.

As can be seen in Table 2, the C₁O₆H₃ bond angles in the ANt-2X⁻ complexes are large than the corresponding angles in the AZW-2X⁻ complexes. These angles are below 91° in the AZW-2X⁻ complexes, but are above 99° in the neutral alanine complexes. The X-H distances increase with the atomic size of the halogen atoms; this trend is caused by the gradual decrease in the proton affinities. As reported by many researchers, the proton-transferred conformer (zwitterionic form) of the alanine molecule does not exist in the gas phase, as intermolecular interactions have no effect in this state. However, the zwitterionic structure [NH₃⁺X₂-CHCH₃-COO⁻] of the alanine complexes with halogen anionic stabilizers have been located in this study.

The total and relative energies of the stationary points for the ANt-2X⁻ and AZW-2X⁻ complexes at the B3LYP level of theory are given in Table 3.

It can be observed that for the alanine complexes with a 2X⁻ stabilizer, the zwitterionic conformers are more stable than the neutral conformers.

In conclusion, halogen anionic stabilizers used to stabilize alanine zwitterions were examined using DFT method. The zwitterionic conformer of alanine [NH₃⁺-CHCH₃-COO⁻] does not exist in the gas phase. However, complexes with the halogen stabilizer have structures of the proton transferred zwitterion conformer, [NH₃⁺X₂-CHCH₃-COO⁻]. That is, the four halogen anions examined (2F⁻, 2Cl⁻, 2Br⁻, 2I⁻) were effective in stabilizing the alanine zwitterions. The halogen anions have large dissociation energies; in particular, the dissociation energy of the 2F⁻ anion is above 600 kJ/mol, suggesting that these anions can be used to stabilize

the alanine zwitterion in the gas phase. Furthermore, the proton affinity is a decisive factor that affects the degree of stabilization. Anionic halogens with smaller sizes have larger dissociation energies and proton affinities, thereby resulting in greater stabilization of the zwitterion alanine.

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