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Molecular Mechanic Studies on the Zwitterionic and Neutral Conformation Stability of Biological Active Peptide

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Conformational analysis,
DFT

Abstract: In this current work the conformational properties of the L-Alanyl-L-Glutamine dipeptide which was biological active dipeptide has been investigated by theoretical conformational analysis method using program which is based on Molecular Mechanic calculations in order to determine the structure function relation. The calculations on zwitterionic and neutral form of Ala-Gln which is formed side and main chain torsion angles let us to define their preferable energetically conformers. The side and main chain status of the stable conformations of Ala-Gln were acquired based on the results of the conformational analysis. By using Ramachandran maps, the global conformation, which has the lowest energy of the dipeptide, has been determined. The goal of this work was to explain structure-activity relationship by investigating the bioactive conformation of biological active peptide.

Biyolojik Aktif Peptidin Zwitterionik ve Nötr Konformasyon Kararlılığı üzerine Moleküler Mekanik Araştırmalar

Anahtar Kelimeler
Zwitteriyonik,
Nötral,
Ala-Gln,
Konformasyon analizi,
DFT

Özet: Bu çalışmada, biyolojik aktif dipeptid olan L-Alanil-L-Glutamin dipeptidin konformasyonel özellikleri, yapı fonksiyonu ilişkisini belirlemek için Moleküler Mekanik hesaplamalara dayanan program kullanılarak, teorik konformasyon analizi yöntemi ile araştırılmıştır. Ala-Gln dipeptidinin zwitterionik ve nötr formda yan zincir torsiyon açılarının bir fonksiyonu olarak hesaplanması, enerjik olarak tercih edilen konformasyonlarını belirlememizi sağlar. Elde edilen konformasyonel analiz sonuçlarına bağlı olarak, dipeptide ait stabil konformasyonların yan zincir kalıntılarının göreli pozisyonları elde edilmiştir. Dipeptide ait en düşük enerji konformasyonu Ramachandran haritaları kullanılarak belirlenmiştir. Bu çalışmanın amacı biyolojik aktif dipeptidin biyoaktif konformasyonunu açıklığa kavuşturmak için yapı-aktivite ilişkisini araştırmaktır.

1. Introduction

Since the electronic and vibrational properties of biologically active peptides are related to its conformational structure, it is very important to know the conformational characteristics of this peptides, to investigate their structural functional properties. Alanyl is a hydrophobic molecule and it can support the body converts glucose to energy, at the same time helps the liver and removes various toxins from the body. Glutamine is necessary for immune system for cell proliferation, it may move through the respiratory tract and increase the function of the stimulated immune cells. Within the muscle, it is important for repair, particularly after trauma. To improve mental function, to control blood sugar levels and to maintain muscle mass, it is also utilized by the body. L-alanyl-L-glutamine (Ala-Gln) dipeptide which is an substantial biological active molecule has various, effective, and important biological activities such as; decreasing effect of infection, dehiscence, abcess, mortality in critically ill patients and length of stay after operation[1], preventive effect of the inhibition of migration, apoptosis[2], improving effect of replenishment of depleted glutathione stores, nitrogen balance, immune defenses, growth performance of piglets[3-7], increasing effect of protein synthesis, ameliorates immune function[3-5], protective effect of intestinal barrier permeability[3-5], and also shows effective

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properties on control of diabetes in liver transplanted patients, survival and activities of digestive enzyme, enzymatic antioxidative status[8-9]. It is also used as an immunomodulator in addition to knowing that chemotherapy side effects are diminised.

Besides, the gastrointestinal mucosal protective and antioxidant activities are known. In the gas-phase amino acids, especially exist in the neutral form, however in the water, amino acids are present in the zwitterionic form. A zwitterion form (at neutral pH values, the amino group (-NH3+) own a positive charge and the carboxyl group (COO-) possess a negative charge) is a structure which has no overall electrical charge, but has apart parts such as positively and negatively charged parts, that exist even in the solid state. These charged moieties cause much stronger ionic forces between the ion and its neighbors or surroundings, rather than weaker hydrogen bonds or other intramolecular forces.

Due to the ionic attractions take more energy to break, melting points of these molecules escalate. In water, the ionic attractions between the ions in the solid amino acid are altered by strong attractions between polar water molecules and the zwitterions. Biological activity is also influenced by the pH of the water as it determines whether or not certain functional groups are charged, and hence it engenders structural modifications. This makes it possible formation of multiple peptide conformations. Due to this structural modification, the biological functions of these peptides are changed. By determining the spatial structures and the full of low-energy conformational states of biological active peptides allow us to understand the relation between structure- function mechanisms. In present study, the results of conformational analysis of zwitterionic and nutral form of Ala-Gln dipeptide are reported.

2. Method

Molecular Mechanic method employs an appropriate algorithm to find the molecular structure of a local energy minimum which correspond to stable conformers of the molecule

Molecular mechanic method with the great computational speed allows us to determine conformational energy and structural searching for peptides. Theoretical conformational analysis method, which is based on the Molecular Mechanics approach, is substantial for conformational compatibility. Calculation of biological active peptide has been performed by the method of theoretical conformational analysis with the help of nonvalent, electrostatic and torsional interactions and energy of the hydrogen bonds. Nonvalent interactions are identified by Lennard-Jones potential with the parameters suggested in [10]. By using Coulombs law which introduces monopole approximation in the partial charges on the atoms in molecule, electrostatic interactions are determined [10]. Hydrogen bonds are described by Morze potential which the value of permittivity is taken to be 10 for zwitterionic surrounding. Amino acid main and side chain dihedral angles are taken from [10] in order to identify torsional potentials and values of rotation barriers. The conformations are performed by the number of rotational degrees of freedom of single bonds of the backbone and side chains for the each amino residue of dipeptide. The rotational angels of each amino acide residue is characterized by the backbone φ, ψ and side chain χ_1, χ_2... dihedral angles. The φ and ψ dihedral angles are located in low-energy region R (φ,ψ=(−180°–0°), B (φ=(−180°)–0°; ψ=0°–180°), L(φ,ψ=0°–180°) and P(φ=0°–180°; ψ=(−180°)–0°) of the conformational map.

Experimental parameters for bond lengths, angles and dihedrals are taken as suggested by Corey and Pauling [11]. The spatial structures and the full complement of low-energy conformational states of the dipeptides was carried out by using program proposed by Godjayev et al.[12]. This study is a review of the structural and energy shift in comparison with zwitterionic and neutral form of biologically active peptid molecule.

3. Results and Discussion

3.1. Structure of zwiterrionic form

The initial conformations of the Ala-Gln peptide were formed by combining low-energy structures of each amino acide residues. B, R and L areas for Alanin and Glutamine were defined and the values of φ and ψ dihedral angles were taken from these regions. Side chains dihedral angles values for Alanin was teken 60°, 180°, -60° for χ_1 and for Glutamine was teken60°, 180°, -60° for χ_1 andχ_2, 90°,-90° for χ_3. 1134 conformers for Ala-Gln dipeptide were investigated for zwitterionic form, one by one. The most stable conformation of zwitterionic forms are in L_3B_{131} region and extended (e) shape on Ramachandran maps.

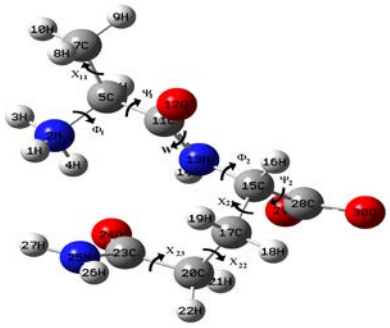


Figure 1. The global conformation of the zwitterionic form of the Ala-Gln dipeptide obtained with LB region.

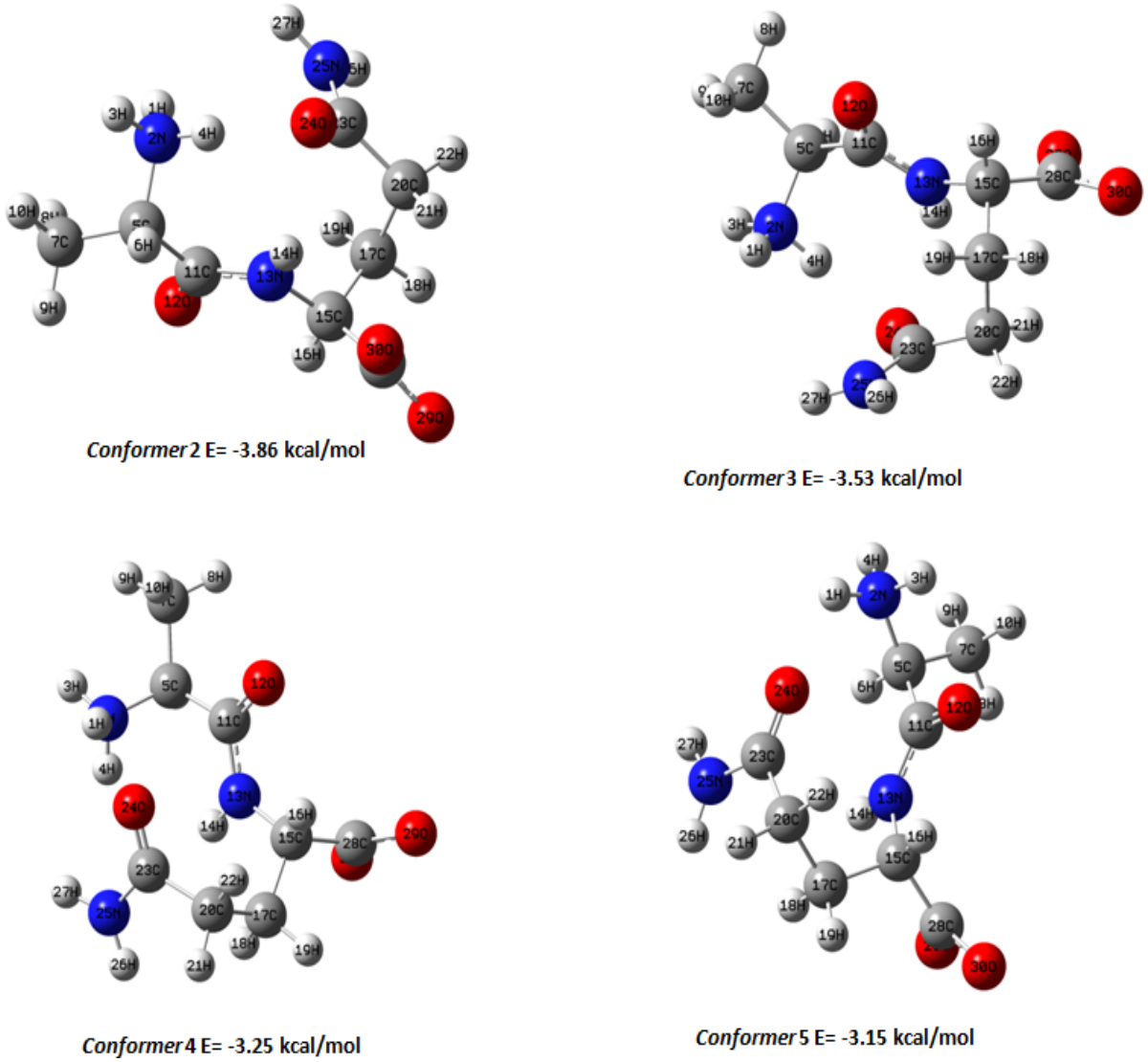


Figure 2. The four stable conformers of zwitterionic form of Ala-Gln dipeptide in 1134 conformers.

Table 1. The dihedral angle values obtained for the zwitterionic form of the Ala-Gln molecule.

	Energy kcal/mol		PHI1	CH11	PSI1	W2	PHI2	CH21	CH22	CH23	PSI2
	Exp.		58.6[a]	55.9[a]	103.67[a]		162.9[b,xray] 164[b,neu]	65.7[b,xray] 66.15[b,neu]	175.5[b,xray] 175.5[b,neu]	167.1[b,xray] 167.2[b,neu]	167.2[b,xray] 167[b,neu]
1	**-4.19**	IN	50.000	60.000	60.000	180.000	-150.000	60.000	-60.000	90.000	150.000
		OUT	**56.874**	**58.332**	**72.521**	**180.708**	**-150.636**	**58.468**	**-74.807**	**102.292**	**158.172**
2	-3.86	IN	50.000	60.000	60.000	180.000	-100.000	60.000	-60.000	90.000	-60.000
		OUT	56.462	58.026	73.122	181.236	-142.786	56.333	-77.555	101.143	-14.082
3	-3.53	IN	50.000	180.000	60.000	180.000	-100.000	60.000	-60.000	90.000	140.000
		OUT	55.532	177.827	72.875	181.193	-141.336	57.582	-78.330	101.154	193.492
4	-3.25	IN	50.000	180.000	60.000	180.000	-90.000	-60.000	60.000	-90.000	-40.000
		OUT	47.485	178.133	65.707	182.911	-97.420	-70.857	65.541	-108.085	-42.634
5	-3.15	IN	-115.000	180.000	140.000	180.000	-150.000	-60.000	60.000	-90.000	150.000
		OUT	-61.156	178.409	129.447	184.417	-114.909	-65.381	70.432	-114.725	146.992
6	-3.05	IN	-115.000	180.000	140.000	180.000	-90.000	-60.000	60.000	-90.000	-40.000
		OUT	-55.901	180.218	139.178	183.263	-111.407	-67.759	68.718	-114.666	-50.091
7	-2.85	IN	-115.000	180.000	140.000	180.000	-100.000	-60.000	60.000	-90.000	140.000
		OUT	-60.351	178.556	140.600	182.613	-110.561	-68.381	68.942	-114.573	139.268
8	-2.83	IN	-115.000	60.000	140.000	180.000	-100.000	-60.000	60.000	-90.000	-60.000
		OUT	-66.795	57.559	143.351	182.835	-108.871	-67.242	67.388	-113.261	-58.166
9	-2.80	IN	-90.000	60.000	-50.000	180.000	-90.000	60.000	-60.000	90.000	-40.000
		OUT	-44.621	58.646	-57.608	178.340	-146.040	67.626	-67.613	106.194	-16.729
10	-2.74	IN	-90.000	180.000	-50.000	180.000	-100.000	60.000	-60.000	90.000	140.000
		OUT	-54.214	173.103	-64.600	178.222	-141.799	67.720	-69.290	105.841	165.607

a: Ref [15]; b: Ref [16]

Table 2. The total energy calculated for the zwitterionic Ala-Gln molecule and the contribution terms that make up this energy.

		Total Energy (kcal/mol)	shape	E van der Walls (kcal/mol)	E electrostatic (kcal/mol)	E torsion (kcal/mol)
1	**LB1**	**-4.19**	**e**	**-6.77**	**1.15**	**1.43**
2	LR2	-3.86	e	-6.56	1.08	1.62
3	LB2	-3.53	e	-6.17	1.17	1.48
4	LR1	-3.25	e	-5.83	1.46	1.12
5	BB1	-3.15	e	-5.27	0.81	1.31
6	BR1	-3.05	e	-5.18	0.99	1.13
7	BB2	-2.85	e	-5.14	1.01	1.28
8	BR2	-2.83	e	-4.89	1.01	1.04
9	RR1	-2.80	f	-5.46	1.49	1.17
10	RB2	-2.74	f	-5.40	1.46	1.20

The most stable structure for zwitterionic form is obtained by χ11=60°, χ21=60°, χ22=-60° and χ23=90° is given in Figure 1. Dihedral angles of the ten possible stable conformations before and after energy optimization and relative total energies are showed in Table 1. Van der Waals, electrostatic, torsional energies which generate total energy for ten conformers are given Table 2. The possible four conformers which have low energy are also demonstrated in Figure 2.

3.1. Structure of neutral form

1134 conformers for Ala-Gln dipeptide were investigated for neutral form, one by one. The most stable conformation of neutral form are determined in LR1 region and extended (e) shape on Ramachandran maps.

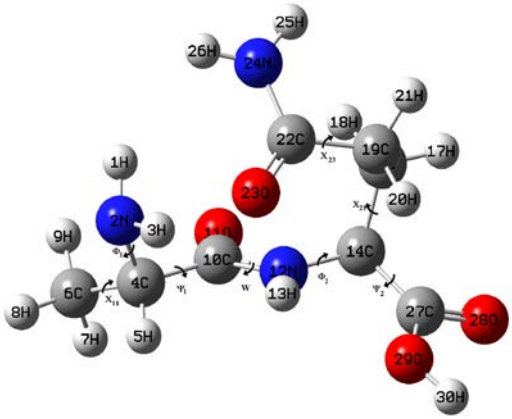
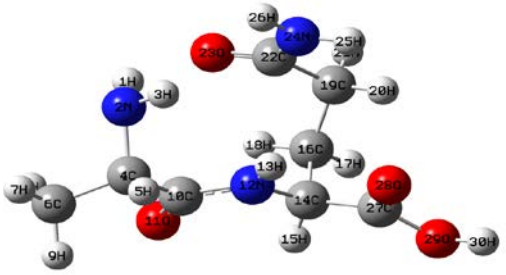
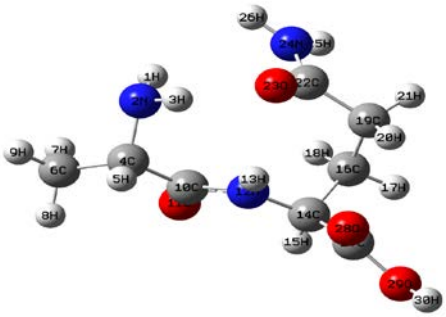


Figure 3. The global conformation of the neutral form of Ala-Gln dipeptide with the atom numbering in LR1 region.

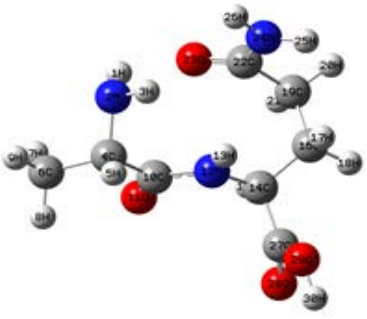
The most stable structure for neutral form is obtained by χ_{11}=180°, χ_{21}=60°, χ_{22}=-60° and χ_{23}=90°. is given in Figure 3. Dihedral angles of the ten possible stable conformations before and after energy optimization and relative total energies are showed in Table 3. The total energy with the sum of Van der Waals, electrostatic, torsional energies for ten conformers are given in Table 4. The possible four conformers which have low energy are also demonstrated in Figure 4.



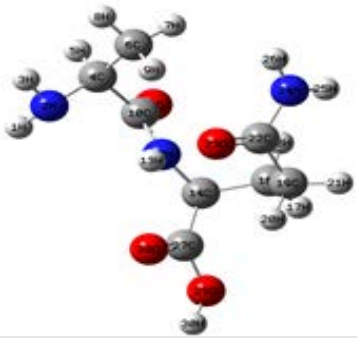
Conformer 2 **E= -0.18 kcal/mol**



Conformer 3 **E= 0.04 kcal/mol**



Conformer 4 **E= 0.14 kcal/mol**



Conformer 5 **E= 1 kcal/mol**

Figure 4. The four more stable conformation of the neutral form of Ala-Gln dipeptide with their energy.

Table 3. The dihedral angle values obtained for the neutral form of the Ala-Gln molecule.

	Energy kcal/mol		*PHI1*	*CH11*	*PSI1*	*W2*	*PHI2*	*CH21*	*CH22*	*CH23*	*PSI2*
	Exp.		**58.6[a]**	**55.9[a]**	**103.67[a]**		**162.9[b,xray] 164[b,neu]**	**65.7[b,xray] 66.15[b,neu]**	**175.5[b,xray] 175.5[b,neu]**	**167.1[b,xray] 167.2[b,neu]**	**167.2[b,xray] 167[b,neu]**
1	**-0.30**	IN	50.000	180.000	60.000	180.000	-90.000	60.000	-60.000	90.000	-40.000
		OUT	**49.409**	**179.261**	**75.708**	**180.713**	**-145.769**	**55.251**	**-76.967**	**104.510**	**-18.820**
2	-0.18	IN	50.000	180.000	60.000	180.000	-150.000	60.000	-60.000	90.000	150.000
		OUT	50.275	179.713	71.746	180.870	-150.316	58.251	-76.214	101.434	156.782
3	0.04	IN	50.000	-60.000	60.000	180.000	-100.000	-60.000	180.000	-90.000	140.000
		OUT	33.546	-60.754	72.750	182.115	-158.090	-61.953	178.574	-104.179	134.931
4	0.14	IN	50.000	180.000	60.000	180.000	-100.000	180.000	60.000	-90.000	-60.000
		OUT	43.119	179.003	81.467	179.776	-104.962	181.206	63.119	-103.098	-61.689
5	1.00	IN	-90.000	180.000	-50.000	180.000	-100.000	60.000	-60.000	90.000	140.000
		OUT	-60.953	175.387	-49.048	178.043	-144.085	65.390	-71.154	106.662	171.051
6	1.05	IN	-90.000	-60.000	-50.000	180.000	-100.000	60.000	-60.000	90.000	-60.000
		OUT	-64.941	-66.141	-49.923	178.843	-146.962	66.835	-68.998	108.171	-5.951
7	1.15	IN	-115.000	180.000	140.000	180.000	-100.000	-60.000	-60.000	90.000	-60.000
		OUT	-173.569	181.382	140.187	182.105	-105.842	-54.222	-59.392	109.009	-56.236
8	1.16	IN	-90.000	-60.000	-50.000	180.000	-150.000	-60.000	-60.000	90.000	150.000
		OUT	-47.417	-61.987	-57.606	182.820	-105.073	-52.647	-58.335	108.879	145.591
9	1.19	IN	-90.000	180.000	-50.000	180.000	-90.000	-60.000	-60.000	-90.000	-40.000
		OUT	-45.628	177.701	-57.620	179.613	-117.776	-65.404	-71.739	-80.926	-45.310
10	1.20	IN	-115.000	180.000	140.000	180.000	-90.000	-60.000	60.000	90.000	-40.000
		OUT	-172.000	181.492	141.077	180.729	-107.307	-70.041	66.530	60.204	-48.035

a: Ref [15]; b: Ref [16]

Table 4. The total energy calculated for the zwitterionic Ala-Gln molecule and the contribution terms that make up this energy.

		Total Energy kcal/mol	shape	$E_{van\ der\ Walls}$ kcal/mol	$E_{electrostatic}$ kcal/mol	$E_{torsion}$ kcal/mol
1	**LR1**	**-0.30**	**e**	**-6.53**	**4.65**	**1.58**
2	LB1	-0.18	e	-6.06	4.41	1.47
3	LB2	0.04	e	-5.50	4.41	1.12
4	LR2	0.14	e	-6.22	5.32	1.04
5	RB2	1.00	f	-5.52	5.31	1.21
6	RR2	1.05	f	-5.96	5.62	1.39
7	BR2	1.15	e	-5.27	5.51	0.91
8	RB1	1.16	f	-5.66	5.90	0.92
9	RR1	1.19	f	-6.13	6.42	0.90
10	BR1	1.20	e	-5.51	5.60	1.10

Table 5. Hydrogen bonds and hydrogen bonding energies for zwitterionic form

Atoms	Hydrogen Bond ($\AA$)	Hydrogen bonding energy (kcal/mol)
4-24	1,92	-1,36
14-24	2,24	-0,70
14-29	2,32	-0,56

Table 6. Interaction energies(kcal/mol) between side and main chains for zwitterionic form.

Ala-main	Ala-side	Gln-main	Gln-side	
2,44	0,60	-3,34	-4,39	**Ala-main**
	0	-0,32	-0,15	**Ala-side**
		2,08	-1,20	**Gln-main**
			-1,32	**Gln-side**

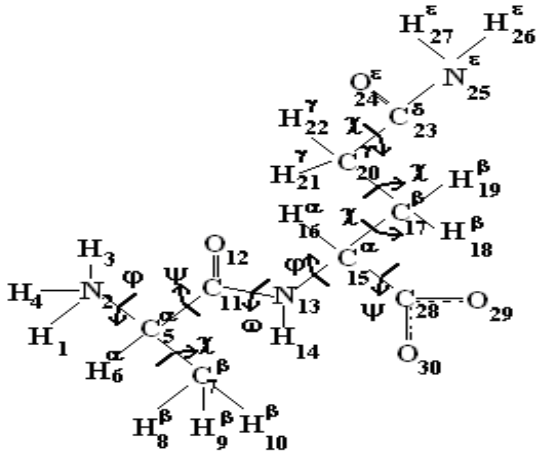


Figure 5. The most stable conformation of the zwitterionic form of Ala-Gln dipeptide.

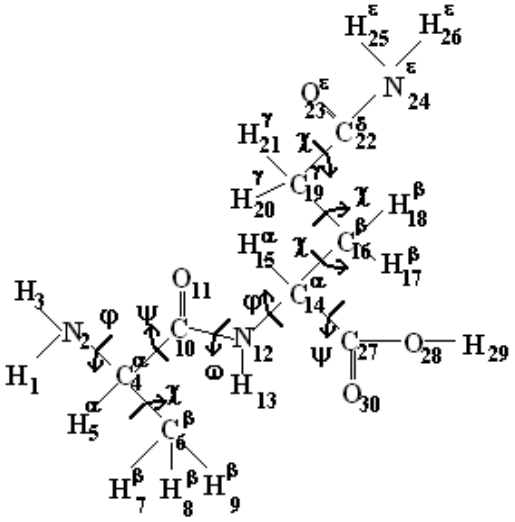


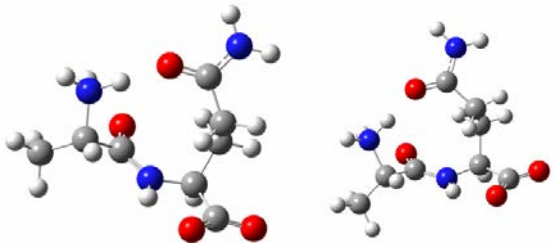
Figure 6. The most stable conformation of the neutral form of Ala-Gln dipeptide.

Table 7. Hydrogen bonds and hydrogen bonding energies for neutral form

Atoms	Hydrogen Bond (Å)	Hydrogen bonding energy (kcal/mol)
3-23	1,95	-0,95
13-23	2,25	-0,49
13-28	2,29	-0,45

Table 8. Interaction energies (kcal/mol) between side and main chains for neutral form.

Ala-main	Ala-side	Gln-main	Gln-side	
4,76	0,81	-2,95	-4,68	Ala-main
	0	-0,29	-0,15	Ala-side
		4,65	-0,28	Gln-main
			-1,79	Gln-side

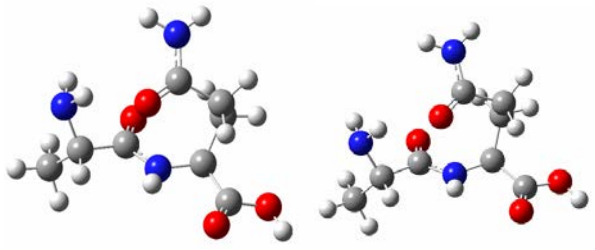


(a) (b)

Figure 7. Comparison of theoretical conformational analysis (a) and DFT/B3LYP 6-31++G(d,p) (b) optimized results of zwitterionic form.

Table 9. Calculated energies (kcal/mol) of Zwitterionic form of Ala-Gln

Zwitterionic Ala-Gln	DFT-RB3LYP 6-31G++(d,p)	DFT-RB3LYP 6-31G(d,p)
Energy	-779.11536503au -488894,89kcal/mol	-779.07174977au -488867,5229kcal/mol



(a) (b)

Figure 8. Comparison of theoretical conformational analysis (a) and DFT/B3LYP 6-31++G(d,p) (b) optimized results of neutral form.

Table 9. Calculated energies (kcal/mol) of Neutral form of Ala-Gln

Nötral Ala-Gln	DFT-RB3LYP 6-31G++(d,p)	DFT-RB3LYP 6-31G(d,p)
Energy	-779.15006391au -488916.6651kcal/mol	-779.11134355au -488892.368kcal/mol

4. Conclusion

The calculated energy, which was performed theoretically conformational analysis method, for zwitterionic form in 1134 conformational region is given (Etot = -4.19 kcal/mol) as the sum of the EvdW = -6.77 kcal/mol, Eel = 1.15 kcal/mol and Etor=1.43 kcal/mol energies. We have determined optimized values of the dihedral angles of backbone and side chains of zwitterionic Ala-Gln dipeptide; its values are φ1=56.87, c11=58.33, Ψ1=72.52 W=180.70, φ2=-150.63, c21=58.46, c22=-74.80, c23=102.29, Ψ2=158.17. The most stable conformation is in LB1 region with the (e) shape on Ramachandran maps. The total energy of global conformation of neutral Ala-Gln molecule which was calculated by [12] (Etot = -0.30 kcal/mol) is given as the sum of the van der Waals (EvdW = -6.53 kcal/mol), electrostatic (Eel = 4.65 kcal/mol), and torsional interactions (Etor=1.58 kcal/mol) energies. The optimized values of the dihedral angles of backbone and side chains of neutral the Ala-Gln dipeptide are found to be; , φ1=49.40, c11=179.26, Ψ1=75.70 W=180.71, φ2=-145.76, c21=57.25, c22=-76.96, c23=104.51, Ψ2=18.82 In 1134 conformers for neutral Ala-Gln molecule the most stable conformation is in LR1 region with extended (e) shape on Ramachandran maps. The experimental dihedral values for alanine and glutamine amino acides were also added in Table 1 and 3. When the theoretical and experimental values are compared, the zwitterionic form is more compatible with the experimental values in Table 1.

When we compare zwitterionic and neutral forms of dipeptide, we obtained that the remarkable contribution on global conformation was Van der Waals interaction with -6.77 kcal/mol and -6.53 kcal/mol for zwiterionic and neutral form, respectively. The electrostatic and torsion interaction provide detrimental effect on global conformation. The calculated values of electrostatic interaction are 1.15 kcal/mol and 4.65kcal/mol for zwiterionic and neutral form, respectively. This detrimental effect was observed more in the neutral form. While a hydrogen bond between the hydrogen and Oksigen atoms (H4-O24) gives rise to global conformation with the contribition of -1,36 kcal/mol for zwitterionic form, in neutral form this hydrogen bond (H3-O23)has a little weak contribution of global conformation of -0,95kcal/mol., see in Table 5. and Table 7.When we analyze the interaction energies (kcal/mol) between side and main chains for zwitterionic form, we concluded that Glutamine side chain and Alanin main chain interaction ensures stable conformation of the dipeptide with -4,39kcal/mol energy in Table 6. This interaction was obtained a little bit higher than zwitterionic form with-4,68kcal/mol. in Table 8. The main chain dihedral angles of conformer2 and conformer3 for zwitterionic and neutral Ala-Gln determined as 2 (φ1=50o, ψ1=60o, w2=180o, φ2=-100o, ψ2=-60o), 3(φ1=50o, ψ1=60o, w2=180o, φ2=-100o, ψ2=140o) and 2 (φ1=50o, ψ1=60o, w2=180o, φ2=-150o, ψ2=150o), 3(φ1=50o, ψ1=60o, w2=180o, φ2=-100o, ψ2=140o) have an total energy -3.86, -3.53 and -

0.18, 0.04 kcal/mol, respectively in Table 1 and Table 3.

The result of theoretical conformation analysis programs were used as starting geometry for quantum chemical calculations. [13-14]. Determination of conformational probabilities of biological macromolecules of zwiterrionic and neutral form of Ala-Gln dipeptide is very considerable to grasp their functions of a drug. The more effective structural analogues may be synthesis based on this conformational analysis results. The conformational energy and structure shifts are found to be in good qualitative agreement for zwitterionic and neutral form of Ala-Gln-dipeptide.

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