A computational study of phenylalanine interaction with guanidinium cation

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1. Introduction

Intermolecular interactions are known to play a key role in many aspects of chemistry and biology, being crucial to phenomena as protein-ligand interaction and molecular recognition.[1-3] Among the different intermolecular forces, those involving aromatic moieties are crucial in protein structure. It is believed that aromatic groups interact in a different manner than aliphatic units in the side chains of aminoacids, and can provide specificity in protein folding.[2, 4]

If an aromatic group is involved in an interaction, it usually corresponds to one of the following types: π···π, XH···π or cation···π. Cation···π interactions are strong interactions in the gas phase and have been recognized as one of the structural motifs conditioning the structure in proteins. Since the initial works from Dougerthy and col. the cation···π interaction is regarded as one key factor in determining the characteristics of proteins.[5, 6] The importance of cation···π interactions in proteins is easily understand taking into account that some amino acids as phenylalanine, tyrosine, tryptophan and histidine bear an aromatic unit in their side chains, whereas other amino acids as arginine, lysine and histidine possess cationic groups depending on the pH. Thus, interaction between side chains of these amino acids is often observed in protein structure suggesting their relevance as a stabilizing motif.[3, 5]

Though the cation···π interactions are known to be strong interactions in gas phase, this has not to be true in solution. Different studies give contradictory results ranging from an important contribution to protein stabilization to an almost negligible effect. These discrepancies are generally addressed to solvent effects, depending on the degree of exposure of the cation···π contact to the solvent.[7-11] Theoretical methods are especially well suited for studying this kind of effect, since the progressive hydration of a given cation···π interaction can be modeled, providing information at a microscopic level which is usually non affordable from experiment. In this work a first step is taken to understand the cation···π interaction between the guanidinium cation, present in the side chain of arginine, and phenylalanine both in its neutral and zwtterionic forms. As a first step, the conformational space of phenylalanine has been studied by means of computational methods, as well as how it is affected by the presence of a single guanidinium cation.

2. Computational Details

Taking into account the conformational flexibility of phenylalanine, a strategy has to be applied to perform an as thorough as possible exploration of its most relevant conformations. In the present work, the conformational search has been carried out by employing a force field method as included in the Macromodel program.[12] A Multiple Minimum Monte Carlo procedure was followed using the MMFFs force field, retaining all structures within 42 kJ/mol to the global minimum.

The structures thus obtained have been subsequently optimized with the B97D functional together with the 6-31+G* basis set. After optimization, frequency calculations at the same level of calculation have been carried out in order to

characterize the stationary points as minima. Finally, energies were recomputed at the MP2/aug-cc-pVDZ level of calculation.

The same procedure has been followed in the case of the complex formed by guanidinium cation and phenylalanine. However, as more than one molecule is present, complexation energies have been obtained at the MP2/aug-cc-pVDZ//B97D/6-31+G* level by applying the counterpoise method to avoid basis set superposition error.[13, 14] All calculations were performed with Gaussian09.[15]

3. Results

Figure 1 shows the optimized structures found for the phenylalanine molecule by carrying out the conformational search plus subsequent optimization at the B97D/6-31+G* level as indicated in computational details. Table 1 lists several geometrical parameters of these minima, whereas in Table 2 energetic information is gathered for all minima as obtained at the MP2/aug-cc-pVDZ level of calculation. Zero point energy corrections and thermal corrections at 298 K have been included by using the frequencies obtained at the B97D/6-31+G* level.

As observed in Figure 1, a total of 23 different minima were found following the procedure described above, of which ten are in an interval of 10 kJ/mol with respect to the most stable one. All structures are within energy differences smaller than 22 kJ/mol. Considering the structures shown in Figure 1 and the data in Tables 1 and 2, it can be observed that the most stable structure corresponds to an arrangement where the acid hydrogen in the carboxylic group of phenylalanine points towards the amine unit, forming an intramolecular hydrogen bond at a distance of about 1.9 Å. Also, one of the hydrogen atoms of the amino group points toward the phenyl ring, being located at about 3.2 Å from its geometrical center. Among the other structures, only in other four an intramolecular OH···N hydrogen bond is observed, three of them among the ten most stable minima. The rest of minima found correspond to different structural arrangements differing in the mutual position of the carboxylic and amino groups and the phenyl ring. Most structures present the amino group oriented towards the phenyl ring.

In general, these results are in agreement with previous ones found in literature, as those from Kim employing the M062X functional with the 6-311+G* basis set.[16] So, our conformational search is able to reproduce the results already obtained by other authors, also revealing another higher energy structures.

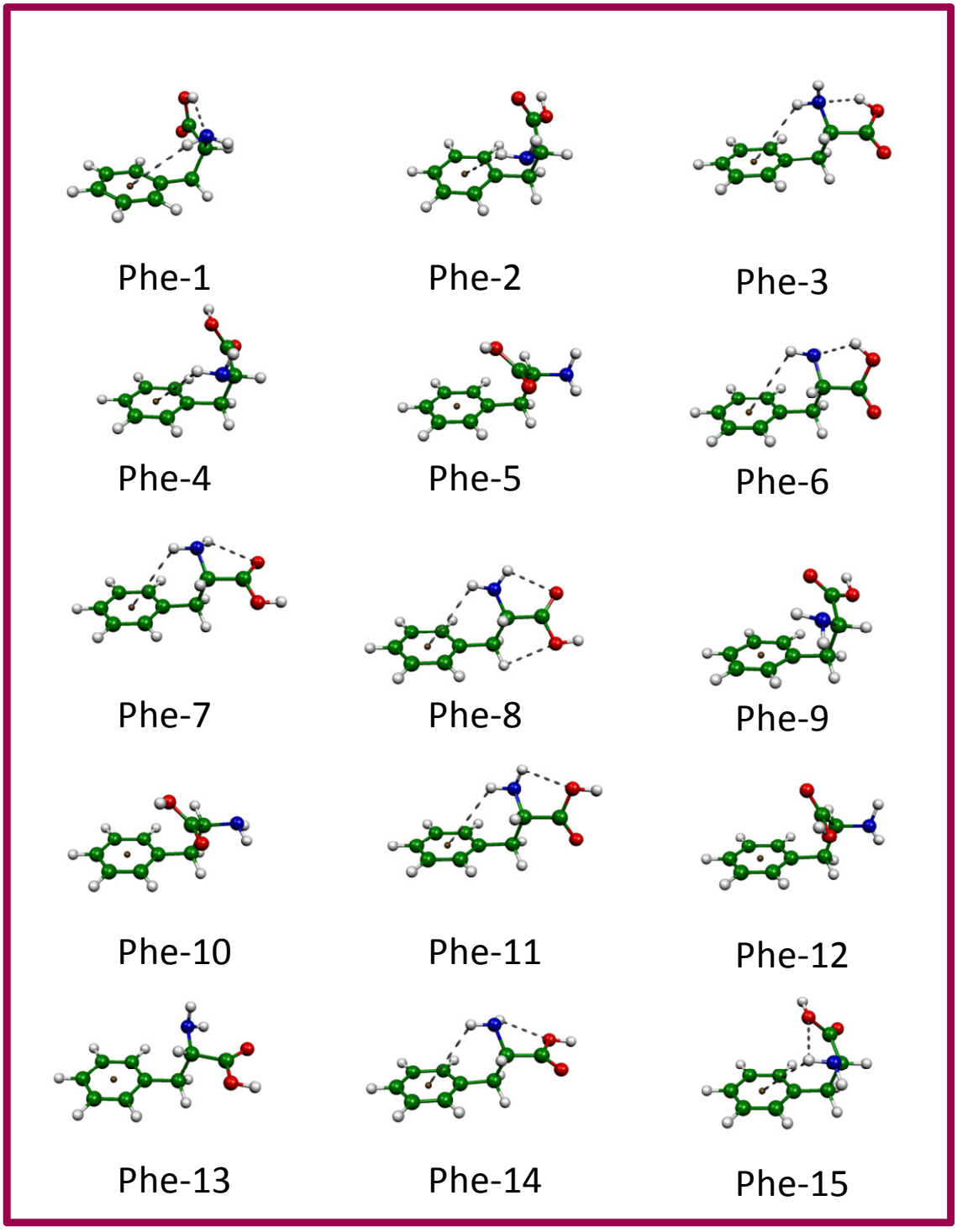

Figure 1a. Minimum energy structures found for phenylalanine molecule following the procedure described in computational details (optimized at the B97D/6-31+G* level).

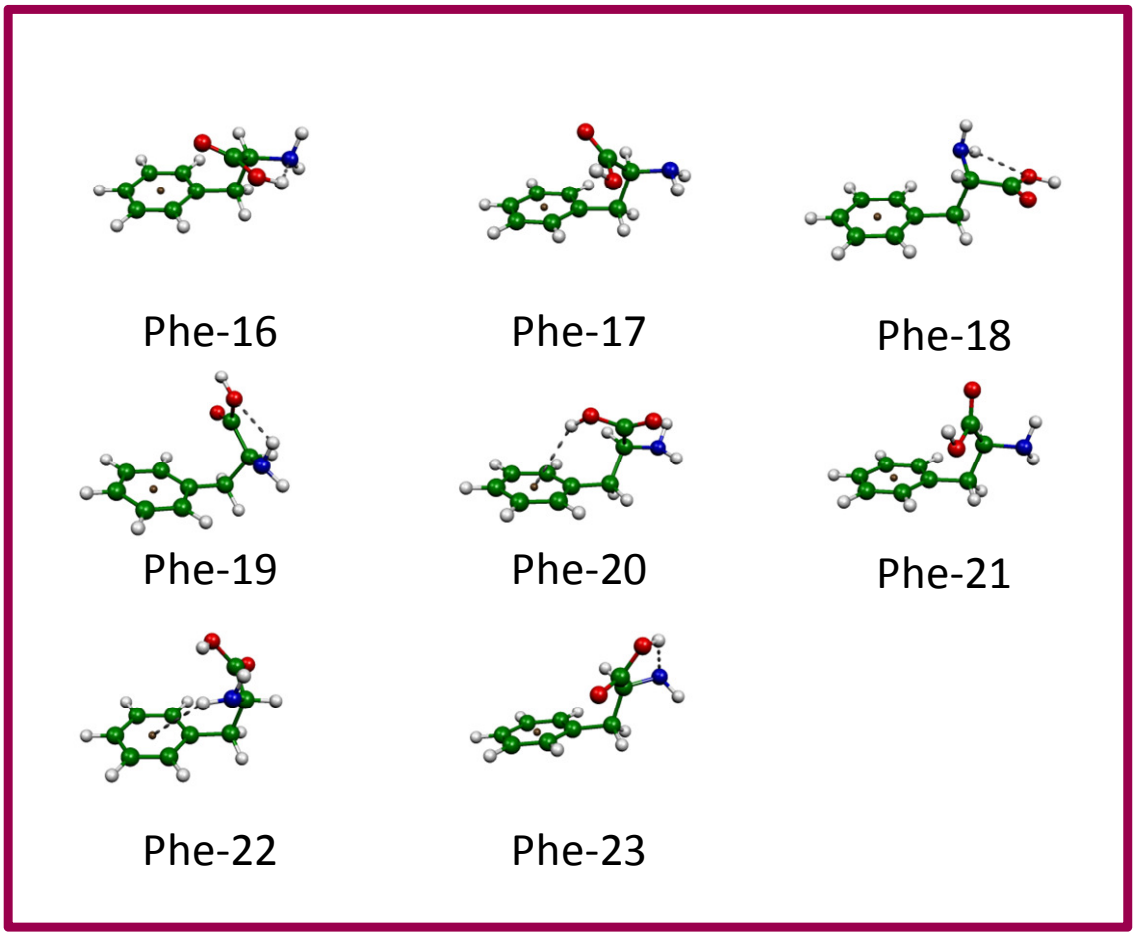

Figure 1b. Minimum energy structures found for phenylalanine molecule following the procedure described in computational details (optimized at the B97D/6-31+G* level).

Table 1. Selected geometrical parameters of the minima found for phenylalanine shown in Figure 1. Distances in Å, angles in degress. X indicates the phenyl ring center.

	φ_{CCCN}	$\varphi_{CCC\text{-}C=O}$	$R_{OH\cdots N}$	$R_{NH1\cdots X}$	$R_{NH2\cdots X}$	$R_{N\cdots X}$
Phe-1	52.7	-43.9	1.88	4.63	3.17	3.93
Phe-2	62.8	118.6	4.40	3.35	4.85	4.00
Phe-3	-64.2	-75.0	1.92	4.55	3.26	3.98
Phe-4	63.1	-61.0	3.73	3.33	4.84	3.99
Phe-5	-177.0	67.1	4.24	5.48	5.85	5.16
Phe-6	-60.9	-39.4	1.91	3.53	4.01	3.98
Phe-7	-60.4	89.8	4.28	3.27	4.49	3.99
Phe-8	-62.2	134.0	4.34	4.83	3.30	3.91
Phe-9	60.5	104.9	4.31	4.25	3.52	4.08
Phe-10	-179.2	69.3	4.17	5.49	5.52	5.21
Phe-11	-64.3	-7.7	3.68	4.75	3.24	3.95
Phe-12	-177.1	-126.8	3.83	5.49	5.85	5.17
Phe-13	-71.3	96.9	4.38	4.75	4.83	4.11
Phe-14	-60.6	-92.3	3.69	3.28	4.37	3.98
Phe-15	61.8	-68.1	3.65	4.38	3.45	4.08
Phe-16	-177.5	-86.0	1.96	5.88	5.38	5.19
Phe-17	-179.7	-113.4	3.71	5.51	5.51	5.22
Phe-18	-70.3	-104.9	3.79	4.66	4.84	4.09
Phe-19	67.9	-54.9	3.66	4.88	4.66	4.12
Phe-20	-170.4	106.3	3.87	5.52	5.84	5.16
Phe-21	-177.1	-144.7	3.85	5.84	5.39	5.17
Phe-22	60.8	-67.8	2.29	3.25	4.79	3.94
Phe-23	-134.6	-31.5	1.91	5.03	5.44	5.04

Table 2. Relative energies (kJ/mol) of the minima found for phenylalanine obtained at the MP2/aug-cc-pVDZ level. Zero point energy corrections and thermal corrections at 298 K obtained from frequencies calculated at the B97D/6-31+G* level

	D_0[(a)]	H^{298}	G^{298}		D_0	H^{298}	G^{298}
Phe-1	0	0	0	Phe-13	10.61	12.15	5.71
Phe-2	2.15	3.04	0.18	Phe-14	11.54	12.79	8.05
Phe-3	2.72	3.12	1.47	Phe-15	11.76	12.55	10.12
Phe-4	5.39	6.14	3.59	Phe-16	13.60	14.42	10.15
Phe-5	6.00	7.33	2.32	Phe-17	14.57	15.99	11.49
Phe-6	6.37	7.14	3.50	Phe-18	15.18	16.95	8.01
Phe-7	7.90	8.93	5.31	Phe-19	16.67	17.96	12.72
Phe-8	8.87	10.00	5.46	Phe-20	18.13	19.44	15.35
Phe-9	9.62	10.63	7.80	Phe-21	19.24	20.74	14.56
Phe-10	9.71	10.96	6.84	Phe-22	20.27	20.76	19.40
Phe-11	10.23	11.33	6.57	Phe-23	21.26	21.83	17.92
Phe-12	10.58	11.83	7.67				

(a) D_0 indicates energy plus zero point correction

However, the interest of this work relies on the guanidinium···phenylalanine interaction. Figure 2 shows the minimum energy structures found for the complex formed by guanidinium and phenylalanine following the procedure indicated in computational details. It is worth noting that including the guanidinium cation facilitates the appearance of complexes with the zwitterionic form of phenylalanine. Also, the presence of the cation reduces the number of possible minima in the energy interval chosen in the conformational search, so 14 minima were found when guanidinium is present, as shown in Figure 2. Table 3 lists selected geometrical parameters of these complexes whereas Table 4 summarizes energetic data.

The most stable minima found presents guanidinium interacting simultaneously with the NH_2 and the C=O groups of the phenylalanine molecule. Also, a cation···π contact is observed between one of the NH_2 groups of guanidinium and the phenyl ring. As observed from data in Table 3, the carbon atom in guanidinium is located at 4.13 Å from the phenyl ring center, whereas distances to C=O carbon and nitrogen atom are around 4 Å both. This disposition is similar to the most stable minimiun observed in other cation···phenylalanine complexes.[17, 18] The complexation energy of this structure amounts to -116.6 kJ/mol at the MP2/aug-cc-pVDZ after inclusion of the ZPE correction obtained with the B97D/6-31+G* method. Therefore, the interaction between guanidinium and phenylalanine is quite intense in the gas phase, especially when compared to the interaction with benzene which amounts to around 60 kJ/mol.

The second most stable structure presents guanidinium in zwitterionic form, with an energy difference with respect to G-Phen-1 of only 1.5 kJ/mol, suggesting both structures could simultaneously exist in a sample. The third minimum energy structure is similar to that observed in G-Phen-2, with the difference of the proton not being transferred to the amino group in phenylalanine. The basic structural pattern of G-Phen-zw1 and G-Phen-2 is the same, but the structures differ by about 3.3 kJ/mol favorable to the zwitterionic form.

The rest of the fourteen minima located exhibits different structural arrangements depending on the conformation of phenylalanine molecule and how guanidinium cation is attached to it. As indicated above, the number of minima is reduced by the presence of guanidinium, so only 6 minima are within an energy difference of 10 kJ/mol with respect to the global minimum (there were 10 structures below 10 kJ/mol for phenylalanine molecule). Overall, a cation···π contact is only observed in four of the minima found, namely G-Phe-1, G-Phe-3, G-Phe6, G-Phe-9, where guanidinium cation interacts with the phenyl ring in a T-like structure by using one of its NH_2 groups, whereas the other two NH_2 units are engaged in other contacts with the amino nitrogen of carboxylic oxygen atoms. In other structures, even when guanidinium does not interact with the phenyl ring, the NH_2 group does in a sort of chainlike hydrogen bonds as G-Phen-2 or G-Phen-4, for example.

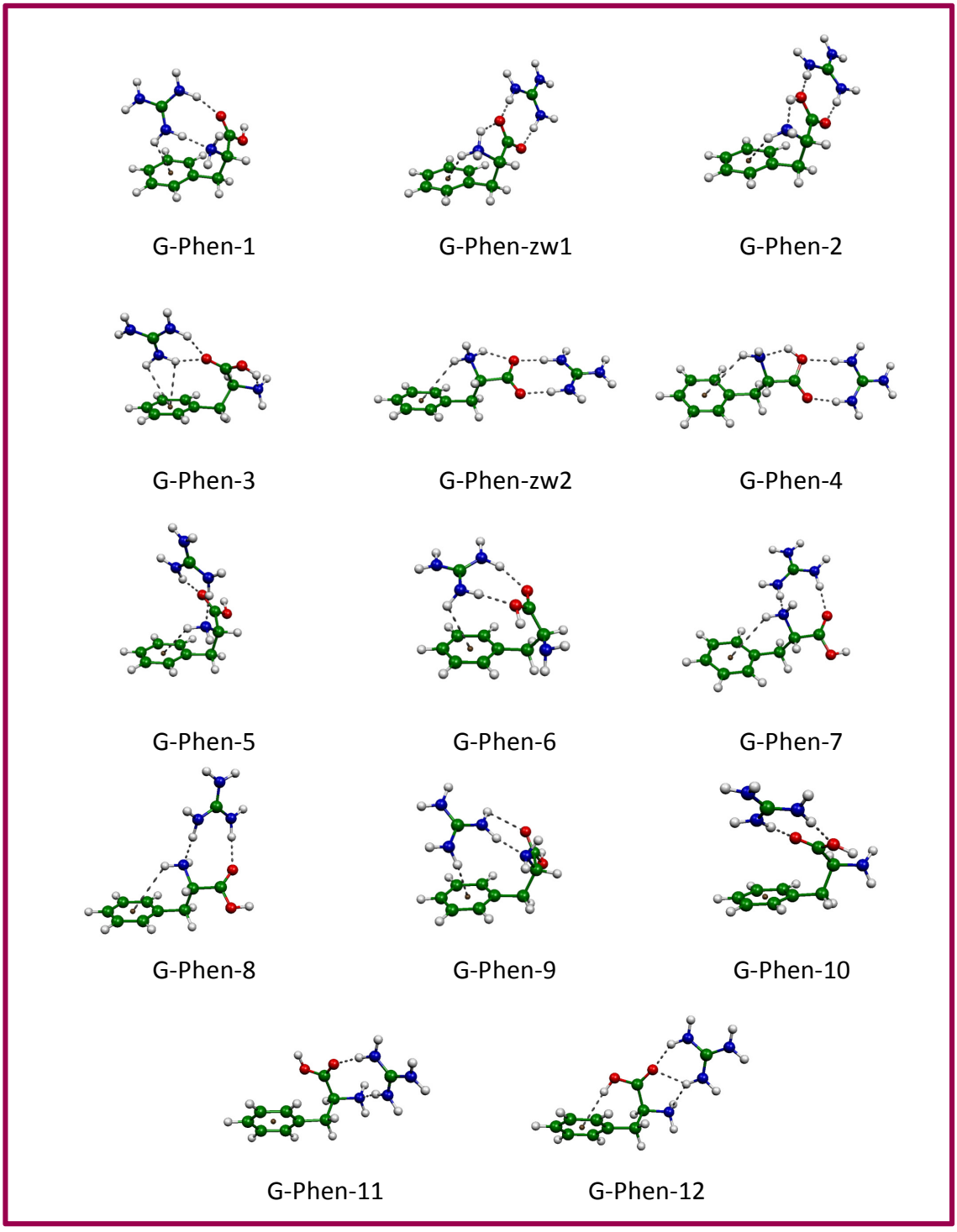

Figure 2. Most stable structures found for the complex formed by phenylalanine and guanidinium cation.

Table 3. Selected geometrical parameters of the minima found for phenylalanine guanidinium complex shown in Figure 1. Distances in Å, angles in degress. X indicates the phenyl ring center. C_g is the carbon in guanidinium.

	φ_{CCCN}	$\varphi_{CCC-C=O}$	$R_{OH\cdots N}$	$R_{NH1\cdots X}$	$R_{N\cdots X}$	$R_{Cg\cdots X}$	$R_{Cg\cdots CO}$	$R_{Cg\cdots N}$
G-Phe-1	53.1	124.8	4.35	4.09	3.93	4.13	4.33	3.74
G-Phe-zw1	49.2	132.9	1.04	4.57	3.79	7.11	4.01	6.17
G-Phe-2	51.5	-51.6	1.67	3.22	3.91	6.90	4.16	6.24
G-Phe-3	-176.7	-74.5	1.83	5.40	5.26	4.33	4.45	6.77
G-Phe-zw2	-55.0	-73.8	1.03	4.30	3.81	9.10	4.00	6.19
G-Phe-4	-61.3	-67.6	1.70	3.29	3.94	9.27	4.18	6.26
G-Phe-5	59.1	114.9	4.34	3.25	3.98	6.47	4.38	3.63
G-Phe-6	73.3	-68.4	1.79	4.29	4.33	4.16	3.97	5.98
G-Phe-7	-56.6	103.8	4.35	3.13	3.85	6.38	4.49	3.70
G-Phe-8	-57.4	113.0	4.34	3.15	3.90	7.43	4.34	3.64
G-Phe-9	52.0	125.5	4.35	4.07	4.01	3.97	4.76	3.81
G-Phe-10	173.5	-78.2	1.79	5.40	5.21	6.21	4.17	6.33
G-Phe-11	-179.9	80.2	4.30	5.44	5.18	7.16	4.53	3.79
G-Phe-12	-172.2	118.2	3.88	5.40	5.19	7.63	4.41	3.72

Table 4. Relative energies (kJ/mol) of the minima found for phenylalanine guanidinium complex obtained at the MP2/aug-cc-pVDZ level. Zero point energy corrections and thermal corrections at 298 K obtained from frequencies calculated at the B97D/6-31+G* level

	D_0[(a)]	ΔH^{298}	ΔG^{298}		D_0	ΔH^{298}	ΔG^{298}
G-Phe-1	0.00	0.00	0.00	G-Phe-6	11.41	10.94	10.82
G-Phe-zw1	1.53	1.79	-2.68	G-Phe-7	12.05	13.26	5.11
G-Phe-2	4.82	5.41	-0.74	G-Phe-8	13.71	14.70	7.97
G-Phe-3	5.91	6.75	1.92	G-Phe-9	17.37	18.17	15.01
G-Phe-zw2	6.38	7.29	0.69	G-Phe-10	17.54	19.10	8.99
G-Phe-4	8.72	10.03	1.46	G-Phe-11	20.59	22.05	13.22
G-Phe-5	10.63	11.49	4.82	G-Phe-12	24.73	26.13	17.11

(a) D_0 indicates complexation energy plus zero point correction

As indicated above, the presence of guanidinium cation makes some zwitterionic structure stable, to the point that the second and fifth most stable structures correspond to zwitterionic phenylalanine. In zwitterioninc phenylalanine, guanidinium interacts with the carboxylate oxygens and no stable structure exhibiting guanidinium···phenyl interaction is found. However, there is a sort of cation-π interaction via the ammonium group in phenylalanine.

In summary, the presence of guanidinium cation promotes important changes on preferred structures of phenylalanine molecule, even allowing the presence of stable complexes formed with zwitterionic phenylalanine. Though it could be expected cation···π interaction to be an important interaction in this system, it is scarcely present in the minima found since guanidinium interacts preferentially with the oxygens of the carboxylic group or amino nitrogen, though as a matter of fact a cation···π interaction it is observed in the most stable structure found for the complex.

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