F. Kiani et al. / Hacettepe J. Biol. & Chem., 2014, 42 (2), 263-274

Determination of acidic dissociation constants of L-phenylalanyl-glycine and L-alanyl-L-alanine in water using ab initio methods

L-fenilalanil-gilisin ve L-alanil-L-alaninin sudaki asidik ayrışma sabitlerinin ab initio yöntemleri ile belirlenmesi

Research Article

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ABSTRACT

The acid dissociation constant is one of the fundamental properties of organic molecules. In this study, density functional theory (DFT) calculations with B3LYP combined with 6-31+G(d) basis set were applied for determination of acidic dissociation constants of L-phenylalanyl-glycine and L-alanyl-L-alanine. An ab initio procedure for accurately calculating aqueous-phase pKa values of the L-phenylalanyl-glycine and L-alanyl-L-alanine is presented. Formation of intermolecular hydrogen bonds between the existent species and water has been analyzed using Tomasi's method. In this way, it was determined that in alkaline aqueous solutions the cation, anion, and neutral species of L-phenylalanyl-glycine and L-alanyl-L-alanine are solvated with some molecules of water. Furthermore, the correlation between the pKa values of these dipeptides was investigated theoretically, and a comparable agreement was found with the experimental results.

Key Words

Acidity constant, L-phenylalanyl-glycine, L-alanyl-L-alanine, solvation free energy, ab initio methods.

ÖZET

Asidik ayrışma sabiti organik bileşiklerin en temel özelliklerinden birisidir. Bu çalışmada, yoğunluk fonksiyon teori (DFT) hesaplamaları 6-31+G(d) ile B3LYP birleştirilmiş temel setler L-fenilalanil-glisin ve L-alanil-L-alaninin asidik ayrışma sabitlerinin belirlenmesinde uygulanmıştır. L-fenilalanil-glisin ve L-alanil-L-alaninin sulu-faz pK_a değeri hesaplamaları için ab initio yöntemi sunulmuştur. Mevcut türler ve su arasında oluşan moleküller arası hidrojen bağlarının oluşumu Tomasi yöntemi kullanılarak analiz edilmiştir. Bu şekilde, su molekülleri ile solvate olmuş L-fenilalanil-glisin ve L-alanil-L-alaninin katyonu, anyon ve nötr türleri bazik sulu çözeltilerde belirlenmiştir. Ayrıca, bu dipeptidlerin pKa değerleri arasındaki ilişki teorik olarak incelenmiş ve kıyaslanabilir uyumlu deneysel sonuçlar bulunmuştur.

Anahtar Kelimeler

Asitlik sabiti, L-fenilalanil-glisin, L-alanil-L-alanin, çözünme serbest enerjisi, ab initio yöntemleri.

Article History: Received: Nov 11, 2013; Revised: Dec 27, 2014; Accepted: Mar 25, 2014; Available Online: Jun 13, 2014.

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INTRODUCTION

Peptides are very attractive as therapeutics, their diverse biological application and potentially high potency and target specificity [1]. In recent years, an increasing activity in design and synthesis of new peptide-based drugs is expected, as a result of combined advances in proteomic research and biotechnology [2].

Peptide structures that have been characterized have come from analysis of NMR or other experimental data in many cases supplemented with theoretical modeling [3].

Proton transfer reactions constitute an important class of chemical reactions and have been studied extensively over a long period of time [4]. The acid dissociation constant, pK_a, determines the degree of dissociation at a given pH. Acidic dissociation constant have many applications in chemical, biological, environmental, and pharmaceutical research because the important physicochemical properties, like lipophilicity, solubility, and permeability, are all dependent on pK_a [5]. Studies of the acidity of organic compounds are important and play a very significant role in the evaluation of activity, reaction mechanisms and structure of organic compounds [6,7]. However the accurate experimental determination of pK_a values is not a trivial task in many cases and is a experimental challenge [8]. This is the case for species such as reaction intermediates, strong acids, and weak acids. As a result, determining pK_a values computationally has become an issue of great interest. In this regard theoretical effort has been devoted to accurately modeling acid-base equilibria in solution and calculating the associated pKa values [9-23].

The prediction of pK_a values of solvated molecules has been an important issue in the computational chemistry community over many years [24-55]. This is due to the fact that, proton transfer is the most frequently occurring reaction in nature, and often encountered reaction in technological processes. Proton plays an important role in energy conversion in living cells and fuel cells facilitate ion exchange in biological and synthetic membranes, catalyze chemical reactions at the active site of proteins and in synthetic reactions[56].

As pKa equals to $\Delta G/2.303RT$, where ΔG is a free energy change of the dissociation reaction either in a gas or solution phase, activity of compound can be determined by the ΔG values [57].

Kinds of polarizable continuum models have been applied to calculate free energy differences for cations, neutral compounds and their anions. On the basis of solvation free energies, the pKa values were obtained for the compounds in question aqueous solutions using thermodynamic equations; involving the combined experimental and calculated data [58].

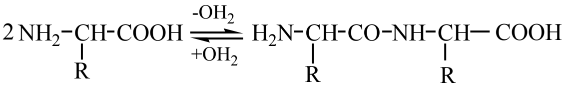
This paper deals with the influence of factors such as the self consistent reaction field (SCRF) model applied, choice of a particular thermodynamic equations, atomic radii used to build a cavity in the solvent (water), optimization of geometry in water, inclusion of electron correlation, the dimension of the basis set on the salvation free energies and on the calculated pK_a values. pK_a values of L-phenylalanyl-glycine and L-alanyl-L-alanine were determined in aqueous solution by ab initio method at room temperature. In order to explain the acidic dissociation constants obtained, we investigated the molecular conformations and solute–solvent interactions of the cation, anion and neutral species of L-phenylalanyl-glycine and L-glanyl-L-glanine using ab initio and density functional theory (DFT) methods.

Computational Method

Figures 4 and 5 show the structure of L-phenylalanyl glycine and L-alanyl-L-alanine and their cations and the practical numbering system adopted for performing the calculations. In this study, structures were optimized by the semi empirical PM3 method in program Hyperchem version 8.0.8 for windows. All calculations were performed using Gaussian 98w version 5.2. DFT calculations were done using the hybrid exchange–correlation functional of Becke, Lee, Yang, and Parr (B3LYP) and the Gaussian 6-31G(d) basis set. To analyze the solvent effects on all species involved in the selected ionization reaction, the polarized continuum model (PCM) of Tomasi et al. was applied [57].

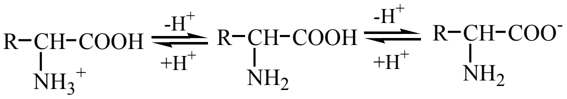
RESULTS AND DISCUSSION

Peptides are formed by binding amino acids together through an amid linkage. Hydrolysis of peptides results in free amino acids [59,60]:



$$2\,NH_2-\underset{R}{CH}-COOH \underset{+OH_2}{\overset{-OH_2}{\rightleftharpoons}} H_2N-\underset{R}{CH}-CO-NH-\underset{R}{CH}-COOH$$

Fully protonated L-phenylalanyl-glycine and L-alanyl-L-alanine have two acid groups: ammonium and carboxyl. A proton can be lost from any one of the two groups to give different ionized species; the loss of a proton from the carboxyl group is most probable, and from the ammonium group least probable. In aqueous solution amino acids and peptides are present, depending on pH, as cations, zwitterions or anions.



$$R-\underset{NH_3^+}{CH}-COOH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} R-\underset{NH_2}{CH}-COOH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} R-\underset{NH_2}{CH}-COO^-$$

Their side chain R, can vary small or bulky, hydrophobic or hydrophilic, polar, charged or neutral. The dissociation constants of amino acids and peptides can be determined, for example, by titration of the acid. Therefore, this concept of microscopic ionization constants k_1 and k_2 may be applied, where k1 involving the carboxyl proton is:

$$k_1 = \frac{[H^+][NH_3^+CHRCOO^-]}{[NH_3^+CHRCOOH]} \qquad (1)$$

and k_2 involving the ammonium proton is:

$$k_2 = \frac{[H^+][NH_2CHRCOOH]}{[NH_3^+CHRCOOH]} \qquad (2)$$

It can be shown that for a dibasic acid the first ionization constant K_1 is the sum k_1+k_2 and second ionization constant K_2 is $\frac{k_{12}.k_{21}}{k_{12}+k_{21}}$.

The chemical interpretation of the changes is not straightforward, even though from model compounds the carboxyl proton is predicted to be the most acidic. Calculations involving the microscopic constants indicate the first and second K corresponds to exclusive removal of the carboxyl proton and a small proton loss from ammonium acidic site, on determined by NMR spectroscopy studies [57].

The acidity of the free carboxyl groups and the basicity of the free amino groups are lower in peptides than in the corresponding free amino acids [60].

The different models of molecules (zwitterions and unzwitterions) were investigated by the G98 program. Considering eqs. (1) and (2), different reactions including cationic, neutral and anionic species were tested, but some of the reactions were not considered further because the estimated error in its acidic dissociation constants were unacceptable. The models finally chosen for the studied system and the calculated values of the acidic dissociation constants of peptides are listed in Table 1.

Solvent-solute Interactions

Ionic product of water

It is well-known that all aqueous solutions contain hydrogen (H^+) and hydroxyl (OH^-) ions. In pure water these ions are derived completely from the ionization of the water molecules:

$$H_2O \leftrightarrow H^+ + OH^- \qquad (3)$$

Table 1. Values of pKa for protonation of L-phenylalanyl-glycine and L-alanyl-L-alanine obtained using the Tomasi Method at the B3LYP/6-31+G(d) level of theory, at 298.15 K.

Species (Experimental)[a]	Selected Equations	pK_a (Calculated)[b]	pK_a (Experimental)[a]
L-phenylalanyl-glycine	$H_2L+(H_2O) \leftrightarrow HL + H_3O^+$	3.1504	3.7037 (I=0.1,$NaClO_4$)
	$HL(H_2O)_3 + H_2O \leftrightarrow L\text{-}(H_2O)_3 + H_3O^+$	7.6443	7.5236 (I=0.1,$NaClO_4$)
L-alanyl-L-alanine	$H_2L+(H_2O)_2 \leftrightarrow HL(H_2O) + H_3O^+$	3.2570	3.30 (I=0.1,$NaClO_4$)
	$HL(H_2O)_3 + H_2O \leftrightarrow L\text{-}(H_2O)_3 + H_3O^+$	8.0361	8.14 (I=0.1,$NaClO_4$)

[a] Experimental data collected from Ref [64,65]
[b] This work

Considering that the H^+ ion is hydrated, appearing predominantly as $H3O^+$, the autoprotolysis of water is better represented by reaction:

$$2H_2O \leftrightarrow H_3O^+ + OH^- \quad (4)$$

Taking into account that water is only a little dissociated and to simplify the discussion we shall make the approximations of replacing the activities in acidity constants by the numerical values of the molar concentrations, consequently:

$$K_w = [H_3O^+][OH^-] \quad (5)$$

At 298.15 K, $K_w = 1.008 \times 10^{-14}$, shows that only a few of the water molecules are ionized.

Conventionally, eq. (4) and (5) are those more used in studies of acid-base equilibria in aqueous medium. On the other hand, the solvation of anions is effective in protic solvents where hydrogen bonds may be formed between the proton of the solvent and the lone pairs of electrons of the anion [57]. The total energies of the single and solvated OH- ion have been calculated in water at the B3LYP/6-31+G(d) level of theory, using Tomasi's model.

To illustrate, Figure 1 shows the structures of the hydroxyl ion solvated with one and also two water molecules together.

It can be observed that the distance between the O1- atom of hydroxyl group The H5 atom of the water (Figure 1a) is equal to 1.557 Å, whereas the bond angle (A_{bond}) that forms the involved atoms ($O_{3-}H_{5-}O_{1-}$) in the hydrogen-bond-donor (IHB) is 177.8°. Furthermore, Figure 1b shows the distance and bond angle formed by the involved atoms (H_2O_4, $O_1H_2O_4$) in the IHB are 1.905 Å and 171.6°, respectively.

The data allows that the IHBs between the OH^- ion and the water molecules of solvation belong to the class of moderate or strong H bonds. The calculated total energy values show the striking decrease of the total energy of the OH^- ion when its solvation increases. For each solvation water molecule, the OH ion decreases its relative energy by 200, 222 kJ mol^{-1}respectively.

Considering these facts and in order to provide a more satisfactory representation of the protolysis of water, the reaction has been shown as follows:

$$3H_2O \leftrightarrow H_3O^+ + OH^-(H_2O) \quad (6)$$

The selected reaction considers that both H+ and OH- ions are hydrated with one water molecule. Moreover, indicating with K_N the equilibrium constant of the reaction of Eq. (6) and taking into account Eqs. (4) and (5), it is inferred that [57]:

$$K_w = K_N [H_2O]$$

where $[H_2O]$ is the molar concentration of water. Consequently, at 298.15 K, it was calculated that:

$$K_N = \frac{K_w}{[H_2O]} = 1.831 \times 10^{-16} \quad (7)$$

Similarly, the total energies of the single and solvated L-phenylalanyl-glycine and L-alanyl-L-alanine specimen (cationic, neutral and anionic) were calculated in water at the B3LYP/6-31+G(d) level of the theory, using Tomasi's model.

Table 2 summarizes the variations of the total energy (kJ mol^{-1}) of the specimen per water molecule as a function of the total number of solvation water molecules.

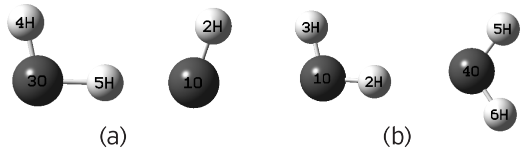


Figure 1. Optimized structure of: a) the hydroxyl ion solvated with one water molecule b) two water molecules together.

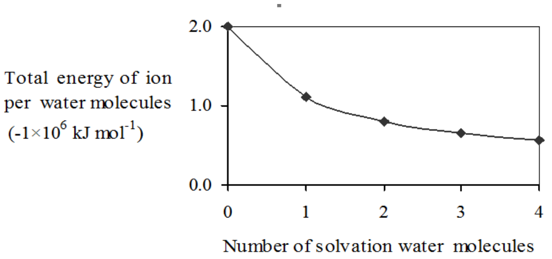


Figure 2. Plot of the total energy (kJ.mol^{-1}) of a solvated L-phenylalanyl-glycine anion anions per water molecule against the total number of solvation water molecules.

Table 2. Calculated total energy using the Tomasi Method at the B3LYP/6-31+G(d) level of theory for cationic, neutral, and anionic species of L-phenylalanyl-glycine and L-alanyl-L-alanine at 298.15 Ka.

No.	Solvated Species	G^0_{sol} (Hartree)	G^0_{sol}/molecule (Kj mol^{-1})	No.	Solvated Species	G^0_{sol} (Hartree)	G^0_{sol}/molecule (Kj mol^{-1})
	L-phenylalanyl-Glycine				L-alanyl-L-alanine		
0	H_2L^+	$-7.6329 \times 10^{+2}$	$-2.0040 \times 10^{+6}$	0	H_2L^+	$-5.7157 \times 10^{+2}$	$-1.5006 \times 10^{+6}$
1	$H_2L^+(H_2O)$	$-8.3974 \times 10^{+2}$	$-1.1024 \times 10^{+6}$	1	$H_2L^+(H_2O)$	$-6.4801 \times 10^{+2}$	$-8.5067 \times 10^{+5}$
0	HL	$-7.6287 \times 10^{+2}$	$-2.0029 \times 10^{+6}$	0	HL	$-5.7112 \times 10^{+2}$	$-1.4995 \times 10^{+6}$
1	$HL(H_2O)$	$-8.3930 \times 10^{+2}$	$-1.1018 \times 10^{+6}$	1	$HL(H_2O)$	$-6.4756 \times 10^{+2}$	$-8.5008 \times 10^{+5}$
2	$HL(H_2O)_2$	$-9.1574 \times 10^{+2}$	$-8.0143 \times 10^{+5}$	2	$HL(H_2O)_2$	$-7.2399 \times 10^{+2}$	$-6.3361 \times 10^{+5}$
0	L^-	$-7.6242 \times 10^{+2}$	$-2.0017 \times 10^{+6}$	0	L^-	$-5.7067 \times 10^{+2}$	$-1.4983 \times 10^{+6}$
1	$L^-(H_2O)$	$-8.3885 \times 10^{+2}$	$-1.1012 \times 10^{+6}$	1	$L^-(H_2O)$	$-6.4711 \times 10^{+2}$	$-8.4949 \times 10^{+5}$
2	$L^-(H_2O)_2$	$-9.1529 \times 10^{+2}$	$-8.0103 \times 10^{+5}$	2	$L^-(H_2O)_2$	$-7.2354 \times 10^{+2}$	$-6.3322 \times 10^{+5}$
3	$L^-(H_2O)_3$	$-9.9172 \times 10^{+2}$	$-6.5094 \times 10^{+5}$	3	$L^-(H_2O)_3$	$-7.9997 \times 10^{+2}$	$-5.2508 \times 10^{+5}$
4	$L^-(H_2O)_4$	$-1.0682 \times 10^{+3}$	$-5.6089 \times 10^{+5}$	4	$L^-(H_2O)_4$	$-8.7587 \times 10^{+2}$	$-4.5992 \times 10^{+5}$
	Water						
0	H_3O^+	$-7.6862 \times 10^{+1}$	$-2.0180 \times 10^{+5}$	0	OH^-	$-7.5952 \times 10^{+1}$	$-1.9941 \times 10^{+5}$
1	$H_3O^+(H_2O)$	$-1.5330 \times 10^{+2}$	$-2.0124 \times 10^{+5}$	1	$OH^-(H_2O)$	$-1.5240 \times 10^{+2}$	$-2.0006 \times 10^{+5}$
0	$H_3O^+(H_2O)_2$	$-2.2973 \times 10^{+2}$	$-2.0105 \times 10^{+5}$	2	$OH^-(H_2O)_2$	$-2.2885 \times 10^{+2}$	$-2.0028 \times 10^{+5}$
1	H_2O	$-7.6434 \times 10^{+1}$	$-2.0068 \times 10^{+5}$	3	$OH^-(H_2O)_3$	$-3.0529 \times 10^{+2}$	$-2.0039 \times 10^{+5}$
2	$(H_2O)_2$	$-1.5287 \times 10^{+2}$	$-2.0068 \times 10^{+5}$	4	$OH^-(H_2O)_4$	$-3.8173 \times 10^{+2}$	$-2.0044 \times 10^{+5}$

[a] *N*: total number of solvation water molecules; G^0_{sol}, total free energy in solution; G^0_{sol}/molecule, total energy of solvated species per water molecule; H_2L^+, cation species; HL, neutral; L^-, anion species.

Figures 2, 3 and Table 2 show that marked increase of the total energies of ions when their solvation increases.

The data show that the water, exerting its hydrogen-bond-donor (HBD) capability, forms IHBs with the L-phenylalanyl-glycine and L-alanyl-L-alanine anions. These hydrogen bonds have been classified as strong, moderate and weak bonds, according to their lengths, angles and energies [61].

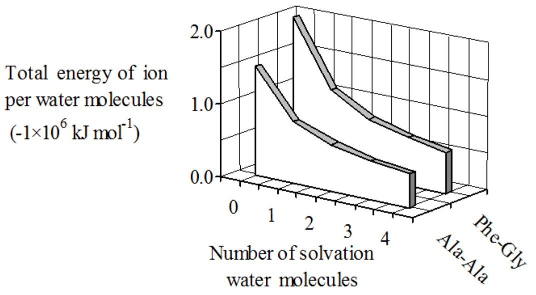


Figure 3. Plot of the total energy (kJ.mol^{-1}) of a solvated L-phenylalanyl-glycine and L-alanyl-L-alanine anions per water molecule against the total number of solvation water molecules.

First Ionization Constant of L-phenylalanyl-glycine

It was selected that in alkaline solutions L-phenylalanyl-glycine suffers a reaction of partial neutralization as follows:

$$H_2L+(H_2O) + OH\text{-}(H_2O) \leftrightarrow HL + 3H_2O \quad K_{Cl} \tag{8}$$

In this reaction $H_2L^+(H_2O)$ is L-phenylalanyl-glycine cation solvated with one water molecule and HL represents L-phenylalanyl-glycine neutral. The previous reaction is characterized by an equilibrium constant, KCl, which was theoretically determined.

By combining Eq. (8) and (4) we obtain the reaction of Eq. (9), which defines the first ionization constant of L-phenylalanyl-glycine (K_{a1}), and considers the solvation of the neutral L-phenylalanyl-glycine:

$$H_2L+(H_2O) \leftrightarrow HL+H_3O^+ \tag{9}$$

It is evident that the constants K_{C1}, Kw and Kal are related by the following equation:

$$K_{a1} = K_{C1} \times K_w \tag{10}$$

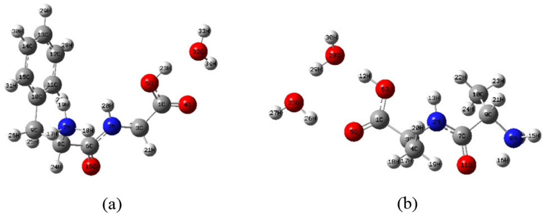

(a) (b)

Figure 4. Optimized structure of: (a) L-phenylalanyl-glycine (b) L-alanyl-L-alanine cation solvated with one and two molecules of water, respectively and practical numbering system adopted for carrying out the calculations.

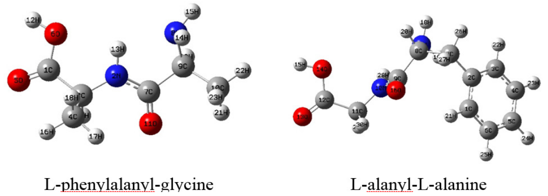

L-phenylalanyl-glycine L-alanyl-L-alanine

Figure 5. Optimized structures of L-phenylalanyl-glycine and L-alanyl-L-alanine for carrying out the calculations.

The above equation was used to determine theoretically the value of the first ionization constant of L-phenylalanyl-glycine in water. Table 3 summarizes the optimized values of molecular properties of $H_2L^+(H_2O)$ cation (Figure 4a), the HL and $HL(H_2O)_3$ neutral molecule (Figure 5 and Figure 6) obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method in water at 298.15 K.

It must be noted that in the formation of the neutral L-phenylalanyl-glycine, the neutral molecules practically do not have the structure that characterizes the solvated L-phenylalanyl-glycine cation (Figure 4a and Table 3).

Obviously, the formation of the neutral L-phenylalanyl-glycine implies that the electronic density of the N_7 atom decreases (in absolute value) with respect to the N_{17} atom of the L-phenylalanyl-glycine cation (Table 3). Furthermore, the negative atomic charge of O_{14}- (qO_{14}-) of the neutral L-phenylalanyl-glycine decreases (in absolute value). These facts help to explain that the H15 atom of the carboxyl (O_{14}-H_{15}) group bound to C_{12} neutral L-phenylalanyl-glycine is more acidic than the H_{23} atom of the carboxyl (O_5-H_{23}) group bound to the C_1 atom of the solvated L-phenylalanyl-glycine cation Also, it shoulde be remarked that the nucleophilic attack of the O^- atom of OH^- ion on the H_{17} atom of the NH_3 group bound to N_7 of the L-phenylalanyl-glycine cation produced the neutral L-phenylalanyl-glycine (Figure 4a).

It is reasonable to observe that the molecular volume of the neutral L-phenylalanyl-glycine molecule solvated with three water molecules is approximately the sum of the molecular volumes of the specimen that form it, (i.e. neutral L-phenylalanyl-glycine molecule with three water molecules).

On the other hand, the pKal value theoretically obtained ($pK_{a1} = 3.1504$) has an adequate agreement with the experimental pK_{a1} value ($pK_{a1} = 3.7037$). We conclude that this fact constitutes a strong support for the reactions and equilibria of ionization selected here [62].

Second Ionization Constant of L-phenylalanyl-glycine

Here, it is selected that the neutral $HL(H_2O)_3$ suffers a total neutralization as follows:

$$HL(H_2O)_3 + OH^-(H_2O) \leftrightarrow L^-(H_2O)_3 + 2H_2O \qquad (11)$$

In the above reaction $L^-(H_2O)_3$ represents the L-phenylalanyl-glycine anion solvated with three water molecules. The reaction described in Eq. (11) is characterized by another equilibrium constant, K_{C2}, which was also theoretically determined. Combining Eqs. (6) and (11) the second ionization reaction of L-phenylalanyl-glycine was obtained:

$$HL(H_2O)_3 + H_2O \leftrightarrow L^-(H_2O)_3 + H_3O^+ \qquad (12)$$

The equilibrium constant K_{a2} that characterizes

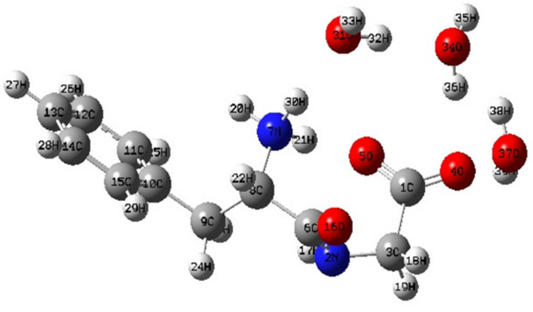


Figure 6. Calculated structure for neutral L-phenylalanyl-glycine solvated with three water molecules, at the B3LYP/6-31+G(d) level of theory and using the Tomasi's method in water at 298.15 K.

Table 3. Calculated structural magnitudes using Tomasi's Method at the B3LYP/6-31+G(d) level of theory for the cation, neutral molecule, and anion of L-phenylalanyl-glycine at 298.15 Ka.

Species	Calculated magnitudes			
L-Phenylalanyl-Glycine	$H_2L^+(H_2O)$	HL	$HL(H_2O)_3$	$L^-(H_2O)_3$
K_{C1}	1.2389✢10^{8}	-		-
K_{C2}	-	-	3.8629×10^{12}	-
K_{a1}	2.2684✢10^{8}	-	-	-
K_{a2}	-	-	7.0730×10^{-4}	-
a_0	5.22	4.93	5.08	5.53
D-$H_{15}C_{11}N_{10}C_9$	-	-	-	-64.2001
D-$H_{18}C_3N_2C_1$	-	-	121.5710	-
D-$H_{19}H_{18}C_8C_7$	-	39.5255	-	-
D-$H_{21}N_7C_6N_2$	-	-	-25.1274	-
D-$H_{22}C_3N_2C_1$	-121.0970	-	-	-
D-$H_{23}C_4C_3C_2$	-	-179.6076	-	-
D-$H_{24}C_4C_3C_2$	-	-	-	179.7206
D-$H_{25}C_{11}C_{10}C_9$	-	-	2.9969	-
D-$H_{26}C_7C_2C_1$	-	156.6501	-	-
D-$H_{27}C_{11}C_{10}C_9$	3.2485	-	-	-
D-$H_{28}N_{19}C_8C_7$	-	-	-	-170.3993
D-$H_{29}C_{13}C_{12}C_{11}$	-179.4392	-	-	-
D-$H_{33}H_{31}N_7C_6$	-	-	-60.0273	-
D-$O_{18}C_9C_8C_7$	-	-	-	50.7267
D-$O_{36}O_{14}C_{12}C_{11}$	-	-	-	143.7153
D-$O_{37}O_4C_1O_5$	-	-	113.3426	-
qC_4	-	-0.279	-	-0.279
qN_{17}	-	-1.023	-	-
qO_5	-0.713	-	-0.688	-
qO_{18}	-	-	-	-0.576
qO_{31}	-	-	-1.112	-
qO_{32}	-1.034	-	-	-
qO_{33}	-	-	-	-1.173
qO_{34}	-	-	-1.143	-
qO_{36}	-	-	-	-1.128
qN_7	-1.175	-	-1.314	-
qO_{14}	-	-0.64	-	-
qN_{19}	-	-	-	-0.051
qO_4	-0.561	-	-0.7	-
qO_{13}	-	-0.524	-	-0.74
qO_{14}	-	-0.64	-	-0.892
d-$H_{17}N_2$	-	-	0.9958	-
d-$H_{18}N_7$	1.0280	-	-	-
d-$H_{18}N_{17}$	-	1.0171	-	-
d-$H_{19}N_7$	1.0362	-	-	-
d-$H_{23}C_4$	-	1.0874	-	-
d-$H_{35}O_{34}$	-	-	0.9477	-
d-$O_{31}N_7$	-	-	2.8102	-
d-$O_{33}O_{13}$	-	-	-	2.8604
A-$H_{15}O_{14}C_{12}$	-	106.7905	-	-
A-$H_{17}N_7C_6$	140.2734	-	-	-
A-$H_{19}N_{17}C_8$	-	110.6040	-	-
A-$H_{20}N_7C_6$	-	-	147.4262	-
A-$H_{22}C_5C_4$	-	-	-	120.3190
A-$H_{23}C_4C_3$	-	119.8122	-	-
A-$H_{24}C_9C_8$	-	-	107.5134	-
A-$H_{26}C_9C_8$	108.5804	-	-	-
A-$H_{30}N_7C_6$	-	-	98.9769	-
A-$H_{33}H_{32}O_5$	135.4133	-	-	-
A-$H_{35}O_{33}O_{13}$	-	-	-	7.9675
A-$H_{39}O_{37}O_4$	-	-	23.2798	-
A-$O_{18}C_9C_8$	-	-	-	118.8220
A-$O_{34}O_4C_1$	-	-	103.8018	-

[a] K_{c1} and K_{c2}, equilibrium constants of equations; K_{a1} and K_{a2}, first and second acidic dissociation constant between the indicated atoms (A°); a_0, bohr radius (A°); q, total atomic charge (Muliken) (au); d, bond lengths between the indicated atoms (A°); A, bond angle(°).

Appendix A

Table A. Calculated structural magnitudes using Tomasi;s Method at the B3LYP/6-31+G(d) level of theory for the cation, neutral molecule, and anion of L-alanyl-L-alanine at 298.15 K.

Species		**Calculated magnitudes**		
L-Alanyl- L-Alanyl	**$H_2L^+(H_2O)_2$**	**$HL(H_2O)$**	**$HL(H_2O)_3$**	**$L^-(H_2O)_3$**
K_{c1}	5.4884×10^{10}	-		-
K_{c2}	-	-	5.0253×10^{7}	-
K_{a1}	5.5323×10^{-4}	-	-	-
K_{a2}	-	-	9.2013×10^{-9}	-
a_0	4.90	4.59	4.85	4.77
$D\text{-}H_{12}N_2C_1O_5$	-	-	-	159.2719
$D\text{-}H_{13}N_8C_7N_2$	-	-	-	-127.2252
$D\text{-}H_{15}C_3N_2C_1$	-	-	-	117.7279
$D\text{-}H_{16}N_8C_7N_2$	177.1977	-	-	-
$D\text{-}H_{19}C_9N_8C_7$	-	-57.839866	-	117.6243
$D\text{-}H_{20}C_6C_3N_2$	59.9646	-	-	-
$D\text{-}H_{24}C_{10}C_9N_8$	61.2735	-	-	-
$D\text{-}H_{25}O_{23}O_5C_1$	-	179.496677	-	-14.30668
$D\text{-}H_{26}O_{25}O_5C_1$	175.2279	-	-	-
$D\text{-}H_{27}O_{26}O_6C_1$	-	-	-	-76.0497
$D\text{-}H_{28}O_{27}O_{11}C_7$	-	-	168.0081	-
$D\text{-}H_{29}O_{28}O_6C_1$	0.3605	-	-	-
$D\text{-}H_{30}O_{29}O_6C_1$	-	-	-	164.6838
$D\text{-}H_{31}O_{30}O_6C_1$	-	-	1.3821	-
$D\text{-}H_{32}O_{30}O_6C_1$	-	-	110.4960	-
$D\text{-}O_{23}O_5C_1C_6$	-	-	-	18.3831
qH_{32}	-	-	0.544	-
qO_{23}	-	-	-	-1.084
qO_{25}	-1.095	-	-	-
qO_{26}	-	-	-	-1.173
qH_{27}	0.545	0.218	-	0.624
qO_{27}	-	-1.082	-1.122	-
qO_{28}	-1.108	-	-	-
qO_{29}	-	-	-	-1.089
qO_{30}	-	-	-1.060	-
$d\text{-}H_{12}N_2$	-	-	-	0.9951
$d\text{-}H_{16}N_8$	1.0656	-	-	-
$d\text{-}H_{19}C_9$	-	-	-	1.0931
$d\text{-}H_{20}C_4$	1.0952	-	-	-
$d\text{-}H_{20}C_{10}$	-	-	-	1.0834
$d\text{-}H_{21}C_9$	1.0938	1.015411	-	-
$d\text{-}H_{24}C_{10}$	1.0959	-	-	-
$d\text{-}H_{25}O_{23}$	-	-	-	0.9507
$d\text{-}H_{26}O_{25}$	0.9775	-	-	-
$d\text{-}H_{28}O_{26}$	-	-	-	0.9473
$d\text{-}H_{29}O_{28}$	0.9931	-	-	-
$d\text{-}H_{30}O_{29}$	-	-	-	0.9471
$d\text{-}H_{31}O_{30}$	-	-	0.9531	-
$d\text{-}O_{23}O_5$	-	-	-	2.9417
$d\text{-}O_{27}O_{11}$	-	-	2.9099	-
$A\text{-}H_{12}N_2C_1$	-	-	-	79.0694
$A\text{-}H_{17}C_3N_2$	107.6592	-	-	-
$A\text{-}H_{19}C_9N_8$	-	-	-	111.6171
$A\text{-}H_{20}C_4C_3$	111.0751	-	-	-
$A\text{-}H_{24}C_{10}C_9$	111.3895	-	-	-
$A\text{-}H_{25}O_{23}O_5$	-	-	-	94.3957
$A\text{-}H_{26}O_{25}O_5$	17.8454	-	-	-
$A\text{-}H_{28}O_{26}O_6$	-	-	-	100.8375
$A\text{-}H_{29}O_{27}O_{11}$	-	109.017477	3.1813	-
$A\text{-}H_{30}O_{28}O_6$	118.8520	-	-	-
$A\text{-}H_{31}O_{29}C_1$	-	-	-	8.7106
$A\text{-}O_{23}O_5C_1$	-	-	-	125.2970
$A\text{-}O_{27}O_{11}O_7$	-	-	145.8465	-
$A\text{-}O_{28}O_6C_1$	114.0515	-	-	-

[a] K_{c1} and K_{c2}, equilibrium constants of equations; K_{a1} and K_{a2}, first and second acidic dissociation constant between the indicated atoms (A°); a_0, bohr radius (A°); q, total atomic charge (Muliken) (au); d, bond lengths between the indicated atoms (A°); A, bond angle(°).

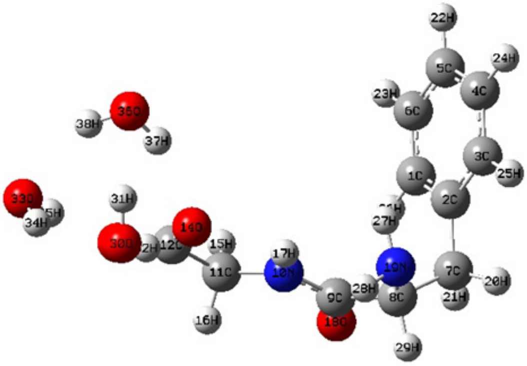


Figure 7. Calculated structure for the L-phenylalanyl-glycine anion solvated with three water molecules, at the B3LYP/6-31+G(d) level of theory and using the Tomasi's method in water at 298.15 K.

the above reaction is linked with constants K_{C2} and K_N by the following equation:

$$K_{a2} = K_{C2} \times K_N \quad (13)$$

This equation is similar to Eq. (10) and it was used to obtain the value of the second ionization constant of L-phenylalanyl-glycine in water. Table 3 gives the values of the molecular parameters and properties calculated for the L-$(H_2O)_3$ anion, in water at 298.15 K, while Figure 7 shows the structure of this anion.

The L-phenylalanyl-glycine anion solvated with three water molecules possesses various structural characteristics that are different to those of the L-phenylalanyl-glycine cation and neutral L-phenylalanyl-glycine molecule solvated with one and three water molecules, respectively. Thus, the acid-base reactions and salvation of species change the structures. From Figure 3 and Table 3, it can be observed that dihedral angles are partically different. Furtheremore, the negative charges of O_{13}- and O_{14}- atoms are high (in absolute value), and formed three moderate HBD, one is O_{14} and H_{32} (O_{14}-H_{32}), another is O14 and H_{37} (O_{14}-H_{37}) and the other is O_{13} and H_{35} (O_{13}-H_{35}).

It must be noted that the pK_{a2} value calculated theoretically (pK_{a2} = 7.6443) is in reasonable agreement with the experimentally determined pK_a (pK_{a2} = 7.5236) [62].

Similarly with L-phenylalanyl-glycine total energies and molecular parameters were obtained for L-alanyl-L-alanine system, using the Tomasi's method at the B3LYP/6-31+G(d) level of theory for anion, cation and neutral species at 298.15 K.

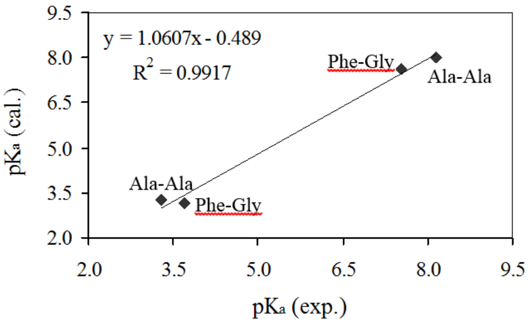


Figure 8. Comparison of calculated and experimental dissociation constants (pK_a) in zero ionic strength.

The resulting values are shown in the Tables 1, 2 and appendix A. Many acid-base reactions were considered for the mentioned system. The values of acidic dissociation constants for all of the reactions were calculated using a computer program, but the reactions were not further considered because the estimated error in their acidic dissociation constants was unacceptable. The models finally selected models and their acidic dissociation constants are listed in Table 1.

CONCLUSIONS

In this study, pKa values of L-phenylalanyl-glycine and L-alanyl-L-alanine was successfully calculated with high accuracy by using ab initio methods. The B3LYP/6-31+G(d) is employed and found to be sufficiently accurate in predicting the acidities of the considered molecules. For this various acid-base reactions were selected the solvation of the hydrogen, hydroxyl ions and other cations or anions in water, which possess a high hydrogen-band-donor capability were studied. It was observed that the nucleophilic attack on the hydrogen atoms of the COOH and NH_3^+ groups of L-phenylalanyl-glycine and L-alanyl-L-alanine by the OH^- and the hydrated OH^- ion with a water molecule gives the corresponding species.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Chemistry Department of Islamic Azad University, Arak branch, for support and contribution in the experimental works.

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