Vol.58, n.3: pp. 477-486, May-June 2015
http://dx.doi.org/10.1590/S1516-8913201500424
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Thermodynamic Study of Asparagine and Glycyl-Asparagine Using Computational Methods

Farhoush Kiani, Hannaneh Behzadi and Fardad Koohyar*
Department of Chemistry; Faculty of Science; Ayatollah Amoli Branch, Islamic Azad University; Amol - Iran

ABSTRACT

This work aimed to develop an ab initio procedure for accurately calculating pK_a *values and applied it to study the acidity of asparagine and glycyl-asparagine. DFT methods with B3LYP composed by 6-31+G(d) basis set were applied for calculating the acidic dissociation constant of asparagine and glycyl-asparagine. The formation of intermolecular hydrogen bonds between the available species and water was analyzed using Tomasi's method. Results showed that in alkaline solutions, the cation, anion and neutral species of asparagine and glycyl-asparagine were solvated with one, two, three and four molecules of water, respectively. There was an excellent similarity between the experimentally attained* pK_a *values and the theoretically ones in this work.*

Key words: Dissociation constant, DFT, Ab initio, atomic charge, asparagine, cation

INTRODUCTION

Amino acids are biologically important organic compounds, which are made from amine ($-NH_2$) and carboxylic acid (-COOH) functional groups, along with a side chain specific to each amino acid. About 500 amino acids are known and can be classified in many ways. In the form of proteins, amino acids comprise the second largest component (after water) of human muscles, cells other tissues. Outside proteins, amino acids perform critical roles in the processes such as neurotransmitter transport and biosynthesis (Wagner and Musso 1983; Weber et al. 1994). The attachment of the amine and the carboxylic acid group together to the first (alpha-) carbon atom is very important in biochemistry of amino acids. Peptides are distinguished from the proteins on the basis of size, and as a benchmark can be understood to contain approximately 50 amino acids. There has been an increasing activity in designing and synthesising new peptide- based drugs combining the advances in proteomic research and biotechnology (Catsch and Harmuth-Hoene 1976). Peptide structures that have been determined have come from the analysis of NMR or other experimental data, in many cases supplemented with theoretical modeling (Beachy et al. 1997).

Asparagine is the ß-amide of aspartic acid and can be found in animal protein. Asparagine is one of the principal, and often the most abundant, amino acids involved in the transport of nitrogen. Asparagine is very active in converting one amino acid into another (amination and transamination) when the need arises. Asparagine serves as an amino donor in liver transamination processes.

Asparagine can be found in eggs, whey, beef, dairy, fish, seafood, lactalbumin, and poultry (animal sources) and also in legumes, potatoes, asparagus, nuts, soy, and seeds (plant sources). Poly-L-asparagine (PASN) nanocapsules can be used as an anticancer targeted drug delivery

---

[*] Author for correspondence: fardadkoohyar@yahoo.com

system. Also, L-asparagine is used in food, pharmaceutical, and medical industries.

On the basis of solvation free energies, the pK_a values were acquired for the compounds in question using thermodynamic equation, involving the combined experimental and calculated data. Acid dissociation constant (K_a, pK_a = -logK_a) is an important property of organic compounds, which has extensive effects on many biological and chemical systems. The pK_a values (for aqueous solutions) are especially useful because of their environmental and pharmacological applications (Stumm and Morgan 1996; Thomas 2000). Some of the properties of drugs such as solubility, extent of binding, and rate of absorption are related to their pK_a values. In addition, the determination of dosage forms and the regimes of drugs are also related to their pK_a values (Thomas 2000). A number of methods, both experimental and theoretical, have been employed to calculate the pK_a values (Donkor and Kratochvil 1993; Silva et al. 1999; Silva et al. 2000; Adam 2002; Pliego and Riveros 2002; Jang et al. 2003; Soriano et al. 2004; Murlowska and Sadlej-Sosnowska 2005; da Silva et al. 2006). The correlation of theoretical and experimental data can allow the development of predictive models to determine the pK_a of the compounds for which no experimental data are yet available (Young 2001). For aqueous solutions, the theoretical determination of the pK_a values remains a challenging problem for the computational chemists. The ab initio calculation of pK_a values in the solvents other than water is easily achieved using continuum solvation models (Fu et al. 2004). Water is a very challenging solvent to model because there are many hydrogen bonds between water molecules that are not considered in continuum solvation models (Cramer and Truhlar 1999). A number of different approaches have been developed to deal with the problem of calculating pK_a values in aqueous solutions. A common practice is to incorporate the experimentally determined values for the free energies of the solvated proton into pK_a calculations.

The dissociation constant, for any weakly acidic or basic groups, is one of the most important physicochemical properties of small molecules and macromolecules. It is generally expressed as the pK_a of each group. This is a major factor in the pharmacokinetics of drugs and in the interactions of proteins with other molecules. Lee and Crippen (2009) surveyed the sources of experimental pK_a values for both the protein and small molecule cases and current methods for predicting them. Of particular concern was an analysis of the scope, statistical validity, and predictive power of methods as well as their accuracy.

For the acids with moderate strength, the calculation of the relative values of the pK_a was possible using simple ab initio or DFT methods such as Hartree-Fock or B_3LYP, provided an isodesmic reaction was used (Namazian and Heidary 2003; Namazian et al. 2004; Namazian et al. 2006; Namazian and Halvani 2006).

Progress in the computational chemistry has led to the development of composite theoretical techniques such as the complete basis set (CBS) and Gaussian-type (GN) methods, which have allowed to accurately predict the thermodynamic properties of molecules of practical significance, including the pK_a values (Alexeev et al. 2005). However, it is difficult to achieve the pK_a values with theoretical methods largely due to errors associated with accuracy. A wide range of computational techniques, including ab initio, density functional theory and high-accuracy composite methods are employed. The pK_a is equal to $\triangle G/2.303RT$, where $\triangle G$ is a free energy change of the dissociation reaction either in a gas or solution. Therefore, acidity of a compound can be determined by the $\triangle G$ value (Mohle and Hofmann 1998; Liptak et al. 2002; Hudaky and Perczel 2004; Kelly et al. 2006; Tosso et al. 2009).

This work attained the molecular conformations and solute-solvent interactions of the cation, anion, and neutral species of asparagine and glycyl-asparagine, using ab initio and density functional theory methods (DFT). The structure of neutral species of asparagine and glycyl-asparagine are shown in Figure 1.

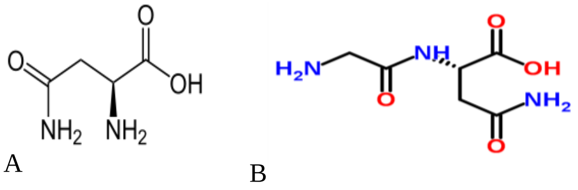


Figure 1 - Molecular structures of asparagine (A) and glycyl-asparagine (B).

COMPUTATIONAL METHODS

This work deals with the influence of factors such as the Self-Consistent Reaction Field (SCRF) model applied, choice of a particular thermodynamic equation, atomic radii used to build a cavity in the solvent (water), geometry optimization of species in water, inclusion of electron correlation, and the dimension of the basis set on the solvation free energies and on the calculated pK_a values.

All the calculations were performed using Gaussian 98. The acidities of asparagine and glycyl-asparagine were calculated using the Hyperchem, version 7 for windows and the hybrid exchange-correlation functional of Becke (1993); (B3LYP) (Lee et al. 1988). Free energies of solvation were calculated using the solvent model chemistry of Tomasi et al (Miertus and Tomasi 1982). In this study, the polarized continuum model (PCM) was applied to analyze the solvent effects on all the species involved in the selected ionization reactions.

In addition, to shed light on the experimental pK_a values of asparagine and glycyl-asparagine in water, some conformers were examined by the program. Finally, the solvation of the specimen was selected by the means of intermolecular hydrogen bonds (IHB) that involved one molecule of the mentioned specimen and some molecules of water.

RESULTS AND DISCUSSION

Acidic dissociation constant is a valuable and fundamental quantity in chemical, biological, environmental, and pharmaceutical studies because the important physicochemical properties, such as lipophilicity, solubility, and permeability, are all dependent on the pK_a (Wan and Ulander 2006). A number of methods, both experimental and theoretical, have been employed to calculate the pK_a values (Trevor and Nelaine 2006). The correlation of theoretical and experimental data can allow the development of predictive models to determine the pK_a of compounds for which no experimental data are yet available.

To shed the light on the experimental pK_a values of amino acids and dipeptides in water, several conformers were tested by the program, but some conformers were not considered further because the estimated error in its acidic dissociation constants was unacceptable. The solvation of the species was selected by the means of intermolecular hydrogen bonds that involved one molecule of the mentioned species and some molecules of water. (Table 1). Some models were finally chosen for the studied system. The calculated values of the acidic dissociation constants for asparagine and glycyl-asparagine are listed in Table 2.

Table 1 - Calculated total energy using the Tomasi method at the B3LYP/6-31+G(d) level of theory for cationic, neutral, and anionic species of asparagine and glycyl-asparagine at 298.15 K[a].

N	Solvated species	G^0_{sol} (Hartree)	G^0_{sol}/molecule KJ.mol^{-1}×10^{-6}	Solvated species	G^0_{sol} (Hartree)	G^0_{sol}/molecule KJ.mol^{-1}×10^{-6}
		Asparagine			Glycyl-asparagine	
0	H_2L^+	-492.918727	-1.294157	H_2L^+	-700.975600	-1.840411
1	$H_2L^+(H_2O)$	-569.360291	-0.747427	$H_2L^+(H_2O)$	-777.423130	-1.020562
2	$H_2L^+(H_2O)_2$	-645.832237	-0.565210	$H_2L^+(H_2O)_2$	-853.750667	-0.747174
3	$H_2L^+(H_2O)_3$	-722.269495	-0.474079	$H_2L^+(H_2O)_3$	-856.231065	-0.562008
0	HL (UZ)	-492.496649	-1.293049	HL (UZ)	-700.518430	-1.839210
1	$HL(H_2O)$ (UZ)	-568.920819	-0.746850	$HL(H_2O)$ (UZ)	-776.959375	-1.019953
2	$HL(H_2O)_2$ (UZ)	-645.367217	-0.564803	$HL(H_2O)_2$ (UZ)	-853.397028	-0.746864
3	$HL(H_2O)_3$ (UZ)	-722.469405	-0.474210	$HL(H_2O)_3$ (UZ)	-855.811852	-0.561733
0	HL (Z)	-492.507189	-1.293077	HL (Z)	-700.523831	-1.839225
1	$HL(H_2O)$ (Z)	-568.952840	-0.746892	$HL(H_2O)$ (Z)	-776.966210	-1.019962
2	$HL(H_2O)_2$ (Z)	-645.354552	-0.564792	$HL(H_2O)_2$ (Z)	-779.336015	-0.682048
3	$HL(H_2O)_3$ (Z)	-722.759122	-0.474400	$HL(H_2O)_3$ (Z)	—	—
0	L^-	-492.050859	-1.291879	L^-	-700.080163	-1.838060
1	$L^-(H_2O)$	-568.488471	-0.746283	$L^-(H_2O)$	-776.513625	-1.019368
2	$L^-(H_2O)_2$	-644.920508	-0.564412	$L^-(H_2O)_2$	-852.971201	-0.746491
3	$L^-(H_2O)_3$	-722.926700	-0.474510	$L^-(H_2O)_3$	-855.279382	-0.561383

[a] N: total number of salvation 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.

Table 2 - Values of pK_a for the protonation of asparagine and glycyl-asparagine obtained using the Tomasi method at the B3LYP/6-31+G(d) level of theory, at 298.15 K[a].

Species	Selected equations	pK$_a$ (calculated)	pK$_a$ (experimental)	ref
Asparagine	$H_2L^+(H_2O) + OH^-(H_2O) \rightleftharpoons HL(H_2O)_2 + H_2O$	2.723527422	2.01 (I = 0)	b
	$HL + OH^-(H_2O) \rightleftharpoons L^- + 2H_2O$	8.700170342	8.80 (I = 0)	b
Glycylasparagine	$H_2L^+(H_2O) + OH^- \rightleftharpoons HL(H_2O) + H_2O$	2.447560050	2.94 (I = 0)	b
	$HL(H_2O) + OH^-(H_2O) \rightleftharpoons L^- + 3H_2O$	8.391184488	8.44 (I = 0)	b

[a] I, ionic strength; [b] (Dean 1999).

First ionization constant of asparagine and glycyl-asparagine

It was assumed that in alkaline solutions, asparagine (a) and glacyl-asparagine (b) underwent a reaction of partial neutralization as follows:

a: $H_2L^+(H_2O) + OH^-(H_2O) \rightleftharpoons HL(H_2O)_2 + H_2O \quad K_{C1a}$ (1)
b: $H_2L^+(H_2O) + OH^- \rightleftharpoons HL(H_2O) + H_2O \quad K_{C1b}$ (2)

In the above reactions, $H_2L^+(H_2O)$ was the asparagine (a) and glycyl-asparagine (b) cations solvated with one water molecule, $HL(H_2O)_2$ represented neutral asparagine solvated with two water molecules, and $HL(H_2O)$ represented neutral glycyl-asparagine solvated with one water molecule. The majority reactions were described by equilibrium constants, K_{C1a} and K_{C1b}, which were theoretically determined.

Considering these actualities and to provide a more satisfactory representation of the protolysis of water, the following reactions could be shown:

$3H_2O \rightleftharpoons H_3O^+ + OH^-(H_2O) \quad K_N$ (3)
$2H_2O \rightleftharpoons H_3O^+ + OH^- \quad K_W$ (4)

The above chosen reactions showed that both H^+ and OH^- ions were hydrated with one water molecule. On the other hand, the K_N and K_W could be presented as the equilibrium constant of the reactions of eqs. 3 and 4, respectively (Blanco et al. 2005) and the following equation was obtained:
$K_W = K_N\,[H_2O]$
As a result at 298.15 K, it was calculated that:

$$K_N = \frac{Kw}{[H2O]} = 1.831 \times 10^{-16} \qquad (5)$$

By combining eqs 1 and 3 and also, 2 and 4, the reactions of eqs 6 and 7, respectively were obtained, which defined the first ionization constants of asparagine (K_{a1a}) and glycyl-asparagine (K_{a1b}). These reactions (eqs 6 and 7) considered the salvation of the neutral asparagine and glycyl-asparagine:

$H_2L^+(H_2O) + 2H_2O \rightleftharpoons HL(H_2O)_2 + H_3O^+ \quad (K_{a1a})$ (6)
$H_2L^+(H_2O) + H_2O \rightleftharpoons HL(H_2O) + H_3O^+ \quad (K_{a1b})$ (7)

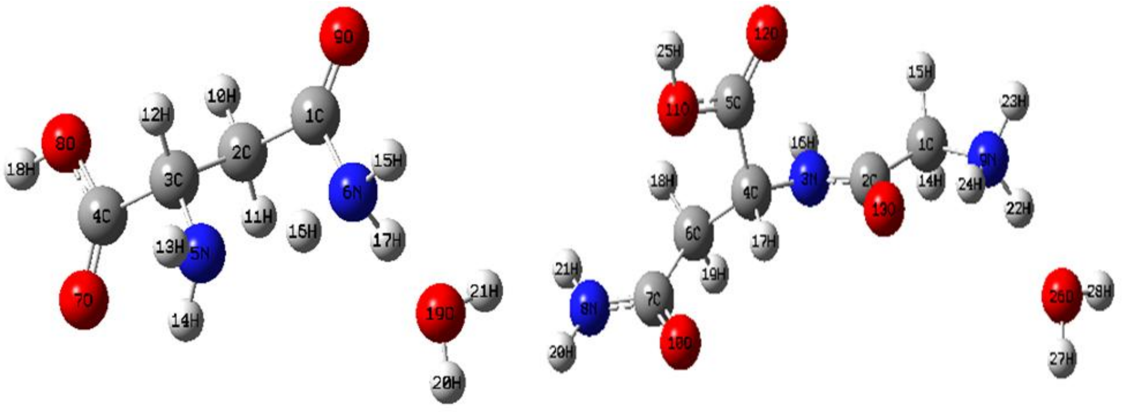
It was evident that the constants K_{C1}, (K_W or K_N) and K_{a1} were related by:

a: $K_{a1a} = K_{C1a} \times K_N$ (8)
b: $K_{a1b} = K_{C1b} \times K_W$ (9)

The above equations were used to theoretically determine the value of the first ionization constants of asparagine and glycyl-asparagine in the water. Table 3 summarizes the optimized values of asparagine molecular properties: $H_2L^+(H_2O)$ cation (Fig. 2), $HL(H_2O)_2$ (Fig. 3) and HL neutral and also, L^- anion (Fig. 4). Table 4 summarizes the optimized values of glycyl-asparagine molecular properties: $H_2L^+(H_2O)$ cation (Fig. 2), $HL(H_2O)$ neutral (Fig. 3) and also, L^- anion (Fig. 4). These data were obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method in water at 298.15 K.

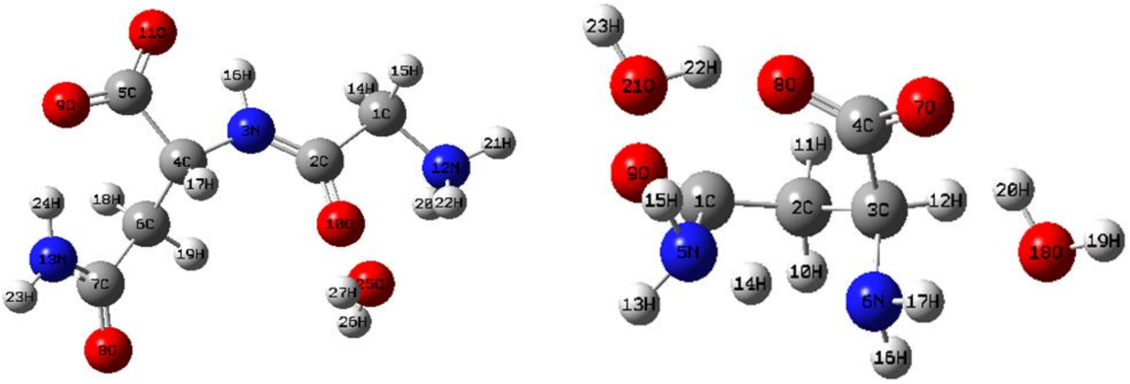
Obviously, the formation of the neutral asparagine implied that the electronic density of the O_8 atom increased notably (in absolute value) with respect to the O_8 atom of the asparagine cation; for glycyl-asparagine the electronic density of O_{11} atom of neutral glycyl-asparagine decreased with respect to O_{11} atom of glycyl-asparagine cation (Tables 3 and 4).

Also, the pK_a values (asparagine and glycyl-asparagine) theoretically obtained (pK_{a1}=2.723527422 and 2.447560050) was comparable with the experimental pK_{a1} value (pK_{a1}= 2.01 and 2.942) (Dean 1999) (Table 2).



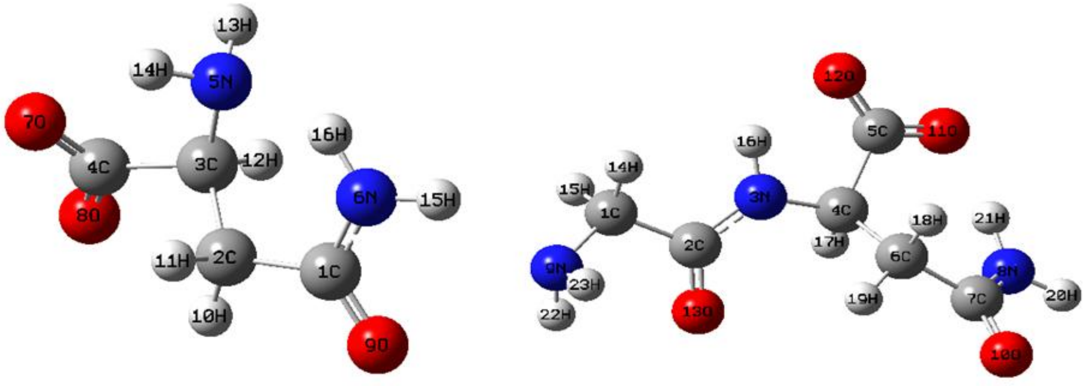
Asparagine$^+$(H_2O) **Glycyl-asparagine$^+$(H_2O)**

Figure 2 - Optimized structures of the asparagine and glycyl-asparagine cations solvated with a water molecule and practical numbering system adopted for carrying out the calculations.



Glycyl-asparagine(H_2O):Z **Aparagine(H_2O)$_2$:Z**

Figure 3 - Calculated structure for neutral glycyl-asparagine solvated with a water molecule and asparagine solvated with two water molecules, at the B3LYP/6-31+G(d) level of theory and using the Tomasi's method in water at 298.15 K.



Asparagine$^-$ **Glycyl-asparagine$^-$**

Figure 4 - Calculated structures for the asparagine and glycyl-asparagine anions solvated at the B3LYP/6-31+G(d) level of theory and using 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 asparagine at 298.15 K[a].

species	**calculated magnitudes**			
Asparagine	**$H_2L^+(H_2O)$**	**$HL(H_2O)_2$:Z**	**HL:Z**	**L^-**
K_{C1}	1.032248427E	—	—	—
K_{C2}	—	10892844.74	—	—
K_{a1}	0.001890047	—	—	—
K_{a2}	—	0.001092535	—	—
a_0	4.47	4.71	4.21	4.43
D-$H_{23}O_{21}O_8C_4$	—	146.912921	—	—
D-$H_{18}O_8C_4C_3$	179.057045	—	—	—
D-$H_{16}N_6C_1O_9$	139.786326	—	2.019005	165.757152
D-$H_{15}N_6C_1O_9$	17.866349	—	19.160111	11.279323
D-$H_{12}C_3C_2$C1	62.26246	-169.480961	67.319874	57.168249
D-$O_9C_1C_2C_3$	-113.585056	158.381581	179.154974	-177.83229
D-$O_7C_4C_3C_2$	106.42019	-170.531262	109.39067	100.679265
D-$N_6C_1C_2C_3$	64.004805	—	-130.457367	46.756758
D-$C_4C_3C_2C_1$	179.829157	-50321834	-678059	174.710647
qO_7	-0.499158	-0.725011	-0.684435	-0.738731
qO_8	-0.613253	-0.753435	-0.673143	-0.733404
qO_9	-0.391297	-0.420807	-0.632159	-0.64443
qO_{18}	—	-1.120972	—	—
qO_{19}	-1.050043	—	—	—
qO_{21}	—	-1.089813	—	—
qH_{13}	0.475792	0.544567	0.521493	0.139429
qH_{14}	0.461261	0.524363	0.514484	0.418694
qH_{15}	0.525609	0.569862	0.51046	0.442906
qH_{16}	0.538423	0.451349	0.468271	0.466954
qH_{17}	0.59413	0.464361	0.457708	—
qH_{18}	0.571264	—	—	—
qH_{19}	—	0.550341	—	—
qH_{20}	0.548149	0.555152	—	—
qH_{21}	0.539301	—	—	—
qH_{22}	—	0.569353	—	—
qH_{23}	—	0.541788	—	—
qC_4	0.634641	0.530538	0.582474	0.614094
qN_5	—	—	-1.011769	—
qN_6	—	—	—	-0.985445
$dO_{19}H_{17}$	1374542	—	—	—
$dH_{22}O_8$	—	1.70508	—	—
$dO_{21}H_{15}$	—	1.85034	—	—
$dH_{20}O_7$	—	1.75876	—	—
A-$N_6H_{17}O_{19}$	173.61775	—	—	—
A-$O_8H_{22}O_{21}$	—	146.81886	—	—
A-$O_7H_{20}O_{18}$	—	159.32543	—	—
A-$N_6H_{17}O_{18}$	—	150.51406	—	—

[a] K_{C1} and K_{C2}, equilibrium constants of equations; K_{a1} and K_{a2}, first and second acidic dissociation constants of species in water; D, dihedral angle between the indicated atoms (Å); a_0, bohr radius (Å); *q*, total atomic charge (Muliken) (au); *r*, bond lengths between the indicated atoms; *d*, distance of the IHB between the indicated atoms (Å); A, H-bond angles (°)

Second ionization constant of asparagine and glycyl-asparagine

It was assumed that the neutral species [a: HL (asparagine) and b: HL(H2O) (glycyl-asparagine)] underwent total neutralization as follows:

a: $HL + OH^-(H_2O) \rightleftharpoons L^- + 2H_2O \quad K_{C2a} \qquad (10)$

b: $HL(H_2O) + OH^-(H_2O) \rightleftharpoons L^- + 3H_2O \quad K_{C2b} \qquad (11)$

In the above reactions, L^- represented the asparagine and glycyl-asparagine anions. The reactions in eqs. 10 and 11 were characterized by other equilibrium constants, K_{C2a} and K_{C2b}, which were also theoretically determined. Combining eqs. 3 and 10, or eqs. 3 and 11, the second ionization reaction of asparagine and glycyl-asparagine were obtained:

a: $HL + H_2O \rightleftharpoons L^- + H_3O^+ \; K_{a2a} \qquad (12)$

b: $HL(H_2O) + OH^-(H_2O) \rightleftharpoons L^- + 3H_2O \quad K_{a2b} \qquad (13)$

The equilibrium constants K_{a2a} and K_{a2b} that characterized the above reactions could be shown as the bellow:

$$K_{a2a} = K_{C2a} \times K_N \quad (14)$$

$$K_{a2b} = K_{C2b} \times K_N \quad (15)$$

These equations were used to obtain the value of the second ionization constant of asparagine and glycyl-asparagine in the water. Tables 3 and 4 gave the values of the molecular parameters and properties calculated for the asparagine and glycyl-asparagine anions, while Fig. 4 showed the structures of these anions.

Table 4 - 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 glycyl-asparagine at 298.15 K[a].

Species	**Calculated magnitudes**		
Glaycylasparagine	**$H_2L^+(H_2O)$**	**$HL(H_2O)$:Z**	**L^-**
K_{C1}	3.5398059914E	—	—
K_{C2}	22188460.35	—	—
K_{a1}	0.840236312	—	—
K_{a2}	0.002236597	—	—
a_0	4.45	4.92	4.71
D-$H_{28}O_{26}N_9C_1$	98.911331	—	—
D-$H_{25}O_{11}C_5C_4$	178.144058	—	—
D-$H_{23}N_{13}C_7C_6$	—	176.819709	68.836556
D-$H_{20}N_{12}C_1C_2$	—	71.707056	172.876346
D-$H_{19}C_6C_4N_3$	28.535559	34.237177	39.862201
D-$H_{17}C_4N_3C_2$	-34.613443	44.969897	37.744216
D-$H_{16}N_3C_2C_1$	-11.787683	-10.679138	-7.337679
D-$O_{13}C_2C_1N_9$	2.396071	—	—
D-$N_{13}C_7C_6C_4$	—	43.702099	28.831115
D-$N_{12}C_1C_2O_{10}$	—	14.420993	9.263684
D-$O_{11}C_5C_4N_3$	175.556334	3.250177	-170.951848
D-$O_9C_5C_4N_3$	—	-177.529946	-152.192156
D-$O_8C_7C_6C_4$	—	-137.049416	25.946372
D-$C_7C_6C_4N_3$	144.673189	153.34975	160.79036
D-$C_6C_4N_3C_2$	-152.1412	-75.986182	-81.253423
D-$C_5C_4N_3C_2$	81.978	161.859427	155.021316
D-$C_4N_3C_2C_1$	-166.899212	-176.946294	-169.936786
qH_{20}	0.464249	0.559145	0.458379
qH_{21}	0.458497	0.538894	0.488222
qH_{22}	0.582976	0.522696	0.416356
qH_{23}	0.529432	0.468335	0.429676
qH_{24}	0.516094	0.490234	—
qH_{25}	0.58526	—	—
qH_{26}	—	0.584184	—
qH_{27}	0.535119	0.530651	—
qH_{28}	0.545533	—	—
qO_8	—	-0.641581	—
qO_9	—	-0.706624	—
qO_{10}	-0.590917	-0.666158	-0.64908
qO_{11}	-0.624096	-0.691954	-0.72685
qO_{12}	-0.492909	—	-0.709506
qO_{13}	-0.557833	—	-0.649026
qO_{25}	—	-1.069674	—
qO_{26}	-1.061871	—	—
qN_9	-1.018491	—	-0.928076
qN_{12}	—	-1.012724	—
qC_5	0.821836	0.782567	0.758133
$dO_{26}H_{22}$	1.75454	—	—
$dO_{25}H_{20}$	—	1.80475	—
$dH_{27}O_{10}$	—	2.11273	—
A-$N_9H_{22}O_{26}$	172.83495	—	—
A-$O_{25}H_{20}N_{12}$	—	143.16933	—
A-$O_{10}H_{27}O_{25}$	—	128.15459	—

[a] K_{C1} and K_{C2}, equilibrium constants of equations; K_{a1} and K_{a2}, first and second acidic dissociation constants of species in water; D, dihedral angle between the indicated atoms (Å); a_0, bohr radius (Å); *q*, total atomic charge (Muliken) (au); *r*, bond lengths between the indicated atoms; *d*, distance of the IHB between the indicated atoms (Å); A, H-bond angles (°)

Obviously, the formation of the neutral asparagine implied that the electronic density of the N_5 atom decreased notably (in absolute value) with respect to the N_6 atom of the asparagine anion, and also for glycyl-asparagine the N_{12} of neutral electronic density decreased with respect to N_9 atom of glycyl-asparagine anion (Tables 3 and 4).

It must be noted that the pk_{a2} value (asparagine and glycyl-asparagine) theoretically calculated (pK_{a2} = 8.700170342 and 8.391184488) was relatively comparable with the experimentally determined pK_a (pK_{a2} = 8.8 and 8.44) (Dean 1999).

Similarly with Asparagine, total energies and molecular parameters were obtained for glycyl-asparagine system, using Tomasi's method at the B3LYP/6-31+G (d) level of theory for the anion, cation, and neutral species at 298.15 K. The resulting values are shown in the Tables 1 and 2.

Tables 3 and 4 show the distances and angles of internal hydrogen bounds (IHBs). These values indicated that the IHB of the cation, neutral and anion of asparagine and glycyl-asparagine was attached to the class of moderate IHBs. According to Blanco et al. (2005), the properties of the moderate hydrogen bonds have the following classification: bond lengths of H·B is between (1.5 and 2.2) Å and the bond angle is 130° to 180°. For weak hydrogen bonds, the bond length and angle are (2.2 to 3.2) Å and 90° to 150°, respectively, and for strong hydrogen bonds are (1.2 to 1.5) Å and 175° to 180°, respectively. It was noteworthy that hydrogen bonding played an important role in proton transfer from the side chain to the backbone amide oxygen atom where hydrogen bonding made the ions much more stable (Kiani et al. 2010). The data about IHBs of species could be used to develop the processes and products involving nanodrugs in pharmaceutical industries.

Figure 5 showed that for asparagine and glycyl-asparagine anions, the total energy of solvated species increased by increasing of number of water molecules. It showed that the solvation of asparagine and glycyl-asparagine molecules was thermodynamic process.

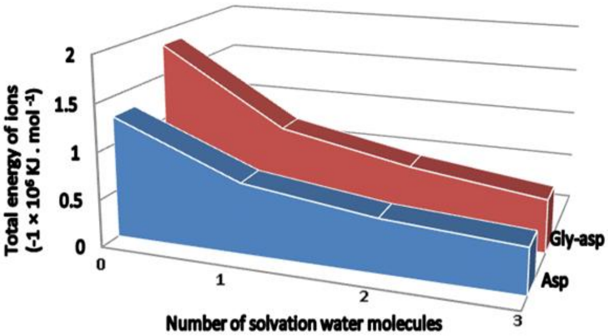


Figure 5 - Plot of the total free energy (KJ·mol^{-1}) of solvated asparagine and glycyl-asparagine anions per water molecule against the total number of solvation water molecules.

CONCLUSION

In this work, the pK_a value of asparagine and glycyl-asparagine were successfully calculated with high accuracy by using ab initio method. The calculations performed at the B3LYP/6-31+G(d) levels of theory using Tomasi's method allowed to prove that cations, neutral molecules, and anions formed IHBs with some molecules of water. Therefore, the various acid-base reactions were determined and the best reaction to adopt (to match) to acidic dissociation constants (pK_{a1} and pK_{a2}) were selected. The calculated results showed very good agreement with the experimental data in all studied solvents.

ACKNOWLEDGEMENTS

Thanks are gratefully extended to the Faculty of Chemistry, Ayatollah Amoli Branch, University of Islamic Azad University, for its valuable help with this work.

REFERENCES

Adam KR. New density functional and atoms in molecules method of computing relative pK_a values in solution. *J Phys Chem A.* 2002; 106: 11963-11972.

Alexeev Y, Windus TL, Zhan CG, Dixon DA. Accurate heats of formation and acidities for H_3PO_4, H_2SO_4, and H_2CO_3 from ab initio electronic structure calculations. *Int J Quantum Chem.* 2005; 102: 775-784.

Beachy MD, Chasman D, Murphy RB, Halgern TA, Friesner RA. Accurate ab initio quantum chemical determination of the relative energetics of peptide conformations and assessment of empirical force fields. *J Am Chem Soc.* 1997; 119: 5908-5920.

Becke AD. Density-functional thermochemistry. III. The role of exact exchange. *J Chem Phys.* 1993; 98: 5648-5652.

Blanco SE, Almandoz MC, Ferretti FH. Determination of the overlapping pK_a values of resorcinol using UV-visible spectroscopy and DFT methods. *Spectrochimica Acta Part A.* 2005; 61: 93-102.

Cramer CJ, Truhlar DG. Implicit solvation models: equilibria, structure, spectra, and dynamics. *Chem Rev.* 1999; 99: 2161-2200.

da Silva G, Kennedy EM, Dlugogorski BZ. Ab initio procedure for aqueous-phase p*K*a calculation: the acidity of nitrous acid. *J Phys Chem A.* 2006; 110: 11371-11376.

Dean JA. Lange's Handbook of Chemistry, 15th Ed; McGraw-Hill: New York, 1999.

Donkor KK, Kratochvil B. Determination of thermodynamic aqueous acid-base stability constants for several benzimidazole derivatives. *J Chem Eng Data*. 1993; 38: 569- 570.

Fu Y, Liu L, Li R, Liu R, Guo Q. First-principle predictions of absolute pK_a's of organic acids in dimethyl sulfoxide solution. *J Am Chem Soc*. 2004; 126: 814-822.

Hudaky P, Perczel A. Conformation dependence of p*K*a: Ab initio and DFT investigation of histidine. *J Phys Chem A*. 2004; 108: 6195-6205.

Jang YH, Goddard WA, Noyes KT, Sowers LC, Hwang S, Chung DS. p*K*a values of guanine in water: density functional theory calculations combined with poisson-boltzmann continuum-solvation model. *J Phys Chem B*. 2003; 107: 344-357.

Kelly CP, Cramer CJ, Truhlar DG. Adding explicit solvent molecules to continuum solvent calculations for the calculation of aqueous acid dissociation constants. *J Phys Chem A*. 2006; 110: 2493-2499.

Kiani F, Rostami AA, Sharifi S, Bahadori A, Chaichi MJ. Determination of acidic dissociation constant of glycine, valine, phenylalanine, glycylvaline, and glycylphenylalanine in water using ab initio methods. *J Chem Eng Data.* 2010; 55: 2732-2740.

Lee AC, Crippen GM. Predicting pK_a. *J Chem Inf Model*. 2009; 49(9): 2013-2033.

Lee C, Yang W, Parr RG. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys Rev B*. 1988; 37: 785-789.

Liptak MD, Gross KC, Seybold PG, Feldgus S, Shields GC. Absolute p*K*a determinations for substituted phenols. *J Am Chem Soc*. 2002; 124: 6421-6427.

Miertus S, Tomasi J. Approximate evaluations of the electrostatic free energy and internal energy changes in solution processes. *Chem Phys*. 1982; 65: 239-245.

Mohle K, Hofmann HJ. Stability order of basic peptide conformations reflected by density functional theory. *J Mol Model*. 1998; 4: 53-60.

Murlowska K, Sadlej-Sosnowska N. Absolute calculations of acidity of C-substituted tetrazoles in solution. *J Phys Chem A*. 2005; 109: 5590-5595.

Namazian M, Halvani S. Calculations of pK_a values of carboxylic acids in aqueous solution using density functional theory. *J Chem Thermodyn*. 2006; 38: 1495-1502.

Namazian M, Heidary H. Ab initio calculations of pK_a values of some organic acids in aqueous solution. *J Mol Struct (Theochem)*. 2003; 620: 257-263.

Namazian M, Halvani S, Noorbala MR. Density functional theory response to the calculations of pK_a values of some carboxylic acids in aqueous solution. *J Mol Struct (Theochem)*. 2004; 711: 13-18.

Namazian M, Kalantary-Fotooh F, Noorbala MR, Searles DJ, Coote ML. Moller-Plesset perturbation theory calculations of the pK_a values for a range of carboxylic acids. *JMol Struct(Theochem)*. 2006; 758: 275-278.

Pliego JR, Riveros JM. Theoretical calculation of pK_a using the cluster-continuum model. *J Phys Chem A*. 2002; 106: 7434-7439.

Silva CO, da Silva EC, and Nascimento MAC. Ab initio calculations of absolute pK_a values in aqueous solution I. carboxylic acids. *J Phys Chem A*. 1999; 103: 11194-11199.

Silva CO, da Silva EC, Nascimento MAC. Ab initio calculations of absolute pK_a values in aqueous solution II. Aliphatic alcohols, thiols, and halogenated carboxylic acids. *J Phys Chem A*. 2000; 104: 2402-2409.

Soriano E, Cerdan S, Ballesteros P. Computational determination of pK_a values. A comparison of different theoretical approaches and a novel procedure. *J Mol Struct (Theochem)*. 2004; 684: 121-128.

Stumm W, Morgan JJ. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters; Wiley-Interscience: New York, 1996.

Thomas G. Medicinal Chemistry: An Introduction; John Wiley and Sons: West Sussex, 2000.

Tosso RD, Zamora MA, Survire FD, Enriz RD. Ab initio and DFT study of the conformational energy hypersurface of cyclic gly-gly-gly. *J Phy Chem A*. 2009; 113: 10818-10825.

Trevor NB, Nelaine MD. Computational determination of aqueous p*K*a values of protonated benzimidazoles (Part 1). *J Phys Chem B*. 2006; 110: 9270-9279.

Wagner I, Musso H. New naturally occurring amino acids. *Angew Chem Int Ed Engl*. 1983; 22(22): 816-828.

Wan H, Ulander J. High–throughput p*K*a screening and prediction amenable for ADME profiling. *Expert Opin Drug Metab Toxicol*. 2006; 2: 139-155.

Weber G, Schörgendorfer K, Schneider-Scherzer E, Leitner E. The peptide synthetase catalyzing cyclosporine production in *Tolypocladium niveum* is encoded by a giant 45.8-kilobase open reading frame. *Current Genetics*. 1994; 26(2): 120-125.

Young DC. Computational Chemistry: A Practical Guide for Applying Techniques to Real-World Problems; John Wiley and Sons: New York, 2001.

Received: November 08, 2014;
Accepted: March 25, 2015.