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DFT/B3LYP Study of Thermochemistry of D-Glucosamine, a Representative Polyfunctional Bioorganic Compound

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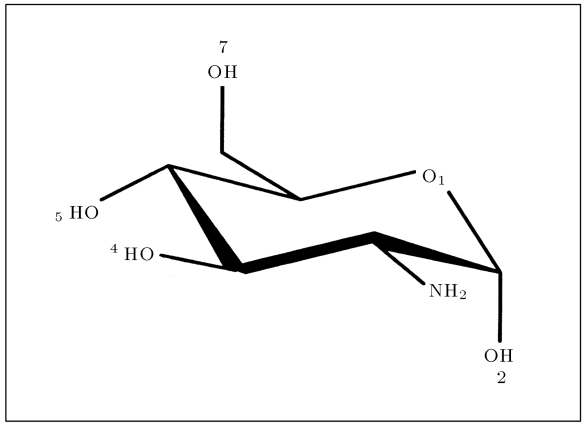
D-glucosamine, as a representative polyfunctional compound, is a bioactive amino sugar. In this study, the gas phase thermochemical properties of D-glucosamine, including its Metal Ion Affinity (MIA), metal binding sites, Anion Affinity (AA), acidity and proton affinity, have been explored, using the Density Functional Theory (DFT) and a 6-311++G**basis set. The summary of the MIA and AA results (in kcal/mol^{-1}) are: Li^+= 67.6, Na^+= 51.1, K^+= 37.3, Mg^{2+}= 207.9, Ca^{2+}= 150.4, Zn^{2+}= 251.2, Cl^-= 27.4, CN^-= 28.0. The acidity values calculated at different sites, including four -OH groups and one -NH_2 group, range from 344.0 to 373.0 kcal/mol^{-1}. These results, surprisingly, indicate how drastically the presence of multiple -OH and -NH_2 groups may vary the thermochemical properties of a polyfunctional bioorganic compound, such as D-glucosamine.

INTRODUCTION

D-glucosamine (GlcN, Scheme 1) is an amino-monosaccharide precursor of the disaccharide unit of glycosaminoglycan, which is the building block of proteoglycans, being the ground substance of articular cartilage [1]. This bioactive sugar is present in the matrix of the connective tissues and gastrointestinal mucosal membranes. It could be combined with other glycosaminoglycans, since it helps to maintain the viscosity in articulation and stimulates cartilage recovery [2-6]. GlcN and chitosan oligomers of low molecular weight (with a degree of polymerization up to 7) were shown to be absorbed easily into the human intestine [7] because of their low molecular weight.

Since the early 1990s, GlcN has been widely promoted for the treatment of human osteoarthritis and has shown significant effects on improving both the symptoms and the structure [8–10]. GlcN not only regulates the synthesis of proteoglycan [11] and glycosaminoglycan [12], but also suppresses the excess production of NO [13]. Therefore, it possibly provides the structure modifying effects of therapeutic efficiency in osteoarthritis. Moreover, GlcN has an inhibitory effect on the neutrophil functions, thereby, probably exhibits anti-inflammatory actions in osteoarthritis symptoms [14].



Scheme 1. Chemical structure of $^4C_1$ chair conformation of D-glucosamine (2-amino-2-deoxy-D-glucose).

Because GlcN is commonly used as dietary supplement in human food and has obtained a great deal of attention from the public as a potential treatment for osteoarthritis, the study of the various characteristics of this biomolecule is of great interest [15].

GlcN contains -NH_2, hemiacetal and -OH groups (Scheme 1), which have a good ability to coordinate

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with metal ions and anions that are often involved in forming hydrogen bond networks. Therefore, strong interactions between GlcN and ions may be expected. It is well known that some essential ions, such as Na^+ and K^+, play important roles in our life and may interact with many biological molecules. Since gaining insights about these interactions experimentally is difficult, current study can be useful for understanding the physiological function of GlcN by studying the coordination between GlcN and these metal ions, although the complexation geometry in the biological environment is quite different from that of these complexes. To the best of the authors' knowledge, this represents the first report about the thermochemical properties of GlcN and the effect of metal cations and anions on them in the gas phase.

The Density Functional Theory (DFT) has proven to be a very efficient and relatively inexpensive method for the prediction of different molecular properties, including fundamental thermochemical properties, such as gas-phase acidity, anion affinity and metal ion affinity [16]. Also, much previous work has clearly shown that the B3LYP approach can be used to obtain reliable results for the systems of interest in this work [17-19].

The objective of this work is mainly to characterize the low-energy conformers of different GlcN-ion complexes. Another goal of the present study is to determine the acidities of different labile hydrogens in D-glucosamine. The first step of the work concerns the computation of stable conformers of GlcN-ion complexes in order to locate the preferred coordination site of the ion. Then, the computed absolute and relative Metal Ion Affinities (MIA), Anion Affinities (AA), acidity and Proton Affinity (PA) of GlcN are reported.

COMPUTATIONAL DETAILS

As the first step of the work, an ensemble of low-energy conformers for each of the compounds of interest is generated through molecular dynamic simulations using MMF force field provided within the Spartan '06V102' package. The most stable conformer was then chosen for further optimization at the DFT level, using the B3LYP nonlocal exchange functionals and the 6-311++G** basis set [20-22], which is also done in the Spartan program. Vibrational frequencies were calculated on the optimized structures, which provided zero point vibrational energies for enthalpy correction to 298.15 k.

In this study, the following gas-phase thermochemical properties of D-glucosamine have been determined as follows [17]:

1. Metal ion affinity (MIA, M: Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+})

$$GlcN + M^{n+} \rightarrow GlcNM^{n+}, \qquad -\Delta H_{rxn} = MIA. \tag{1}$$

2. Anion affinity (AA, A: Cl^-, CN^-)

$$GlcN + A^- \rightarrow GlcNA^-, \qquad -\Delta H_{rxn} = AA. \tag{2}$$

3. Acidity

$$GlcNH \rightarrow GlcN^- + H^+, \qquad \Delta H_{rxn} = \Delta H_{acid}. \tag{3}$$

RESULTS AND DISCUSSION

D-Glucosamine: Structure and Conformer

A total of fifty five conformers of D-glucosamine, within a relative energy range of 10 kcal/mol^{-1}, were obtained using Spartan '06V102'. The relatively large number of conformers for this molecule is due to the possibility of various hydrogen bond features. The lowest-energy conformer of GlcN was selected and optimized using Spartan at a B3LYP/6-311++G** level of theory and the structure is presented in Figure 1. Its electronic energy at the B3LYP/6-311++G** level of theory is given in Table 1.

As indicated in Figure 1, many hydrogen bonds can form among the hemiacetal, hydroxyl and amino groups in GlcN. With six donors (one acetal, four -OHs and one -NH_2) and six acceptors (four from -OHs and one from -NH_2), the hydrogen bond combinations in GlcN are complex and various types of hydrogen bonds may coexist in one conformer. An acceptor may interact with one or more donors in the molecule and, consequently, multiple hydrogen bonds are often

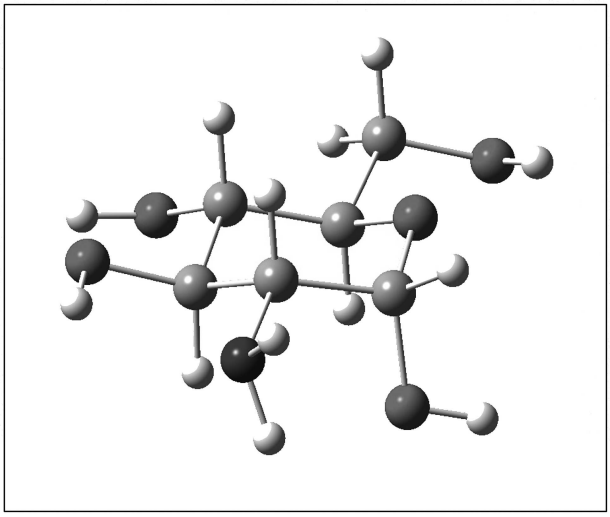


Figure 1. The lowest-energy conformer of the GlcN.

Table 1. B3LYP/6-311++G** thermochemical properties of GlcN[a].

	GlcN	GlcN-Li^+	GlcN-Na^+	GlcN-K^+	GlcN-Mg^{2+}	GlcN-Ca^{2+}	GlcN-Zn^{2+}	GlcN-Cl^-	GlcN-CN^-
Energy (cal/mol)	-667.5328	-674.9277	-829.7029	-1267.3545	-867.1068	-1344.6799	-2446.2659	-1127.8801	-760.4668
ΔE(0 K)		-66.51	-50.26	-36.8	-206.42	-149.1	-249.93	-27.04	-27.94
ΔE(298 K)		-67	-50.37	-36.7	-207.27	-149.79	-250.61	-26.75	-27.45
ΔH(298 K)		67.6	51.0	37.3	207.9	150.4	251.2	27.3	28.0
$q_{O\text{-}1}$	-0.216	-0.213	-0.220	-0.221	-0.270	-0.297	-0.288	-0.202	-0.390
$q_{HO\text{-}1}$	0.277	0.303	0.297	0.295	0.362	0.357	0.347	0.199	0.574
q_N	-0.260	-0.277	-0.280	-0.293	-0.234	-0.491	-0.236	-0.345	-0.277
q_{HN}	0.246	0.282	0.260	0.266	0.333	0.330	0.336	0.240	0.247
q_{HN}	0.246	0.284	0.269	0.259	0.310	0.290	0.353	0.230	0.239
$q_{O\text{-}3}$	-0.241	-0.233	-0.360	-0.345	-0.086	-0.090	-0.083	-0.274	-0.270
$q_{HO\text{-}3}$	0.282	0.307	0.325	0.315	0.304	0.299	0.308	0.276	0.278
$q_{O\text{-}4}$	-0.202	-0.228	-0.394	-0.372	-0.297	-0.367	-0.288	-0.216	-0.212
$q_{HO\text{-}4}$	0.267	0.316	0.355	0.347	0.417	0.348	0.425	0.255	0.251
$q_{O\text{-}5}$	-0.110	-0.028	-0.028	-0.033	-0.079	-0.274	-0.057	-0.104	-0.089
$q_{O\text{-}6}$	-0.224	-0.321	-0.338	-0.337	-0.364	-0.363	-0.365	-0.255	-0.255
$q_{HO\text{-}6}$	0.245	0.301	0.311	0.307	0.345	0.343	0.351	0.258	0.261
q_M		0.607	0.861	0.960	0.743	1.396	0.606	-0.756	-0.426,-0.513
HO-1...HN-2	2.40	2.36	2.48	2.48	2.39	2.47	2.32	2.50	2.40
HN-2...HO-3	2.30	2.26	2.15	2.13				2.25	
HO-3...HO-4	2.40							2.36	2.47
HO-4...HO-3		2.25							
HO-5...HO-1	2.50	2.62	2.65	2.60	2.68	2.69			2.50
HO-5...HO-6	2.39							2.27	
HO-6...HO-3					1.79	1.79	1.81		
HO-6...HO-4		1.95	1.82	1.82					

a: All energies are in kcal mol^{-1} and all bond lengths are in Å.

found in the GlcN conformers. As can be seen from Figure 1, calculations show that this conformer of GlcN is the most stable because all of the hydroxylic hydrogens are incorporated in the hydrogen bonding. As is well known, the high electronegativity difference between H and O causes this hydrogen bond to be very strong.

Coordination Mode

The lowest-energy conformers of $GlcNM^{n+}$ and $GlcNA^-$ complexes are given in Figures 2 and 3. In principle, cations can coordinate with D-glucosamine at many different positions: Two oxygens (Figure 2b), nitrogen and oxygen (Figure 2a) or at nitrogen and two or more oxygens in a multi-coordinated mode (Figure 2d). The first two cases are observed in monovalent metal ions and the last one in divalent cations.

GlcN can also capture anions by hydrogen bonding donors, which are hydrogen atoms of the hydroxylic groups. All these possible coordination modes (Figures 2 and 3) of the most stable conformer of the D-glucosamine molecule have been explored. Results from the B3LYP/6-311++G** full energy optimization of cation- and anion-complexed GlcN are reported in Tables 1 and 2 and Figures 2 and 3. The vibrational analysis indicates that all these structures are minima.

As indicated in Figure 2, in complexes with monovalent cations, GlcN exists as a bidentate chilate. Calculations show that Li^+ prefers to locate between N-2 and O-3 and closer to the oxygen O-3. It should be pointed out that this trend is in accordance with the hard-soft acid-base concept [23]. Whereas Na^+ and K^+, in their most stable conformers, are placed between two oxygens, and interestingly, in each

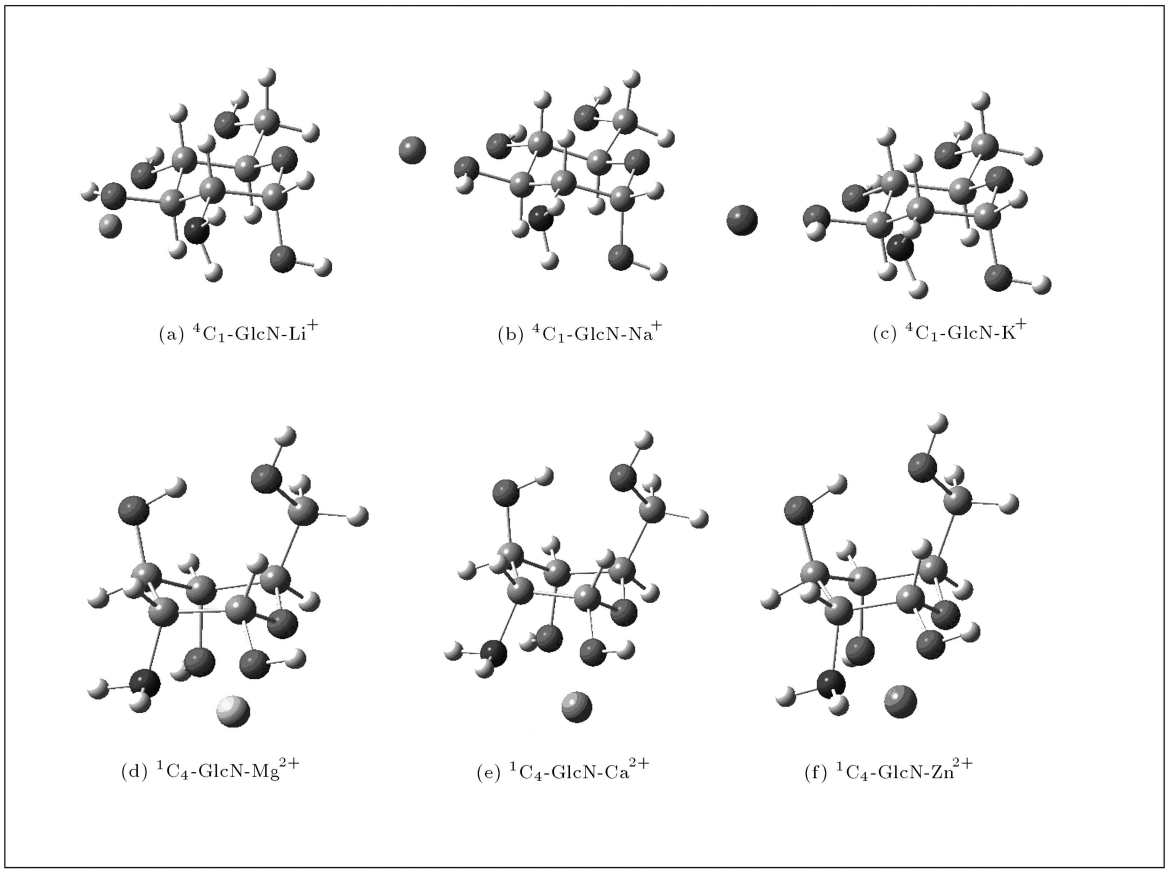


Figure 2. B3LYP/6-311++G** lowest-energy conformers for $GlcNM^{n+}$.

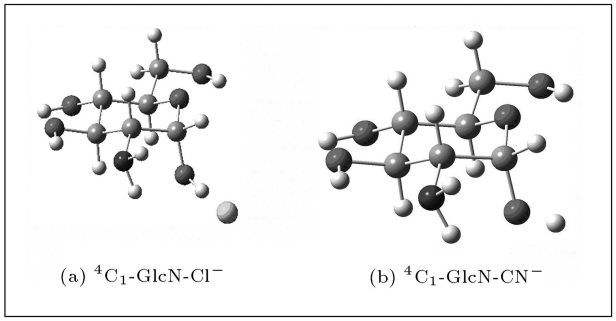


Figure 3. B3LYP/6-311++G** lowest-energy conformers for $GlcNA^-$.

complex, the distance of the metal cation from the oxygens is the same (Table 2).

In case of divalent cations, such as Mg^{2+}, calculations show that GlcN changes its configuration from $^4C_1$ to $^1C_4$ in the chair form, in order to have maximum interaction with the cations. The -CH_2OH and other -OH groups, except the anomeric group, go to axial positions and, thus, GlcN exists as a tetradentate chilate (see Figures 2d to 2f). It is worth noting that the M^{n+}-O distances depend on the metal ion radius. For instance, as seen in Table 2, Li^+-O3, Na^+-O3, K^+-O3, Mg^{2+}-O5 and Ca^{2+}-O5 distances are 1.85, 2.23, 2.60, 2.05 and 2.34 Å, respectively. However, in the case of the Zn^{2+} cation, the situation is slightly different, because of the presence of d orbitals.

For both anions considered in this study, the first and second most stable structures found by MMFF method were selected for further optimization at B3LYP/6-311++G** (Figure 3). As illustrated in Figures 3a and 3b, Cl^- and CN^- ligands are coordinated to GlcN at the HO-1 site by means of an intermolecular hydrogen bonding. Table 1 shows that, for both anionic ligands, the greatest interaction between the GlcN and the anion occurs from HO-1. As will be discussed in the Acidity Section, this is because HO-1 is the most acidic site of GlcN, in comparison with all other hydroxylic hydrogens, resulting in stronger hydrogen bonding.

Metal Ion Affinity (MIA)

Since the GA scaffold is the base of a common drug and due to its role in the chelation of cations in biological systems, the binding enthalpy between GlcN and cations (denoted by MIA) was also calculated in the present work. These results, together with donor-atom to cation distances, are given in Tables 1 and 2.

Table 2. B3LYP/6-311++G** distances (Å) between metal ions, anions and coordinating atoms in their lowest-energy conformers.

	M. . . O-1	M. . . N-2	M. . . O-3	M. . . O-4	M. . . O-5
GlcN+Li$^+$		2.03	1.85		
GlcN+Na$^+$			2.24	2.24	
GlcN+K$^+$			2.60	2.61	
GlcN+Mg^{2+}	2.51	2.09		2.04	2.05
GlcN+Ca^{2+}	2.54	2.41		2.36	2.34
GlcN +Zn^{2+}	2.95	2.00		2.01	2.06
GlcN+CN $^{-*}$	1.60				
GlcN+Cl $^{-*}$	2.04				

* In these cases, the given distances are between anions and hydrogen of hydroxyl group.

It is obvious that MIA is strongly dependent on the "radius" and the "charge" of cations. As indicated in Table 1, the GlcN molecule interacts with smaller cations, e.g. Li^+, stronger than with larger cations, e.g. K^+, and therefore, stronger interactions lead to higher MIA. For example, MIAs of GlcN, with Li^+, Na^+ and K^+, are 67.6, 51.0 and 37.3 kcal/mol^{-1}, respectively. It should be pointed out that this trend in the calculated MIAs is in accord with the hard-soft acid-base concept [23]. The better matched the donor is with the acceptor, the stronger is the complexation. For example, for a GlcN-Li^+ complex, the hard-hard electrostatic interaction between Li^+ and OH results in the stronger metal complexation, as compared with GlcN-Na^+ and GlcN-K^+ complexes. It can be concluded that the MIA of GlcN is considerably different from that of the monofunctional molecules, such as ethanol, whose MIA with Li^+ and Na^+ are 39.2 ± 1.9 and 26.3 ± 1.3 kcal/mol^{-1}, respectively (http://webbook.nist.gov/chemistry).

The higher charge of divalent cation also results in interaction with more lone pairs of donors and, consequently, permits extra stabilization of the complex through a higher electrostatic interaction [20]. For example, the calculated binding enthalpy for Mg^{2+} is much larger than that for Na^+ (Table 1), due to the larger charge-to-size ratio of Mg^{2+}, with respect to that of Na^+. Mg^{2+} interacts with the GlcN molecule more strongly than Ca^{2+} because of its smaller radii.

Atomic charges at donor atoms, predominantly on those incorporated in metal complexes, differed from those of GlcN, according to the coordinates of M^{n+} in the conformers (Table 1). For each $GlcNM^+$, the charges of these atoms in the complex were comparably higher than non-coordinated atoms, except O-6 in GlcN-Li^+. For instance, in the GlcN-Na^+ complex, the charges of oxygen coordinated with sodium (e.g., O-3 and O-4) were 0.360 and -0.394, whereas the other atoms, which were not involved in the complex, had lower charges (e.g., $q_{O\text{-}1}$= -0.220, $q_{N\text{-}2}$= -0.280, $q_{O\text{-}5}$= -0.028 and $q_{O\text{-}6}$= -0.338).

Interestingly, when increasing the cation radius from Li^+ to K^+, the MIA of GlcN decreases. For instance, as seen in Table 1, the net charges of Li^+, Na^+ and K^+ in the corresponding $GlcNM^+$ are 0.607, 0.861 and 0.960. This trend suggests that the covalent character of M^+-GlcN decreases from Li^+ to K^+. This inclination in the bond character is in good accordance with the calculated MIAs for these three metal cations.

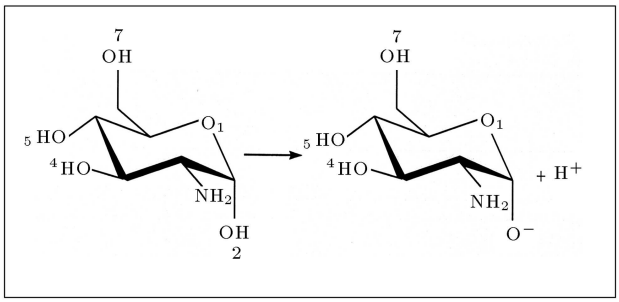
In GlcN-M^{2+} complexes, only O-3 and O-6 as donor atoms were not included in the complexes, which indicates minimum and maximum negative charges, except Ca^{2+} with a maximum at N-2 (Table 1). The charges at O-5 for Mg^{2+} and Zn^{2+} were -0.079 and -0.056, but for Ca^{2+}, was -0.274. This can be explained by the greater distance between O-5 and the cation in the case of Ca^{2+} (2.05Å in Mg^{2+}, 2.34 Å in Ca^{2+} and 2.06 Å Zn^{2+}, Table 2). For the divalent cations, Mg^{2+} and Ca^{2+}, one can see the same trend in the bond character as seen for the monovalent cations Li^+, Na^+ and K^+. For example, as seen in Table 1, the net charges of Mg^{2+} and Ca^{2+} in the corresponding $GlcNM^{2+}$ are 0.743 and 1.396, which are in accordance with the calculated Mg^{2+} and Ca^{2+} affinities.

Acidity

With the elimination of solvent effects, the gas phase acidity of organic compounds was extensively investigated in order to study the intrinsic factors (i.e., the electronic effects of various groups) that influence the acidity of an organic compound [24,25]. The gas phase acidity of different -OH groups of GlcN (as shown in Scheme 2) have been calculated and summarized in Table 3. Acidities were conducted at B3LYP/6-311++G** and have been determined according to Equation 3. In GlcN, the acidity of all -OH groups (i.e., HO-1, HO-3, HO-4 and HO-6) was determined.

Table 3. B3LYP/6-311++G** hydrogen bond length (Å) in different $GlcN^-$.

	GlcN-HO-1	GlcN-HN	GlcN-HO-3	GlcN-HO-4	GlcN-HO-6
HO-1...HN-2	2.36				2.33
HN-2...HO-1		2.15	2.26	2.21	
HN-2...HO-3	2.22	2.12			2.33
HO-3...HO-4	2.32	2.46	2.05		2.30
HO-3...HN-2			2.63		
HO-4...HO-3				2.09	
HO-4...HO-6				1.68	
HO-5...HO-6	2.07	2.10	2.21		2.31



Scheme 2. Schematic reaction of acidity of GlcN.

As seen in Table 3, these calculated acidities are in the range of ca. 343–373 kcal/mol^{-1}.

Table 3 also shows that, based on the predicted acidity values, the HO-1 is the most favored deprotonation site. The more (less) acidic positions of the GlcN are those that generate the most (least) stable anionic species. Figure 4 illustrates that, for the deprotonated HO-1 group, the π-delocalization effect and conformational flexibility play a key role in the stability of this site. This can lead preferentially to an opening of the cyclic structure of GlcN and the making of a carbonyl group in the anion, as opposed to a hydroxyl group in the parent neutral. For deprotonated HN-2, HO-3 and HO-4 groups, calculations show that $GlcN^-$ changes its configuration from $^4C_1$ to $^1C_4$ in the chair form to have maximum hydrogen bonds with the negative atom (Table 3). For deprotonated HO-6 group, $GlcN^-$ remains at the $^4C_1$ form, since changing the configuration is energetically unfavorable.

Referring to Table 4, ΔH_{acid} = 346.0 kcal mol^{-1} for HO-4, which is a surprising result, as it shows that hydrogen bonding can make the acidity of an alcohol as strong as an organic acid. For instance, the acidities of 2-propanol and acetic acid are 375.1 and 343.2 kcal mol^{-1} in the gas phase, respectively [26,27]. Theory shows that the length of hydrogen bond between HO-6.... O-4 is 1.69Å, which is much stronger than a normal hydrogen bond.

As seen in Table 4, for the -NH_2 group, one has: ΔH_{acid} = 365.3 kcal mol^{-1}. The acidities of 1-propanamine and 2-propanamine are 398.4 and 397.2 kcal mol^{-1} in the gas phase, respectively [28]. In comparison with these linear amines, this is a surprising result, as it shows that hydrogen bonding can make the acidity of a primary amine even stronger than an alcohol.

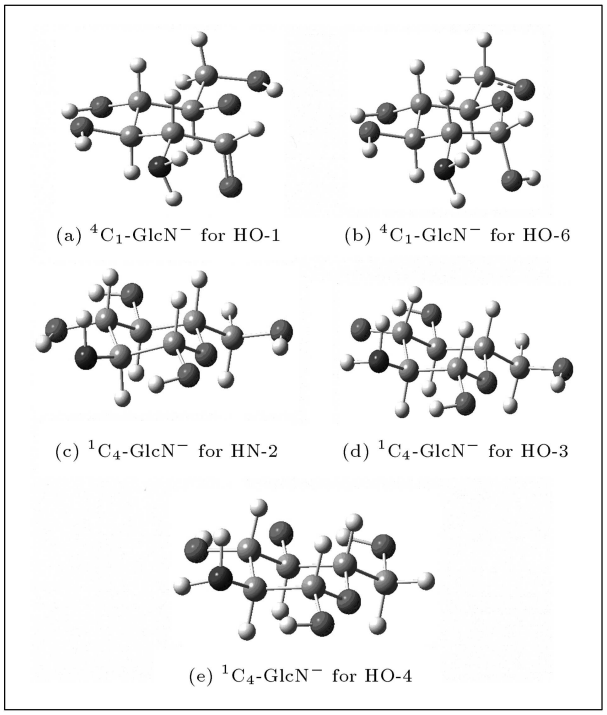


Figure 4. B3LYP/6-311++G** structures of $GlcN^-$ for different acidic hydrogens.

Anion Affinity (AA)

The anion affinity of GlcN with two anions are studied with B3LYP/6-311++G** methods and the results are summarized in Table 1. The optimized bond lengths with this method are given in Table 2. Unlike $GlcNM^{n+}$ conformers, which are strongly bonded with lone pairs of donor groups, here, the calculations show a drastic decrease in the binding enthalpy, because of the

Table 4. Computed ΔH_{acid} values at 298 K in kcal mol^{-1}. The basis set for all calculation levels is 6-311++G**.

	E (cal/mol)	ΔE (0K)	ΔE (298 K)	ΔH (298K)
GlcN-HO-1	-666.9686	345.11	345.39	344.4
GlcN-HN	-666.9361	364.78	365.25	365.3
GlcN-HO-3	-666.9601	350.32	350.87	351.5
GlcN-HO-4	-666.9686	345.11	343.02	346.0
GlcN-HO-6	-666.9245	371.59	372.35	372.9
Ethanol [26]				378.3
2-Propanol [26]				375.1
Acetic Acid [27]				343.2
2-Propanamine [28]				397.2
1-Propanamine [28]				398.4

weaker intermolecular hydrogen bonding. Both anions interact with GlcN via their -OH groups (Figure 3). For example, AAs of GlcN with Cl^- and CN^- are 27.3 and 28.0 kcal mol^{-1}, respectively. As discussed before, both anions interact with the most acidic hydrogen (HO-1). The net charges of hydrogen of HO-1 are 0.574 in GlcN-CN^- and 0.199 in GlcN-Cl^-. Based on these net charges, some partial deprotonation in GlcN-CN^- should be observed, as seen in Figure 3. Interestingly, no interaction between carbon atom and GlcN was observed in the case of CN^-, as hydrogen bonding cannot exist.

Proton Affinity (PA)

Based on Equation 5, the proton affinity of GlcN was calculated for its NH_2 group, which was expected to be its most basic site.

$$GA + H^+ \rightarrow GAH^+, \qquad \Delta H_{rxn} = -PA. \tag{4}$$

The most stable conformers of the protonated GlcN ($GlcNH^+$) are shown in Figure 5, whereas in $GlcNH^+$, the configuration changes from the $^4C_1$ form in the GlcN (Figure 1) to $^1C_4$ form, to have two strong hydrogen bonds. Therefore, all of the functional groups go to axial positions, except anomeric -OH (HO-1). This permits the structure to make two strong hydrogen bonds (1.78 and 1.86 Å). Calculation shows that $-\Delta H_{rxn}$ (i.e., PA) of GlcN is 225.5 kcal mol^{-1}. It is interesting that this value indicates that the proton affinity of GlcN (a secondary amine) is about 5 kcal mol^{-1} more than that of the isopropyl amine [29]. This higher PA of GlcN is due to the presence of various hydrogen bonds in $GlcNH^+$, which are absent in the isopropyl ammonium ion.

CONCLUSION

A detailed computational study concerning the most stable structure of D-glucosamine and its binding properties with cations (H^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, and Zn^{2+}) and anions (Cl^- and CN^-) were performed by means of the B3LYP/6-311++G** method. These calculations enabled the prediction of the gas-phase thermochemical properties of these compounds, including metal ion affinity, anion affinity, acidity and proton affinity. This high-level DFT study, interestingly, indicates that, in a bioorganic compound like GlcN, intramolecular interactions (mainly, multiple hydrogen bonding) are important factors in determining the thermochemical properties of itself, as well as its complexes.

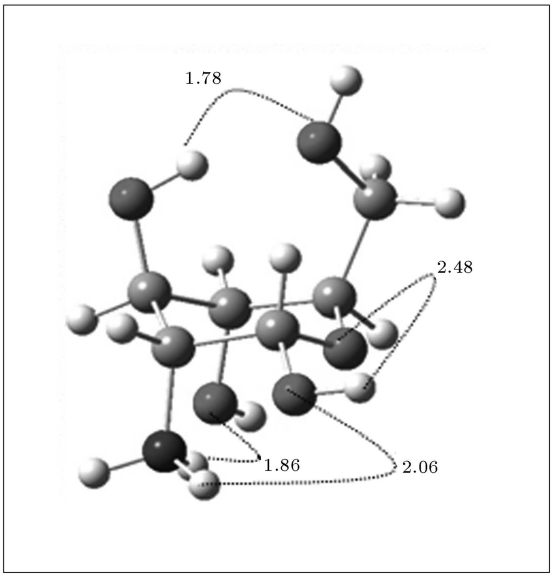


Figure 5. The lowest-energy conformers of the $^1C_4$-$GlcNH^+$.

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