Protonation sequence of linear aliphatic polyamines from intramolecular atomic energies and charges

Ignacy Cukrowski^{*(a)} and Chérif F. Matta^(b)

(a) Department of Chemistry, University of Pretoria, Lynnwood Road, Pretoria 0002, South Africa. Fax: +2712 420-4687, Tel: +2712 420-3988, E-mail: ignacy.cukrowski@up.ac.za

(b) Department of Chemistry and Physics, Mount Saint Vincent University, Halifax, NS, Canada B3M 2J6; and Department of Chemistry, Dalhousie University, Halifax, NS, Canada B3H 4J3. Fax: +1-(902)-457-6134, Tel: +1-(902)-457-6142, E-mail: cherif.matta@msvu.ca

Abstract

The analysis of intramolecular atomic energies and charges, obtained from QTAIM, strongly and unambiguously suggests that the most stable HL^+ form of a linear triamine $H_2N(CH_2)_2NH(CH_2)_3NH_2$ (L) has the secondary nitrogen atom protonated, contradicting earlier reports. This is supported by the $G(aq)$ value (at the RX3LYP/6-311++G(d,p) level of theory in solvent, CPCM/UAKS) which is lower by about 4 kcal mol⁻¹ (equivalent to three log units of a protonation constant log K_H) when compared with remaining HL^+ tautomers. The most stable H_2L^{2+} form of L found from QTAIM has two terminal nitrogen atoms protonated, which is in agreement with the existing views. A new rule governing the protonation sequence of linear polyamines is proposed stating that at thermodynamic equilibrium the protonation sequence in a solution results in the formation of tautomers which (i) minimizes the differences in the atomic energies and net atomic charges of both terminal atoms, N and C, in all partially protonated forms, HL^+ and H_2L^{2+} , and (ii) have a symmetrical distribution of the atomic energies and net atomic charges on the terminal fragments $-CH_2-NH_2$ of a linear molecule. Several conditions, involving the atomic energies and charges of the terminal N- and carbon atoms, are defined and incorporated in a protocol which results in a definite prediction of the protonation sequence of linear aliphatic triamines.

Keywords *Protonation sequence, DFT, QTAIM, linear aliphatic polyamines, atomic energies, atomic charges*

1. Introduction

The protonation sequence of linear aliphatic polyamines (LAP) in solution has attracted the interest for decades [1-8] but it is still a matter of controversy. It is a challenging task because different parts of LAPs are so alike that the basicities of the different nitrogen atoms are very similar. In addition, the number of possible combinations in protonation sequences increases quickly with an increase in a number of nitrogen atoms particularly when combined with different lengths of $-(CH_2)_k-$ fragments between the nitrogen atoms. The same applies to uncertainty in the proposed protonation sequences of LAPs and there are many contradicting reports, even when the same experimental technique was used. For instance, in case of $H_2N(CH_2)_mNH(CH_2)_nNH_2$, (L) where $m,n = 2-4$, all three possible protonation sites have been proposed for the first protonation step, $H^+ + L = HL^+$. An equilibrium mixture of tautomers involving the protonated primary and secondary nitrogen atoms being at almost statistical ratio was suggested for the monoprotonated forms of 1,4,7-triazaheptane (2,2-tri) [1] and 1,4,7,10-tetraazadecane [2] by Paoletti *et al.* from thermochemical studies. Hague and Moreton [6], from ^{13}C NMR studies, have concluded that in the monoprotonated forms of 1,4,8-triazaoctane (2,3-tri) the proton is shared equally by all the nitrogen atoms. However, according to Delfini *et al.*, [4] who have also used ^{13}C NMR, a tautomeric equilibrium between species with protonated primary and secondary nitrogen atoms does not take place in the first protonation step. They postulated that (i) in all five triamines examined by them (with different combinations of m and n , $m,n = 2-4$) the protonation of the secondary nitrogen occurs only after the protonation of the two primary (terminal) nitrogen atoms is complete, and (ii) in the case of the asymmetric triamines (such as 2,3-tri) the protonation of the nitrogen attached to the longer aliphatic chain starts first. Clearly, the interpretation of experimental data reported to date prove to be difficult and the protonation sequence involving the first protonation step still must be regarded as highly uncertain, even in case of simple triamines. It is generally accepted, however, that the H_2L^{2+} form of triamines involves two terminal nitrogen atoms [1,3,6,8] followed by the protonation of the secondary nitrogen atom when H_3L^{3+} is formed.

This paper reports a theoretical (computational) investigation of the protonation sequence in water of linear aliphatic triamines with 2,3-tri used as a representative example (a fully deprotonated form of 2,3-tri is shown in Fig. 1). The quantum theory of atoms in molecules (QTAIM) was used to analyze calculated electron densities and “wavefunctions” to obtain atomic properties such as atomic energies and atomic populations/charges. The analysis of QTAIM atomic energies $E(\Omega)$ and atomic electron populations $N(\Omega)$ or, equivalently, atomic charge $q(\Omega)$ distributions within each protonated form of this molecule is used to predict the protonation sequence in water. For comparison, an analogous QTAIM study is also performed on the relevant gas structures of 2,3-tri. This is a first attempt of this kind of analysis hence new hypotheses had to be formulated to interpret the data in a meaningful way. The trends found for the atomic energies and charges are compared with trends found for Gibbs free energies computed for all possible protonated forms of 2,3-tri in the vacuum phase and in solvent.

2. Computational Methods

All electronic structure calculations were performed with the aid of Gaussian 03, revision D01 [9]. GaussView 4.1.2 [10] was utilized as a molecular builder and for visualization. All structures have been fully optimized without constraints at the RX3LYP/6-311++G(d,p) level of theory. Since the ultimate aim of this work is to rationalize and to predict the protonation sequence in aqueous solution, we have used the Conductor-like Polarizable Continuum Model (CPCM) [11-15] in conjunction with the united atom (UA) cavity-model in-tagged with Kohn-Sham (KS) radii (UAKS) [16,17] with water as a solvent ($\epsilon = 78.39$). The same set of molecules was also optimized at the RB3LYP/6-31+G(d,p) level of theory in a gas phase. A tight gradient convergence criterion with ultrafine integration grid was used in all calculations. All reported geometries belong to genuine minimum energy conformations (imaginary frequencies are not present).

The atomic energies and charges were computed in both the vacuum phase and the solvated state using QTAIM [18] implemented in AIMALL [19].

3. Results and Discussion

3.1 G(aq) and the Protonation Sequence in Solvent

There are three possible protonation sites in 2,3-tri, namely N1, N4, and N8 (Fig. 1) and all possible protonated forms were energy-minimized in solvent. A full set of optimized structures is shown in Fig. S1, Supplementary Information, and the relevant $G(\text{aq})$ values are shown in Table 1. From the analysis of data seen in Table 1 and assuming that at the thermodynamic equilibrium the lowest energy form of H_nL^{n+} predominates in a solution (with the lowest $G(\text{aq})$ value), the following protonated forms might be proposed as most stable in solvent (water)

where the values in parentheses are the % Boltzmann distribution mole fractions in aqueous solution at 25°C.

On average, the Gibbs free energy of $(\text{HL}^+)_{\text{N4}}$ (see structure (a) in Fig. 2) is smaller by 4 kcal mol⁻¹ when compared with both tautomers with the primary (terminal) nitrogen atoms being protonated and is the only monoprotonated form that exists in solution at 25°C (Table 1). The difference in $G(\text{aq})$ values is equivalent to about 3 log units of a protonation constant $\log K_{\text{H}}$ (1.36 kcal mol⁻¹ is equivalent to 1 log K_{H} unit). One can argue that this difference should be seen as large and sufficient to support eq. 1 as the resultant first protonation step. Surprisingly, this is in contrast to earlier experimental reports [1,2,4,6,8] as the formation of the monoprotonated form with the secondary nitrogen being protonated as a predominant or the only form was not proposed. Instead, either a mixture of two tautomers in case of 2,2-tri [1] or three tautomers in case of 2,3-tri [6,8] was suggested, or the formation of a single monoprotonated form with a nitrogen atom attached to the longer aliphatic chain was proposed [2].

Following on reasoning used for the first protonation step, $(\text{H}_2\text{L}^{2+})_{\text{N1,N4}}$ should be excluded because it has the highest $G(\text{aq})$ value (by about 3 kcal mol⁻¹) among all possible tautomers. The difference $\delta G(\text{aq}) = G(\text{H}_2\text{L}^{2+})_{\text{N1,N8}} - G(\text{H}_2\text{L}^{2+})_{\text{N4,N8}} = 0.5 \text{ kcal mol}^{-1}$ is rather

small and translates into $\sim 70\%$ of $(\text{H}_2\text{L}^{2+})_{\text{N4,N8}}$ and $\sim 30\%$ of $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$ at the present level of theory. This energy difference is too small and close to the accuracy of the electronic structure calculations and one can consider these two tautomers as equally possible. This is also a rather surprising result as it is against generally accepted and experimentally supported view that the second protonation step results in two terminal nitrogen atoms being protonated and one would expect that the $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$ tautomer should have a significantly lower energy $G(\text{aq})$ when compared with the two other possible protonated forms. In general, the analysis of $G(\text{aq})$ values contradicts previous experimental reports and when used as a predictive tool, has not resulted in an unambiguous interpretation of the protonation sequence.

3.2. QTAIM-Based Analysis in Solvent

QTAIM [18] is used here as a predictive tool of the protonation sequence from properties of the atoms in the molecules such as atomic charges and atomic energies. The molecular virial ratios ($\gamma = -V/T$) in solvent for L, three tautomers of HL^+ and H_2L^{2+} , as well as H_3L^{3+} were 2.00469 , 2.00497 ± 0.00001 , 2.00530 ± 0.00004 , and 2.00570 , respectively. These departures of γ from the ideal value of 2 indicate a disagreement between the total molecular energies and the corresponding kinetic energies ($1 - \gamma$) of $\sim 0.5\%$. A simple correction was applied to satisfy the molecular virial theorem by scaling all raw integrated atomic kinetic energies by the molecular $(1 - \gamma)$ [20].

We begin by analyzing the fully deprotonated (L) and fully protonated (H_3L^{3+}) ligand, since these are the only possible forms that can exist leaving no ambiguity related to the placement of protons. It is assumed that these two forms have characteristics which could be used to predict the most likely protonated forms, HL^+ and H_2L^{2+} , in solution.

3.2.1. Analysis of the Nitrogen Atoms

The terminal nitrogen atoms (N1 and N8) in the fully deprotonated, L, and fully protonated, H_3L^{3+} , forms of 2,3-tri have almost the same net atomic charges in solvent (Table 2). The charge differences, $\delta q(\text{N}) = q(\text{N1}) - q(\text{N8})$, in L and H_3L^{3+} are -0.003 and $-0.002 e$, respectively, and they can be regarded as rather small. Similar charge differences between terminal nitrogen atoms we found only for $(\text{HL}^+)_{\text{N4}}$ ($0.004 e$) and

$(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$ ($-0.003 e$). However, more than one order of magnitude larger $\delta q(\text{N})$ values are observed for $(\text{HL}^+)_{\text{N1}}$ and $(\text{HL}^+)_{\text{N8}}$, 0.059 and $-0.064 e$, as well as $(\text{H}_2\text{L}^{2+})_{\text{N1,N4}}$ and $(\text{H}_2\text{L}^{2+})_{\text{N4,N8}}$, 0.053 and $-0.051 e$, respectively.

A similar pattern applies to atomic energy distribution of the terminal nitrogen atoms in solvent. Small differences $\delta E(\text{N}) = E(\text{N1}) - E(\text{N8})$ of -0.5 and $2.4 \text{ kcal mol}^{-1}$ were found in L and H_3L^{3+} , respectively. Figure 3 shows relative atomic energies of nitrogen atoms for all the H_nL^{n+} forms of 2,3-tri, where the lowest energy of the N4-atom in H_3L^{3+} was set to zero. Besides L and H_3L^{3+} , small $\delta E(\text{N})$ values (Figure 3) are observed only for $(\text{HL}^+)_{\text{N4}}$ and $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$, -0.4 and $0.1 \text{ kcal mol}^{-1}$, respectively, but $\delta E(\text{N})$ values are *orders of magnitude larger* in $(\text{HL}^+)_{\text{N1}}$ and $(\text{HL}^+)_{\text{N8}}$ (-82.0 and $81.4 \text{ kcal mol}^{-1}$, respectively) and in $(\text{H}_2\text{L}^{2+})_{\text{N1,N4}}$ and $(\text{H}_2\text{L}^{2+})_{\text{N4,N8}}$ (-76.6 and $78.7 \text{ kcal mol}^{-1}$, respectively) when compared with $\delta E(\text{N})$ values in L , $(\text{HL}^+)_{\text{N4}}$, $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$, or H_3L^{3+} . The analysis of atomic energies is consistent with the net atomic charge analysis.

It is seen in Figure 3 that the lowest atomic energy nitrogen atoms are observed in the fully protonated form indicating that H_3L^{3+} is the most stable form of the 2,3-tri. The first protonation step results in the decrease of the energy of the secondary nitrogen atom by about 70 kcal mol^{-1} which is accompanied by the small decrease in the energy of the terminal nitrogen atoms, by about 11 kcal mol^{-1} . The energy of the terminal nitrogen atoms in $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$ is lower by about 100 and 90 kcal mol^{-1} when compared with relevant terminal nitrogen atoms in L and $(\text{HL}^+)_{\text{N4}}$, respectively. The observed energy differences between unprotonated and protonated nitrogen atoms are very large and in favor of more stable protonated nitrogen atoms, hence one would expect that the protonation reaction should be a spontaneous process. This conclusion correlates very well with the fact that after dissolving a free ligand L in water the solution becomes highly basic, because a spontaneous protonation of nitrogen atoms takes place and the equilibrium mixture of L and HL^+ is formed instantly ($\text{L} + \text{H}_2\text{O} = \text{HL}^+ + \text{OH}^-$).

3.2.2. Analysis of the Carbon Atoms

Analysis of net atomic charges on the C2- and C7-atoms (they are bonded to the terminal N1- and N8-atoms, respectively) in L and H_3L^{3+} reveals (Table 3) that $\delta q(\text{C}) =$

$q(\text{C2}) - q(\text{C7})$ is -0.004 and -0.003 e , respectively, which is as small as observed for the terminal nitrogen atoms. Similar net charge differences between terminal carbon atoms we found only for $(\text{HL}^+)_{\text{N4}}$ (-0.005 e) and $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$ (-0.002 e). However, more than an order of magnitude larger $\delta q(\text{C})$ values are observed for $(\text{HL}^+)_{\text{N1}}$ and $(\text{HL}^+)_{\text{N8}}$, -0.075 and -0.070 e , as well as $(\text{H}_2\text{L}^{2+})_{\text{N1,N4}}$ and $(\text{H}_2\text{L}^{2+})_{\text{N4,N8}}$, -0.077 and 0.069 e , respectively.

Atomic energies distribution of the carbon atoms follows the trend found for their charge distribution. Small differences $\delta E(\text{C}) = E(\text{C2}) - E(\text{C7})$ of -5.9 and -3.8 kcal mol^{-1} were found in L and H_3L^{3+} , respectively. Similar in value $\delta E(\text{C})$ were again observed only in $(\text{HL}^+)_{\text{N4}}$ (-5.3 kcal mol^{-1}) and $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$ (-6.3 kcal mol^{-1}). However, $\delta E(\text{C})$ of about ± 30 kcal mol^{-1} was found in all remaining protonated forms of 2,3-tri. In general, the charge and atomic energy analyses of the N- and carbon atoms are totally consistent and in full agreement.

3.2.3. Charge Distribution within the Molecule

Schwarzenbach [3] proposed decades ago that the electrostatic repulsion between the two charges in H_2L^{2+} would result in their moving to the terminal nitrogen atoms (as far from one another as possible). This view is generally accepted in the studies of triamines and results obtained here are in full agreement. The QTAIM-based studies showed, however, that the intramolecular charge distribution which results in the terminal nitrogen atoms having virtually the same charges, exists not only in H_2L^{2+} , as postulated by Schwarzenbach, but also in L and H_3L^{3+} as well as in all the most stable partially protonated forms of polyamine, here $(\text{HL}^+)_{\text{N4}}$ and $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$. Clearly, according to results obtained from QTAIM, all the protonated forms of 2,3-tri, which we consider here as most stable, have an even charge distribution on the both extended ends (involving N- and carbon atoms) of the linear molecule.

The proposal made by Schwarzenbach can now be quantified using the QTAIM data and, tentatively, one might generalize it for linear aliphatic triamines as *at thermodynamic equilibrium the protonation sequence in a solution results in the formation of tautomers which have (i) the differences in the atomic energies and net atomic charges of both terminal atoms, N and C* (here $\delta q(\text{N}) = q(\text{N1}) - q(\text{N8})$ and $\delta q(\text{C}) = q(\text{C2}) - q(\text{C7})$) *in all*

partially protonated forms, HL^+ and H_2L^{2+} , being smallest, and (ii) a symmetrical distribution of the atomic energies and net atomic charges on the terminal fragments – CH_2-NH_2 of a linear molecule. Since the same charge distribution rules are observed also in fully deprotonated as well as fully protonated forms of triamine 2,3-tri, it is reasonable to assume that these rules should hold also for all linear aliphatic triamines, and most likely also polyamines of a type $H_2N(CH_2)_m\{NH(CH_2)_n\}_kNH_2$, where k , m , and n are natural numbers with $k \geq 1$ and $m, n \geq 2$.

3.2.4. Protonation Sequence

If the smallest δq and δE values are used as a guide then $(HL^+)_{N4}$ of 2,3-tri is most stable among the monoprotated forms and must be seen as either the only form present in a solution at the equilibrium, or at least as a largely predominant one. For the second protonation reaction, the formation of $(H_2L^{2+})_{N1,N8}$ (see structure (b) in Fig. 2) can be predicted with high certainty where the terminal N1 and N8 must be seen as preferentially protonated atoms. From that follows that the QTAIM-established sequence of protonation of 2,3-tri is

It is clear that the QTAIM-based analysis (i) does not support the first step in the protonation sequences proposed from the thermochemical or ^{13}C NMR studies where either all three possible HL^+ forms were suggested to be present at the same (or similar) concentration ratio, or the terminal nitrogen atom of the longer aliphatic chain was to be protonated first, and (ii) strongly supports and correlates well with the generally accepted protonation sequence reported to date for the second protonation reaction where two terminal nitrogen atoms are involved.

3.2.5 Charge and Energy Distributions between Terminal Nitrogen and Carbon Atoms

It is seen in Tables 2 and 3 that the net charges of nitrogen and carbon atoms change

on protonation except the charge on C6, which remains virtually the same in all protonated forms. Let us analyze now the interatomic charge differences $\Delta\delta q(\text{C},\text{N})$ between the terminal N- and carbon atoms, $\delta q(\text{C2},\text{N1}) = q(\text{C2}) - q(\text{N1})$ and $\delta q(\text{C7},\text{N8}) = q(\text{C7}) - q(\text{N8})$, in all possible protonated forms of 2,3-tri – see Table 4(a). It is seen that the interatomic charge differences are almost equal in value in L and H_3L^{3+} , $\Delta\delta q(\text{C},\text{N}) = \delta q(\text{C2},\text{N1}) - \delta q(\text{C7},\text{N8})$ is -0.002 and -0.001 e , respectively. Small values of $\Delta\delta q(\text{C},\text{N})$ are also observed in $(\text{HL}^+)_{\text{N4}}$ (-0.009 e) and $(\text{H}_2\text{L}^{2+})_{\text{N1},\text{N8}}$ (0.001 e), which were suggested above as most stable protonated forms. However, *orders of magnitude larger values* are observed for the remaining protonated forms, e.g. $\Delta\delta q(\text{C},\text{N}) = -0.133$ and -0.130 e in $(\text{HL}^+)_{\text{N1}}$ and $(\text{H}_2\text{L}^{2+})_{\text{N1},\text{N4}}$, respectively. The difference $\Delta\delta q(\text{C},\text{N})$ can also be seen as an intramolecular terminal dipole between the terminal positively charged carbon atom and negatively charged nitrogen atom. From the above analysis it would follow that all the most stable protonated forms of 2,3-tri tend to have terminal dipole values as small and equal in value as possible.

The atomic energy differences $\Delta\delta E(\text{C},\text{N})$ between the terminal N- and carbon atoms, $\delta E(\text{C2},\text{N1}) = E(\text{C2}) - E(\text{N1})$ and $\delta E(\text{C7},\text{N8}) = E(\text{C7}) - E(\text{N8})$, were also analyzed – see Table 4(b). It is clear that the pattern in the atomic energy distribution follows that found for the net atomic charges. The deprotonated and fully protonated as well as the most stable partially protonated forms of 2,3-tri found above, $(\text{HL}^+)_{\text{N4}}$ and $(\text{H}_2\text{L}^{2+})_{\text{N1},\text{N8}}$, all have small the $\Delta\delta E(\text{C},\text{N})$ values. An order of magnitude larger $\Delta\delta E(\text{C},\text{N})$ values, in the range of ± 35 kcal mol^{-1} , are observed for all other tautomers and this strongly supports the sequence of protonation of 2,3-tri in solvent proposed above.

3.3. Protonation Sequence in a Gas Phase

As a test case, all the protonated forms of 2,3-tri were optimized in the gas phase (even if not the lowest energy conformers in the gas phase [21-23] for the sake of making a direct comparison with the linear and completely antiperiplanar conformers investigated in solvent). The $G(\text{g})$ values obtained at the RB3LYP/6-31+G(d,p) for HL^+ and H_2L^{2+} are shown in Table 5. It is seen that the $G(\text{g})$ value for $(\text{HL}^+)_{\text{N4}}$ is significantly lower (by more than 7 kcal mol^{-1}) when compared with the energies of the two remaining HL^+ tautomers. Even much larger

differences are observed between H_2L^{2+} tautomers with $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$ being the lowest energy tautomer. Clearly, results seen in Table 5 can be seen as being in full agreement with the protonation sequence proposed from the QTAIM-based analysis in solvent.

It appears that the analysis of $G(\text{g})$ values obtained for the antiperiplanar conformers could be used as a predictive tool of the protonation sequence in solvent. It was then of importance to find out if an analogous QTAIM-based analysis performed on the gas-structures predicts similar trends between the atomic energies and net atomic charges, as found in solvent. A full set of data for the net charges and atomic energies of N- and carbon atoms is shown in Tables S1-S2 in Supplementary Information. It is seen in Table S1 that the small energy differences between the terminal nitrogen atoms of -1.2 , -3.5 , 0.4 , and $4.9 \text{ kcal mol}^{-1}$ are observed only for L, $(\text{HL}^+)_{\text{N4}}$, $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$, and H_3L^{3+} , respectively, and this coincides fully with the smallest $G(\text{g})$ values obtained for these structures. Up to two orders of magnitude larger $\delta E(\text{N})$ differences (between -198 and 96 kcal mol^{-1}) are observed for the remaining protonated forms which have significantly larger $G(\text{g})$ values. A similar trend is also observed in case of the terminal carbon atoms, where the $\delta E(\text{C})$ values for the lowest in the Gibbs free energy structures, L, $(\text{HL}^+)_{\text{N4}}$, $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$, and H_3L^{3+} , are -5.3 , -4.5 , -8.5 , and $-2.8 \text{ kcal mol}^{-1}$, respectively (Table S2). Order of magnitude larger differences are observed for the remaining protonated forms, where $\delta E(\text{C})$ varies between -71 to 63 kcal mol^{-1} .

Surprisingly, a full consistency is not observed for the charge distribution. Based on the solvent analyses, one would expect to see the small charge differences between the terminal nitrogen atoms to be also present in all the gas-structures where the smallest differences in atomic energies $\delta E(\text{N})$ are observed. This does not hold in case of $(\text{HL}^+)_{\text{N8}}$, where a very small charge difference of $0.003 e$ is also observed, even though the $G(\text{g})$ value of this tautomer is larger, by about 7 kcal mol^{-1} , when compared with $(\text{HL}^+)_{\text{N4}}$. However, $\delta E(\text{N})$ of this tautomer is large, $95.9 \text{ kcal mol}^{-1}$, whereas the $\delta E(\text{N})$ values for the tautomers with the smallest $G(\text{g})$ values are order of magnitude smaller. From that it would follow that a small value of $\delta q(\text{N})$ is a necessary but not sufficient condition for the prediction of the protonation sequence in solvent from the data obtained in the gas phase. Interestingly, the small charge differences between the terminal carbon atoms correlate fully with the small $G(\text{g})$ values; on average, the absolute $\delta q(\text{C})$ values of about $0.003 e$ are observed only for tautomers with the

smallest $G(g)$ values and up to two orders of magnitude larger differences are observed in the remaining tautomers.

The interatomic charge differences between the terminal N- and carbon atoms were also analyzed – see Table 6(a). All tautomers with the smallest $G(g)$ values, together with L and H_3L^{3+} , also have small $\Delta\delta q(C,N)$ values (between 0.001 and 0.012 e). All the remaining tautomers have an order of magnitude larger $\Delta\delta q(C,N)$ values except $(HL^+)_{N1}$. The differences in the atomic energies between the terminal N- and carbon atoms follow a similar pattern; the tautomers with the smallest $G(g)$ value are characterized by the small $\Delta\delta E(C,N)$ values – see Table 6(b). Interestingly, three possible H_2L^{2+} tautomers have small $\Delta\delta E(C,N)$ values; all of them are below 10 kcal mol⁻¹.

From the $G(g)$, net atomic charge, and atomic energy distributions in the gas phase it is evident that a full correlation exists only between the minimum Gibbs free energy values and atomic energies of N- and carbon atoms; there is not a single higher Gibbs free energy structure which has small the $\delta E(N)$ and $\delta E(C)$ values. Interestingly, we found a higher Gibbs free energy tautomer which had a small charge difference between the terminal nitrogen atoms (see $\delta q(N)$ for $(HL^+)_{N8}$ in Table S1). Clearly, the analysis of atomic charges in the gas-optimized antiperiplanar conformers could not result in an unambiguous prediction of the protonation sequence in solvent when performed separately. But in case of the analysis performed in solvent, an excellent correlation was found between the charge differences ($\delta q(N)$, $\delta q(C)$ and $\Delta\delta q(C,N)$) and atomic energy distributions for the terminal N- and carbon atoms (all obtained from the QTAIM-based analysis), but unfortunately the $G(aq)$ values have not followed these trends fully.

3.4. Conformational Considerations

It is known that polyamines are flexible molecules and can attain almost any conformational structure (depending on the environment). There are many examples of all-trans (i) free ligands, such as α,ω -diamines (1,2-Ethylenediamine [24], 1,3-Propanediamine [25] and 1,4-Butanediamine [25]), (ii) fully protonated polyamines (2,2-tri [26], 2,3-tri [27]), and (iii) partly protonated polyamines [26]. Only the geometries with all N-C-C-C or N-C-C-N dihedral angles of 180° (all-trans conformers) were considered here; a full conformational

analysis was not performed in this work because (i) linear structures were reported in crystal structures, as indicated above, and (ii) it is impractical in view of the combinatorial increase of possible principal conformers with the length of the chain.

It is important to stress here that the above examples of the reported structures not only support the use of completely antiperiplanar conformers in solvent, but most importantly they can be used in a full support of the proposed protonation sequence. It is evident from [26] that (i) as expected from the earlier literature reports and our results reported here, both terminal nitrogen atoms are protonated in the H_2L^{2+} form of 2,3-tri and (ii) the secondary nitrogen atom is protonated in the HL^+ form of triamine 2,2-tri, as it is proposed in this work for 2,3-tri. Interestingly, we were not able to find a crystal structure of any triamine with a primary nitrogen atom being protonated, as postulated earlier [1,2,4,6,8].

4. Conclusions

The analysis of all the results, obtained in the gas and aqueous phase, leads to the following conclusions when the theoretical prediction for the protonation sequence of linear all-trans aliphatic triamines. The most probable protonated form can be characterized by:

- (i) The smallest differences in the atomic energies between the terminal nitrogen atoms, $\delta E(N)$, and carbon atoms bonded to the terminal nitrogen atoms, $\delta E(C)$. This appears to be an overriding factor and can be seen as a necessary and sufficient condition (it was met in the gas phase and solvent).
- (ii) The smallest differences in the net atomic charges between the terminal carbon atoms, $\delta q(C)$ (it was met in both phases) and between the terminal nitrogen atoms, $\delta q(N)$ (it was met fully only in solvent in this study).
- (iii) The smallest differences in interatomic energies $\Delta\delta E(C,N)$ and charges $\Delta\delta q(C,N)$ between the terminal nitrogen and carbon atoms (these conditions were fully met only in solvent).
- (iv) In case when two (or more) tautomers have small and comparable $\delta q(N)$, $\Delta\delta E(C,N)$ or $\Delta\delta q(C,N)$ values, then the selection of the most likely protonation site must be supported by the small $\delta E(C)$ and $\delta E(N)$ values.

The combination of the above conditions may constitute a scheme for the prediction of the protonation sequence in linear polyamines. The present study suggests that the atomic energies and charges of the carbon atoms might be more important in establishing the protonation sequence than that of the terminal nitrogen atoms; one can see that the conditions (i) and (ii) are fully met in the gas phase and solvent only in case of carbon atoms. This is in contrast to the previous considerations where rationalization of a protonation sequence was focused at a charge distribution among nitrogen atoms.

Gibbs free energies obtained in the gas phase, $G(g)$, can be used to predict the protonation sequence in solvent. However, the minimum $G(aq)$ value has not been observed for the H_2L^{2+} tautomer selected from the QTAIM-based analysis. Several levels of theories and solvation models were tested but each time the computed $G(aq)$ values have not changed the trend reported in this work - it is possible that the $G(aq)$ values are highly dependent on the solvation model used. Fortunately and conveniently, the atomic energies vary by orders of magnitude and hence an unambiguous prediction of the most likely protonated forms is possible. Because the same trends in the atomic energy distributions were observed in the gas phase and solvent, we conclude that it is likely a good predictor of protonation sequences.

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Figure Captions

Fig. 1 Structure of the free ligand, 1,4,8-triazaoctane, optimized at the RX3LYP/6-311++G(d,p) level of theory in solvent (CPCM/UAKS).

Fig. 2 Most likely partly protonated forms of 1,4,8-triazaoctane found from the QTAIM-based analysis of atomic energies and charges: (a) $(\text{HL}^+)_{\text{N4}}$ and (b) $(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$.

Fig. 3 Relative atomic energy distribution for the nitrogen atoms in all H_nL^{n+} forms of 1,4,8-triazaoctane (optimized at the RX3LYP/6-311++G(d,p) level of theory in solvent, CPCM/UAKS) with indicated protonated nitrogen atoms (the atomic energy of the N4-atom in H_3L^{3+} was set to zero).

Table 1 Gibbs free energies, $G(\text{aq})$, (RX3LYP/6-311++G(d,p), CPCM/UAKS), of all protonated forms of 2,3-tri and differences, $\delta G(\text{aq})$ in kcal mol⁻¹, calculated against the lowest-energy protonated form of a relevant tautomer which is shown in bold.

H_nL^{n+}	$G(\text{aq})$ / au	$\delta G(\text{aq})$	Relative Boltzmann Population
$(\text{HL}^+)_{\text{N1}}$	-364.03334	3.8	0.2%
$(\text{HL}^+)_{\text{N4}}$	-364.03944	0.0	99.7%
$(\text{HL}^+)_{\text{N8}}$	-364.03295	4.1	0.1%
$(\text{H}_2\text{L}^{2+})_{\text{N1,N4}}$	-364.47386	3.1	0.4%
$(\text{H}_2\text{L}^{2+})_{\text{N1,N8}}$	-364.47808	0.5	30.0%
$(\text{H}_2\text{L}^{2+})_{\text{N4,N8}}$	-364.47883	0.0	69.7%

Table 2 Net charges and atomic energies (RX3LYP/6-311++G(d,p), CPCM/UAKS) of nitrogen atoms in all H_nL^{n+} forms of 2,3-tri. Lowest energies of nitrogen atoms of each H_nL^{n+} form are shown in bold.

H_nL^{n+}	Atom	$q(N) / e$	$E(N) / au$	$\Delta E_1(N)^a$	$\Delta E_2(N)^b$	$\delta E(N)^c$	$\delta q(N)^d$
L	N1	-1.034	-55.15373	32.4	121.7		
	N4	-1.015	-55.20539	0.0	89.2	-0.5	-0.003
	N8	-1.032	-55.15296	32.9	122.1		
$(HL^+)_{N1}$	N1	-0.973	-55.29969	0.0	30.1		
	N4	-1.003	-55.21930	50.4	80.5	-82.0	0.059
	N8	-1.031	-55.16897	82.0	112.1		
$(HL^+)_{N4}$	N1	-1.024	-55.17137	90.9	110.6		
	N4	-0.965	-55.31628	0.0	19.7	-0.4	0.004
	N8	-1.028	-55.17072	91.3	111.0		
$(HL^+)_{N8}$	N1	-1.034	-55.16895	81.4	112.1		
	N4	-1.011	-55.22073	48.9	79.6	81.4	-0.064
	N8	-0.970	-55.29866	0.0	30.7		
$(H_2L^{2+})_{N1,N4}$	N1	-0.974	-55.31468	10.4	20.7		
	N4	-0.966	-55.33124	0.0	10.3	-76.6	0.053
	N8	-1.028	-55.19265	87.0	97.2		
$(H_2L^{2+})_{N1,N8}$	N1	-0.973	-55.31486	0.1	20.6		
	N4	-0.999	-55.23515	50.1	70.6	0.1	-0.003
	N8	-0.970	-55.31495	0.0	20.5		
$(H_2L^{2+})_{N4,N8}$	N1	-1.024	-55.18999	88.9	98.9		
	N4	-0.967	-55.33166	0.0	10.0	78.7	-0.051
	N8	-0.972	-55.31541	10.2	20.2		
H_3L^{3+}	N1	-0.975	-55.33348	8.9	8.9		
	N4	-0.969	-55.34761	0.0	0.0	2.4	-0.002
	N8	-0.973	-55.33732	6.5	6.5		

^{a)} relative energy, in kcal mol⁻¹, calculated versus lowest energy nitrogen atom within a molecule

^{b)} relative energy, in kcal mol⁻¹, calculated versus lowest energy nitrogen atom, N4 in H_3L^{3+} , among all protonated forms of 2,3-tri

^{c)} $\delta E(N) = E(N1) - E(N8)$ in kcal mol⁻¹

^{d)} $\delta q(N) = q(N1) - q(N8)$ in e

Table 3 Net charges and atomic energies (RX3LYP/6-311++G(d,p), CPCM/UAKS) of carbon atoms in all H_nL^{n+} forms of 2,3-tri

H_nL^{n+}	Atom	$q(C) / e$	$E(C) / au$	$\Delta E(C)_1$ a	$\Delta E(C)_2$ b	$\delta E(C)^c$	$\delta q(C)^d$
L	C2	0.346	—	119.4	139.5	-5.9	-0.004
	C3	0.325	—	103.1	123.2		
	C5	0.341	—	115.8	135.9		
	C6	0.025	—	0.0	20.1		
	C7	0.350	—	125.3	145.4		
$(HL^+)_{N1}$	C2	0.275	—	88.3	101.4	-37.5	-0.075
	C3	0.341	—	110.8	123.9		
	C5	0.337	—	115.3	128.4		
	C6	0.025	—	0.0	13.1		
	C7	0.350	—	125.9	139.0		
$(HL^+)_{N4}$	C2	0.358	—	125.7	137.7	-5.3	-0.005
	C3	0.257	—	76.2	88.2		
	C5	0.273	—	89.8	101.8		
	C6	0.026	—	0.0	12.0		
	C7	0.359	—	131.0	143.0		
$(HL^+)_{N8}$	C2	0.347	—	116.6	133.4	25.2	0.070
	C3	0.323	—	99.7	116.6		
	C5	0.350	—	115.2	132.1		
	C6	0.038	—	0.0	16.9		
	C7	0.278	—	91.3	108.2		
$(H_2L^{2+})_{N1,N4}$	C2	0.282	—	97.4	100.1	-34.4	-0.077
	C3	0.279	—	89.5	92.2		
	C5	0.264	—	87.2	89.8		
	C6	0.026	—	0.0	2.6		
	C7	0.360	—	131.8	134.4		
$(H_2L^{2+})_{N1,N28}$	C2	0.277	—	85.3	95.3	-6.3	-0.002
	C3	0.340	—	107.6	117.6		
	C5	0.347	—	114.8	124.7		
	C6	0.038	—	0.0	10.0		
	C7	0.279	—	91.6	101.6		
$(H_2L^{2+})_{N4,N8}$	C2	0.354	—	120.6	130.1	25.2	0.069
	C3	0.252	—	69.4	78.9		
	C5	0.284	—	89.2	98.7		
	C6	0.035	—	0.0	9.5		
	C7	0.286	—	95.3	104.9		
H_3L^{3+}	C2	0.283	—	92.3	92.3	-3.8	-0.003
	C3	0.275	—	83.3	83.3		
	C5	0.276	—	86.9	86.9		
	C6	0.036	—	0.0	0.00		
	C7	0.286	—	96.1	96.1		

^{a)} relative energy, in kcal mol⁻¹, calculated versus lowest energy carbon atom within a molecule

^{b)} relative energy, in kcal mol⁻¹, calculated versus lowest energy carbon atom, C6 in H_3L^{3+} , among all protonated forms of 2,3-tri

^{c)} $\delta E(C) = E(C2) - E(C7)$ in kcal mol⁻¹

^{d)} $\delta q(C) = q(C2) - q(C7)$ in e .

Table 4 Charge (part a, in e) and atomic energy (part b, in kcal mol⁻¹) differences between terminal C- and nitrogen atoms in all H_nL^{n+} forms of 2,3-tri optimized at the RX3LYP/611++G(d,p) level of theory in solvent (CPCM/UAQS)

(a)

Ligand	$\delta q(\text{C2}, \text{N1})$	$\delta q(\text{C7}, \text{N8})$	$\Delta \delta q(\text{C}, \text{N})$
L	1.380	1.382	-0.002
$(\text{HL}^+)_{\text{N1}}$	1.248	1.381	-0.133
$(\text{HL}^+)_{\text{N4}}$	1.378	1.387	-0.009
$(\text{HL}^+)_{\text{N8}}$	1.381	1.248	0.133
$(\text{H}_2\text{L}^{2+})_{\text{N1}, \text{N4}}$	1.257	1.387	-0.130
$(\text{H}_2\text{L}^{2+})_{\text{N1}, \text{N8}}$	1.250	1.249	0.001
$(\text{H}_2\text{L}^{2+})_{\text{N4}, \text{N8}}$	1.378	1.258	0.120
H₃L³⁺	1.258	1.259	-0.001

(b)

Ligand	$\delta E(\text{C2}, \text{N1})$	$\delta E(\text{C7}, \text{N8})$	$\Delta \delta E(\text{C}, \text{N})$
L	34826.5	34826.7	-2.2
$(\text{HL}^+)_{\text{N1}}$	34873.9	34838.9	35.0
$(\text{HL}^+)_{\text{N4}}$	34842.5	34845.2	-2.7
$(\text{HL}^+)_{\text{N8}}$	34836.9	34874.6	-37.7
$(\text{H}_2\text{L}^{2+})_{\text{N1}, \text{N4}}$	34887.6	34859.7	28.0
$(\text{H}_2\text{L}^{2+})_{\text{N1}, \text{N8}}$	34884.3	34885.5	-1.2
$(\text{H}_2\text{L}^{2+})_{\text{N4}, \text{N8}}$	34854.7	34890.2	-35.5
H₃L³⁺	34900.1	34904.3	-4.2

Table 5 Gibbs free energies, $G(g)$ at the RB3LYP/6-31+G(d,p) level of theory, of all protonated forms of 2,3-tri and differences, $\delta G(g)$ in kcal mol⁻¹, calculated against the lowest-energy protonated form shown of a relevant tautomer which is shown in bold.

H_nL^{n+}	$G(g)$ / au	$\delta G(g)$
$(HL^+)_{N1}$	-364.01036	7.8
$(HL^+)_{N4}$	-364.02275	–
$(HL^+)_{N8}$	-364.01143	7.1
$(H_2L^{2+})_{N1,N4}$	-364.23212	33.2
$(H_2L^{2+})_{N1,N8}$	-364.28505	–
$(H_2L^{2+})_{N4,N8}$	-364.25867	16.6

Table 6 Charge (part a, in e) and atomic energy (part b, in kcal mol^{-1}) differences between terminal C- and nitrogen atoms in all H_nL^{n+} forms of 2,3-tri optimized at the RB3LYP/61+G(d,p) level of theory in a gas phase

(a)

Ligand	$\delta q(\text{C2},\text{N1})$	$\delta q(\text{C7},\text{N8})$	$\Delta\delta q(\text{C},\text{N})$
L	1.473	1.461	0.012
$(\text{HL}^+)_{\text{N1}}$	1.473	1.483	-0.011
$(\text{HL}^+)_{\text{N4}}$	1.533	1.522	0.011
$(\text{HL}^+)_{\text{N8}}$	1.497	1.337	0.160
$(\text{H}_2\text{L}^{2+})_{\text{N1},\text{N4}}$	1.374	1.568	-0.194
$(\text{H}_2\text{L}^{2+})_{\text{N1},\text{N8}}$	1.361	1.354	0.007
$(\text{H}_2\text{L}^{2+})_{\text{N4},\text{N8}}$	1.585	1.371	0.213
H_3L^{3+}	1.383	1.382	0.001

(b)

Ligand	$\delta E(\text{C2},\text{N1})$	$\delta E(\text{C7},\text{N8})$	$\Delta\delta E(\text{C},\text{N})$
L	10881.3	10885.4	-4.1
$(\text{HL}^+)_{\text{N1}}$	11020.7	10868.8	151.9
$(\text{HL}^+)_{\text{N4}}$	10921.2	10922.2	-1.0
$(\text{HL}^+)_{\text{N8}}$	10896.0	10935.4	-39.4
$(\text{H}_2\text{L}^{2+})_{\text{N1},\text{N4}}$	10942.7	10949.7	-6.9
$(\text{H}_2\text{L}^{2+})_{\text{N1},\text{N8}}$	10933.3	10942.2	-8.9
$(\text{H}_2\text{L}^{2+})_{\text{N4},\text{N8}}$	10946.6	10949.3	-2.7
H_3L^{3+}	10945.7	10953.4	-7.7

Fig. 1 Structure of the free ligand, 1,4,8-triazaoctane, optimized at the RX3LYP/6-311++G(d,p) level of theory in solvent (CPCM/UAKS)

(a)

(b)

Fig. 2 Most likely partly protonated forms of 1,4,8-triazaoctane found from the QTAIM-based analysis of atomic energies and charges: (a) $(HL^+)_{N4}$ and (b) $(H_2L^{2+})_{N1,N8}$.

Fig. 3 Relative atomic energy distribution for the nitrogen atoms in all $H_n L^{n+}$ forms of 1,4,8-triazaoctane (optimized at the RX3LYP/6-311++G(d,p) level of theory in solvent, CPCM/UAKE) with indicated protonated nitrogen atoms (the atomic energy of the N4-atom in H_3L^{3+} was set to zero)