

Potentiometric Study of a Benzene-based Tripodal Triamine as Chelator for Zn(II) Ion

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Abstract

The complexation of a C_3 -symmetrical tripodal triamine ligand, N^1, N^3, N^5 -tris(2-aminoethyl)benzene-1,3,5-tricarboxamide (TAT, L) with Zn(II) ion was investigated at an ionic strength of 0.1 M NaClO₄ and 25 ± 1 °C in aqueous medium. Formation of complex species [ZnL(H₂O)]²⁺ and [ZnL(OH)]⁺ were depicted in solution, where the ligand is tri-coordinated through three amine N-atoms and their stability constants were calculated with the HYPERQUAD 2000 computer program. The p*K*_a of the zinc-bound water molecule is 7.84, making the Zn(TAT) complex a viable model of carbonic anhydrase.

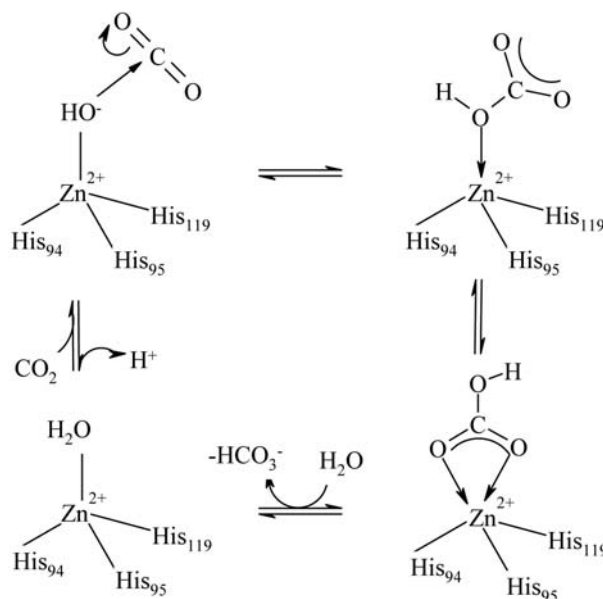
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1. Introduction

Carbonic anhydrases (CA), the first zinc containing metalloenzyme catalyzes the reversible hydration of carbon dioxide in a very efficient way (Scheme 1), and thus plays an important role in respiration and intracellular CO₂/HCO₃⁻ equilibrium.¹ X-ray diffraction studies demonstrate that the active site of CA consists of a zinc(II) ion coordinated by the imidazole groups of the three histidine residues.^{2,3} The water molecule coordinated to the zinc ion has a low p*K*_a (ca. 7) and is deprotonated at physiological pH. The resulting hydroxide ion is a potent nucleophile, which attacks CO₂ to give a coordinated bicarbonate anion. Expulsion of the bicarbonate ion from the active site, with concomitant coordination of a water molecule to Zn(II), completes the catalytic cycle (Scheme 1).^{2,3}

In order to elucidate the mechanism of zinc(II) involvement in hydrolytic enzymes, small-molecules structurally mimicking the active sites of CA have been synthesized as models and their properties are investigated.⁴⁻⁸ Many models were found to be valuable in confirming the Lewis acid behaviour of the zinc in the enzyme and the coordination modes of substrates in CA. However, in many cases it was found that models get dimerisation⁹ and

also, fail to reproduce the high catalytic efficiency of enzyme presumably due to the irreversible binding of the product (bicarbonate) to the metal center.¹⁰



Scheme 1. Schematic representation of the reversible hydration of carbon dioxide by CA.

Keeping the above facts in mind, we have synthesized a C_3 -symmetry tripodal-amine chelator, N^1, N^3, N^5 -tris(2-aminoethyl)benzene-1,3,5-tricarboxamide (TAT, L), which has three long ethylenediamine arms connected to a rigid aromatic platform through amide linkages. The chelating ability of ligand TAT towards Zn(II) was studied by potentiometric method, and the stability constants of the complexes depicted in water as well as the coordination modes of the ligand TAT were explained. Molecular modeling calculations were carried out to propose the 3D-model structure of the ligand TAT and its Zn(II) complexes.

2. Experimental

2.1. Materials and Measurements

All the chemicals: 1,3,5-benzenetricarboxylic acid, absolute ethanol, ethylenediamine and diethyl ether used for the synthesis of ligand TAT were obtained from Sigma-Aldrich Chemicals Ltd., whereas the chemicals for potentiometric study were obtained from Ranbaxy Chemicals Ltd., India. Elemental analyses (CHN) data was obtained with an Exeter Analytical CE-440 elemental analyzer. Infrared and ^1H NMR spectra were recorded on a Perkin Elmer Model RX-I FT-IR and Bruker AMX-300 spectrophotometer, respectively.

2.2. Synthesis of TAT

The synthesis of ligand TAT was reported earlier.¹¹ In our adopted procedure, first the triethylbenzene-1,3,5-tricarboxylate was prepared by passing dry HCl gas into a hot solution of 1,3,5-benzenetricarboxylic acid in absolute ethanol. The acidified mixture was refluxed for 5 hrs and then was kept overnight at an ambient condition resulting needle like crystals, which were filtered off, washed with ether and then dried under *vacuo* over CaCl_2 . 1.5 g Triethylbenzene-1,3,5-tricarboxylate (5.1 mol) was added slowly into a freshly distilled ethylenediamine (20 ml). The mixture was stirred under nitrogen atmosphere initially at 0 °C for 30 minutes and then at room temperature for 24 h. Excess of ethylenediamine was removed by rota evaporator leaving a sticky solid mass. Addition of cold diethyl ether (100 ml) gave powder like solid, which was filtered off under nitrogen atmosphere. The product was washed with ether and then dried under *vacuo* to get pale white solid. Yield: 65% (1.11 g), IR (ν , cm^{-1}): 3449, 3235, 3047, 2850, 1643 and 1559; ^1H NMR (D_2O , δ , ppm): 2.71 (t, 6H, $-\text{CH}_2\text{NH}_2$), 3.35 (t, 6H, $-\text{CH}_2\text{NHCO}-$) and 8.00 ppm (s, 3H, Ar-H); CHN (%): found (Calc.) C = 53.61 (53.56), H = 7.43 (7.19), N = 24.54 (24.98).

2.3. Titration Procedure

The apparatus used, the experimental details, the calibration techniques, and the titration procedures were

as described before.^{12,13} Double distilled water was used for the preparation of all the solutions. Stock solutions of 0.01 M ligand and zinc(II) (from its nitrate salt) were prepared. KOH solution (0.1 M) was made from carbon dioxide free double distilled water and was standardized against potassium hydrogen phthalate. HClO_4 solution (0.0987 M) was prepared and its accurate concentration was determined by titrating with 0.1 M KOH. The protonation constants of the ligand and the stability constants of the metal complexes were determined by potentiometric titrations at 25 ± 1 °C maintained from a double wall glass jacketed titration cell connected to a constant temperature circulatory bath. For all titrations, the observed pH was measured as $-\log [\text{H}^+]$ using a ThermoOrion 720A+ pH meter equipped with a combined glass electrode. The electrode was calibrated to read pH according to the classical method. A standard hydrochloric acid solution was titrated with a standard KOH solution and the calculated hydrogen ion concentrations ($\text{p}K_w = 13.77 \pm 0.05$) was used to convert the pH-meter reading to hydrogen ion concentration. The ionic strength was maintained to 0.1 M with addition of appropriate amount of 1 M NaClO_4 solutions. Titrations with L:M = 1:0, 1:1 and 1:2 were carried out, where for each titration 51 data were recorded between the pH ranges of ~ 2 to ~ 11.5 . The protonation and stability constants were calculated using computer program Hyperquad 2000.¹⁴ The species distribution curves were obtained from the measured equilibrium constants using the program HySS.¹⁵

2.4. Computational Methods

All the calculations were carried out on a Pentium IV 3.0 GHz machine with a Windows-XP operating system. The systematically search to global minimum energy conformer of the ligand was obtained by using CON-FLEX¹⁶ with MM3 molecular mechanics in molecular modeling software CAChe version 6.1.1.¹⁷ Then, the minimized structure was further optimized through semi-empirical method by applying PM3 Hamiltonian. Semi-empirical methods represent a “middle road” between the mostly qualitative results available from molecular mechanics and the computationally time-consuming quantitative results available from *ab initio* methods; and with minimal computational time this method can able to give relatively good results in predicting molecular structure and calculating various electronic and thermodynamic properties. Furthermore, the proposed 3D-model structures of the $[\text{ZnL}(\text{H}_2\text{O})]^{2+}$ and $[\text{ZnL}(\text{OH})]^+$ complexes were also obtained by the similar method as followed for the free ligand TAT.

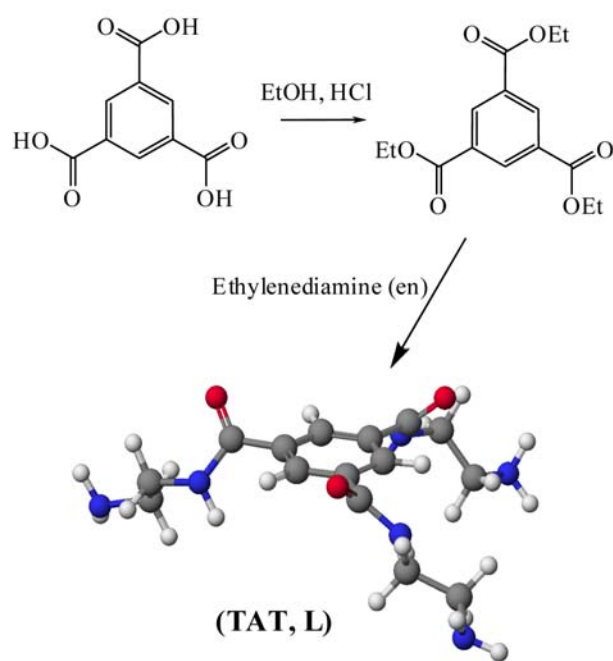
The ^1H NMR of the ligand from its PM3 optimized structure was calculated by applying TNDO/2 (Typed Neglect of Differential Overlap) semi-empirical method (with convergence limit 0.01 and maximum iterations 50) using the program HyperNMR Version 7.0.¹⁸ The refer-

ence shielding constant (ppm) taken for proton was 23.951 ppm.

3. Results and Discussion

3.1. Synthesis of Ligand

The ligand was synthesized from 1,3,5-benzenetricarboxylic acid in two steps as presented in Scheme 2, and was characterized through elemental analyses, IR and ^1H NMR spectral data. The ligand TAT is soluble in water but insoluble in common organic solvents. The infrared spectrum of TAT taken as KBr pellet gave characteristics $\nu_{\text{C=O}}$ (amides), ν_{NH} (amines) and $\nu_{\text{Ar-H}}$ (aromatic) bands respectively at 1643, 3449 and 3047 cm^{-1} . The ^1H NMR spectrum gave two triplets at 2.71 and 3.35 ppm assigned for methylene protons attached to amine and amide functionals respectively. A singlet at 8.00 ppm was observed due to the aromatic protons. The elemental (C, N and H) analyses are consistent with the formulation of the compound ($\text{C}_{15}\text{H}_{24}\text{N}_6\text{O}_3$). The optimized structure of the ligand obtained through conformational search using CONFLEX with MM3 molecular mechanics calculation followed by semi-empirical PM3 method is shown in Scheme 2. The optimized structure indicates that the three pendant arms are forming a large cavity due to the rigid benzene ring with the three coordinating amine groups are aside. No steric repulsion was detected between the three pendant arms. The ^1H NMR spectrum for the ligand TAT was also obtained theoretically using semi-empirical TNDO/2 method, which gave peaks for the methylene groups



Scheme 2. Synthesis of ligand TAT (L). The 3D-model structure of TAT is obtained through semi-empirical PM3 methods.

attached to the amine and amide groups at 3.271 and 3.576 ppm respectively. Peak due to aromatic protons was observed at 6.155 ppm. Peaks at 3.072 and 6.806 ppm for amine and amide protons were not observed experimentally due to deuterium exchange.

3.2. Ligand Protonation Constants

The potentiometric titration curve for the ligand TAT is shown in Fig. 1. Analysis of potentiometric data using the program Hyperquad 2000 gave three protonation constants: 10.29 ± 0.04 , 9.65 ± 0.03 and 8.71 ± 0.01 . These constants are defined by the equation 1.

$$\text{LH}_{n-1} + \text{H}^+ \rightleftharpoons \text{LH}_n \quad K_n = \frac{[\text{LH}_n]}{[\text{LH}_{n-1}][\text{H}^+]} \quad (1)$$

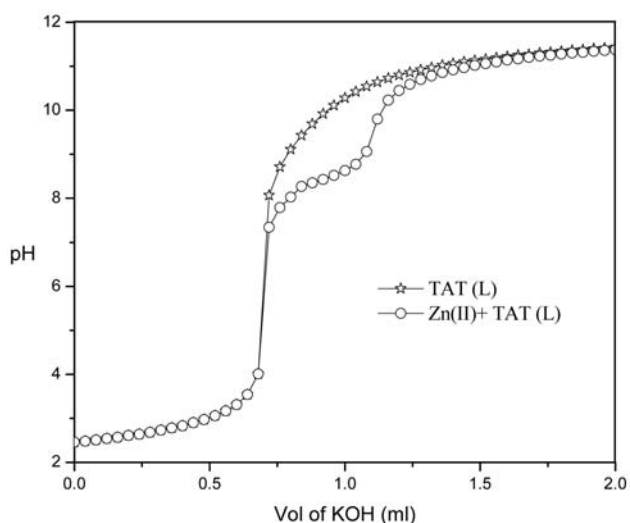


Figure 1. Potentiometric titration curves of free ligand, TAT and in the presence of Zn(II), in 1:1 molar ratio.

The neutral ligand L has six protonation sites: three amine and three amide groups. The protonation constants of amide groups could not be evaluated within the experimental pH because of their very low acidity. Thus, the fully protonated form of the ligand is defined by LH_3^{3+} . The species distribution curve (Fig. 2) indicates that TAT exists in the fully protonated form, LH_3^{3+} , in the acidic solution ($\text{pH} < \sim 6$). Stepwise deprotonation occurs with the rise in the pH, and the species LH_2^{2+} , LH^+ and L starts to form after $\text{pH} \sim 6$, ~ 8 and ~ 9 , respectively. The free ligand species L became predominant after $\text{pH} 10.5$.

The observed protonation constants of TAT (10.29, 9.65 and 8.71) has higher log K values as compared to the rigid tripods like *cis,cis*-1,3,5-triaminocyclohexane (TACH: 10.16, 8.66 and 7.17)¹⁹ and 1,1,1-tris(aminoethyl)ethane (TAME: 10.15, 8.24 and 5.85).²⁰ However, the values are very similar to a more flexible tripodal ligand tris(2-aminoethyl)amine (TREN: 10.13, 9.43 and

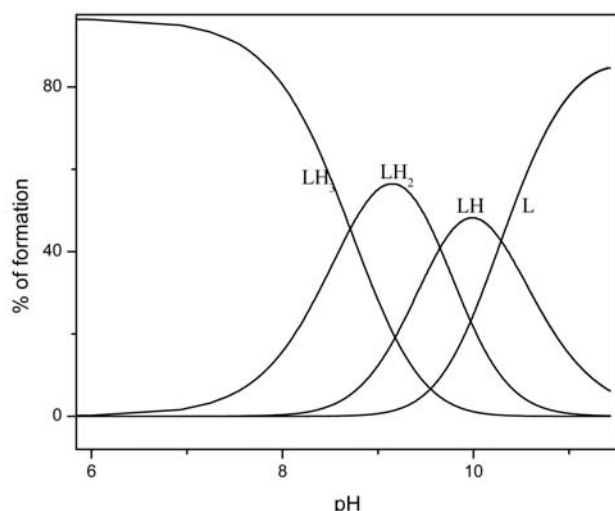


Figure 2. Species distribution curve of the ligand TAT at $\mu = 0.1$ M NaClO_4 and 25 ± 1 °C.

8.43).²¹ This can be ascribed mainly due to the minimum electrostatic repulsion between the amino groups in TAT. Again, the difference between successive deprotonation constants $\Delta \log K_1$ and $\Delta \log K_2$ values for TAT, TACH and TAME are calculated to be 0.64, 1.50, 1.91 and 0.94, 1.49, 2.39 respectively. The lower values of $\Delta \log K_1$ and $\Delta \log K_2$ for TAT as compared to TACH and TAME indicates that the uptake of the second and third proton can be regarded as a favorable process, a phenomenon which seems to be incompatible with a conformational change enforced by steric and electrostatic repulsion after uptake of first proton in case of TACH and TAME.²²

3. 3. Metal Complexes

The potentiometric titration of ligand TAT (L) with Zn(II) ion was carried out in 1:1 and 1:2 metal-ligand molar ratios at $\mu = 0.1$ M NaClO_4 and 25 ± 1 °C in aqueous medium. Deviation of the metal-ligand curve (Fig. 1) from the free ligand curve indicates the formation of metal complexes. Analysis of potentiometric data with the computer program Hyperquad 2000 gave best-fit for the models ZnL and ZnLH_{-1} and their overall stability constants ($\log \beta$) were calculated to be 6.56 ± 0.05 and -1.28 ± 0.03 , respectively. No additional species were detected from the data obtained between 1:1 and 1:2 metal-ligand molar ratios.

The complete formation of the 1:1 complexes with Zn(II) does not occur below pH 7.0. The real reason for this is the high $\text{p}K_a$ of the ligand and moderate stability of the complexes. This is clearly highlighted in the Zn(II):L (1:1) titration and species distribution curve (Fig. 3). The first metal complex species ZnL is formed after pH ~ 7.7 , where the ligand TAT may be coordinated through the three amine nitrogen atoms. Simultaneously, formation of species ZnLH_{-1} was also observed, which predominates at

higher pH after ~ 9.0 . Formation of ZnLH_{-1} indicates that the complex ZnL has lost a proton. This proton from the species ZnL may be released either from a water molecule attached to the metal leaving behind an hydroxide group, or it may come from the ligand. As the ligand contains three amide groups, Zn(II) binding may take place through any one of the deprotonated amide nitrogen atom to give ZnLH_{-1} . But, it is well known that the deprotonation of the amide group is very endothermic and can take place if it is compensated energetically by the formation of a strong bond between the metal and deprotonated amide group.^{12,23} This is the case for the Cu(II) and Ni(II) ions, which profit greatly from ligand field stabilization effects, whereas Co(II) and Zn(II) ions do not profit much from ligand field effects and thus do not promote much deprotonation of amide groups.²⁴ Thus, formation of metal species ZnL and ZnLH_{-1} can be represented by the following equilibrium reactions:

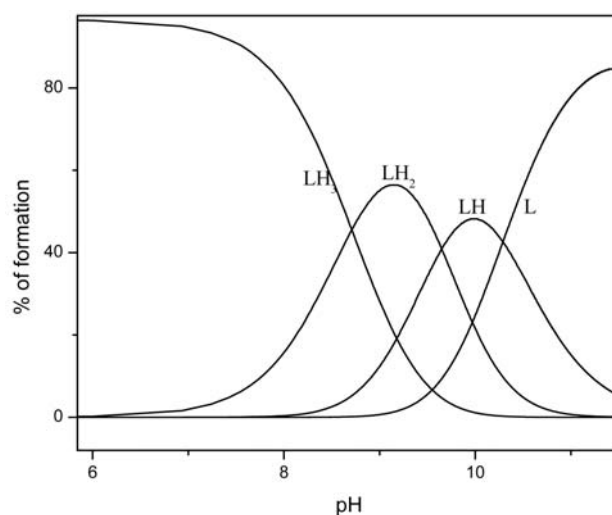
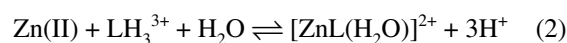


Figure 3. Species distribution curves for Zn(II):L (1:1) system at $\mu = 0.1$ M NaClO_4 and 25 ± 1 °C in aqueous medium.

The obtained stability constants $\log \beta_{\text{ZnL}}$ for TAT shows intermediate binding affinity in comparison to TACH (6.90)²² and TAME (6.60)²², but, appreciably lower than the more flexible TREN (15.05).²⁵ TREN, different from other three tripodal-amines (TAT, TACH and TAME), behaves as a tetra-coordinated ligand by the coordination of epical tertiary nitrogen.²⁵ Secondly, the rigid aromatic ring in TAT may provide an additional steric hindrance for the encapsulation of Zn(II) ion. Nevertheless, the most important thing is that all these tripodal-amines showed higher stability than the tris(imidazole) model systems of CA.²⁶ Furthermore, the calculated $\text{p}K_a$ [$\text{p}K_a = \log \beta(\text{ZnL}) - \log \beta(\text{ZnLH}_{-1})$] value of the coordinated water

molecule in Zn(TAT) is 7.84. This value is similar to that of the analogous water molecule in CA (~7) and its model compound TACH (7.95).²⁷ Also, the ligand TAT shows a lack of dimerization. These observations suggested that TAT can be implemented as a model compound for the investigation of catalytic activities for CO₂ hydration or hydrolytic cleavages.

The proposed 3D-model structures of the Zn(TAT) complexes were obtained through molecular modeling calculations using molecular mechanics (MM) followed by semi-empirical PM3 method in *vacuo*. The optimized structures of ZnL(H₂O) and ZnL(OH) (Fig. 4) predicted a distorted tetrahedral geometry. The calculated Zn-O distance is 2.173 and 1.878 Å, whereas the average Zn-N distance is 2.073 and 2.094 Å for the complexes ZnL(H₂O) and ZnL(OH), respectively. The calculated average N-Zn-N bond-angle is 112.80 and 109.21 Å, whereas average N-Zn-O bond-angle is 105.81 and 109.53 Å for the complex above, respectively. All these calculated values are found to be similar to that determined by single X-ray technique for the Zn(II) complexes with the similar tripodal-amine ligands.^{8,25}

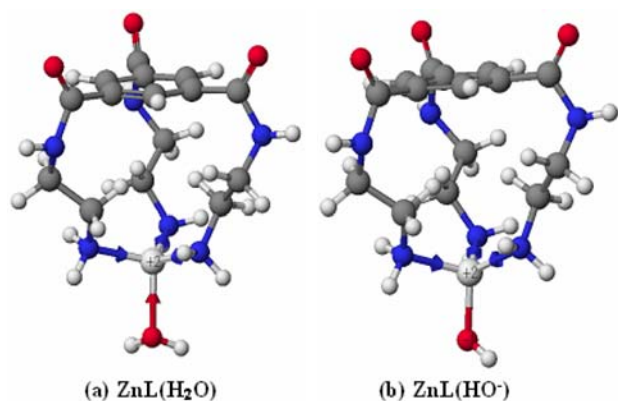


Figure 4. Optimized structures of (a) [ZnL(H₂O)]²⁺ and (b) [ZnL(OH)]⁺ complexes through semi-empirical PM3 method.

4. Conclusions

The present study provides a rigid tripodal-amine ligand, TAT, which can mimic the active site of carbonic anhydrase (CA) adequately. TAT formed two complex species [ZnL(H₂O)]²⁺ and [ZnL(OH)]⁺ in the solution, where the ligand is tri-coordinated through three amine N-atoms. No dimeric complexes were observed with TAT. The pK_a of the zinc-bound water molecule was found to be 7.84. The coordination behaviour of the ligand and TAT makes the Zn(TAT) complex a viable model of carbonic anhydrase and also, it can be exploited in terms of catalytic activities for CO₂ hydration or hydrolytic cleavages.

5. References

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Povzetek

Raziskovali smo kompleksacijo C_3 -simetričnega troveznega triaminskega liganda *N1,N3,N5*-tris(2-aminoetil)benzen-1,3,5-trikarboksamida (TAT, L) s cinkovimi ioni pri ionski moči 0,1 M NaClO₄ in temperaturi 25 ± 1 °C v vodni raztopini. Opisali smo nastanek kompleksnih zvrsti [ZnL(H₂O)]²⁺ in [ZnL(OH)]⁺, v katerih je ligand trovezno koordiniran preko treh aaminskih *N*-donorskih atomov. Z računalniškim programom HYPERQUAD 2000 smo izračunali konstante stabilnosti obeh kompleksnih ionskih zvrsti. pK_a vrednost na cink koordinirane molekule vode je 7,84, zato je Zn(TAT) kompleks primeren model za ogljikovo anhidrazo.