Synthesis, Structure and Molecular Modeling of a ZnII-Phenolate Complex as a Model for ZnII-Containing Tyrosinate Metalloenzymes

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We describe herein the synthesis, crystal structure and 1H NMR properties of the mononuclear [ZnII(L-Br)].2H2O complex containing the hexadentate H2L-Br ligand (H2L-Br = N,N'-bis-(5-bromo-2-hydroxybenzyl)-N,N'-bis-(pyridin-2-ylmethyl)-ethane-1,2-diamine). DFT calculations demonstrate very good agreement between parameters calculated and those determined by X-ray crystallography, and reveal that only the phenolate groups of the H2L-Br ligand participate in the formation of the HOMO, while only one of the pyridine rings contributes to the LUMO formation.

Keywords: ZnII-phenolate complex, crystal structure, 1H NMR, DFT calculation

Introduction

Many ZnII-containing enzymes have been discovered and their role in biological processes studied. Carbonic anhydrase,1,2 carboxypeptidase,3,4 β-lactamase II,6 thermolysin,7 alkaline phosphatase,8 and astacin9,10 are examples of such metalloenzymes. Of these, astacin, a digestive zinc-endopeptidase that is involved in hydrolytic processes should be highlighted since it represents the first example of a zinc enzyme that contains a tyrosine residue coordinated directly to the metal center in the active site.11 In fact, astacin, an endopeptidase isolated from crawfish Astacus astacus represents the prototype for the “astacin family”,12,13 which includes mammalian metallo-endopeptidases14 and developmentally regulated human,15 fruitfly,16 frog17 and sea urchin18 proteins. The X-ray crystal structure of astacin (R-value of 0.162) reveals that the ZnII-ion lies in a trigonal bipyramidal coordination environment with three histidines, a water molecule and a more remote tyrosine as ligands.11 One histidine nitrogen and the tyrosine OH group, at distances of 2.3 and 2.6 Å to the zinc, respectively, are apically connected, whereas the other three ligands are coplanar and 2.1 Å apart from the zinc center. Following our interest in the search for new compounds as structural and functional models for the active site of zinc-containing metalloenzymes,19-21 we report here the synthesis, X-ray structure and molecular modeling using Density Functional Theory (DFT) for the [Zn(L-Br)] complex, where L-Br2– is the deprotonated form of the N,N'-bis-(5-bromo-2-hydroxybenzyl)-N,N'-bis-(pyridin-2-ylmethyl)-ethane-1,2-diamine. Importantly, theoretical calculations have been recently introduced in our group as a strategy for planning the synthesis of new ligands and model complexes.22

Experimental

Abbreviations

H2L-Br = N,N'-bis-(5-bromo-2-hydroxybenzyl)-N,N'-bis-(pyridin-2-ylmethyl)-ethane-1,2-diamine; H2L = (N,N'-bis-
Material and methods

All reagents and solvents were purchased from commercial sources and used as received. 1H NMR spectra were recorded on a Bruker 200 FT spectrometer, in CDCl3 as a solvent. Infrared spectra were recorded with a Perkin Elmer FTIR 2000, in KBr pellets. Elemental analyses were performed with a Carlo Erba instrument model E-1110.

Synthesis of H$_2$L-Br

The H$_2$L-Br ligand was prepared according to the sequence of reactions depicted in Scheme 1, with slight modifications of the method described for the synthesis of the H$_2$bbpen ligand. Ethylenediamine (10.0 mmol) was added dropwise to a THF/methanol - 2:1 solution of 2-hydroxy-5-bromobenzaldehyde (20.0 mmol) while stirring. After 30 min NaBH$_4$ (26.0 mmol) was added, and a few minutes later the deep yellow solution became colorless. Then, 4.0 mol L$^{-1}$ HCl was added to adjust the pH to 7 and the solvent was removed under vacuum at 40 °C. Water was added to the precipitated product which was filtered off and washed with water followed by cold methanol. The white solid obtained (7.5 mmol) was added to a solvent mixture of THF and water 1:1 (100 mL) with 2-chloromethylpyridine hydrochloride (23 mmol) and sodium carbonate (38 mmol). This mixture was refluxed for 18 hours and the THF removed under vacuum at 40 °C. The residual water was decanted off and the product (yellowish oil) was solubilized in a mixture of 2-propanol and ethyl acetate. The H$_2$L-Br ligand precipitated as a white powder after 24 hours (yield = 75%). Anal. Calc. for H$_2$L-Br (Br$_2$C$_{28}$H$_{28}$N$_4$O$_2$): C, 54.92; H, 4.61; N, 9.15%. Found: C, 54.86; H, 4.73; N, 8.99%. IR (KBr pellet) $\nu_{\text{max}}$/cm$^{-1}$: 1593(m), 1569(m), 1480(s), 1456(m), 1431(m), $\nu$(C=C, C=N); 1271(s), 1236(m), $\nu$(C-O).

Synthesis of [Zn(L-Br)].2H$_2$O

The [Zn(L-Br)].2H$_2$O complex was prepared by the reaction of Zn(OAc)$_2$.2H$_2$O (1 mmol) with H$_2$L-Br (1 mmol) in methanol while stirring at 45 °C for 30 min. After evaporation of the solvent, the crude oil was dissolved in chloroform and washed with a NaHCO$_3$ (5% aqueous solution) in a separation funnel. The organic layers were combined, dried with anhydrous Na$_2$SO$_4$ and concentrated at reduced pressure. The resulting colourless oil was crystallized from an ethylacetate/methanol (1:1) solution (yield = 70%), providing suitable crystals for X-ray crystallography analysis. Anal. Calc. for [Zn(L-Br)].2H$_2$O (C$_{28}$H$_{32}$N$_4$O$_4$Br$_2$Zn): C, 47.12; H, 4.52; N, 7.85%. Found: C, 47.35; H, 4.97; N, 7.79%. IR (KBr pellet) $\nu_{\text{max}}$/cm$^{-1}$: 1606(w), 1585(w), 1471(s), 1440(w), 1413(w), $\nu$(C=C, C=N); 1274(s), $\nu$(C-O).

Crystal structure determination

A colorless irregular block was prepared from a big crystal, which was selected from the crystalline sample of the [Zn(L-Br)] complex. The crystal data were measured on an Enraf–Nonius CAD4 diffractometer, using graphite monochromated Mo-K$_\alpha$ radiation ($\lambda$=0.71069 Å), at room temperature. Cell parameters were determined from 25 carefully centered reflections in the q range 8.76–15.30° and refined by the least-squares method. The collected intensities were corrected for Lorentz and polarization effects and for

![Scheme 1](image-url)
absorption (face-indexed method; T_mn 0.28 and T_max 0.64). The structure was solved by direct methods and was refined by the full-matrix least-squares method using SHELXS97 and SHELXL97 computer programs, respectively. All non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms bonded to C atoms were placed at idealized positions using standard geometric criteria, whereas the H atoms of the water molecule of crystallization were found from Fourier map and treated with a riding model. Further relevant crystallographic data are summarized in Table 1. The drawing of molecular structure was made with ORTEP3 program.

**Computational details**

All geometry optimizations were performed with B3LYP hybrid density functional theory in conjunction with the 6-31G (d,p) basis set and LACVP* basis set for the metal using the Spartan 04 program. The calculations were carried out on a 2.6 GHz Athlon PC, with 1 GB RAM and 40Gb HD under the operational system Windows 2000, using the Spartan 04 program.

**Results and Discussion**

**Syntheses**

The H$_2$L-Br ligand was obtained in a good yield and pure enough to be fully characterized and used as a precursor for the synthesis of coordination compounds. The reaction between one equivalent of H$_2$L-Br and one equivalent of Zn(OAc)$_2$·2H$_2$O produced the mononuclear complex [Zn(L-Br)].2H$_2$O. Infrared spectral data reveal that upon coordination of H$_2$L-Br to the zinc there is a general bathochromic shift of ~15 cm$^{-1}$ and a decrease in intensity of the C=N and C=C stretching modes. Consequently, the [Zn(L-Br)] complex presents a higher distorted geometry due to its five-membered ring in the equatorial plane compared to the six-membered ring in [ZnL$_3$]. The average Zn-O bond lengths are 0.063 Å shorter than those in [ZnL$_3$]. The average Zn-O bond lengths in [Zn(L-Br)] (1.997 Å) are 0.058 Å longer in [Zn(L-Br)] when compared to [ZnL$_3$],30 except for the fact that [Zn(L-Br)] contains an ethylenediamine backbone instead of a propane-1,3-diamine backbone in [ZnL$_3$]. Consequently, the [Zn(L-Br)] complex presents a higher distorted geometry due to its five-membered ring in the equatorial plane compared to the six-membered ring in [ZnL$_3$]. The higher distortion in [Zn(L-Br)] can be evidenced by the three trans angles which are 3.5° (N1-Zn-O2), 4.8° (N2-Zn-O1) and 11.1° (N31-Zn-N41) smaller for [Zn(L-Br)] when compared to [ZnL$_3$]. The average Zn-O bond lengths for [Zn(L-Br)] (1.997 Å) are 0.063 Å shorter than those for [ZnL$_3$]. This is attributed to the higher distortion in the coordination sphere and the weaker electron-withdrawing effect of the bromo groups in [Zn(L-Br)]. On the other hand, the average Zn-N$_{py}$ bond lengths are 0.058 Å longer in [Zn(L-Br)] when compared to [ZnL$_3$], whereas the Zn-N$_{amine}$ bonds are identical in both complexes.

Since the [Zn(L-Br)] and the [Zn(bpa)$_2$]$^{19}$ complexes (Hbpa = N-(2-hydroxybenzyl)-N-(pyridin-2-ylmethyl) amine) possess identical coordination environments, a comparison of their structural parameters should also be of interest. Firstly, it should be noted that H$_2$L-Br is a hexadentate N$_4$O$_2$ ligand bound to the Zn$^{II}$-ion in its...
deprotonated form, while in [Zn(bpa)₂] the Hbpa ligand corresponds to the half of H₂L-Br without the ethylenediamine backbone and the bromo substitution in the para-position of the phenolate group (tridentate N₂O-donor). Consequently, in [Zn(L-Br)] the tertiary amine nitrogen atoms must be coordinated in a cis-position to each other excluding the possibility of an inversion center at the zinc. Secondly, in both complexes the N-(2-hydroxybenzyl)-N-(pyridin-2-ylmethyl)amine unity adopts a facial coordination arrangement. However, in [Zn(bpa)₂] the atoms of the same nature (two N amine, two Npyridine and two Ophenolate) are coordinated in trans positions.

Table 1. Crystal data and structure refinement for [Zn(L-Br)].2H₂O

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Formula</td>
<td>C₇₈H₇₈Br₂N₄O₃Zn</td>
</tr>
<tr>
<td>Formula weight</td>
<td>693.73</td>
</tr>
<tr>
<td>T</td>
<td>293(2) K</td>
</tr>
<tr>
<td>λ</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
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</tr>
<tr>
<td>Space group</td>
<td>P2₁/n</td>
</tr>
<tr>
<td>Cell parameters</td>
<td>a = 12.783(1) Å, b = 16.242(2) Å, c = 13.916(2) Å, β = 100.65(1)°</td>
</tr>
<tr>
<td>V</td>
<td>2839.5(6) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>ρ (Density)</td>
<td>1.623 g cm⁻³</td>
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<tr>
<td>μ</td>
<td>3.716 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>1392</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.46 x 0.26 x 0.13 mm</td>
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<tr>
<td>Theta range for data collection</td>
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</tr>
<tr>
<td>Index ranges</td>
<td>-15 ≤ h ≤ 14; 0 ≤ k ≤ 19; 0 ≤ l ≤ 16</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>5203</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>4986 (R(int) = 0.0860)</td>
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<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
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<td>Data / restraints / parameters</td>
<td>4986 / 0 / 343</td>
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<tr>
<td>Goodness-of-fit on F²</td>
<td>0.963</td>
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<tr>
<td>Final R indices</td>
<td>R₁ = 0.0626, wR₂ = 0.1351</td>
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<tr>
<td>R indices (all data)</td>
<td>R₁ = 0.2101, wR₂ = 0.1732</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.778 and -0.779 e.Å⁻³</td>
</tr>
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</table>

Table 2. Selected calculated and experimental bond lengths (Å) and angles (°) for [Zn(L-Br)]

<table>
<thead>
<tr>
<th>Bond</th>
<th>Experimental</th>
<th>Calculated</th>
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<tbody>
<tr>
<td>Zn-O1</td>
<td>1.983(6)</td>
<td>1.997</td>
</tr>
<tr>
<td>Zn-O2</td>
<td>2.011(6)</td>
<td>2.016</td>
</tr>
<tr>
<td>Zn-N1</td>
<td>2.249(7)</td>
<td>2.393</td>
</tr>
<tr>
<td>Zn-N2</td>
<td>2.270(7)</td>
<td>2.412</td>
</tr>
<tr>
<td>Zn-N3</td>
<td>2.206(8)</td>
<td>2.272</td>
</tr>
<tr>
<td>Zn-N4</td>
<td>2.202(8)</td>
<td>2.268</td>
</tr>
<tr>
<td>C5-C6</td>
<td>1.508(12)</td>
<td>1.534</td>
</tr>
</tbody>
</table>

Figure 1. ORTEP of [Zn(L-Br)] with atom-labeling scheme and ellipsoids at 40% probability.
with respect to each other since the molecule has an inversion center. Thus, the Zn-O distances in [Zn(bpa)₂] are ~ 0.1 Å longer than those detected in [Zn(L-Br)], while the Zn-Namine distances are 0.1 Å shorter. This fact is most probably a reflection of distinct trans-influence of the phenolate groups in these complexes. As expected, the Zn-Npyridine bond distances (av. 2.16 Å in [Zn(bpa)₂] and av. 2.26 Å in [Zn(L-Br)]) are comparable and fall into the range of Zn-Npyridine distances observed for other octahedral ZnII complexes already reported in the literature.19,20,29,30

Finally, this structural arrangement of H₂L-Br around the zinc in [Zn(L-Br)] has also been observed in MIII complexes (MIII = V, Mn, Fe, Ga, In) with H₂L¹ and its chloro and bromo derivatives.³³⁻³⁵ An exception is the [Ru(bbpen)]⁺ cation complex which shows two amine nitrogens, two pyridine nitrogens and two phenolate oxygen atoms all as cis pairs.³⁴

The coordination of phenolate moieties in cis positions to the metal center induces a intermolecular bifurcated H bond formation, where the water molecule of crystallization is the donor group (O1W-H1WA 0.88 Å) and the oxygen atoms O1 (H1WA…O1 2.29 Å; O1W…O1 3.00(1) Å; <O1W-H1WA…O1 138.7°) and O2 (H1WA…O2 2.16 Å; O1W…O2 2.84(1) Å; <O1W-H1WA…O2 133.7°) are the acceptors. The bromine atoms are also involved in observed short contacts with neighboring H atoms (H46…Br2 3.01 Å, <C46-H46…Br2 143.0°; H15…Br2 3.04 Å, C15-H15…Br2 130.1°).

¹H NMR spectrum of [Zn(L-Br)].₂H₂O

Since the [Zn(L-Br)] complex is diamagnetic, ¹H NMR was used to investigate the species in CDCl₃ solution. The room-temperature ¹H NMR spectra, 200 MHz (Figure 2) clearly indicate the formation of the complex, and confirm that the symmetric solid-state structure is retained in solution. The assignments of all protons in the ligand and in the corresponding complex are based on the intensity of the signals and spin-spin splitting structure. The ¹H NMR spectra for the free ligand and the corresponding [Zn(L-Br)] complex, depicted in Figure 2, contain seven unique protons resonances in the aromatic region with some small differences, indicating that complexation has taken place. The pyridine H⁶ hydrogen atoms are shifted downfield by 0.37 ppm for [Zn(L-Br)], relative to their positions in the free ligand spectrum. A 0.33 ppm upfield shift is also observed for the phenyl H⁶ hydrogen atoms in the [Zn(L-Br)] spectrum. The most remarkable differences between the ligand and the complex are the resonances in the aliphatic region for the methylene groups. The free ligand,

Figure 2. ¹H NMR spectra (200 MHz) in CDCl₃ of H₂L-Br (top) and [Zn(L-Br)] (bottom). [H₂L-Br]: δ 8.56 (d, 2H₆, J = 4.8 Hz, py); 7.64 (dt, J = 7.6 Hz, 2H, py); 7.26-7.17 (m, 4H, 2H₅, 2H₅'); 7.09 (d, J = 7.8 Hz, 2H₃, py); 6.98 (d, J = 1.9 Hz, 2H', ph); 6.69 (d, J = 8.7 Hz, 2H', ph); 6.36 (d, J = 8.3 Hz, ph); 4.08 (d, J = 17.0 Hz, -CH₂-py); 3.93 (d, J = 11.7 Hz, -CH₂-ph); 3.26 (d, J = 11.7 Hz, -CH₂-py); 3.60 (d, J = 17.0 Hz, -CH₂-py); 2.68 (s, 4H, NCH₂CH₂N).
H₂L-Br, contains three prochiral CH₂ groups with enantirotopic Hs isolated from the others. The Hs are observed as three singlet peaks shifted upfield to 3.72 (s, 4H, -CH₂-py), 3.63 (s, 4H, -CH₂-ph) and 2.68 ppm (s, 4H, -NCH₂CH₂N⁻). The [Zn(L-Br)] complex also contains three prochiral CH₂ groups, two groups with diastereotopic Hs (-CH aHb-py; -CH aHb-ph), and one with enantirotopic Hs (-NCH₂CH₂N⁻), all isolated from the other Hs. These Hs are observed as two pairs of doublets in the case of the diastereotopic Hs, with high geminal coupling constants at 4.08 (d, 2H, J 17 Hz, -CH₂-py), 3.93 (d, 2H, J 11.7 Hz, -CH₂-ph), 3.60 (d, 2H, J 17.0 Hz, -CH₂-py), and 3.26 ppm (d, 2H, J 11.7 Hz, -CH₂-ph) and a singlet in the case of the enantirotopic Hs at 2.68 ppm (s, 4H, -NCH₂CH₂N⁻).

Theoretical calculations

The results for the principal calculated and experimental (for comparison) structural parameters of the [Zn(L-Br)] complex are shown in Table 2 and the optimized structure is shown in Figure S1 in the Supplementary Information. The maximum variation for the bond lengths is 0.16 Å and for angles is 9.1°. A comparison between the geometric parameters of the model and the experimental data shows that the results are in good agreement. The difference noted is due to the fact that the model complexes were considered in the gas phase while the experimental parameters were measured in crystalline form. The graphical representation of HOMO shows that only the phenolate rings participate in its formation (Figure 3 top). On the other hand, only one of the pyridinic rings contributes to the LUMO formation (Figure 3 bottom). The surface of electrostatic potential shows once again that the electronic density of the complex is localized around the phenolic rings. It can also be noted that the bromide substitutions pull the electronic density to the halogen atom (Figure S2 in the Supplementary Information).

Conclusions

Only recently mononuclear Zn II-phenolate containing complexes have been reported.19,20,29,30 In this paper we described the synthesis, crystal structure and ¹H NMR properties of such a complex. The good agreement between the theoretical and experimental data obtained for [Zn(L-Br)] indicates that the use of DFT is appropriate in the planning and synthesis of new structural and functional models for Zn II-containing phenolate enzymes. Based on this information, the synthesis of further multidentate ligands containing phenol as a coordinating group are under investigation, and will be the subject of further reports.

Supplementary Information

The crystallographic data (atomic coordinates and equivalent isotropic displacement parameters, calculated hydrogen atom parameters, anisotropic thermal parameters and bond lengths and angles) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 261524). Copies of this information may be obtained free of charge from: CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk). Figures S1 and S2 showing the optimized structure and the electrostatic potential surface for [Zn(L-Br)], respectively are available free of charge via internet at http://jbcs.sbibq.org.br

Figure 3. Graphical representation of HOMO (top) and LUMO (bottom) for [Zn(L-Br)].

Acknowledgments

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References