Synthesis spectroscopic studies, molecular structure optimization, and superoxide dismutase activity of copper(II) and zinc(II) bipyridyl assisted supramolecular motifs containing octadentate schiff base

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Schiff base N,N',N'',N'''-tetrasalicylidene-3,3'-diaminobenzidine (H₄tsdb) and its dinuclear [M₂(bpy)₂tsdb]₂DMSO. 8H₂O; bpy=2,2'-bipyridine; M=Cu(II) I, M=Zn (II) 2. complexes have been prepared and characterized by their elemental analysis, FAB-mass, IR, NMR (1H,13C and 1H-1H COSY), UV-vis, ESR (Cu(II) only) spectra and magnetic-moment data. Force field calculations reveal highly distorted octahedral environment around both the metal centers. The Cu-Cu and Zn-Zn distances are found to be 12.28 and 12.37Å, respectively. Superoxide dismutase activity of the complexes has also been studied.

Synthetic and structural studies of dinuclear/polymeric metal complexes have been found important in the development of new synthetic models of several enzymes including superoxide dismutase (SOD). Selective designing of a ligand for a particular metal ion in a desired stereochemistry has been discussed by Fenton et al. The utility of ligands containing N, O donors lies in their rigidity, coplanarity and the bite angle. Such ligands have been exploited by Srivastava et al. to prepare metal complexes as models of SOD. Additionally, such ligands have also provided important models for the elucidation of reversible oxygen binding in hemoglobin. It will not be out of place to mention that the progress in the development of SOD models is a continuous research effort from Lippard, Fridovich to Yamauchi et al. which prompted us recently to prepare and characterize a schiff base, bis-salicylidene-4,4'-biphenylenediamine and its dinuclear Cu(II) and Zn(II) complexes in addition to our earlier study on synthetic models of SOD. The present work deals with the synthesis and characterization of an octadentate ligand (H₄tsdb Scheme 1) which bears two sets of N₂O₂ donor sites separated by a biphenylene group and its dinuclear complexes of Cu(II) and Zn(II) for the evaluation of their superoxide dismutase activities.

Experimental

Solvents and other materials purchased from Sigma-Aldrich were used without further purification. Precursor complexes [Zn(bpy)(CH₃CO₂)₂]·H₂O and [Cu(bpy)(CH₃CO₂)₂]·H₂O were prepared using literature procedure. The micro analytical and FAB mass data were obtained from a Carlo Erba Elemental Analyser 1108 and a JEOL SX 102/DA-6000 mass spectrometer respectively [m-nitrobenzyl alcohol was used as a matrix]. However, molar conductance of complexes was measured on a Scientronic digital conductivity bridge in DMSO. Magnetic susceptibility measurement of the Cu(II) complex was done using Faraday technique on a locally built magnetometer. IR (KBr pellets) and UV/vis spectra (DMSO) were recorded on a JASCO FT IR-5300 and a Shimadzu UV1601 spectrophotometer respectively whereas 1H and 13C NMR spectra (DMSO-d₆) of the ligands and Zn(II) complex was recorded on a JEOL Fx-90Q FT NMR spectrometer at 25°C using (CD₃)₂Si as reference. ESR spectra were recorded on a Varian E-109 x band ESR spectrometer using TCNE (g=2.0027) as an external calibrant. Cyclic voltammogram of the Cu(II) complex was obtained using an electrochemical interface SI 1287 potentiostat.

Synthesis of ligand (H₄tsdb)

N,N',N'',N'''-Tetrasalicylidene-3,3'-diaminobenzidine (H₄tsdb) was prepared by stirring an ethanolic solution of 3,3'-diaminobenzidine (0.214 g, 0.1 mmol) (5 mL) and salicylaldehyde (0.488 g, 0.4 mmol) (10 mL) containing few drops of acetic acid for 4 h then refluxed for additional 2 h. Upon cooling, the yellow microcrystalline compound thus obtained was filtered and washed successively with water, ethanol followed by diethyl ether and crystallized from DMF and dried in vacuo. m.p. 230 ± 1°C Yield: 0.54 g (80%), IR (KBr cm⁻¹): ν(OH), 3452, ν(C=N), 1614, ν(Phenyl), 1570.

1H NMR (DMSO-d₆, 90 MHz δppm): 13.0 (4 OH, s), 9.0 (2 CH, s), 8.93 (2 CH, s) 7.8 (4 H, d), 7.6 (4 H, m), 7.3 (4 H, d), 7.2 (2 H, s), 7.0-6.8 (8 H, m)
This remarkable change clearly supports that OH groups get deprotonated during coordination with the metal ions. Furthermore, IR spectra of the complexes did not show significant shift in the peak position of ν(C=N) but the shift in the position of peaks observed at 1616-1612 and 3466-3454 cm⁻¹ indicate their non-electrolytic nature in solution. The colour of the ligand (H₄tsdb) changes from yellow to orange upon heating which is consistent with the thermochromic nature of the earlier reported Schiff bases.

Peaks observed at 1616-1612 and 3466-3454 cm⁻¹ in the IR spectra of the complexes were assigned to ν(C=N) and ν(H₂O) respectively. Though the presence of H₂O in the spectra of the complexes did not allow us to identify the coordination of deprotonated OH group, yet upon comparison of ¹H NMR spectrum of the free ligand with ¹H NMR of its Zn(II) complex it was found that the signal initially observed at δ 13.0 ppm in the spectrum of the free ligand disappeared from the spectrum of the complex. This remarkable change clearly supports that OH groups get deprotonated during coordination with the metal ions. Furthermore, IR spectra of the complexes did not show significant shift in the peak position of ν(C=N) but the shift in the position of peaks observed at δ 164.0 and 163.6 ppm in the ¹³C NMR spectrum of free ligand to δ 163.7 and 162.6 ppm in the spectrum of its Zn(II) complex indicates that >C=N groups of the ligand coordinate with the metal ions. However, presence of two peaks due to >C=N groups was
further substantiated by signals observed at δ 9.0 and 8.93 ppm in 1H NMR spectrum of the Zn(II) complex. Since the position of bipyridyl protons are overlapped with the >C=N and aromatic protons, 1H-1H COSY spectrum of the Zn(II) complex was recorded. It is evident that the signals observed at δ 9.2, 9.1, 8.7, 8.5, 8.0, 7.6 as well as δ 7.4, 6.7 and 6.5 ppm could be assigned to –CH=N and 3.3', 6.6', 5.5' 4.4' of bpy as well as aromatic protons respectively. Peaks observed at δ 143.2, 136.2, 123.1, 114.6 and 113.0 ppm in the 13C NMR spectrum of Zn (II) complex were assigned to bipyridyl carbons where as peaks observed at δ 139.7, 138.1, 134.4, 125.5 and 119.5 ppm were assigned to aromatic carbons.

The UV-vis spectrum of the ligand in DMSO (10⁻⁴ M) shows intra-ligand transition at λ 375 nm (ε 9500 dm³ mol⁻¹ cm⁻¹). However, Cu(II) complex in DMSO (10⁻⁴ M) shows d-d transitions at λ 520², 630 (ε 400, 600 dm³ mol⁻¹ cm⁻¹) along with a charge-transfer transition at 440 nm (ε 4100 dm³ mol⁻¹ cm⁻¹).

In the spectrum of Zn(II) complex, charge-transfer transition appears at 412 nm (ε 2100 dm³ mol⁻¹ cm⁻¹). Other peaks observed at λ 344, 380, 360 nm were assigned to intra-ligand transitions.

Magnetic moment (μ₀∥) value is found to be 1.9 BM/Cu(II) ions which indicates octahedral geometry around Cu(II) ions.

Distorted structure of the Cu(II) complex was supported by its E.S.R. spectrum recorded in solid state both at RT and LNT which shows anisotropic effect with g tensors as g₁, g₂ and g₃ observed respectively at 2.36, 2.20 and 2.06 in consistence with the earlier report. Solution spectrum of the complex shows similar pattern and hyperfine splitting neither due to ⁶⁵Cu nor ¹⁴N nuclei could be observed.

For further structural support several attempts were made to grow single crystals suitable for X-ray crystallography but crystals could not be grown even
after several months under different conditions. Therefore, attempts have been made to simulate the structures of the complexes by force field calculations. Various studies have proven that such computations can be successfully employed to transition metal complexes. In the present MM2 force field calculations a few additional terms like charge-dipole interaction, torsional and non-bonded constrains etc. have been incorporated. Molecular geometry optimization was carried out using Chem3D Ultra (ver.6.0) program package which employs a modified version of Allinger’s MM2 force field for molecular mechanical calculations.

Selected bond lengths and bond angles are listed in Table 1. The space-filling model of this complex reveals the twisted shape of the molecule. The molecule is a homodinuclear complex with an approximate C₂ symmetry. The Cu-Cu non-bonded distance is 12.28 Å which is large enough to disallow any electronic interaction between them.

Each Cu(II) center is hexacoordinated with substantial distortion from octahedral geometry (Fig. 1). The two Cu-O bonds are trans to each other making the O-Cu-O angle 171.9°. The two bond lengths are identical (1.33 Å) and so are the Cu-N bond distances (2.00-2.02 Å). An interesting feature of the molecule is that the aromatic rings in the spacer group are twisted (dihedral angle 68.0°). The bite angle of the bpy group is 81.8° where as the angle subtended by one of the nitrogens of bpy N(13) and a nitrogen of the imine group N(51) is larger (101.7°) (Fig. 2). The two pyridyl rings in a bipyridine unit are coplanar. The five-membered chelate rings are planar while the six-membered ones acquire a boat conformation.

The structure of the Zn(II) complex (Fig. 2) is similar to that of the Cu(II) complex except for the small variation in bond lengths and angles due to Zn(II) ion, the only remarkable difference observed is in the interplanar angle between the two phenyl rings of the spacer group. The rings are almost perpendicular to each other making an angle of 98.3°. From the similarities as found for the structures of complexes 1 and 2 it can be naively concluded that the geometry imparted at the metal centers is mainly due to the ligand framework and not due to the electronic properties of the metal (Fig. 3).

On comparing the SOD activities (55-13 μm) reported for mononuclear complexes containing bipyridine/phenanthroline along with dipeptides as additional ligands, the activity shown by presently reported Cu-Cu dinuclear complex (37.5 μm) could be
considered worthwhile, though Zn-Zn activity was found to be less as (105.0 μm). However, greater activity of Cu(II) complex as compared to corresponding Zn(II) complex again emphasized the functional requirement of the Cu(II) ion for SOD activity.

In various of complexes which act as superoxide dismutase enzyme, either there is one electron oxidation followed by reduction of metal ion or formation of a superoxide complex occurs which then get reduced to peroxide by another superoxide ion. To explore the mechanism of action, absorption spectrum of complex I was recorded in the presence and absence of alkaline DMSO (alkaline DMSO act as a source of O2−). The spectrum gave two peaks at 441 and 319 nm. These peaks got suppressed in alkaline DMSO containing buffer (pH 8.6). However, upon addition of NBT which acts as O2− scavenger, these peaks were reverted to their original position. Thus, this experiment indicates that O2− is initially attached to the metal complex which later gets reduced by another O2− ion.

Cyclic voltammogram of the Cu(II) complex recorded in DMF (10−4 M) using TBAP (0.1 M) as supporting electrolyte, Ag/Ag+ as reference and Pt as working electrodes and at a scan rate 100 mV sec−1 shows a broad metal based peak centered at -0.35V which is comparable to that reported for other Cu(II) dinuclear complexes.

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