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Synthesis, Characterization and Crystal Structure of Dimeric Copper(II) Complex Bearing Mixed Ligands Acetylacetone and Biimidazole: DNA Binding and Cleavage Studies

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Transition metal complexes capable of cleaving DNA under physiological conditions are of interest for various applications in nucleic acid chemistry, in the development of metal-based anticancer agents.[1-4] Many studies suggest that DNA is the primary intracellular target of antitumor drugs, because the interaction between small molecules and DNA can cause DNA damage in cancer cells.[5] Among the transition metal based DNA cleaving agents, copper(II) complexes show efficient chemical nuclease activity. Recent reports have shown that heterocyclic base based copper(II) complexes show efficient DNA cleavage activity by oxidative and hydrolytic pathways.[6,7] Such complexes may show DNA cleavage both in the presence (chemical nuclease) or absence (hydrolytic) of a reducing agent. The present work stems from our interest to explore the synthesis, crystal structure, DNA binding and oxidative DNA cleavage activity of redox active copper(II) complex $[Cu(biim)(acac)H_2O)]_2[Cu(acac)_2(ClO_4)_2]$ in which Cu(II) ions is in two different coordination environment, coordinated by acetylacetone and *N,N*-donor heterocyclic base biimidazole. Similar types of copper(II) complexes have been reported, in that one of the copper(II) ions, is coordinated to actylacetone and bipyridine moiety. This class of cytotoxic and antineoplastic compounds are known under the trade name CASIOPEINAS®.[8,9]

Copper(II) complex was synthesized in high yield and characterized. The IR spectrum of the complex show broad band at 3433 cm^{-1} indicating the presence of water molecules in the complex. Weak band at 3286 cm^{-1} is attributed to N-H stretching vibrations and a sharp band at 1315 cm^{-1} is attributed to N-H bending vibration. The absorption at 1664 cm^{-1} is corresponding to the C=O group. The complex showed strong bands at 1089 cm^{-1} and 626 cm^{-1} possibly due to the perchlorate ions.[10] The band at 920 cm^{-1} which is normally assigned to the rocking mode of coordinated water[11] confirms the presence of coordinated water in the complex.

The electronic spectrum of the complex in acetonitrile exhibited the broad low energy band at 615 nm (ε = 670) is assigned to the d–d transition for Cu(II) ions and the intense high energy absorption band observed at 275 nm (ε = 6430) is attributed to the intraligand π-π* transition in the coordinated ligands.[12]

The copper(II) complex was characterized by single-crystal X-ray diffraction technique. ORTEP view of the complex was shown in Figure 1. Selected bond lengths (Å) and bond angles (°) are listed in Table S1. As depicted in Figure 1, two different Cu^{2+} complexes, $[Cu(biim)(acac)H_2O)]^+$ and half molecule of $[Cu(acac)_2]$ are co-crystallized in the asymmetric unit along with a ClO_4^- in the lattice. The complex crystallized in the triclinic crystal belonging to the space group $P\bar{1}$. The Cu(II) ions are in the complex exhibited distorted square pyramidal (molecule A) and square planar (molecule B) geometry. In a molecule A the acac and biim ligands are coordinated in a bidentate fashion to the copper ion Cu(1) through oxygen (O16 and O12) and nitrogen (N2 and N8) atoms forming with the equatorial plane and oxygen (O19) of an aqua ligand at the elongated axial site (Cu(1)-O19 = 2.342 Å) forming square pyramidal CuN_2O_3 coordination geometry. While the other copper(II) ion (molecule B) has bonded to four oxygen atoms (O20, O24, $O20^i$, $O24^i$ [at i = 1−x, 1−y, 1−z]) of two acac ligands making square planar CuO_4 coordination geometry. The uncoordinated perchlorate anion form two long axial bonds (Cu-O28 =

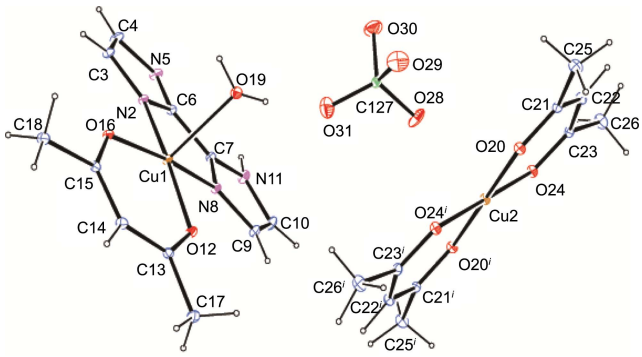

Figure 1. ORTEP view of copper(II) complex.

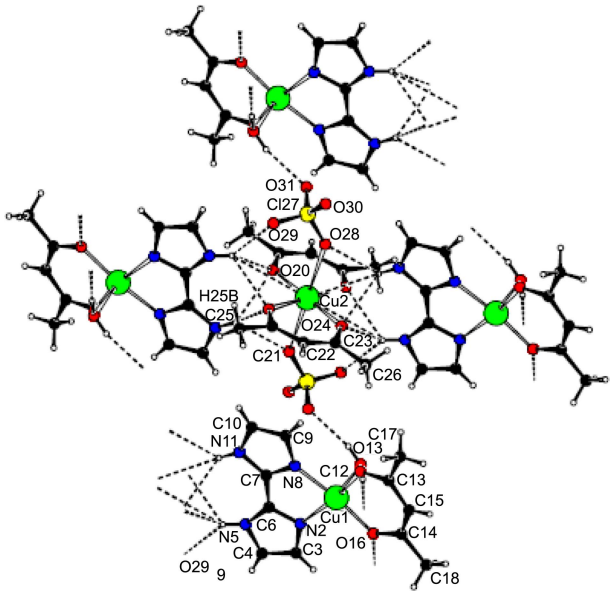

Figure 2. Crystal Packing diagram of copper(II) complex.

2.543 Å) making elongated octahedral in a crystal packing. The perchlorate anion not only assists the packing of the complex in two different geometries, but also it participates in bridging of molecules A and B. The Cu(1) was displaced from the mean equatorial planes toward the axially coordinated O19. The extent of distortion in the square-pyramidal coordination geometry can be observed from the av. τ value of 0.1 in a molecule A.[13] The Cu(1)–Cu(2) separations within the unit is 7.254 Å.

The one dimensional chain is linked by the weak hydrogen-bonds which formed by axially coordinated aqua hydrogen atoms. These chains are extended into two-dimensional network by O-H···O intermolecular hydrogen-bonds (Fig. 2). The rare two centered bifurcated hydrogen bonding[14,15] is observed between O31^{i} and O16^{ii} atoms with angle of 112.2° (symmetry position at *i* = 1–x, 1–y, 1–z, *ii* = 1–x, 2–y, –z). The hydrogen bonding interactions gives rise to a two dimensional network having well-defined channels. We thus consider that many hydrogen bonding interaction stabilize the molecules and crystal packing. Details of the hydrogen bonding interactions with symmetry code are given in Table S2.

The ESR spectrum of copper(II) complex in dimethylformamide shows four lines due to hyperfine splitting with nuclear hyperfine spin 3/2 and the observed $g_{\|}$ value (avg) is 2.31, the $g_{\perp}$ value is 2.02, and the $A_{\|}$ value is 160 G; The relation $g_{\|} > g_{\perp}$ is typical of Cu(II) having one unpaired electron in a $d_{x^2-y^2}$ orbital. Room-temperature magnetic moment studies of the copper(II) complex shows μ_{eff} value of 1.77 B.M. which is nearer to the spin-only value of the copper(II) ion. These values are in agreement with the distorted square pyramidal geometry indicated by the structural data. The electrochemical properties of the copper(II) complex was studied by cyclic voltammetry in dimethylformamide containing 10^{-1} M tetra(*n*-butyl)ammonium perchlorate (TBAP). The cyclic voltammogram of copper(II) complex (Fig. S1) shows two successive irreversible reduction wave at $E^{1}_{pc} = -0.64$ V & $E^{2}_{pc} = -0.81$ V which may be assigned to the reduction of $Cu^{II}Cu^{II}/Cu^{II}Cu^{I}/Cu^{I}Cu^{I}$.

The copper(II) complex were tested against four pathogenic bacteria (Staphylococus aureus, Bacillus subtilis Escherichia coli and Pseudomonas aeruginosa) and two fungi (Aspersillus niger, Candila albicans) to test their inhibition efficacy as antimicrobial agents. The experimental results compared with standard drugs were displayed in Table S3 which shows that the complex exhibit inhibition efficiency for all bacteria and fungi. Complex shows higher antifungal activity for Candila albicans than that of Aspersillus niger which may be due to chelation of metal complex. The complex exhibits comparatively similar antimicrobial activities with respective standard drugs.

To investigate the mode of binding between the complex and Calf Thymus DNA, UV-absorption studies was carried out, which shows intense absorption peaks at 234 and 298 nm in the UV region. On addition of increasing amounts of DNA to the complex, both two characteristic peaks decreased gradually with the maximum hypochromicity of 18% and 11%, respectively, suggesting the strong interaction between complex and DNA. The isobestic point at 248 nm also proved the formation of the new complex between DNA and complex. In the absorption peak at 234 nm an obvious bathochromism (~3 nm) was found, which demonstrated that the complex probably bind to DNA via an intercalative mode.[16] The intrinsic binding constant value K_b was calculated as 3.6×10^5 M^{-1} which is relevant to that of other typical intercalators.[17] The strong DNA binding nature of the complex may be due to the π-π* interaction through the heterocyclic ring of the nitrogen bases.

In order to further investigate the interaction mode between the complex and DNA, the fluorescence titration experiments are performed. If the complex added to the ethidium bromide (EB)-DNA system replace the bound EB, the emission intensity will be reduced. The fluorescence quenching of EB bound to DNA by complex, in which the fluorescence intensity at 600 nm (λ_{ex}) 548 nm) of EB in the bound form was plotted against the compound concentration. The linear Stern-Volmer quenching constant K value obtained for Cu(II) complex is 0.777, which reveals that the complex have stronger affinity for DNA. The K_{app} value is found to be 1.39 $\times 10^5$ M^{-1} and this result indicates that copper(II) complex binds to DNA by partial intercalation mode.[18-20]

Figure 3(a) shows the results of the agarose gel electrophoresis separations of plasmid pBR322 DNA by the complex in the presence of mercaptopropionic acid as a reducing agent. As observed in lanes 1-3, MPA, complex alone does not induce obvious DNA cleavage, when complex (100 μM) coexists with mercaptropropionic acid (MPA), 50% of DNA is converted from Form I to Form II (lane 4) a prominent DNA scission is observed and the conversion found to be maximum at 200 μM of complex (lane 5). The cleavage ability of the complex might be due to the presence of Cu^{2+} ions which can promote the probability of double strand scission immediately after the DNA has undergone a single

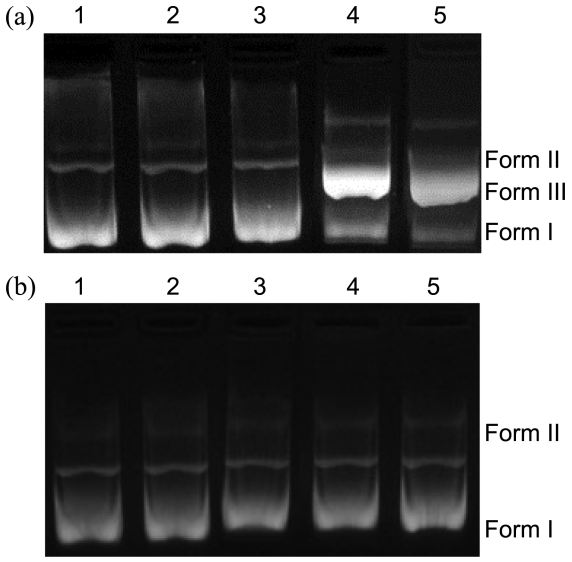

Figure 3. (a) Cleavage of SC pBR322 DNA (0.2 μg, 33.3 μM) by copper(II) complex in the presence MPA (200 μM) as reducing agent in 50 mM Tris–HCl/50 mM NaCl buffer (pH 7.2). Lane 1, DNA control; lane 2, DNA + MPA; lane 3, DNA + complex (200 μM); lane 4, DNA + MPA+ complex (100 μM); lane 5, DNA + MPA + complex (200 μM). (b) Supercoiled pBR322 DNA (0.2 μg, 33.3 μM) treated with 200 μM complex and 200 μM MPA in the presence of different radical scavengers. Lane 1, DNA control; lane 2, DNA + MPA + 20 mM DMSO; lane 3, DNA + MPA + 20 mM DMSO + complex (200 μM); lane 4, DNA + MPA + 20 mM KI; lane 5, DNA + MPA+ 20 mM KI + complex.

strand break. To reveal the DNA cleavage mechanism by copper(II) complex with various quenchers like hydrogen peroxide scavenger (KI) and hydroxy radical scavenger (DMSO) were used and the results are illustrated in Figure 3(b). No significant cleavage was observed in the presence of DMSO (lane 3) and KI (lane 5). The significant increase in the DNA cleavage activity by the complex in the presence of MPA and the inhibition of activity in the presence of DMSO and KI suggest that this reaction was preferentially proceeded by a hydroxy radical mechanism with ·OH species[21] or copper-oxo species as the cleavage active species.[22] Further studies on the reaction mechanism as well as on sequence selectivity of the copper(II) complex are in progress.

In summary mixed ligand copper(II) complex containing bidentate ligands, *viz*., acetylacetonate and 2,2'-biimidazole, have been synthesized and characterized. Interestingly, the complex contain two types of copper(II) ions with square pyramidal and square planar geometry co-crystallized in the asymmetric unit with strong intermolecular interaction. The complex show efficient DNA binding ability, the binding constant value is consistent with other typical intercalators. The synthesized complex has significant oxidative chemical nuclease activity which could induce scission of pBR322 supercoiled DNA effectively to linear form and the cleavage mechanism proceeding by a hydroxy radical. Microbial studies revealed that the complex is useful as antibacterial and antifungal agents.

Experimental

Synthesis. A methanol solution (10 mL) of $Cu(ClO_4)_2{\cdot}6H_2O$ (0.33 g, 2 mmol) was initially reacted with acetylacetone (0.02 g, 2 mmol) treated with NaOH (0.040 g, 1.0 mmol) in water (10 mL) under magnetic stirring at room temperature. After 30 min, a 20 mL methanolic solution of the biimidazole (0.15 g, 2 mmol) was added to the solution and the resulting mixture was stirred for 2 h at room temperature. The green colored solid was isolated and washed with cold aqueous methanol and finally dried over P_4O_{10} (Yield: 67%). Anal. Calc. (%) for $C_{32}H_{44}Cl_2Cu_3N_8O_{18}$; C, 35.25; H, 4.07; N, 10.28; Cu, 17.49. Found (%): C, 34.95; H, 4.24; N, 10.42; Cu, 17.19. FT-IR, cm^{-1} (KBr disc): 3433br, 3286br, 1664s, 1575s, 1315s, 1089vs, 920s, 626s, 431m. μ_{eff} (solid, 298 K): 1.77 B.M.

Single crystal X-ray structure was determined using Bruker SMART APEX-II CCD diffractometer with graphite monochromated MoKα radiation (0.71037). Crystal data for $[Cu(biim)(acac)H_2O)]_2[Cu(acac)_2(ClO_4)_2]$ ($C_{32}H_{44}Cl_2Cu_3N_8O_{18}$) at 296(2) K: M_r = 1090.27, Triclinic, space group P-1, a = 8.3342(17) Å, b = 9.1617(18) Å, c = 14.720(3)Å, α = 91.82(3)°, β = 97.80(3)°, γ = 95.21(4)°, V = 1107.8(4) $Å^3$, Z = 1, ρ_{calcd} = 1.634 mg m^{-3}, Final R indices R1 = 0.0484, wR2 = 0.1548, GOF = 1.097 with [$I > 2\sigma$ (I)], Crystallographic data have been deposited with Cambridge Crystallographic Date Centre: Deposition number CCDC-909586 for the complex.

Copies of the date can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Date Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; Fax: +441223336033; e-mail: deposit@ccdc.cam.ac.uk).

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Supporting Information. Selected bond lengths (Å) and bond angles (°), potential hydrogen bonds present in the crystal packing, cyclic voltammogram of copper(II) complex, and anti-microbial activity for the complex are available free of charge on the Web at http://www.csj.jp/journals/bcsj/.

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