

Original Research Article

Synthesis, Characterization and DNA Cleavage of Copper(II) Complex with D,L-Dithiothreitol

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Abstract

Purpose: To study deoxyribonucleic acid (DNA) shearing capability of copper(II) complex of dithiothreitol (DTT) and to evaluate its potential application in cancer therapy.

Methods: A parrot green complex was synthesized by grinding copper acetate monohydrate and DTT in 1:2 molar ratio in a mortar until no fumes of acetic acid were observed. The complex was characterized using attenuated total reflectance-Fourier transform infra-red (ATR-FTIR), and x-ray diffraction (XRD) techniques. Further information was also collected through Karl Fischer titration, thermogravimetric analysis (TGA) and magnetic moment. Cleavage of DNA was determined by agarose gel electrophoresis. The gel was then stained, analyzed and photographed under ultraviolet (UV) light.

Results: ATR-FTIR confirmed the formation of copper(II) complex with DTT by binding through thiol group based on the disappearance of the thiol (-SH) stretching peak at 2545 cm⁻¹. The crystalline structure was elucidated by a sharp intense peak at 38.520 in XRD spectrum while the octahedral geometry of complex was inferred from a magnetic moment of 1.72 B.M. The results for water content obtained by Karl Fischer titration and TGA revealed that water molecules are not part of the coordination sphere of the complex. Cleavage study of DNA showed that the complex completely sheared the circular DNA compared to pure DTT.

Conclusion: Solvent free synthesis of Copper(II)-DTT complex has been successfully achieved, and an anhydrous complex with octahedral geometry obtained. The complex has a greater potential to shear DNA molecule than pure DTT.

Keywords: DNA shearing, Copper(II) complex, Dithiothreitol, Attenuated total reflectance-Fourier transform infra-red, Karl Fischer titration, Magnetic moment

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INTRODUCTION

D,L-Dithiothreitol, Cleland's reagent, has vast applications including biochemical synthesis of thiol proteins, chemical peptide synthesis and the study of protein chemistry[1-4]. DTT is also found to be present in high concentration in samples of biomolecules which are studied as targets or models for biological heavy metal ion binding and

reactivity. It is noted that DTT is a strong chelating agent [3], but only a few studies have been reported for its interactions with different metal ions [5-10].

Deoxyribonucleic acid (DNA) is observed to be a target for most of the anticancer and antiviral therapies [11,12]. Thus the synthesis of molecules, capable of binding and cleaving DNA,

is performed with an aim to find their utility in the design and development of synthetic restriction enzymes; and furthermore their capability to investigate the structure of DNA itself [13]. Reedijk *et al* have investigated the complex [Cu(II) (pyrimol)Cl] having efficient self-activated DNA cleavage [14].

In the present study we have synthesized a stable Cu (II)-DTT complex. The obtained parrot green product was analyzed using ATR-FTIR, XRD, TGA, Magnetic moment and Karl Fischer titration for different structure related properties; and then electrophoresis for DNA shearing. These techniques provided sound evidence for the binding of copper ions with DTT and also its shearing potential.

EXPERIMENTAL

Chemicals

D,L-dithiothreitol (DTT), copper acetate monohydrate and DNA pUC18 were purchased from Phyto Technology Laboratories, Riedel-de-Han and Fermentas, respectively. All of these chemicals were of analytical grade and used without further purification.

Procedure for complex formation

Copper acetate monohydrate (0.3993 g, 0.002 mol) and DTT (0.617 g, 0.004 mol) were weighed using analytical balance (ATX224 Schמדzu) and placed in the mortar. The mixture was ground with pestle until no fumes of acetic acid were observed.

The color change of mixture was primary indication of copper(II)-DTT complex formation. The color of copper acetate monohydrate was green and DTT was white while the complex was of parrot green color.

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) studies

ATR-FTIR spectrum was obtained on an ATR-FTIR spectrophotometer (Lambda7600, Australia). The spectra were produced after scanning 60 times at 4 cm⁻¹ resolution transmission and wavelength range of 4000 to 4500 cm⁻¹.

X-ray diffraction (XRD) studies

The XRD patterns were recorded using an X'PERT PRO X-ray diffractometer (PANalytical, Netherland) employing CuK α radiation ($\lambda = 1.541$

Å). The 2 theta (2 θ) scanning range was 20-80 degree.

Assessment of magnetic moment

Magnetic susceptibility value of Cu(II)-DTT complex was measured at room temperature in solid state by using magnetic susceptibility balance (Sherwood Scientific UK, Model MBS-Auto).

Karl Fischer titration

The percentage of water in the synthesized complex was determined using Karl Fisher titrator (Metrohm, USA model 841 titrando).

Thermal analysis

The simultaneous TG-DTA spectra were measured by using SDT Q 600 (TA Instruments USA) with a sample weight 2-3 mg under nitrogen flow with increase in temperature of 10 °C/min between 41.5 °C to 700 °C.

DNA cleavage detection

The cleavage of DNA was determined using Maxicell electrophoretic gel system (Florida, USA) and BIORAD Power Pac 3000 (USA). The experiments were performed by incubation of the sample containing 30 μ M pUC18 DNA, 50 μ M copper(II)-DTT complex and 500 μ M 2-mercaptoethanol in 50 mM Tris-HCl buffer (pH 7.2) at 35 °C for 90 min. Pure DTT (50 μ M) and two controls, that is, pUC18 DNA (30 μ M) and copper acetate monohydrate (50 μ M) were separately incubated by using 500 μ M 2-mercaptoethanol in 50 mM Tris-HCl buffer (pH 7.2) at 35 °C for 90 min.

After incubation, the samples were loaded on 1.5 % agarose gel and subjected to electrophoresis for 2 h at 80V. After electrophoresis, the gel was stained with 0.5 mg/ml ethidium bromide, analyzed and photographed under UV light [15].

RESULTS

ATR-FTIR spectra

The infrared spectra of pure DTT and its Cu(II) complex are given in Figure 1. The spectrum of DTT shows an absorption peak at 2545 cm⁻¹ for -SH group. This peak is absent in the spectrum of Cu(II)-DTT complex. It indicates that -SH group of DTT is involved in bond formation with Cu(II) ion. Also the absorption in the region of 3400-3200 cm⁻¹ confirmed the presence of -OH

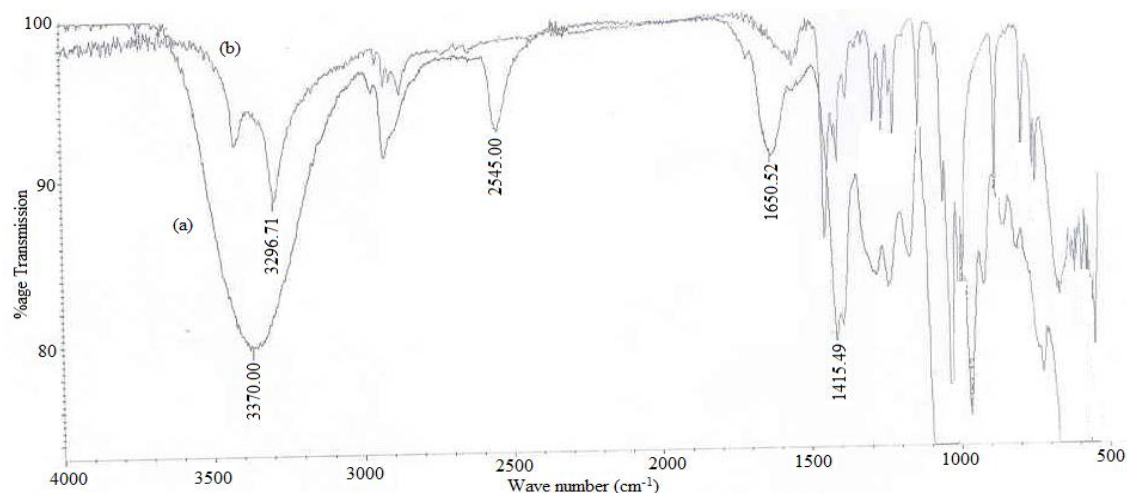


Figure 1: ATR-FTIR Spectrum: (a) pure DTT (b) Copper(II)-DTT complex

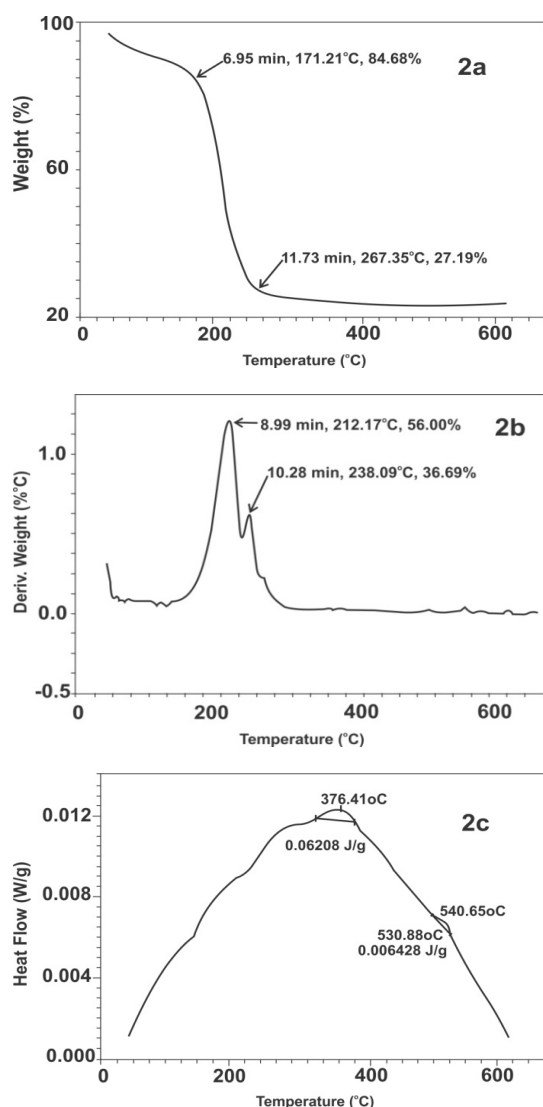


Figure 2: Thermal spectra of Cu(II)-DTT complex.
Note: **2a** = TGA of Copper (II)-DTT; **2b** = DTA of Copper (II)-DTT = **2c**: DSC of Copper (II)- DTT

group in both the DTT and its complex.

XRD

X-ray diffraction spectrum of DTT showed various intense peaks at 20.527, 23.455, 26.663, 28.287, 33.045, 34.487 and 36.766. In X-Ray Diffraction spectrum of Copper(II)-DTT complex, a single sharp intense peak at 38.520 was appeared.

Magnetic moment

The effective magnetic moment of the synthesized complex is 1.72 BM.

Thermal characteristics

The thermogravimetric analysis (TGA) of the complex (Figure 2a) indicates two degradation steps in the range 50-171.21 °C and 171.21-267.35 °C. The derivative thermo gravimetric analysis (DTA) curve of the complex (Figure 2b) shows two endothermic peaks at 212.17 °C and 238.09 °C. The differential scanning calorimetric (DSC) curve (Figure 2c) also shows two endothermic peaks at 376.41 °C and 530.88 °C.

Electrophoresis

The result of electrophoresis is shown in Figure 3 in the form of agarose gel image. Large numbers of additional small bands can be seen which confirm the fragmentation of DNA by the newly synthesized copper(II) complex.

DISCUSSION

DNA shearing is an important phenomenon owing to its applications in the field of biology

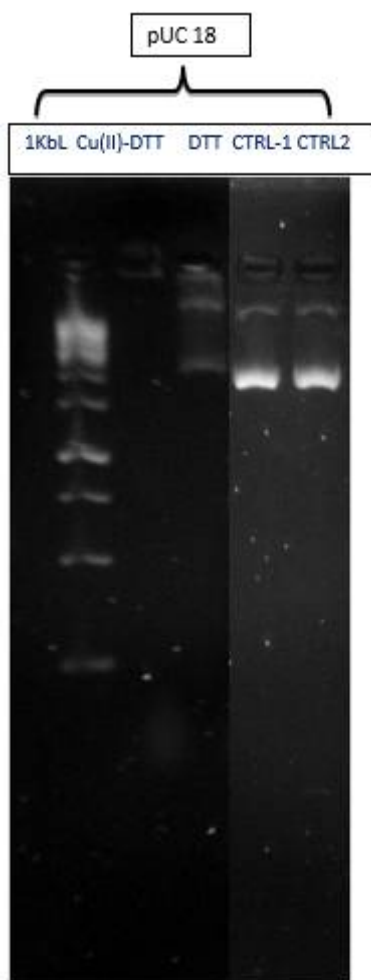


Figure 3: DNA shearing using Cu(II)-DTT and DTT

including cancer treatment. Studies of DNA shearing are focus of many researchers especially during the last two decades. Krezel *et al* synthesized different complexes of DTT with different metals and studied through potentiometric titrations, UV–Visible and NMR spectroscopy. They proposed that addition of copper(II) to a DTT solution, copper(II) ions are reduced to copper(I) ions and DTT is oxidized to its cyclic disulphide [10]. Our method remained better in preparing copper(II)-DTT complex as no reduction of copper(II) ion into copper(I) or conversion of DTT into cyclic disulphide was observed.

IR spectrum of DTT and its complex as shown in Figure 1 indicates the disappearance of the thiol(-SH) stretching peak at 2545 cm^{-1} for Copper(II)-DTT complex because of deprotonation of the -SH group of DTT molecule after forming a covalent bond with Copper (II) ion through the Sulphur atom. The non-involvement of the -OH group of DTT in copper ion co-ordination is evident from the fact that the -OH stretching frequencies of DTT and its complex

appear in the range of $3200 - 3400\text{ cm}^{-1}$. Mandal *et al* and Dokken *et al* reported the similar findings for copper sulfur linkage [16,17].

We tried to isolate the crystals suitable for single x-ray diffraction but it was unsuccessful. However the structural information was determined by powder XRD. Copper(II) complex showed a sharp intense peak at 38.520 which indicates the crystalline nature of the complex. Mishra *et al* reported the similar results for the crystalline nature of synthesized complex [18].

The effective magnetic moment value is similar to already reported copper(II) complexes. Revankar *et al* reported the magnetic moment value for copper (II) complex as 1.72 BM suggesting a distorted octahedral geometry around the central copper atom [19].

Percentage of water in copper(II) complex is very small which indicated that water molecules are not the part of complex rather it is the water adsorbed on the surface of the complex. Chohan *et al* reported that in copper(II) complexes with chromone-derived compounds, Karl Fisher titration values do not equate with any whole number of water molecules [20].

The TGA of the complex (Figure 2a) showed two thermal degradation steps. In the first thermal degradation step at $171.21\text{ }^{\circ}\text{C}$ weight loss corresponded to the loss of water molecule. Maximum weight loss took place at $267.35\text{ }^{\circ}\text{C}$ indicating the loss of Cu(II)-DTT molecule from the complex. The DTA curve of the complex showed two endothermic peaks at 212.17 and $238.09\text{ }^{\circ}\text{C}$ which were also assigned to loss of water and Cu(II)-DTT molecule from the complexes. The DSC curve (Figure 2c) also showed two endothermic peaks at $376.41\text{ }^{\circ}\text{C}$ and $530.88\text{ }^{\circ}\text{C}$. Chohan *et al* and Chandraleka *et al* reported the thermal degradation of copper(II) complexes and their results well supported our work [20,21].

Metals have been explored for their various biological applications along with other metals of its class. In the present study, the Cu(II)-DTT complex also showed very interesting effects due to its interaction with DNA. The circular DNA molecules and the smaller ones moved faster on gel during electrophoresis. The DNA damage was assessed by visualizing the pattern of DNA bands after treatment with copper complex and that for the pure compounds. Copper complex completely shears the circular DNA pUC18 in such a way that even the ethidium bromide does not intercalate in DNA and illuminates under UV. However the pure DTT shows no affection

pUC18. Pragathi *et al* studied DNA cleavage activity by Cu(II) complex with Schiff's base and found that the copper complex exhibited DNA shearing property even without the presence of an oxidant [22]. Neves *et al* presented hydrolytic cleavage of DNA by Fe (III) and Fe (II) complexes [23].

CONCLUSION

Parrot green crystals of copper(II)-DTT complex has been successfully prepared and structurally corroborated by different techniques. DNA shearing using copper(II) complex is superior to that using DTT. These results support the significance of copper(II) complex of a naturally occurring amino acid in the field of medicine including in cancer therapy. Cu(II)-DTT may serve as a potent artificial/synthetic nuclease with various biological applications. Similar type of complexes may be suitable for DNA shearing, the advantage being that they would more economical and potentially achieve better therapeutic outcomes.

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