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M. Sc. Thesis

Title of the Thesis:

Interaction studies of the zinc and copper complexes of curcumin with calf thymus DNA using spectroscopic methods

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Abstract

Curcumin is a polyphenolic diketone from turmeric. In this study two mononuclear complexes Zn(II)-Cur (1) and Cu(II)-Cur (2) (Cur = Curcumin, (1*E*,6*E*)-1,7-bis (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione) have been synthesized and characterized by elemental analysis (CHN), FT-IR, ^{1}H NMR and UV-Vis techniques. The interactions of the complexes with calf thymus DNA (CT- DNA) were investigated by absorption spectroscopy, fluorescence spectroscopy, circular dichroism and viscosity measurements. The binding constants of the complexes to CT-DNA calculated from UV-vis spectra were found to be 1.26×10^{5} M^{-1}(Cu(II)- Cur) and 6.61×10^{4} M^{-1}(Zn(II)- Cur) respectively. This indicating that DNA-complexes bonding have an affinity less than the classical intercalators, but it is comparable to complexes which bind to DNA mainly via groove binding mode. The binding affinity of Cu(II)-Cur complex to DNA was higher than that of Zn(II)-Cur. The results of CD spectra showed that binding of both complexes to DNA induced conformational changes in DNA (B DNA to A DNA). Moreover as the relative viscosity of CT-DNA showed an inappreciable increase in the presence of varying amounts of complexes. Fluorimetric studies showed that fluorescence enhancement was initiated by a static process in the ground state. As well as the thermodynamic data are lowering than those of previously reported for an intercalator and confirmed the non-intercalating interaction mode. Therefore experimental results suggested that both complexes interact with CT-DNA via groove-binding mode. Previous spectral data and molecular modeling calculations showed curcumin binds in the minor groove of the DNA double helix.

Key words: CT-DNA; Curcumin, Cu(II)-Cur complex, Zn(II)-Cur complex, Multispectroscopic techniques, Groove binding.

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Abbreviations and Symbols

LUMO	lowest unoccupied molecular orbital
HUMO	highest unoccupied molecular orbital
CT – DNA	calf thymu DNA
CD	circular dichroism
K_D	dynamic enhancement constant
K_B	bimolecular enhancement constant
K_F	binding constant
τ_0	lifetime
MLCT	Metal-to-ligand charge transfer
Cur	Curcumin
AD	Alzheimer's disease
acac–	Acetylacetonate
appc	2-acetylpyridine-pyridine-2-carboxamidrazone
atpc	2-acetylthiophene-pyridine-2-carboxamidrazone

Chapter One

Introduction

1.1. Bioinorganic chemistry

Bioinorganic chemistry is best considered as understanding all aspects of the role of metal ions in biology and has been traditionally heavily involved in understanding their processing, incorporation into protein and the nature and function of metaloproteins. Advances in our understanding of how cells process metals and the genetic basis of disease is naturally expanding the traditional directions of bioinorganic chemistry toward an appreciation of its medical importance especially with respect to the role of metalloproteins in human health and disease [1].

Inorganic or metal-containing medicinal compounds may contain either (a) chemical elements essential to life forms-iron salts used in the treatment of anemia- or (b) nonessential/toxic elements that carry out specific medicinal purposes- platinum containing compounds as antitumor agents or technetium and gadolinum complexes as medicinal diagnostic tools. Introducing metal ions into a biological system may be carried out for therapeutic or diagnostic purposes, although these purposes overlap in many cases.

In 1991, Peter Sadler noted that most elements of the periodic table, up to and including bismuth with an atomic number of 83, have potential uses as drugs or diagnostic agents. Inorganic compounds have found usage in chemotherapeutic agents such as [2]:

1. Anticancer agents like cis-[Pt $(NH_3)_2Cl_2$]

2. The gold-containing antiarthritic drug Auranofin.

3. Metal-mediated antibiotics like bleomycin, which requires iron or other metals for activity

4. Technetium-99m and other short-lived isotopes used as radiopharmaceuticals in disease diagnosis and treatment.

5. Magnetic resonance imaging (MRI)-enhancing gadolinium compounds.

6. Antibacterials, antivirals, antiparasitics, and radiosensitizing agents.

1.2. Medicinal chemistry

Medicinal chemistry involves the study of the interaction of drugs with biological systems at the molecular level, and the design and synthesis of such drugs. Medicinal chemistry requires intimate knowledge of the metabolism and stability, as well as target interactions of the drug [1].

Medicinal applications of metals can be traced to almost 5000 years back but the lack of experience of traditional medicinal chemists and pharmacologists in dealing with biologically active metal complexes, poses a substantial activation energy barrier to their identifying active metal complexes and shepherding them to the clinic. This factor retards the development of metallo-pharmaceuticals. However, it provides enterprising transition metal chemists with opportunities to pioneer the development of exciting new drugs [3].

1.3. Medicinal inorganic chemistry

Inorganic chemistry is playing a role in the biotechnology revolution currently on going worldwide. AnorMed and Kinetek pharmaceuticals, in Canada, currently have metal complexes in clinical trials. The field of inorganic chemistry in medicine may usefully be divided into two main categories - drugs which target metal ions in some form, whether free or protein-bound, and secondly, metal-based drugs where the central metal ion is usually the key feature of the mechanism of action. Metal-based drugs are a commercially important sector of the pharmaceutical business. Applications continue to grow and approaches to further clinically useful agents are ever more sophisticated [1].

1.4. Copper

It is the 29th element on the Periodic Table, located between nickel and zinc in the first row of transition elements. Copper has eleven known isotopes, of which only two, ^{65}Cu and ^{63}Cu, are present in significant amounts, with natural abundances of 30.91 and 69.09% respectively, resulting in an atomic weight of 63.546. The ground state electronic configuration of elemental copper is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^1$ or [Ar] $3d^{10} 4s^1$ [4]. Common oxidation states of copper include the less stable copper (I) state, Cu^+; and the more stable copper (II) state, Cu^{2+}, which forms blue or blue-green salts and solutions. Under unusual conditions, a +3 state and even an extremely rare +4 state can be obtained [4].

1.4.1. Biological application of copper

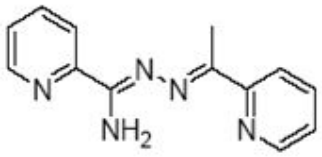
Though the use of copper dates back to antiquity, the recognition of its biological importance is of a more recent origin. Copper has been found to be required essential trace element for the normal functioning of plants, animals and most microorganisms. As a trace metal, it is present in tissues and fluids at parts per million (ppm) or parts per billion (ppb) concentrations [5]. Issues pertaining to the absorption, transport, and function of Cu in the body may be relevant to an investigation of the pharmacology and biodistribution of Cu-NSAIDs. The following section briefly discusses the various biological role of Cu, its biodistribution and its function in inflammation. Copper was first shown to be an essential biological element in the 1920s when anemia was found to result from Cu-deficient diets in animals [6] and addition of Cu salts corrected this affliction [6,7]. It is now recognized as an essential trace element for many biological functions [8]. It serves as a catalytic component in many enzymes, e.g. it is an important constituent of metalloproteins (exhibiting oxidative reductase activity, e.g. oxidases or hydroxylases) [9], and in such enzymes as lysyloxidase (required for connective tissue) and cytochrome oxidase (electron

transport protein) [10]. Copper also influences specific gene expression in mammalian cells [11,12], nerve myelation and endorphin action [13], with Cu deficiency impairing immunity [14-16]. The role of trace metallic elements, such as Cu in inflammation, is of great interest given their function as co-factors in metabolic processes involving articular/connective tissue and the immune system [17] and their effect on PG synthesis [18,19-22].

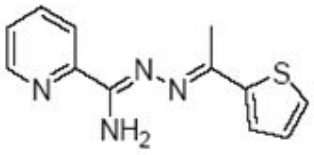
1.4.2. Copper containing anticancer agents

The use of copper for the treatment of cancer dates back to early 1980 with the report by Petering [23] on the activity of copper thiosemicarbazones. More recent work has involved the use of copper complexes of carboxamidrazones [24] and carboxylates [25]. The carboxamidrazones are interesting because they have similar structures to the thiosemicarbazones which were the first copper complexes to be reported for anticancer activity. Both classes of copper complexes have been explored by Padhye et al [24,25]. The carboxamidrazone chemistry involves the use of the ligands 2-acetylpyridine-pyridine-2-carboxamidrazone (appc) and 2-acetylthiophene-pyridine-2-carboxamidrazone (atpc). These ligands are readily synthesized by refluxing pyridine-2-carboamidrazide with excess 2-acetyl pyridine or 2-acetyl thiophene, respectively, in EtOH for 2 hours. The molecular structures of appc and atpc are shown in (Fig1.1).

The copper complexes of these ligands were also readily synthesized by adding equimolar amounts of appc or atpc and $CuCl_2$dihydrate to methanol and refluxing for 1 hour. The products precipitated out of solution as dark green crystalline material. The molecular structures of [Cu (appc) Cl_2], A, and [Cu (atpc) Cl_2], B, are shown in (Fig1.2).



A



B

Fig. 1.1. Molecular Structure of A) appc and B) atpc.

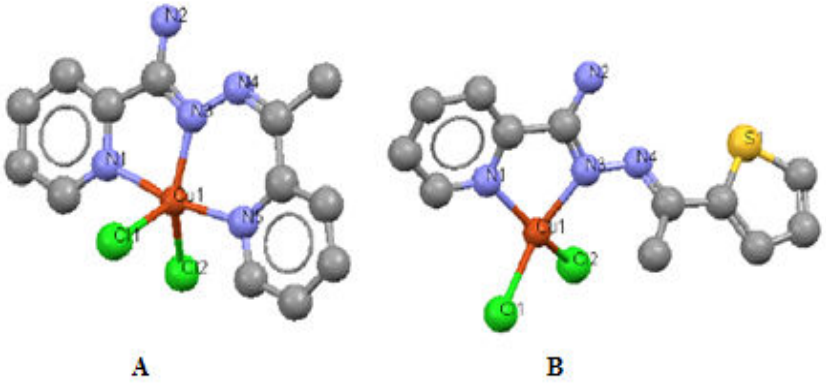


Fig. 1.2. Molecular Structures of Copper Carboxamidrazones.

The copper carboxylate chemistry involves the use of the ligand 5-amino-1-tolylimidazole-4-carboxylic acid, shown in (Fig 1.3), which can readily be obtained by the alkaline hydrolysis of ethyl-5-amino-1-tolylimidazole-4-carboxylate. The copper complex was then synthesized by reacting two equivalents of 5-amino-1-tolylimidazole-4-carboxylic acid with one equivalent of copper nitrate in methanol at pH 7. The product precipitates as the pure green solid shown in (Fig1.4).

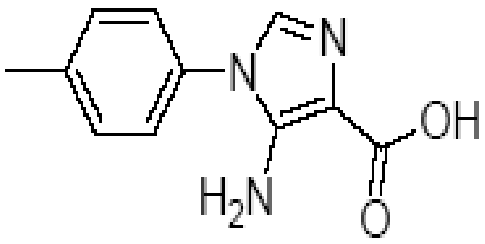


Fig. 1.3. Molcular Structure of 5-amino-1-tolylimiddazole-4-carboxylic acid.