In the Name of God

Faculty of Chemistry
Department of Inorganic Chemistry

M.Sc.Thesis

Title of the Thesis:
Spectroscopic studies on the interaction of a water soluble morin derivative and bovine serum albumine

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Abstract

In the present investigation, an attempt has been made to study the interaction of sodium morin-5′-sulfonate (NaMSA) and its two new complexes of zinc(II) and copper(II) with the transport proteins, bovine serum albumin (BSA) employing UV-vis, fluorometric and circular dichroism (CD) techniques. In the first part, the experimental results indicated that the quenching mechanism of BSA by the NaMSA was a static procedure. Various binding parameters were evaluated. The negative value of ΔH, positive value of ΔS and the negative value of ΔG indicated that electrostatic interactions and hydrogen bonding play major roles in the binding of the NaMSA and BSA. Based on Forster's theory of non-radiation energy transfer, the binding distance, r, between the donor (BSA) and acceptor (NaMSA) was evaluated. The results of CD and UV–vis spectroscopy showed that the binding of this complex to BSA induced conformational changes in BSA. In the secound part, NaMSA complexes with Zn^{+2} and Cu^{+2} were synthesized and characterized by elemental analysis, IR and Uv/vis spectroscopy and ^{1}H NMR spectroscopy. The binding interactions between these two complexes and bovin serum albumin (BSA) were investigated. In fluorimetric studies, the binding constants, K_b were calculated and indicated that Cu-NaMSA complex has higher affinity to bind to BSA. The results of CD spectra showed that binding of the two complexes to BSA induced conformational changes in BSA. The values of K_b from quenching flurescence results were found and indicated that there is a strong binding force between Cu-NaMSA and Zn-NaMSA complexes and BSA.

ABBREVIATIONS

bipy	2,2 □- bipyridine
Phen	1,10-phenanthroline
2,9- dmp	2,9-dimethyl-1,10-phenanthroline
DDP	dichlorodiammineplatinum
LUMO	lowest unoccupied molecular orbital
HUMO	highest unoccupied molecular orbital
BSA	bovine serium albumine
CT–DNA	calf thymu DNA
MSA	morin-5□- sulfonic acid
NaMSA	sodium salt of morin-5'-sulfonic acid
CD	circular dichroism
Trp	tryptophan
K_{sv}	stern-volmer constant
K_q	quenching constant
K_b	binding constant
τ_0	lifetime
MLCT	Metal-to-ligand charge transfer
FTRE	foster non-radiative energy transfer theory
MRE	mean residue ellipticity

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CHAPTER ONE

INTRODUCTION

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 metalloproteins. In a 'steady-state' environment all essential metals are incorporated in the right place at the right time and the organism functions normally. Alternatively, genetic factors may lead to failure to incorporate and subsequent metabolic disorders may be caused by free metal ions. 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].

1.1 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 [2].

1.2 Medicinal inorganic chemistry

Inorganic chemistry is playing a role in the biotechnology revolution currently on going world wide. 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 [1].

1.3 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] $3d^{10} 4s^1$ [3]. 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 [3].

1.3.1. Biological applications of copper

While iron, on account of the solubility of its ferrous form, was widely available in the reducing environment of the early Earth, copper, which was present as highly insoluble cuprous sulfides, must have been poorly bioavailable [4]. Copper is present in a large number of enzymes, many involved in electron transfer, activation of oxygen and other small molecules such as oxides of nitrogen, methane and carbon monoxide, superoxide dismutation, and even, in some invertebrates, oxygen transport. The routinely encountered oxidation states are Cu(I) and Cu(II), and as with iron, the reduced form can catalyse Fenton chemistry with hydrogen peroxide. Cu(I) can form complexes with coordination numbers 2, 3 or 4, while Cu(II) prefers coordination numbers 4, 5 or 6. Whereas four-coordinate complexes of Cu(II) are square-planar, the corresponding Cu(I) complexes are tetrahedral. Among the divalent elements of the transition series, Cu(II) forms the most stable complexes. In terms of the HSAB classification Cu(II) is ‘hard’, while Cu(I) is ‘soft’ underlined by its preference for sulfur ligands. Both forms have fast ligand exchange rates. It appears that throughout the living world intracellular concentrations of ‘free’ copper are maintained at extremely low levels, most likely because intracellular copper metabolism is characterized by the use of copper chaperone proteins to transport copper towards their target proteins (cytochrome oxidase, superoxide dismutase (SOD) and the multi-copper

oxidases, whose copper is inserted in the Golgi apparatus) [4]. 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 [5] and their effect on PG synthesis [6,7-10].

1.3.2. Copper containing anticancer agents

The use of copper for the treatment of cancer dates back to early 1980 with the report by Petering [11] on the activity of copper thiosemicarbazones. More recent work has involved the use of copper complexes of carboxamidrazones [12] and carboxylates [13]. 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. [12, 13] 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 (Fig. 1.1).

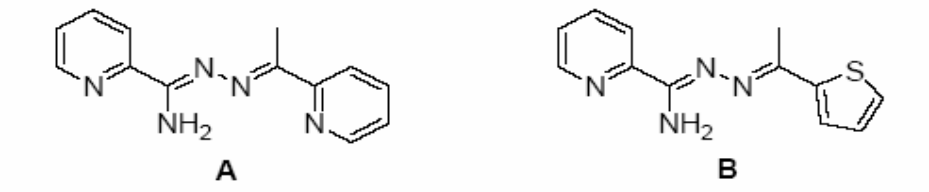

Fig. 1. 1 Molecular structure of A) appc and B) atpc.

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], and [Cu (atpc) Cl_2], are shown in (Fig. 1.2).

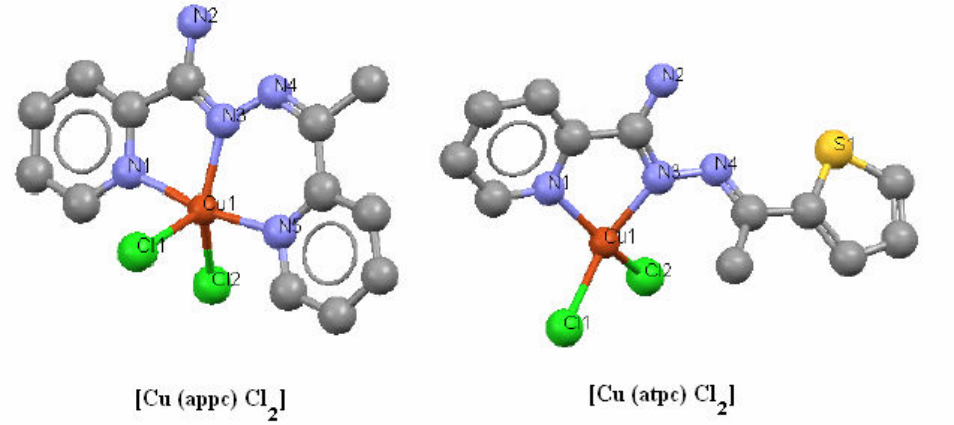

Fig. 1. 2 Molecular structures of copper carboxamidrazones.

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 bis(5-amino-1-tolylimidazole-4-carboxylate)Cu(II) shown in (Fig. 1.4).

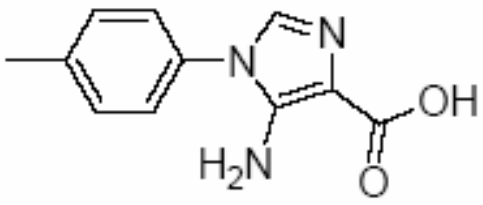

Fig. 1. 3 Molcular structure of 5-amino-1-tolylimiddazole-4-carboxylic acid.

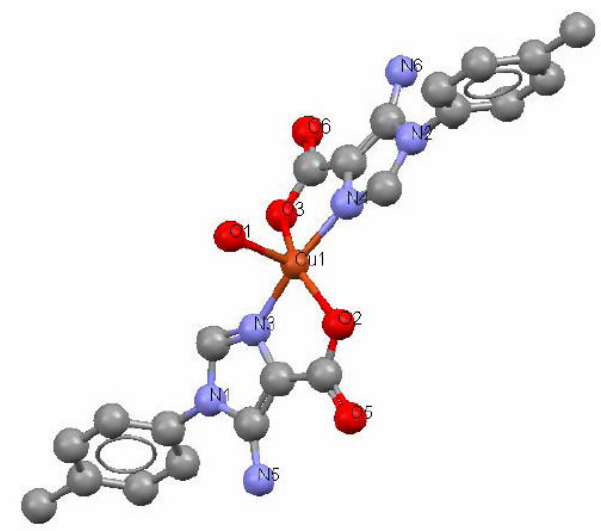

Fig. 1. 4 Molecular structure of bis(5-amino-1-tolylimidazole-4-carboxylate)Cu(II).

There has been some recent work done trying to elucidate the mechanism of action of copper intracellularly. Zou [14] and Somasundaram [15] have explored reactive oxygen species formation and DNA damage leading to cell p53 upregulation, respectively. It is believed that reactive oxygen species can induce oxidative stress in cells which leads to the initiation of apoptosis [16].These reactive oxygen species are believed to induce apoptosis by interacting with intracellular macromolecules, by attacking the cell membrane, and causing DNA strand breaks [14]. The data obtained by Zou indicates that increased levels of copper in the cell, known as copper overload, become quite toxic and leads to the formation of reactive oxygen species which cause a decrease in glutathione activity, cellular redox state changes, and DNA damage leading to cell death [14].Somusandaram explored DNA damage caused by copper to a further extent. They found that Cu(I) complexes cause cell cycle arrest leading to apoptosis[15].Their data indicate that the DNA damage caused by Cu(I) leads to the activation of the tumor suppressor gene p53 leading to apoptosis[15].The tumor suppressor p53 gets activated in cells under stress especially when DNA damage occurs[17].

1.4. Zinc

It is the 30th element on the Periodic Table, located between copper and Ga in the first row of transition elements. The ground state electronic configuration of elemental Zinc is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^2$ or [Ar] $3d^{10} 4s^2$. Zinc is, in some respects, chemically similar to

magnesium, because its ion is of similar size and its only common oxidation state is +2. Zinc is the 24th most abundant element in the Earth's crust and has five stable isotopes. Impure zinc metal was not produced in large scale until the 13th century in India, while the metal was unknown to Europe until the end of the 16th century. Alchemists burned zinc in air to form what they called "philosopher's wool" or "white snow". The element was probably named by the alchemist Paracelsus after the German word Zinke. German chemist Andreas Sigismund Marggraf is normally given credit for discovering pure metallic zinc in 1746. A variety of zinc compounds are commonly used, such as zinc carbonate and zinc gluconate (as dietary supplements), zinc chloride (in deodorants), zinc pyrithione (anti-dandruff shampoos), zinc sulfide (in luminescent paints), and zinc methyl or zinc diethyl in the organic laboratory. It is somewhat less dense than iron and has a hexagonal. The chemistry of zinc is dominated by the +2 oxidation state. When compounds in this oxidation state are formed the outer shell s electrons are lost, which yields a bare zinc ion with the electronic configuration or [Ar] $3d^{10}$.

The metal is hard and brittle at most temperatures but becomes malleable between 100 and 150°C. Above 210°C, the metal becomes brittle again and can be pulverized by beating. Zinc is a fair conductor of electricity. Five isotopes of zinc occur in nature. 64Zn is the most abundant isotope (48.63% natural abundance). This isotope has such a long half-life, at 4.3×1018 a, ^{70}Zn (0.6%), with a half life of 1.3×10^{16} a is not usually considered to be radioactive.

1.4.1. Biological applications of zinc

After iron, zinc is the second most abundant trace element in the human body: an average adult has ~3 g of Zn, corresponding to a concentration of about 0.6 mM. Some 95% of zinc is intracellular. It is essential for growth and development in all forms of life, and has been proposed to have beneficial therapeutic and preventative effects on infectious diseases, including a shortening of the length of the common cold in man.

Zinc is found in more than 300 enzymes, where it plays both a catalytic and a structural role. It is the only metal to have representatives in each of the six fundamental classes of enzymes recognized by the International Union of Biochemistry [18].

The divalent zinc ion is redox inactive, in contrast, for example, to manganese, iron and copper. Its d^{10} configuration means that not only does it have no d-d transitions, and therefore no absorption spectroscopy, but also its complexes are not subject to ligand field

stabilization effects such that Zn^{2+} has no ligand field constraints on its coordination geometry. Coordination number and geometry are therefore dictated only by ligand size and charge. This means that zinc can, in principle, adopt highly flexible coordination geometry. However, in most zinc proteins there is a strong preference for tetrahedral coordination, frequently slightly distorted, which enhances both the Lewis acidity of the zinc centre and the acidity of a coordinated water molecule. Only Cu(II) is a better Lewis acid. A few cases of zinc in five-coordinate distorted trigonal bipyramidal geometry have been reported. Since zinc is of borderline hardness, it can bind oxygen (Asp, Gu, H2O), nitrogen (His) and sulfur (Cys) ligands [18].

Three types of zinc-binding sites have been recognized in zinc enzymes catalytic sites, structural sites and cocatalytic sites. Many of these zinc enzymes are peptidases and amidases, involved in the cleavage of amide bonds—they include peptidases, such a thermolysin and carboxy-peptidases; β-lactamases, which destroy the four-member β-lactam rings in penicillins; and matrix metalloproteinases, which degrade extracellular matrix components such as collagen. Zinc enzymes also participate in the cleavage of the phosphodiester bonds in both DNA and RNA, and their role extends beyond catalysis of hydrolytic reactions to include the important lyase, carbonic anhydrase and the oxidoreductase, alcohol dehydrogenase [18].

1.5. Flavonoids

The Hungarian Nobel laureate Albert Szent-Györgyi discovered flavonoid compounds in 1936.1 Using evidence from his own experiments, he hypothesized that a new vitamin – vitamin P works synergistically with vitamin C in citrus extracts to strengthen capillaries.2 Although the description of flavonoids as vitamins was eventually found to be inaccurate, research into their beneficial potentials continued and has increased dramatically over the past two decades [19-26].

Flavonoids (flavus–yellow), or bioflavonoids, are a ubiquitous group of polyphenolic substances which are present in most plants, concentrated in the seeds, fruit skin or peel, bark and flowers.3 More than 4000 different flavonoids have been identified to date, making them the largest group of plant chemicals. Many fruits and vegetables, especially buckwheat, apple and onion, are some of these sources. Beverages prepared from plant extracts (beer, tea, wine, fruit juice) are the principal source of dietary flavonoid intake [27-32].

1.5.1. Health benefits of flavonoids

Scientific studies conducted in the last few years generated a growing interest in the potentially important role of flavonoids in maintaining human health. A considerable number of plant medicines contain flavonoids, which have been reported by many authors as having anti-bacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti-neoplastic, anti-thrombotic, and vasodilatory actions[33-47]. Overwhelmingly, the pharmacological effects are related to the anti-oxidant activity of flavonoids, arising through their ability to scavenge free radicals. When generated in excess, free radicals can damage biomolecules, and are therefore implicated in the etiology of several diseases and ageing.32 Radical scavenging by flavonoids occurs via electron donation from the free hydroxyls on the flavonoid nucleus with the formation of a less reactive flavonoid aroxyl radical, which is stabilized by resonance and therefore plays only a moderate role in the propagation of radical- induced damage in biological systems. The anti-oxidant activity of flavonoids correlates well with their physiological function in vivo, because oxidative stress is known to participate in the initial process of atherosclerosis leading to coronary heart disease and other patho-physiological events. A number of studies have revealed that flavonoids act as anti-oxidants by scavenging reactive oxygen species [48-57].

Specifically, flavonoids reduce the risk of stroke and heart disease (the so-called French paradox, the lack of a positive correlation between a high intake of saturated fat and the occurrence of coronary heart disease is related at least partly to the consumption of red wine,43 which is rich in flavonoids), protect against age-related vision disorders, relieve hay fever, sinusitis, asthma symptoms, alleviate inflammatory skin conditions, reduce inflammation in joints and muscles, common to rheumatoid arthritis, minimize menopausal hot flushes, shrink hemorrhoids, reduce varicose veins and battle viral infections[33-47, 58].

A considerable number of pharmaceutical preparations containing flavonoids as active substance are commercially available today. For example, Ginkgo biloba leaf extract,45 used in the treatment of symptoms in the early stages of Alzheimer's disease, vascular dementia and memory impairment,46 is the most widely sold phytomedicine in Europe. Some of the commercial pharmaceutical preparations which include the flavonoid rutin are widely used for curing veins diseases. Quercetin, the most biologically active and common dietary flavonoid, is generally used as a dietary supplement.