In the Name of God

Razi University

Faculty of Science
Department of Chemistry

M.Sc.Thesis

Title of the Thesis:

Comparative DNA interaction studies of a food colorant, indigo carmine and its metal complex and DNA interaction studies of food colorant, patent blue v using different instrumental methods

Supervisor:

Dr. N. Shahabadi

By:

Khadijeh Safari Namivandi

March, 2013

Acknowledgments

Thanks God, the merciful and the passionate, for providing me the opportunity to step in the excellent world of science.

I would like to express my special thanks to Dr. Nahid Shahabadi the director of this thesis for her supervision, constructive guidance, excellent suggestion and great disposing efforts.

Special thanks go to my friends and all students in Inorganic research laboratory for providing a friendly environment during my stay in the research laboratory.

Special thanks to my dear father for unconditional love and to my compassionate mother that her prayers have been with me. I would like to thank my dear sister and brothers for encouragement and support thought the years.

Dedicated to:

My Dear

parent

Abstract

The copper (II) complex containing the indigo carmine has been synthesized and characterized by elemental analysis, FT-IR, ^{1}H NMR, conductivity measurement and UV-Vis techniques. The binding interactions between indigo carmine (IC) and its copper complex with calf thymus DNA (CT-DNA) and patent blue v (PBV) were studied by absorption, emission, circular dichroism spectroscopies, viscosity measurements, cyclic voltammetry and cleavage studies by agarose gel electrophoresis. Hyperchromism in the UV absorption band of, increase in the viscosity of DNA were observed for IC and PBV. Furthermore, mentioned compounds induced detectable changes in the CD spectrum of CT-DNA . In fluorimeteric studies, the binding mode of IC and PBV with DNA were studied using neutral red as a fluorescence prob. Cleavage experiments showed that the IC and its Cu(II) complex are not able to perform cleavage of pUC18 plasmid DNA. Hypochromism in the UV absorption band of copper complex, decrease in the viscosity of DNA, stabilization of the right-handed B form of CT-DNA detected by changes in the CD spectrum of CT-DNA were observed for the complex. As an evidence by quenching fluorescence intensity of Hoechst-DNA solution in the presence of increasing amounts of the copper complex, it is able to displace the Hoechst 33258 groove binder to DNA. Finally all results suggest that IC and PBV are able to intercalate into the DNA base pairs and this copper complex is able to interact with DNA via groove binding mode.

Keywords

Indigo Carmine, Patent Blue v, Copper complex, DNA interaction, Groove binding mode, intercalation, Food colorant

.

Table of Contents

Contents **page**

Chapter One: Introduction

1.1. General……... 2

1.2. Medicinal chemistry……. 3

1.3. Medicinal inorganic chemistry…….. 3

1.4. Copper…… 3

1.4.1. Biological applications of copper..... 4

1.4.2. Copper containing anticancer agents……. 4

1.5. Food additives……. 7

1.6. Food colorants……. 8

1.6.1. Indigo carmine (IC)…….. 9

1.6.2. Patent Blue v…….. 10

1.7. Deoxyribonucleic Acid (DNA)……. 11

1.7.1. History of DNA……..... 11

1.7.2. DNA structure…… 12

1.7.3. DNA forms…….. 14

1.7.3.1. The B form of DNA…… 15

1.7.3.2. The A form of DNA…….. 16

1.7.3.3. The Z form of DNA…….. 16

1.7.3.4. The H form of DNA……. 17

1.7.3.5. Other forms of DNA…….. 18

1.8. DNA-drug interaction……..... 18

1.8.1. Covalent binding……. 19

Contents **page**

1.8.2. Non-Covalent binding……. 19

1.8.2.1. Electrostatic interactions…….. 20

1.8.2.2. Groove binding…… 20

1.8.2.3. Intercalation……... 21

1.9. Analytical techniques to evidence DNA-drug interactions…… 22

1.9.1. UV-vis absorption spectroscopy……. 22

1.9.2. Circular dichroism spectroscopy…… 23

1.9.3. Fluorescence emission spectroscopy…… 24

1.9.4. Fluorescent probes…… 26

1.9.4.1. Hoechst 33258…… 26

1.9.4.2. Methylene Blue…….. 26

1.9.4.3. Neutral Red……... 27

1.9.5. Viscosity measurement…….. 28

1.9.5.1. Ostwald capillary viscometer…… 29

1.9.6. Agarose-Gel electrophoresis of DNA plasmid (Cleavage study)……. 29

1.9.6.1. Conformations of Uncut Plasmid DNA…… 30

1.9.7. IR spectroscopy……... 31

1.9.8. NMR spectroscopy…… 32

1.9.9. Voltammetry study…… 33

1.9.9.1. Cyclic Voltammetry…….. 34

1.10. Aim of this study……..... 36

Chapter Two: Materials & Methods

2.1. Materials..... 38

2.2. Apparatus…… 39

Contents **page**

2.3. Various sections of this study…….. 39

2.4. Synthesis of Copper complex…….. 40

2.5. Preparation of buffers..... 40

2.6. Preparation of DNA, Indigo carmine, copper complex and Patent Blue v solutions for interaction……. 40

2.7. Methods…….. 41

2.7.1. UV-Vis absorption measurements..... 41

2.7.2. Circular dichroism measurements..... 41

2.7.3. Viscosity measurements..... 41

2.7.4. Cyclic voltammetry…… 42

2.7.5. Gel electrophoresis experiments…… 42

2.7.6. Fluorescence measurements..... 42

Chapter Three: Results and Discussion

3.1. Synthesis and characterization of copper complex…… 45

3.2. DNA interactions studies of indigo carmine and its copper complex……. 51

3.2.1. Absorption studies…….. 51

3.2.1.1. Determination of binding constant……. 52

3.2.1.2. Effect of ionic strength on the binding of the copper complex and DNA……. 54

3.2.2. Circular dichroic spectral studies…… 54

3.2.3. Viscosity measurements…… 56

3.2.4. Cleavage of pUC18 plasmid DNA by IC ligand and its copper complex…. 57

3.2.5. Cyclic Voltammetry studies…….. 58

Contents **page**

3.2.6. Fluorescence spectra……. 59

3.2.6.1. Competitive binding studies between Hoechst 33258/indigo carmine and Neutral Red (NR)/indigo carmine to DNA…… 59

3.2.6.2. Competitive binding studies between Hoechst 33258/Cu complex and Neutral Red (NR), Methylene Blue/copper complex to DNA 61

3.2.6.3. Quenching mechanism of IC-DNA and enhancement mechanism of its copper complex–DNA…… 63

3.2.6.4. Binding constants and the number of binding sites…… 66

3.2.7. Thermodynamic studies…… 67

3.3. DNA interactions studies of PBV…… 69

3.3.1. UV–Vis absorption spectra…….. 69

3.3.2. Circular dichroic spectral studies……. 70

3.3.3 Viscosity measurements……. 70

3.3.4. Voltammetry studies…….. 71

3.3.5. Spectrofluorimetry studies…….. 72

3.3.5.1. Fluorescence quenching studies…….. 72

3.3.5.2. Binding constants and the number of binding sites…….. 74

3.3.6. Thermodynamic studies……. 74

Conclusion…… 75

Suggestions…….. 75

Reffrences……. 76

List of Figures

Contents **page**

Fig. 1. 1 Molecular structure of appc and atpc……. 5

Fig. 1. 2 Molecular structures of copper carboxamidrazones…….. 6

Fig. 1. 3 Molcular structure of 5-amino-1-tolylimiddazole-4-carboxylic acid……. 6

Fig. 1. 4 Molecular structure of bis(5-amino-1-tolylimidazole-4-carboxylate)Cu(II)… 6

Fig. 1.5. Indigo Carmine……. 10

Fig. 1. 6. E 131; Patent Blue v…… 11

Fig. 1.7. Structures of nucleic acid constituents…… 12

Fig. 1.8.Watson-Crick pairing between purine and pyrimidine bases incomplementary DNA strands…… 13

Fig.1.9. Major and minor grooves formed by DNA helix……. 14

Fig.1.10. Typical form of DNA…… 15

Fig. 1.11. Intra molecular triplex DNA (HDNA) form……. 17

Fig. 1.12. Covalent binding mode…… 19

Fig. 1.13.The schematic drawing for electrostatic binding…….. 20

Fig. 1.14.The schematic drawing for Groove binding…… 21

Fig. 1.15.The schematic drawing for Intercalation binding……. 22

Fig. 1.16. Electro transitions in ultraviolet/visible spectroscopy…….. 23

Fig. 1.17. Schematic CD spectra of the three different structure families of nucleic acids……. 24

Fig. 1.18. Electronic transition energy level diagram…….. 25

Fig. 1.19. Molecular structure of Hoechst 33258…….. 26

Fig. 1.20. Molecular structure of Methylene blue……. 27

Fig. 1.21. Molecular structure of Neutral Red…… 28

Contents **page**

Fig. 1.22.Schematic for increase in DNA length via Intercalation mode……. 28

Fig. 1.23. Ostwald Capillary vicometer…….. 29

Fig. 1.24. Two types of apparatus for gel electrophoresis……. 30

Fig 1.25.Different form of DNA plasmid…….. 31

Fig. 1.26. Approximate position of IR bands of DNA and aqueous solvents…….. 32

Fig. 1.27. Potential time extraction signal in cyclic voltammetric experiment…… 35

Fig. 1.28.Typical cyclic voltammogram for a reversible redox process…… 35

Fig. 3.1. The structures of a) IC b) copper complex…….. 45

Fig. 3. 2. UV spectra of IC and copper complex in Tris-HCl buffer……. 47

Fig. 3.3. The IR spectrum of Indigo Carmine and the IR spectrum of Copper complex …… 48

Fig. 3.4. The 1H NMR spectrum of indigo carmine in DMSO solvent…….. 49

Fig. 3.5. The 1H NMR spectrum of the copper complex in DMSO solvent……. 50

Fig. 3. 6. Absorption spectra of a) Indigo Carmine and b) Copper complex in the absence and presence of increasing amounts of CT-DNA…… 52

Fig. 3. 7. Plot of [DNA]/(εa-εf) vs [DNA] for the titration of DNA with a) IC b) Copper complex……. 53

Fig. 3. 8. Effect of ionic strength on the copper complex–DNA spectra…… 54

Fig. 3.9. The changes of CD spectrum of DNA in the absence and presence of increasing amounts of IC……. 55

Fig. 3. 10. Circular dichroism spectra of CT-DNA (8 10-5) in Tris-HCl in the Presence of increasing amounts of the copper complex…….. 56

Fig. 3. 11. Effect of increasing amounts of IC and its complex, on the viscosity of CT-DNA (5 × 10-5 M) in 50 mM Tris buffer……. 57

Contents **page**

Fig 3.12. Cleavage of DNA in the presence of increasing amounts of a) IC and b) Its copper complex…… 58

Fig. 3. 13. cyclic voltammograms of a) IC and b) Copper complex, in the absence and presence of increasing amounts of CT-DNA……. 59

Fig .3.14. Emission spectra of the Hoechst–DNA complex in the presence of increasing amounts of IC…… 60

Fig.3.15. Emission spectra of the NR–DNA complex in the presence of increasing amounts of IC……. 61

Fig.3.16. Emission spectra of the NR–DNA complex in the presence of increasing amounts of copper complex……. 62

Fig.3.17. Emission spectra of the MB–DNA complex in the presence of increasing amounts of copper complex……. 62

Fig.3.18. Emission spectra of the Hochesct–DNA complex in the presence of increasing amounts of copper complex……. 63

Fig. 3.19. Fluorescence spectra of the copper complex in the absence and presence of the increasing amounts of DNA……... 65

Fig. 3. 20. Stern–Volmer plots of the system for IC –DNA…… 65

Fig. 3. 21. Stern–Volmer plots of the system for copper complex –DNA……. 66

Fig. 3. 22. Absorption spectra of PBV in the absence and presence of increasing amounts of CT-DNA……. 69

Fig. 3. 23. Plots of [DNA]/ (εa −εf) versus [DNA]……. 69

Fig. 3.24. CD spectra of DNA in the presence of increasing amounts of PBV…… 70

Fig. 3. 25. Effect of increasing amounts PBV on the viscosity of calf thymus DNA.. 71

Fig .3.26.Differential pulse voltammograms for PBV at the presence of different

Contents **page**

concentrations of DNA…… 71

Fig.3.27. Emission spectra of the NR–DNA complex in the presence of increasing amounts of PBV……. 72

Fig. 3. 28. Stern–Volmer plots of the system for PBV–DNA…….. 73

List of Tables

Contents **page**

Table 1.1.Certifiable Colors Synthetic……. 10

Table 1.2.Comparison of the Structural Properties of A, B, Z DNAs and Other Forms of DNA…… 18

Table 1.3. Typical chemical shift ranges for proton resonances in NMR spectra of DNA……. 34

Table 3.1. Elemental analysis data for the copper complex…….. 47

Table 3.2. IR data for free ligand and its Cu complex (in cm-1)…….. 47

Table 3.3. Selected 1H NMR spectral data (in ppm)…… 49

Table.3.4. The quenching constants of IC and its complex by CT-DNA at different Temperatures…….. 66

Table 3.5. Binding constants (*Kf*) and number of binding sites (n) of the DNA–IC and DNA-complex system…… 67

Table 3.6. Thermodynamic parameters for the binding of IC and its complex to CT-DNA …….. 68

Table .3.7. The quenching constants of CT DNA-NR by PBV at different Temperatures…… 73

Table .3.8.Binding constants (Kf) and number of binding sites (n) of the PBV-DNA system……. 74

Table .3.9.Thermodynamic parameters for the binding of PBV to CT-DNA……. 75

LIST OF ABBREVIATIONS

A	adenine
T	thymine
C	cytosine
G	guanine
τ_0	lifetime
K_b	binding constant
K_f	binding constant
Kq	fluorophore quenching rate constant
K_E	fluorophore enhancement rate constant
Ksv	quenching constant
K_D	Dynamic enhancement constant
IC	Indigo Carmine
PBV	Patent Blue v
MB	Methylene Blue
NR	Neutral Red
CD	circular dichroism
CV	cyclic voltametry
DPV	differential pulse voltametry
DMSO	dimethyl suloxide
LUMO	lowest unoccupied molecular orbital
HUMO	highest unoccupied molecular orbital
UV-vis	Uv-visible spectroscopy
CT–DNA	calf thymu DNA

Chapter One

Introduction

1.1. General

The field of bioinorganic chemistry, which deals with the study of role of metal complexes in biological systems, has opened a new horizon for scientific research in coordination compounds. A large number of compounds are important from the biological point of view. Some metals are essential for biological functions and are found in enzymes and cofactors required for various processes. For example, hemoglobin in red blood cells contains an iron porphyrin complex, which is used for oxygen transport and storage in the body. Chlorophyll in green plants, which is responsible for photosynthetic process, contains a magnesium porphyrin complex. Cobalt is found in the coenzyme B_{12}, which is essential for the transfer of alkyl groups from one molecule to another in biological systems.

Metals such as copper, zinc, iron and manganese are incorporated into catalytic proteins (the metalloenzymes), which facilitate a multitude of chemical reactions needed for life. Some metals have been used as drugs and diagnostic agents to treat a variety of diseases and conditions. Platinum compounds, cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$), carboplatin and oxaliplatin are among the most widely used cancer therapeutic agents. Gold drugs, myocrisin and auranofin are used for the treatment of rheumatoid arthritis. Another important aspect of medicinal inorganic chemistry is the development of radiopharmaceuticals and diagnostic agents. Many organic compounds used in medicine do not have a purely organic mode of action; some are activated or biotransformed by metal ions including metalloenzyme, others have a direct or indirect effect on metal ion metabolism. The pharmacological activities of these metal compounds depend on the metal ion, its ligands and the structure of the compounds. These factors are responsible for reaching them at the proper target site in the body. Biologically relevant metal complexes have several requirements in terms of their synthetic design. First, a biologically active metal complex should have a sufficiently high thermodynamic stability to deliver the metal to the active site. The metal-ligand binding should be hydrolytically stable.

The kinetics with which the metal ion undergoes ligation or deligation reactions is of great importance.The molecular weight of the metal complex is also critical. The compounds of low molecular weight with neutral charge and some water solubility are soluble in almost any medium and may slip through biological membranes by passive diffusion [1]

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 [2].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 [2].

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, 65Cu and 63Cu, 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 [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 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 [5]. 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) [5]. 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 [6] and their effect on PG synthesis [7-11].

1.4.2. Copper containing anticancer agents

During the past decade after the successful achievement of cis-dichlorodiammine platinum(ll) (cisplatin), a number of derivatives of thiosemicarbazone, such as 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone) copper complexes (Cu-KTS), have been found to exhibit antitumour activity by binding with DNA . In addition, the copper complexes of