Structure and Reactivity of Metal Complexes Bound to DNA

Thesis by
Pratip K. Bhattacharya

In Partial Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy

California Institute of Technology
Pasadena, California
2003
(Defended June 26, 2003)

Acknowledgements

The research outlined in the thesis below would not have been possible without the inspiration and help provided by people too numerous to mention. I am indebted to my advisor, Professor Jacqueline K. Barton for giving me the opportunity to do research in an exciting field. Through her personal example of intellectual curiosity, optimism and integrity, Prof. Barton sets the tone of free thinking and enormous scientific excitement in the group. Being a part of the Barton group is indeed one of the most exciting experiences of my life. Jackie was always available to discuss matters great or small, and her willingness to allow me to pursue the study of topics outside the areas of group expertise strongly influenced the direction of my research. I want to thank Barton group secretary, Maureen Renta and the Chemistry Division secretary, Dian Buchness for their invaluable help in making sure graduate students are not burdened with any administrative tasks.

My thesis committee is also in need of special recognition. I thank Professor Nathan S. Lewis for serving as the Chairman of my committee. My graduate research was greatly influenced by Professor Richard W. Roberts. Rich's timely suggestions regarding DNA base-pair lifetime measurements by NMR spectroscopy during my candidacy exam played a big role in shaping my thesis. Professor Steven L. Mayo was the last faculty member to join my committee. Steve came on board in time for my fourth-year meeting. His expertise has helped me with many of my NMR experiments.

Many Barton group members, past and present, provided their valuable time and knowledge to help me learn new ideas and collect data. Dr. Christine Stinner and Dr. Christopher R. Treadway were instrumental in imparting me with their knowledge of organic and inorganic synthesis respectively. Dr. Megan E. Nuñéz patiently showed me how to use new instruments, run gels and various biochemical techniques. Professor Eric D. A. Stemp readily dispensed his knowledge of photochemistry and photophysics and was a great support.

For my first project in the Barton group with paramagnetic Nickel complexes, Professor Holly J. Lawson was a great collaborator. She helped me in several inorganic synthesis and was always willing to discuss research or other mundane matters. It was a great pleasure to collaborate with Dr. Henrik Junicke on the NMR structure of the mismatch bound to DNA.

For two summers, I was assisted by Julie Cha and Helen Cheung, two talented undergraduate SURF students at Caltech. My best wishes go out to them as they toil through graduate schools. Drs. Matthias Pascaly, Matthias M. Manger, Alexander Schnyder and Jae Yoo donated various ligands and metal complexes for some of the studies detailed in this thesis. Prof. David Vicic was always available to discuss science as well as world affairs. Dr. Elizabeth Boon Carrico, Dr. Kimberly Copeland, Tashica Williams, Sarah Delaney, Donato Ceres, Greg Drummond, Jonathan Hart, Dr. Eva Rueba, Dr. Melanie O'Neill, Dr. Anne Petitjean, Dr. Eylon Yavin, Dr. Chikara Dohno and Dr. Uli Scatzschneider all contributed in various ways to increase both my knowledge of science and research as well as my enjoyment of graduate school.

Other members of the Caltech community have assisted me at various times over the past several years. Dr. Robert Lee, the Manager of NMR Facility at Caltech and Dr. Scott Ross of the Mayo group were tremendous sources of support. Both of them spent countless hours in training me with the NMR techniques, designing pulse sequences and helping me with my various NMR experiments.

It would have been a challenge to survive the graduate school experience without the support of many good friends. My hiking and photography buddies at Alpine and Photography Clubs at Caltech deserve special mention. Despite their busy schedule, Dr. Kent Harris, Dr. Sanjay Kumar, Zohir Chowdhury, Andrew Leavenworth, Eric Barker and Robert Puckett were always there for me whenever I feel like camping and hiking out in one of those gorgeous national parks in California or even crash in their pads at odd hours.

Far from home, I came close to finding family in the residence of James Regan. Jim befriended me during my first year of graduate school, and our philosophies on life, politics, work, and appreciation of nature and wilderness have produced a great friendship. It was a lot of fun camping out with him in the remote parts of San Gabriel and San Bernardino mountain ranges which only a native Angelino and an avid camper like him will know. His kids, Adam and Jeffery were a blast!

Finally, I want to thank my parents in India who through their unconditional love and support certainly made things easier for me during trying times. Their selfless outlook of life and emphasis on intellectual and spiritual growth coupled with attitude of giving has helped me to cope with a new culture and a new vision of life in the United States and forge many lasting relationships.

Abstract

Establishing correlations among structure, dynamics and reactivity is a fundamental problem in biological chemistry. Here, this problem is explored in the context of the design and reactivity of different metallointercalators bound to DNA.

First, the effects of intervening mismatches on DNA structure, dynamics and DNA charge transport reactivity is examined. The π–stacked DNA base pairs mediate charge transport chemistry over long molecular distances in a reaction that is exquisitely sensitive to DNA sequence dependent conformation and dynamics. To examine the long-range charge transport as a function of intervening base mismatches, a series of DNA oligonucleotides were synthesized that incorporate a ruthenium intercalator, $[Ru(phen)(bpy')(dppz)]^{2+}$ (phen = 1,10 phenanthroline; bpy' = 4-butyric acid-4'-methylbipyridine; dppz = dipyrido[3,2-a:2',3'-c]phenazine) linked covalently to the 5' terminus of one strand and containing two 5'-GG-3' sites in the complementary strand. Single base mismatches were introduced between the two guanine doublet steps, and the efficiency of transport through the mismatches was determined through measurements of the ratio of oxidative damage at the guanine doublets distal versus proximal to the intercalated ruthenium oxidant. Differing relative extents of guanine oxidation were observed for the different mismatches. The damage ratio of oxidation at the distal versus proximal site for the duplexes containing different mismatches varies in the order GC ~ GG ~ GT ~ GA > AA > CC ~ TT ~ CA ~ CT. The extent of distal/proximal guanine oxidation in different mismatch-containing duplexes was then compared with the helical stability of the duplexes, electrochemical data for intercalator reduction on different mismatch-containing DNA films, and base-pair lifetimes for oligomers containing the different mismatches derived from ^{1}H NMR measurements of the imino proton exchange rates. The exchange kinetics of the imino protons were measured from selective longitudinal relaxation times, and the effect of the mismatch was observed on the base pair lifetime up to a distance of two neighboring base pairs. The overall order of base-

pair lifetimes in the selected sequence context of the base pair was as follows: GC > GG > AA > CC > TT. While a clear correlation is evident both with helix stability and electrochemical data monitoring reduction of an intercalator through DNA films, guanine damage ratios was found to correlate most closely with base-pair lifetimes. These results underscore the importance of base dynamics in modulating long-range charge transport through the DNA base-pair stack.

In a related ^{1}H NMR structural study of the ruthenium intercalator, $[Ru(phen)(bpy')(dppz)]^{2+}$ covalently tethered to a short eightmer DNA duplex, d(ACGAGCAC)•d(GTICTCGT) with a nine carbon linker, the type of construct used in charge transport experiments, a very fast exchange was observed. Comparison of the NOESY data obtained from the NMR study of this system and control samples comprising of the duplex with only linker and the duplex alone, led to the conclusion that the nine carbon linker is positioned between the second and fourth bases from the point of its origin. The absence of any site specificity of the metal complex in the oligonucleotide complicates the structural characterization by NMR study. This led us to conceive of a more general strategy of obtaining structural information of metal complexes that bind non-specifically to DNA based on paramagnetic NMR.

The selective paramagnetic relaxation of oligonucleotide proton resonances of two short self-complementary oligonucleotides; $d(GTCGAC)_2$ and $d(GT\underline{GC}AC)_2$ by $Ni(phen)_2(L)^{2+}$ where L= dipyridophenazine (dppz), dipyrido[3,2-d:2',3'-f]quinoxaline (dpq) and phenanthrenequinone (phi) was examined to obtain structural insight into the non-covalent binding of these metal complexes to DNA. In the oligonucleotide $d(GTCGAC)_2$, preferential broadening of the G1H8, G4H8, T2H6, and C3H6 proton resonances was observed with $Ni(phen)_2(dppz)^{2+}$, $Ni(phen)_2(dpq)^{2+}$ and $Ni(phen)_2(phi)^{2+}$. In the case of the sequence $d(GT\underline{GC}AC)_2$, where the central two bases are juxtaposed from the previous one, preferential broadening was observed instead for the A5H2 proton resonance. Thus, a subtle change in the sequence of the oligonucleotide

can cause significant change in the binding location of the metal complex in the oligonucleotide. Owing to comparable changes for all metal complexes and sequences in broadening of the thymine methyl proton resonances, the switch in preferential broadening was attributed to a change in site location within the oligomer rather than to an alteration of groove location. Therefore, even for DNA-binding complexes of low sequence-specificity, distinct variations in binding as a function of sequence are apparent and can be monitored using paramagnetic probes.

Finally, ^{1}H NMR spectroscopy was employed to study the binding of $[Rh(bpy)_2chrysi]^{3+}$ (chrysi = 5,6-chrysenquinone diimine), a metal complex which specifically targets mismatches, to a ninemer oligonucleotide $d(GCCTCAGGC)_2$ containing centrally placed CC mismatch. Evidence supports intercalation by the metal complex within the mismatch site (i) upfield chemical shifts and significant broadening of the chrysi resonances and (ii) an increase in duplex melting temperature in the presence of the metal complex. To simplify the NMR spectra, the Δ isomer of $[Rh(d_8\text{-}bpy)_2chrysi]^{3+}$ was employed in NMR experiments with DNA. A break in the connectivity in the NOE walk is observed between T_4 and C_5, thereby marking the binding site of the metal complex at the CC mismatch. Intermolecular NOE's place the metal complex in the major groove of the oligonucleotide.

Thus through a series of experiments in this thesis, attempts have been made to correlate the structure and dynamics of metal complexes bound to DNA. Truly, metal complexes bound to DNA provide an interesting system to study structure, function and dynamics in a single package.

TABLE OF CONTENTS

CHAPTER 1. The Influence of Intervening Mismatches on Long-range Guanine Oxidation in DNA Duplexes. 1

CHAPTER 2. ^{1}H NMR Determination of Base Pair Lifetimes in Oligonucleotides Containing Single Base Mismatch. 30

CHAPTER 3. ^{1}H NMR Studies of Ru(phen)(bpy')$(dppz)^{2+}$ Covalently Tethered to a DNA 66

CHAPTER 4. ^{1}H NMR Studies of Nickel(II) Complexes Bound to Oligonucleotides: A Novel Technique for Distinguishing the Binding Locations of Metal Complexes in DNA 82

CHAPTER 5. ^{1}H NMR Structural Evidence for a Mismatch Specific Intercalator: $[Rh(bpy)_2chrysi]^{3+}$ Bound to $d(GCCTCAGGC)_2$ 107

CHAPTER 6. The Importance of Intercalation in Long-range Guanine Oxidation in DNA Duplexes. 130