Interactions of metal complexes with DNA

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Interactions of Metal Complexes With DNA

A thesis submitted in (partial) fulfilment of the requirements for the award of the degree

Doctor of Philosophy

from

University of Wollongong

by

Jihan Talib

Bachelor of Medicinal Chemistry Advanced (Honours)

School of Chemistry

November 2008
DECLARATION

I, Jihan Talib, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The work has not been submitted for qualification at any other academic institution.

Jihan Talib

4th November 2008
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PUBLICATIONS


Talib, J., Green, C. Davis, K. J., Urathamakul, T., Beck, J. L., Aldrich-Wright, J. R and Ralph, S. F. (2008) A Comparison of the Binding of Metal Complexes to Duplex and Quadruplex DNA. Dalton Trans. 8, 1018-1026

ABSTRACT

Electrospray ionisation mass spectrometry (ESI-MS), absorption spectrophotometry and circular dichroism spectroscopy were used to investigate the non-covalent binding interactions of the nickel complexes \([\text{Ni(phen)}_2(L)]\text{Cl}_2\), \((L = \text{phen, dpq, dpqc and dppz})\) with the 16mer oligonucleotide D2, which has the following base sequence: (GCTGCCAAATACCTCC/GGAGGTATTTGGCAGC). In addition, the extent of unwinding of the negatively supercoiled plasmid pUC9 caused by the nickel complexes, and the extent to which they inhibit \textit{in vitro} synthesis of mRNA, were investigated using gel electrophoresis. The results of these studies showed that DNA binding strengthened as the size of the unique ligand was increased. Comparison of each of the above sets of results with those obtained from identical experiments performed using the analogous ruthenium complexes \([\text{Ru(phen)}_2(L)]^{2+}\) \((L = \text{phen, dpq, dpqc, dppz})\) showed that varying the metal ion had a measurable effect on DNA binding affinity, with the nickel complexes generally interacting more weakly with D2 than the corresponding ruthenium complexes.

ESI-MS/MS and in-source collision-induced dissociation experiments were performed using the tetrameric quadruplex DNA molecule Q5 (TTGGGGGT)_4 and antiparallel dimeric quadruplex Q2 (GGGTTTTTGGGG)_2 in order to determine their gas-phase dissociation profiles. It was found that the gas phase stability of the quadruplex DNA was dependent on its charge state, the number of oligonucleotide strands that make up the quadruplex, and the number of consecutive G-tetrads that it contains. ESI-MS and circular dichroism spectroscopy were also used to examine the non-covalent binding interactions of the octahedral nickel and ruthenium complexes stated above, as well as several square planar platinum complexes with Q5. The platinum complexes studied were
[Pt(en)(phen)]^{2+}, [Pt(en)(3,4,7,8-Me_4phen)]^{2+}, [Pt(en)(4,7-Me_2phen)]^{2+} and [Pt(5,6-Me_2phen)(S,S-dach)]^{2+}. The results obtained from these experiments showed that each of the three groups of metal complexes were able to bind to Q5. In contrast to what was found in experiments involving the duplex DNA molecule D2, the presence of the intercalating dppz ligand in the coordination sphere of both the nickel and ruthenium complexes did not greatly increase their binding affinity towards quadruplex DNA. This observation suggests that intercalative binding interactions may not play as important a role in the binding of metal complexes to quadruplex DNA. ESI-MS was used to analyse mixtures containing the organic drug daunomycin, Q5, and either [Ru(phen)_2(dppz)]^{2+} or [Pt(en)(4,7-Me_2phen)]^{2+}, in order to obtain information about the qDNA binding modes of these metal complexes. The affinity of the above two metal complexes towards parallel tetrameric quadruplexes with different lengths was also compared using ESI-MS in an attempt to shed light on whether they bind to the ends of the quadruplexes or in grooves along their lengths.

The optimal conditions required to obtain ESI mass spectra of the non-covalent adduct formed between the DNA binding domain of mouse transcription factor PU.1, and a short 10mer DNA molecule containing its 5'-GGAA-3' consensus sequence, were determined. ESI-MS was then used to probe the extent of inhibition of formation of this non-covalent complex caused by addition of [Ru(phen)_2(dppz)]^{2+} or [Pt(5,6-Me_2phen)(S,S-dach)]^{2+}. Both metal complexes were shown to inhibit binding of the transcription factor to its DNA recognition site, demonstrating the potential of these complexes for transcription therapy.
ABBREVIATIONS

A adenine
AML acute myeloid leukemia
bip biphenyl
Bqdi 1,2-benzoquinone diimine
Bpy bipyridine
C cytosine
CD circular dichroism
CI chemical ionisation
CID collision induced dissociation
CT-DNA calf thymus DNA
Dach 1,2-diaminocyclohexane
DCM dichloromethane
DMB 4,4’-dimethyl-2,2’-bipyridine
DNA deoxyribonucleic acid
dppm 1,2-bis(diphenylphosphino)methane
dppz dipyrido[3,2-a:2',3'-c]phenazine
dpq dipyrido[3,2-d-2',3'-f]quinoxaline
dpqc dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydrophenazine)
dsDNA double-stranded DNA
DTC diethylthiocarbocyanide
EDTA ethylenediaminetetraacetic acid
EGR1 early growth response factor 1
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>EI</td>
<td>electron ionisation</td>
</tr>
<tr>
<td>en</td>
<td>(1,2-diaminoethane)</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>FD</td>
<td>field desorption</td>
</tr>
<tr>
<td>FDA</td>
<td>food and drug administration</td>
</tr>
<tr>
<td>G</td>
<td>guanine</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione-S-transferase</td>
</tr>
<tr>
<td>HAT</td>
<td>1,4,5,8,9,12-hexaazatriphenylene</td>
</tr>
<tr>
<td>HIF-1</td>
<td>hypoxia inducible factor 1α</td>
</tr>
<tr>
<td>IκB</td>
<td>inhibitor of κB</td>
</tr>
<tr>
<td>ICD</td>
<td>induced circular dichroism</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix assisted laser desorption ionisation</td>
</tr>
<tr>
<td>MGP</td>
<td>4-(guanidylmethyl)-1-10-phenanthroline</td>
</tr>
<tr>
<td>MOPS</td>
<td>3-(N-morpholino)propanesulfonic acid</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>m/z</td>
<td>mass-to-charge</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor-κB</td>
</tr>
<tr>
<td>NH₄OAc</td>
<td>ammonium acetate</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PD</td>
<td>plasma desorption</td>
</tr>
<tr>
<td>phi</td>
<td>9,10-phenanthrenequinone diimine</td>
</tr>
<tr>
<td>phen</td>
<td>1,10-phenanthroline</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>Q-TOF</td>
<td>quadrupole time-of-flight</td>
</tr>
<tr>
<td>qDNA</td>
<td>quadruplex DNA</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>R,R-Me₂trien</td>
<td>2R,9R-diamino-4,7-diazadecane</td>
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<tr>
<td>Sp1</td>
<td>Specificity Protein 1</td>
</tr>
<tr>
<td>Stat3</td>
<td>Signal transducer and activator of transcription</td>
</tr>
<tr>
<td>T</td>
<td>thymine</td>
</tr>
<tr>
<td>TBACl</td>
<td>tetrabutylammonium chloride</td>
</tr>
<tr>
<td>terpy</td>
<td>2,2:6’2”-terpyridine</td>
</tr>
<tr>
<td>TFOs</td>
<td>triplex forming oligonucleotides</td>
</tr>
<tr>
<td>TMPyP₄</td>
<td>[tetra(N-methyl-4-pyridyl-porphine)]</td>
</tr>
<tr>
<td>tpphz</td>
<td>tetrapyridophenazine</td>
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<tr>
<td>yAP-1</td>
<td>yeast Activator Protein 1</td>
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