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Interactions of metal complexes with DNA

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Interactions of Metal Complexes With DNA

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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

Gornall, K. C., Samosorn, S., **Talib, J.,** Bremner, J. B. and Beck, J.L. (2007) Selectivity of an Indolyl Berberine Derivative for Tetrameric G-quadruplex DNA. *Rapid Commun. Mass Spectrom.* 11, 1759-1766.

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

Talib, J., Harman, D., Dillon, C., Beck, J. L., Aldrich-Wright, J. R and Ralph, S. F. (2009) Does the Metal Influence the Non-covalent Binding of Complexes to DNA? *Dalton Trans.* DOI: 10.1039/B814156H.

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 $[Ni(phen)_2(L)]Cl_2$, (L = 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 *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 $[Ru(phen)_2(L)]^{2+}$ (L = 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 $(GGGGTTTTGGGG)_2$ 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

$[Pt(en)(phen)]^{2+}$, $[Pt(en)(3,4,7,8\text{-}Me_4phen)]^{2+}$, $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$ and $[Pt(5,6\text{-}Me_2phen)(S,S\text{-}dach)]^{2+}$. 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 $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$, 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 $[Pt(5,6\text{-}Me_2phen)(S,S\text{-}dach)]^{2+}$. 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

EI	electron ionisation
en	(1,2-diaminoethane)
ESI	electrospray ionisation
FAB	fast atom bombardment
FD	field desorption
FDA	food and drug administration
G	guanine
GSH	glutathione
GST	glutathione-S-transferase
HAT	1,4,5,8,9,12-hexaazatriphenylene
HIF-1	hypoxia inducible factor 1α
IκB	inhibitor of κB
ICD	induced circular dichroism
MALDI	matrix assisted laser desorption ionisation
MGP	4-(guanidylmethyl)-1-10-phenanthroline
MOPS	3-(*N*-morpholino)propanesulfonic acid
MS	mass spectrometry
m/z	mass-to-charge
NF-κB	nuclear factor-κB
NH_4OAc	ammonium acetate
NMR	nuclear magnetic resonance
PD	plasma desorption
phi	9,10-phenanthrenequinone diimine
phen	1,10-phenanthroline

Q-TOF	quadrupole time-of-flight
qDNA	quadruplex DNA
RNA	ribonucleic acid
R,R-Me_2trien	2R,9R-diamino-4,7-diazadecane
Sp1	Specficity Protein 1
Stat3	Signal transducer and activator of transcription
T	thymine
TBACl	tetrabutylammonium chloride
terpy	2,2:6'2''-terpyridine
TFOs	triplex forming oligonucleotides
TMPyP4	[tetra(*N*-methyl-4-pyridyl-porphine)]
tpphz	tetrapyridophenazine
yAP-1	yeast Activator Protein 1

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