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Mass spectrometric studies of non-covalent biomolecular complexes

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Mass Spectrometric Studies of Non-Covalent Biomolecular Complexes

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DECLARATION

I, Thitima Urathamakul, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the Department 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.

Thitima Urathamakul

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PUBLICATIONS

Beck, J.L., Gupta, R., **Urathamakul, T**., Williamson, N.L.; Sheil, M.M., Aldrich-Wright, J. R. and Ralph, S.F. (2003) Probing DNA Selectivity of Ruthenium Metallointercalators Using ESI Mass Spectrometry. *Chem. Commun.*, **5**, 626-7.

Urathamakul, T., Beck, J.L., Sheil, M.M., Aldrich-Wright, J.R. and Ralph, S.F. (2004) A Mass Spectrometric Investigation of Non-Covalent Interactions Between Ruthenium Complexes and DNA. *Dalton Trans.*, **17**, 2683-2690.

Beck, J.L., **Urathamakul, T**., Watt, S.J., Sheil, M.M., Schaeffer, P.M. and Dixon, N.E. (2006) Proteomic Dissection of DNA Polymerisation. *Expert Rev. Proteomics*, **3**, 197-211.

Watt, S.J., **Urathamakul, T**., Schaeffer, P.M., Sheil, M.M., Dixon, N.E. and Beck, J.L. (2006) Electrospray Ionisation Mass Spectrometry of Oligomers of *E. coli* DnaB Helicase and Mutants. *Rapid Commun. Mass Spectrom.*, **21**, 132-140.

ABSTRACT

Electrospray ionisation mass spectrometry (ESI-MS) was employed to investigate non-covalent associations of macromolecules with ligands, metal ions and other macromolecules. Firstly, ESI-MS was used to examine the interactions of six ruthenium compounds with three different DNA sequences (D1, D2 and D3). The relative binding affinities of these ruthenium compounds towards dsDNA was determined to be: $[Ru(phen)_2(dppz)]^{2+} \geq [Ru(phen)_2(dpqMe_2)]^{2+} > [Ru(phen)_2(dpqC)]^{2+} > [Ru(phen)_2(dpq)]^{2+} > [Ru(phen)_2(pda)]^{2+} > [Ru(phen)_3]^{2+}$. This order was in good agreement with that obtained from DNA melting temperature experiments. Competition experiments involving ruthenium compounds and organic drugs were also conducted to obtain information about the DNA binding modes of the ruthenium compounds. These studies provide strong support for the routine application of ESI-MS as a tool for analysis of non-covalent complexes between metallointercalators and dsDNA.

ESI-MS also proved to be a rapid and efficient tool for investigation of interactions between the N-terminal domain of ε (ε186, the exonuclease proofreading subunit of *E. coli* DNA) and three different metal ions (Mn^{2+}, Zn^{2+} and Dy^{3+}). The dissociation constants (K_d) for binding of Mn^{2+}, Zn^{2+} and Dy^{3+} to ε186 were determined from ESI-MS data to be 38.5 x 10^{-6}, 3.7 x 10^{-6} and 2.0 x 10^{-6} M, respectively. Despite binding the least tightly to the protein, incorporation of Mn^{2+} into the enzyme resulted in the highest enzymatic activity as measured by spectrophotometric studies. This suggested that Mn^{2+} is possibly the native metal ion present in ε186. The ability of the metal ions to enhance ε186 enzymatic activity was found to follow the order:

Mn^{2+} >> Zn^{2+} > Dy^{3+}. The results of these experiments also provided evidence that the presence of two divalent metal ions was essential for efficient enzyme-catalysed hydrolysis.

The distribution of different oligomeric forms of wild-type *E. coli* DnaB helicase and DnaB helicase mutants (F102E, F102H, F102W and D82N) was examined using a factory-modified Q-ToF mass spectrometer equipped with a 32,000 *m/z* quadrupole. Previous experiments showed that the heptameric form of the wild-type protein was favoured in the presence of methanol (30% *v/v*). In the current work, mixtures of hexamer, heptamer, decamer and dodecamer were observed in solutions containing 1000 mM NH_4OAc, 1 mM Mg^{2+} and 0.1 mM ATP, pH 7.6. When the proteins were prepared in solutions containing a lower concentration of Mg^{2+} (0.1 mM), only the hexameric form was observed for all proteins except D82N, which showed a mixture of hexamer and heptamer. These observations suggest that the higher order structures were stabilised at high concentrations of Mg^{2+}. In addition, the hexamers of DnaB and mutants ($(DnaB)_6$, $(F102W)_6$ and $(D82N)_6$) formed complexes with four to six molecules of the helicase loading partner, DnaC.

ESI-MS was used in conjunction with hydrogen/deuterium exchange studies to probe the unfolding mechanisms of linear and cyclised DnaB-N (the N-terminal domain of DnaB helicase) containing linkers comprised of different numbers of amino acid residues (3, 4, 5 and 9). The unfolding rates for all the cyclised proteins were about ten-fold slower than for the corresponding linear proteins. These observations suggest that enhancement of protein stability against unfolding could be achieved

through cyclisation. Furthermore, the HDX data showed that all the proteins examined exhibited a rare EX1 mechanism at near neutral pH.

ABBREVIATIONS

ε186	N-terminal domain of ε
A_{420}	Absorbance at 420 nm wavelength
ADP	Adenosine-5′-diphosphate
AMP-PNP	β, γ-imidoadenosine-5′-triphosphate
ATP	Adenosine 5′-triphosphate
BIRD	Blackbody infrared radiative dissociation
bp	Base pair
bpy	2,2′-Bipyridine
BSA	Bovine serum albumin
CD	Circular dichroism
CI	Chemical ionisation
CID	Collision-induced dissociation
Da	Dalton
DAPI	4′,6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
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-tetrahydro)phenazine
$dpqMe_2$	Dipyrido[6,7-d:2′,3′-f]2,3-dimethylquinoxaline
DSC	Differential scanning calorimetry
dsDNA	Double-stranded DNA
DTT	D, L-Dithiothreitol
$Dy(OAc)_3$	Dysprosium(III) acetate

ECD	Electron-capture dissociation
EDTA	Ethylenediaminetetraacetic acid
EI	Electron ionisation
EM	Electron microscopy
EPR	Electron paramagnetic resonance
ESI	Electrospray ionisation
FAB	Fast atom bombardment
FD	Field desorption
FTICR	Fourier transform ion cyclotron resonance
HDX	Hydrogen/deuterium exchange
HSQC	Heteronuclear single quantum correlation
HMQC	Heteronuclear multiple quantum correlation
HX	Hydrogen exchange
ICP	Inductively coupled plasma
IR	Infrared
ITC	Isothermal titration calorimetry
k_{cat}	Turnover number (Michaelis-Menten kinetics)
K_d	Dissociation constant
kDa	Kilo Dalton
KF	Klenow fragment of Pol I (contains exonuclease domain)
kV	Kilovolts
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption ionisation

$Mg(OAc)_2$	Magnesium(II) acetate
MLCT	Metal-to-ligand charge transfer
$Mn(OAc)_2$	Manganese(II) acetate
M_r	Molecular mass
MS	Mass spectrometry
MWCO	Molecular weight cut off
NH	Amide hydrogen
NH_4OAc	Ammonium acetate
NMR	Nuclear magnetic resonance
NTP	Nucleoside triphosphate
PAGE	Polyacrylamide gel electrophoresis
PAP	Purple acid phosphatase
PD	Plasma desorption
Pda	9,10-diaminophenanthrene
PEG	Polyethylene glycol
phen	1,10-Phenanthroline
pm	Picometres
*p*NP-TMP	5′-*p*-nitrophenyl ester of thymidine-5′-monophosphate
Pol I	DNA polymerase I
Pol III	DNA polymerase III
Q-ToF	Quadrupole-time-of-flight
RNA	Ribonucleic acid
SPR	Surface plasmon resonance
SUPREX	Stability of unpurified proteins from rates of H/D exchange
ssDNA	Single-stranded DNA

TMP	Thymidine-5′-monophosphate
Tris-HCl	Tris (hydroxymethyl) amino methane hydrochloride
UV	Ultraviolet
$Zn(OAc)_2$	Zinc(II) acetate

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