2006

Mass spectrometric studies of non-covalent biomolecular complexes

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Mass Spectrometric Studies of Non-Covalent Biomolecular Complexes

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

Doctor of Philosophy

from

University of Wollongong

by

Thitima Urathamakul
Bachelor of Science (Honours)

Department of Chemistry

October 2006
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

24th October 2006
ACKNOWLEDGEMENTS

While this thesis is a culmination of three years’ worth of work and study, my contribution in the form of its writing is but a small part of the overall process. The following is a list of people who have played an integral part in my life over the past several years – people who have provided me with guidance and support both immeasurable and invaluable. In short, people without whom this thesis would not have been possible.

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Last but not least, my dearest husband Min for all his endless support and patience, particularly during the tough time of writing up. Min, thank you for believing in me and for always being there for me.


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 heptamer 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$_4$OAc, 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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ε186</td>
<td>N-terminal domain of ε</td>
</tr>
<tr>
<td>A&lt;sub&gt;420&lt;/sub&gt;</td>
<td>Absorbance at 420 nm wavelength</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine-5′-diphosphate</td>
</tr>
<tr>
<td>AMP-PNP</td>
<td>β, γ-imidoadenosine-5′-triphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine 5′-triphosphate</td>
</tr>
<tr>
<td>BIRD</td>
<td>Blackbody infrared radiative dissociation</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>bpy</td>
<td>2,2'-Bipyridine</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CD</td>
<td>Circular dichroism</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical ionisation</td>
</tr>
<tr>
<td>CID</td>
<td>Collision-induced dissociation</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>DAPI</td>
<td>4′,6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dppz</td>
<td>Dipyrido[3,2-a:2',3'-c]phenazine</td>
</tr>
<tr>
<td>dpq</td>
<td>Dipyrido[3,2-d:2',3'-f]quinoxaline</td>
</tr>
<tr>
<td>dpqC</td>
<td>Dipyrido<a href="6,7,8,9-tetrahydro">3,2-a:2',3'-c</a>phenazine</td>
</tr>
<tr>
<td>dpqMe₂</td>
<td>Dipyrido[6,7-d:2',3'-f]2,3-dimethylquinoxaline</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Double-stranded DNA</td>
</tr>
<tr>
<td>DTT</td>
<td>D, L-Dithiothreitol</td>
</tr>
<tr>
<td>Dy(OAc)₃</td>
<td>Dysprosium(III) acetate</td>
</tr>
</tbody>
</table>
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
$k_{\text{cat}}$ Turnover number (Michaelis-Menten kinetics)
$K_d$ Dissociation constant
$k\text{Da}$ 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$_4$OAc  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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Chemical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMP</td>
<td>Thymidine-5′-monophosphate</td>
</tr>
<tr>
<td>Tris-HCl</td>
<td>Tris (hydroxymethyl) amino methane hydrochloride</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>Zn(OAc)$_2$</td>
<td>Zinc(II) acetate</td>
</tr>
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