Use of electrospray ionization mass spectrometry to study protein conformation and protein-protein interactions

Stephen J. Watt
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Use of Electrospray Ionization Mass Spectrometry to Study Protein Conformation and Protein-Protein Interactions

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

Doctor of Philosophy

from

University of Wollongong

by

Stephen J. Watt
Bachelor of Medicinal Chemistry (Honours)

Department of Chemistry
2005
CERTIFICATION

I, Stephen J. Watt, 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 document has not been submitted for qualification at any other academic institution.

Stephen J. Watt
23\textsuperscript{th} August 2005
For my family
PUBLICATIONS


ABSTRACT

The polymerisation of a polypeptide chain from an encoded genetic sequence allows the formation of structured molecules, known as proteins. These are essential components for a range of processes including molecular recognition, DNA replication and enzymatic functions. In this thesis, the ability of electrospray ionization (ESI) mass spectrometry (MS) to be used as a tool to determine functional properties of proteins has been explored. The coupling of ESI-MS to hydrogen deuterium exchange has been used to show how restriction of the C- and N-termini by cyclization of the polypeptide backbone can affect the ability of a protein to sample unfolded or partially unfolded states. The development of appropriate methodologies for analysis of linear (uncyclized) and cyclized systems identified a slowing of the rate of unfolding due to cyclization. The implication for the unfolding processes of proteins are discussed. An increased thermal stability of the cyclized protein was also demonstrated. This property was used to analyse the ability of ESI-MS to identify changes in protein structure from shifts in ion distributions. Important observations regarding the polarity of ionization used in these experiments are highlighted.

The effect opposite polarity ionization has on the ability to detect conformational changes in proteins and interactions with small ligands was explored using the well-characterized calmodulin-calcium-antipsychotic drug system. Important considerations regarding the binding of metal ions to protein structures are discussed in relation to the ability to unequivocally identify a conformational transition in protein structure from ESI mass spectra. An inability to detect complexes of calcium loaded-calmodulin with the antipsychotic drug trifluoperazine in the negative ion mode was observed, a result believed to be due to the Coulombic repulsions between acidic residues of calmodulin.

Finally, the non-covalent complex and interactions of the E. coli helicase (DnaB) were probed by nanoESI-MS and MS/MS studies. Development of suitable conditions allowed for identification of a previously unresolved heptamer in addition to the expected hexamer. The interaction of DnaB with its loading partner DnaC and the possible roles of ATP and ADP in this interaction were also probed with findings being related to the biological functions of these proteins.
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CHAPTER 1
GENERAL INTRODUCTION

1.1 Protein Conformational Dynamics

1.1.1 The Protein Folding Problem

1.1.2 Energy Landscape Model for Protein Folding

1.1.3 The Formation of Tertiary Structure

1.1.4 Dynamics of a Folded Protein

1.1.5 Techniques Used to Determine Conformational Dynamics of Folded Proteins

1.1.6 Mass Spectrometry Techniques used to Probe Conformational Dynamics of Proteins

1.1.6.1 Development of Mass Spectrometry for the Analysis of Biomolecules

1.1.6.2 The Charge State Distribution

1.1.6.3 Mechanism of ESI
1.1.6.4 Hydrogen Deuterium Exchange and Mass Spectrometry

1.1.6.5 Chemical Modifications

1.1.6.6 Analysis of Protein Conformations from the Charge State Distribution Observed in ESI Mass Spectra

1.2 Non-Covalent Complexes

1.2.1 Techniques to Characterise Non-Covalent Macromolecular Complexes

1.2.2 ESI-MS of Non-Covalent complexes

1.2.2.1 Conditions for Observing Non-Covalent Complexes by ESI-MS

1.2.2.2 Mass Analysers for Detecting Non-Covalent Complexes

1.2.3 Solution and Gas Phase Characteristics of Non-Covalent Complexes Between Biomolecules and Small Molecules

1.2.4 Mass Spectrometry Studies of Large Macromolecular Complexes

1.3 Scope of this Project

CHAPTER 2
MATERIALS AND METHODS

2.1. Materials

2.2 Protein Preparation

2.2.1 DnaB-N

2.2.2 Ribonuclease A and α-Lactalbumin

2.2.3 Calmodulin

2.2.4 DnaB for Preparation of (DnaB)₆ and (DnaB)₆(DnaC)ₓ

2.2.5 DnaC
2.2.6  (DnaB)_6(DnaC)_x Complexes

2.3  Protein Concentration Determination

2.4  Hydrogen/Deuterium Exchange (HDX)
  2.4.1  Preparation of Ammonium Acetate in Deuterium Oxide (D₂O) Solution
  2.4.2  Quenching Method for Deuterium Exchange
  2.4.3  Direct Injection for Deuterium Exchange

2.5  Structure Visualisation

2.6  Mass Spectrometry

CHAPTER 3
DEUTERIUM EXCHANGE CHARACTERISTICS OF LINEAR AND CYCLIZED MUTANTS OF THE N-TERMINAL OF DNAB (DNAB-N)

3.1  Introduction

3.2  Scope of Project

3.3  Results and Discussion
  3.3.1  Development of HDX Techniques for Analysis of DnaB-N
  3.3.2  Unfolding Properties of DnaB-N Mutants
  3.3.3  Effect of Temperature on Exchange Rates
  3.3.4  Confirmation of EX1 Exchange Mechanism

3.4  Conclusions
CHAPTER 4
THERMAL STABILITY OF DNAB-N MUTANTS – COMPARISON OF POSITIVE AND NEGATIVE ION ESI-MS. ERROR! BOOKMARK NOT DEFINED.

4.1 Introduction Error! Bookmark not defined.

4.2 Scope of project Error! Bookmark not defined.

4.3 Results and Discussion Error! Bookmark not defined.

4.3.1 Thermal Denaturing of X-Lin and Cz-DnaB-N Error! Bookmark not defined.

4.3.2 A Mechanism for the Opposite Polarity Ionization of DnaB-N Error! Bookmark not defined.

4.3.3 Opposite Polarity Ionization of α-Lactalbumin Error! Bookmark not defined.

4.3.4 Opposite Polarity Ionization of Ribonuclease A Error! Bookmark not defined.

4.4 Conclusion Error! Bookmark not defined.

CHAPTER 5
COMPARISON OF POSITIVE AND NEGATIVE IONIZATION ESI MASS SPECTRA OF CALMODULIN: INTERACTIONS WITH DIVALENT METALS AND ANTIPSYCHOTIC DRUGS ERROR! BOOKMARK NOT DEFINED.

5.1 Introduction Error! Bookmark not defined.

5.2 Scope of this Chapter Error! Bookmark not defined.

5.3 Results and Discussion Error! Bookmark not defined.

5.3.1 Analysis of apoCaM Error! Bookmark not defined.

5.3.2 Analysis of Calcium-Binding Properties of CaM Using Positive and Negative ion ESI Mass Spectra. Error! Bookmark not defined.

5.3.3 Ca₄CaM-TFP/IPA Interactions Error! Bookmark not defined.
CHAPTER 6
DnaB-DnaC INTERACTIONS
Error! Bookmark not defined.

6.1 Introduction
Error! Bookmark not defined.
6.1.1 The E. coli replisome
Error! Bookmark not defined.
6.1.2 Helicases
Error! Bookmark not defined.
6.1.3 DnaB
Error! Bookmark not defined.

6.2 Scope of this Chapter
Error! Bookmark not defined.

6.3 Results and Discussion
Error! Bookmark not defined.
6.3.1 Establishment of Conditions for the Analysis of DnaB
Error! Bookmark not defined.
6.3.2 Conditions for Favouring (DnaB)$_6$ or (DnaB)$_7$
Error! Bookmark not defined.
6.3.3 Dissociation of (DnaB)$_6$ and (DnaB)$_7$ by nanoESI-MS/MS
Error! Bookmark not defined.
6.3.4 Detecting Quaternary Structural Changes of DnaB
Error! Bookmark not defined.
6.3.5 Associations of the DnaB/DnaC Complex
Error! Bookmark not defined.
6.3.6 Collision-Induced Dissociation of the (DnaB)$_6$(DnaC)$_6$ Complex
Error! Bookmark not defined.
6.3.7 DnaC-ATP/ADP Interactions
Error! Bookmark not defined.

6.4 Conclusions
Error! Bookmark not defined.

REFERENCES
Error! Bookmark not defined.
APPENDIX
Error! Bookmark not defined.
LIST OF FIGURES

1.1 The secondary protein structures .........................................................2
1.2 A schematic diagram of the landscape model for protein folding ........4
1.3 The structure of some commonly observed structural motifs ..............6
1.4 An example of a charge state distribution produced by electrospray ionization mass spectrometry .........................................................12
1.5 A schematic picture of the process of the Ion Evaporation Model and the Charged Residue Model for ESI .........................................................14
1.6 An example of bimodal charge state distribution produced by electrospray ionization mass spectrometry .........................................................22
1.7 A schematic of an electrospray ionization source ..................................31
1.8 A schematic picture of a hybrid quadrupole time-of-flight mass spectrometer ..............................................................33
3.1 The process of cyclization by a split-intein system .................................60
3.2 The NMR determined structures of Cyclized, X-Linear and native DnaB-N ..........................................................62
3.3 Hydrogen deuterium exchange of Cz-DnaB-N comparing direct injection with quenching methods .........................................................66
3.4 A comparison of quenching buffers at pH 2.1 and 3.5 when analysing the hydrogen-deuterium exchange characteristics of Cz-DnaB-N ........68
3.5 A comparison of the HDX exchange characteristics of X-Lin and Cz-DnaB-N ..........................................................70
3.6 First order In plots of X-Lin-DnaB-N and Cz-DnaB-N ............................74
4.1 Positive ion ESI mass spectra of X-Lin- and Cz-DnaB-N at 40 °C and 240 °C ..........................................................82
4.2 Negative ion ESI mass spectra of X-Lin- and Cz-DnaB-N at 40 °C and 240 °C ..........................................................83
4.3 ESI-MS spectra of α-lactalbumin following treatment with DTT ..........91
6.10 ESI mass spectra of DnaC and with mixtures of ATP and ADP recorded on the QToF-2™………………………………………………..153

6.11 Ratios of intensities of DnaC-nucleotide complexes to intensities of unbound DnaC………………………………………………………….155

A.1 The hydrogen deuterium exchange of α-lactalbumin when in holo and apo forms…………………………………………………………..196

A.2 The hydrogen deuterium exchange of ribonuclease A in oxidised and reduced forms…………………………………………………………197

LIST OF TABLES

2.1 The wavelengths and extinction coefficients used to determine concentrations of proteins used in this thesis…………………………50

2.2 The protein structures and the Protein Data Bank identification codes for proteins studies in this thesis……………………………………53

2.3 ESI-MS conditions used for the analysis of protein samples on the Waters Q-ToF2™ mass spectrometer……………………………………55

2.4 MS profile used to analyse protein samples using the Waters Q-ToF2™ mass spectrometer………………………………………………56

2.5 The ESI-MS conditions used for the analysis of DnaB and DnaB/C complexes on the Waters Q-ToF Ultima™………………………………57

3.1 The rates of unfolding of X-Linear and cyclized-DnaB-N at various temperatures…………………………………………………………76

3.2 The rates of unfolding of X-Lin- and Cz-DnaB-N at various pH values……77

A.1 Extent of reduction of disulfide bonds of ribonuclease A following treatment with DTT and CH3CN……………………………………195

ABBREVIATIONS

ADP     Adenosine-5′-diphosphate

AMP-PNP Adenosine 5′-(β,γ-imido)triphosphate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Adenosine-5′-triphosphate</td>
</tr>
<tr>
<td>ATPγS</td>
<td>Adenosine-5′-(γ-thio)-triphosphate</td>
</tr>
<tr>
<td>AS</td>
<td>Aerospray</td>
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<tr>
<td>βγATP</td>
<td>β-γ-methyleneadenosine-5′-triphosphate</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CAD</td>
<td>Collisionally Activated Dissociation</td>
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<tr>
<td>CaM</td>
<td>Calmodulin</td>
</tr>
<tr>
<td>CD</td>
<td>Circular Dichroism</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical Ionization</td>
</tr>
<tr>
<td>CRM</td>
<td>Charged Residue Model</td>
</tr>
<tr>
<td>CryoEM</td>
<td>Cryoelectron Micrograph</td>
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<tr>
<td>CSD</td>
<td>Charge State Distribution</td>
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<tr>
<td>DnaB-N</td>
<td>N-Terminal of DnaB</td>
</tr>
<tr>
<td>ds</td>
<td>Double Stranded</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EI</td>
<td>Electron Ionization</td>
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<tr>
<td>EH</td>
<td>Electrohydrodynamic</td>
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<td>ESI</td>
<td>Electrospray Ionization</td>
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<tr>
<td>FIB</td>
<td>Fast Ion Bombardment</td>
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<tr>
<td>FAB</td>
<td>Fast Atom Bombardment</td>
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<tr>
<td>HDX</td>
<td>Hydrogen Deuterium Exchange</td>
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<tr>
<td>IEM</td>
<td>Ion Evaporation Model</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>IPA</td>
<td>Imipramine</td>
</tr>
<tr>
<td>ITC</td>
<td>Isothermal Titration Calorimetry</td>
</tr>
<tr>
<td>LD</td>
<td>Laser Desorption</td>
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<tr>
<td>MALDI</td>
<td>Matrix Assisted Laser Desorption Ionization</td>
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<td>MHC</td>
<td>Major Histocompatibility Complexes</td>
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<td>MS</td>
<td>Mass Spectrometry</td>
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<tr>
<td>m/z</td>
<td>Mass to Charge</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>oa</td>
<td>Orthogonal Acceleration</td>
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<tr>
<td>OAc</td>
<td>Acetate</td>
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<td>PD</td>
<td>Plasma Desorption</td>
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<td>PDB</td>
<td>Protein Data Bank</td>
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<td>Q-ToF</td>
<td>Quadrupole Time of Flight</td>
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