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## The chaperone action of $\alpha$ -crystallin

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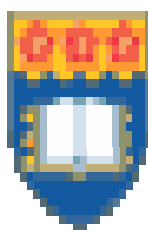
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# **The Chaperone Action of $\alpha$ -Crystallin**

**Arezou Ghahghaei, Master of Science in Chemistry**

Submitted in fulfillment of the requirement  
for Degree of Doctor of Philosophy



Department of Chemistry  
University of Wollongong  
Wollongong, AUSTRALIA

March, 2006

# Declaration of authenticity

This thesis is submitted in accordance with the regulation of the University of Wollongong in fulfillment of the degree of Doctor of Philosophy. It does not include any material previously published by another person except where due reference is made in the text. The experimental work described in this thesis is original and has not been submitted for a degree to any University.

Arezou Ghahghaei

March 2006

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# Table of Contents

## The Chaperone Action of $\alpha$ -Crystallin

|                                  |       |
|----------------------------------|-------|
| Declaration of authenticity..... | i     |
| Acknowledgements.....            | ii    |
| List of Figures .....            | xii   |
| List of Tables.....              | xix   |
| List of abbreviation used .....  | xxi   |
| Abstract.....                    | xxiii |

## Chapter 1

### Introduction

|       |   |    |
|-------|---|----|
| 1.1   | Molecular Chaperones .....                              | 1  |
| 1.1.1 | Small Heat-Shock Proteins (sHsps) .....                 | 4  |
| 1.1.2 | sHsp Structure.....                                     | 5  |
| 1.2   | $\alpha$ -Crystallin .....                              | 8  |
| 1.3   | Structure of $\alpha$ -crystallin .....                 | 9  |
| 1.3.1 | Chaperone action of $\alpha$ -crystallin .....          | 11 |
| 1.4   | Amyloid fibril formation.....                           | 12 |
| 1.5   | Macromolecular crowding .....                           | 18 |
| 1.5.1 | Effects of crowding on equilibria .....                 | 22 |
| 1.5.2 | Effects of crowding on reactions of macromolecules..... | 24 |
| 1.6   | Research purposes .....                                 | 25 |

## Chapter 2



## Materials and Methods

|         |   |    |
|---------|---|----|
| 2.1     | Materials .....   | 28 |
| 2.2     | Methods .....   | 29 |
| 2.2.1   | Isolation and purification of $\alpha$ -, $\beta$ - and $\gamma$ -crystallins from bovine eye lenses .....  | 29 |
| 2.2.2   | Visible Absorption Spectroscopy .....   | 30 |
| 2.2.2.1 | Reduction stress assay .....  | 30 |
| 2.2.2.2 | Heat-stress assay.....  | 31 |
| 2.2.3   | Fibril formation and kinetic evaluation .....   | 32 |
| 2.2.4   | Intrinsic fluorescence spectroscopy.....  | 33 |
| 2.2.5   | ANS binding experiments .....   | 34 |
| 2.2.6   | Circular Dichroism (CD) spectroscopy of $\kappa$ -casein and $\alpha$ -crystallin .....   | 35 |
| 2.2.7   | Nuclear Magnetic Resonance (NMR) Spectroscopy .....   | 36 |
| 2.2.7.1 | $^1\text{H}$ NMR spectroscopy of $\alpha$ -lactalbumin and $\kappa$ -casein in the presence and absence of $\alpha$ -crystallin and dextran ..... | 36 |
| 2.2.7.2 | TOCSY and NOESY experiments .....   | 37 |
| 2.2.8   | Size exclusion HPLC .....   | 38 |
| 2.2.8.1 | Size exclusion HPLC of $\kappa$ -casein in the presence and absence of different concentration of dextran .....                                   | 38 |
| 2.2.8.2 | Time course size exclusion HPLC of $\kappa$ -casein in the presence and absence of 10% w/v dextran .....  | 39 |
| 2.2.9   | Transmission electron microscopy (TEM) of $\alpha_s$ - and $\kappa$ -caseins...   | 40 |

|            |  |    |
|------------|--|----|
| 2.2.10     | Isolation and purification of $\alpha$ A- and $\alpha$ B-crystallin from bovine $\alpha$ -crystallin .....   | 40 |
| 2.2.11     | Protein characterization by sodium dodecyl sulphate polyacrylamide gel electrophoresis.....  | 41 |
| 2.2.12     | Subunit exchange of $\alpha$ A-crystallin.....   | 42 |
| 2.2.12.1   | Labeling of recombinant $\alpha$ A-crystallin with fluorescence probes .....   | 42 |
| 2.2.12.2   | Measurement of the rate of subunit exchange.....   | 43 |
| 2.2.12.2.1 | The effect of 10% w/v dextran on the rate of subunit exchange in $\alpha$ A-crystallin in the presence and absence of reduced ovotransferrin ..... | 44 |

## **Chapter 3**

### **The effect of dextran on the amorphous aggregation of target proteins and the chaperone action of $\alpha$ -crystallin**

|       |  |    |
|-------|--|----|
| 3.1   | Purification of $\alpha$ -crystallin .....   | 47 |
| 3.2   | Visible Absorption Spectroscopy.....   | 48 |
| 3.2.1 | The effect of dextran on the aggregation of reduced ovotransferrin .....   | 49 |
| 3.2.2 | The effect of dextran on the chaperone action of $\alpha$ -crystallin preventing the aggregation of reduced ovotransferrin ..... | 51 |
| 3.2.3 | The effect of dextran on the aggregation of reduced insulin.....   | 54 |
| 3.2.4 | The effect of dextran on the chaperone action of $\alpha$ -crystallin with aggregating reduced insulin .....                     | 56 |

|       |   |    |
|-------|---|----|
| 3.2.5 | The effect of dextran on the chaperone action of $\alpha$ -crystallin with aggregating reduced $\alpha$ -lactalbumin .....  | 56 |
| 3.2.6 | The effect of dextran on the aggregation of heated $\beta_L$ -crystallin .....  | 60 |
| 3.2.7 | The effect of dextran on the chaperone action of $\alpha$ -crystallin with heated $\beta_L$ -crystallin .....   | 62 |
| 3.3   | Intrinsic tryptophan fluorescence spectroscopy .....  | 64 |
| 3.3.1 | Changes in intrinsic tryptophan fluorescence of $\alpha$ -crystallin upon interaction with reduced insulin in the presence of 10% w/v dextran.....                                  | 64 |
| 3.4   | ANS fluorescence studies: Interaction of insulin with $\alpha$ -crystallin in the presence of dextran .....   | 68 |
| 3.5   | Changes in intrinsic tryptophan fluorescence of $\alpha$ -lactalbumin upon interaction with $\alpha$ -crystallin in the presence and absence of 10% w/v dextran .....               | 71 |
| 3.6   | ANS fluorescence studies: Interaction of $\alpha$ -lactalbumin with $\alpha$ -crystallin in the presence and absence of dextran .....   | 74 |
| 3.7   | Unfolding and aggregation of reduced $\alpha$ -lactalbumin in the presence and in the absence of 10% w/v dextran (as monitored by real-time 1D $^1\text{H}$ NMR spectroscopy) ..... | 76 |
| 3.8   | $^1\text{H}$ NMR spectroscopy of $\alpha$ -lactalbumin in the presence and absence of dextran.....  | 82 |
| 3.9   | Discussion.....   | 86 |
| 3.10  | Conclusions .....   | 94 |

## Chapter 4

## **The effect of $\alpha$ -crystallin on the fibril formation of target protein in the presence and absence of dextran**

|         |  |     |
|---------|--|-----|
| 4.1     | Thioflavin T binding assays with $\alpha$ -lactalbumin, $\alpha_s$ - and $\kappa$ -casein ....   | 98  |
| 4.1.1   | Thioflavin T binding assays with $\alpha$ -lactalbumin.....  | 98  |
| 4.1.2   | Thioflavin T binding assays with $\alpha_s$ -casein .....  | 100 |
| 4.1.3   | Thioflavin T binding assay of $\alpha_s$ -casein in the presence of $\alpha$ -crystallin and 10% w/v dextran .....                         | 102 |
| 4.1.3.1 | Transmission electron microscopy (TEM) of reduced $\alpha_s$ -casein in the presence and absence of dextran and $\alpha$ -crystallin ..... | 103 |
| 4.1.4   | Thioflavin T binding assay of $\kappa$ -casein in presence of $\alpha$ -crystallin and 10% w/v dextran .....                               | 104 |
| 4.1.5   | Transmission electron microscopy (TEM) of reduced $\kappa$ -casein in the presence and absence of dextran and $\alpha$ -crystallin .....   | 109 |
| 4.2     | Intrinsic tryptophan fluorescence spectroscopy of $\kappa$ -casein with $\alpha$ -crystallin and 10% w/v dextran .....                     | 111 |
| 4.3     | ANS fluorescence studies: Interaction of $\kappa$ -casein with $\alpha$ -crystallin in the presence and absence of 10% w/v dextran .....   | 113 |
| 4.3.1   | $\kappa$ -Casein in the presence of dextran and $\alpha$ -crystallin.....  | 113 |
| 4.3.2   | Reduced $\kappa$ -casein in the presence of dextran and $\alpha$ -crystallin ..  | 114 |
| 4.4     | Circular Dichroism (CD) spectroscopy of $\kappa$ -casein .....   | 116 |
| 4.5     | $^1\text{H}$ NMR spectra of $\kappa$ -casein aggregation in the presence and absence of 10% w/v dextran after the addition of DTT .....    | 118 |

|      |  |     |
|------|--|-----|
| 4.6  | 1D and 2D $^1\text{H}$ NMR spectroscopy of $\kappa$ -casein in the presence and absence of dextran. ....                               | 121 |
| 4.7  | Size exclusion HPLC.....   | 126 |
| 4.8  | Size exclusion HPLC time-course: change in the aggregate size of $\kappa$ -casein in the presence and absence of 10% w/v dextran ..... | 129 |
| 4.9  | Discussion.....  | 134 |
| 4.10 | Conclusion .....   | 139 |

## Chapter 5

### The effect of dextran on the rate of subunit exchange of $\alpha\text{A}$ -crystallin

|         |  |     |
|---------|--|-----|
| 5.1     | Purification of $\alpha\text{A}$ - and $\alpha\text{B}$ -crystallin .....  | 143 |
| 5.2     | Chaperone activity of $\alpha\text{A}$ - and $\alpha\text{B}$ -crystallin subunits.....  | 145 |
| 5.2.1   | Visible absorption spectroscopy of heated $\beta_{\text{L}}$ -crystallin in the presence of $\alpha$ -, $\alpha\text{A}$ - and $\alpha\text{B}$ -crystallin .....  | 145 |
| 5.2.2   | The chaperone activity of $\alpha$ - and $\alpha\text{A}$ -crystallin in the presence and absence of dextran at 37°C, 42°C and 45°C. ....                          | 147 |
| 5.3     | Subunit exchange rates of $\alpha\text{A}$ -crystallin .....   | 150 |
| 5.3.1   | Labeling of $\alpha\text{A}$ -crystallin with lucifer yellow iodoacetamide (LYI) and 4-acetamido-4'-((iodoacetyl)amino)-stilbene-2,2'-disulfonic acid (AIAS) ..... | 150 |
| 5.3.2   | Fluorescence resonance energy transfer (FRET) measurements during subunit exchange in $\alpha\text{A}$ -crystallin .....   | 153 |
| 5.3.2.1 | Determination of the rate constant of subunit exchange in  |     |

|  |     |
|--|-----|
| labeled $\alpha$ A-crystallin by FRET .....  | 153 |
| 5.3.2.2 The effect of 10% w/v dextran on the rate of subunit exchange of $\alpha$ A-crystallin at 37°C. ....   | 154 |
| 5.3.2.3 The effect of reduced ovotransferrin on the rate of subunit exchange of labeled $\alpha$ A-crystallin in the presence and absence of 10% w/v dextran ..... | 158 |
| 5.3.2.4 The effect of molecular crowding on subunit exchange rate of $\alpha$ A-crystallin at different temperatures.....  | 162 |
| 5.4 Circular Dichroism (CD) spectroscopy of $\alpha$ -crystallin in the presence and absence of dextran at different temperatures .....                            | 169 |
| 5.5 Intrinsic fluorescence of $\alpha$ -crystallin in the presence and absence of dextran at different temperatures.....   | 172 |
| 5.6 Discussion.....  | 175 |
| 5.7 Conclusions .....  | 181 |

## **Chapter 6**

### **The effect of $\alpha$ -crystallin on the heat-induced aggregation of $\beta$ -lactoglobulin**

|   |     |
|---|-----|
| 6.1 The effect of pH on the aggregation of $\beta$ -lactoglobulin.....  | 184 |
| 6.2 The effect of dextran on heated $\beta$ -lactoglobulin at different pH values .....   | 185 |
| 6.3 The chaperone action of $\alpha$ -crystallin in preventing aggregation of heated $\beta$ -lactoglobulin at different pH values..... | 187 |
| 6.4 Chaperone action of $\alpha$ -crystallin in preventing the aggregation of   |     |

|  |     |
|--|-----|
| reduced and heated $\beta$ -lactoglobulin at different pH values ..... | 191 |
| 6.5 Discussion.....  | 194 |
| 6.6 Conclusion .....   | 198 |

## Chapter 7

### Nuclear Magnetic Resonance (NMR) spectroscopic and chaperone studies of dogfish $\alpha$ -crystallin

|   |     |
|---|-----|
| 7.1 $^1\text{H}$ 2D TOCSY and NOESY NMR spectroscopy of dogfish $\alpha$ -crystallin .....                        | 201 |
| 7.2 Chaperone assay of dogfish $\alpha$ -crystallin .....   | 211 |
| 7.2.1 Reduction assay of dogfish $\alpha$ -crystallin .....   | 211 |
| 7.2.2 Heat stress assay of $\beta_L$ -crystallin in the presence and absence of dogfish $\alpha$ -crystallin..... | 213 |
| 7.3 Discussion.....   | 215 |
| 7.4 Conclusion .....  | 217 |

## Chapter 8

### Conclusions and future directions

### Appendix

|  |     |
|--|-----|
| Buffers .....                            | 225 |
| NMR sample buffer.....                   | 225 |
| SDS-Page reagents.....                   | 226 |
| Subunit exchange experiment buffer ..... | 227 |

### References

|                 |     |
|-----------------|-----|
| Reference ..... | 228 |
|-----------------|-----|

# List of Figures

## Chapter 1: Introduction

Figure 1.1: The putative mechanism of chaperone action of  $\alpha$ -crystallin to prevent aggregation and subsequent precipitation of stressed proteins .....6

Figure 2.1: Oligomeric arrangement of subunits in *T. aestivum* Hsp16.9 and *M. jannaschii* Hsp16.5.....7

Figure 3.1: A model for quaternary structure of  $\alpha$ -crystallin ..... 10

Figure 4.1: Modeling of an amyloid fibril structure..... 14

Figure 5.1: X-ray diffraction pattern of PI3-SH3 fibrils..... 15

Figure 6.1: A proposed mechanism for lysozyme amyloid fibril formation ..... 16

Figure 7.1: Representation of macromolecules inside a cell of *Escherichia coli*19

Figure 8.1: Excluded volume available in macromolecular solutions .....21

## Chapter3: The effect of dextran on the amorphous aggregation of target proteins and the chaperone action of $\alpha$ -crystallin

Figure 3.1: Elution profile for lens crystallins on a Sephacryl S-300 column.....48

Figure 3.2: Ovotransferrin aggregation in the presence of dextran (w/v .....50

Figure 3.3: Ovotransferrin aggregation in the presence and absence of  $\alpha$ -crystallin and 10% w/v dextran.....52



|   |    |
|---|----|
| Figure 3.4: Insulin aggregation in the presence of dextran (w/v.....  | 54 |
| Figure 3.5: Insulin aggregation in the presence and absence of $\alpha$ -crystallin and 10% w/v dextran .....   | 56 |
| Figure 3.6: $\alpha$ -Lactalbumin aggregation in the presence of $\alpha$ -crystallin.....  | 58 |
| Figure 3.7: $\alpha$ -Lactalbumin aggregation in the presence of 10% w/v dextran and $\alpha$ -crystallin .....   | 59 |
| Figure 3.8: $\beta_L$ -Crystallin aggregation in the presence of different concentrations of dextran (w/v .....   | 60 |
| Figure 3.9: $\beta_L$ -Crystallin aggregation in the presence and absence of $\alpha$ -crystallin and 10% w/v dextran .....   | 62 |
| Figure 3.10: The intrinsic tryptophan fluorescence spectra of insulin, $\alpha$ -crystallin (1:1 molar ratio) and 10% w/v dextran .....                               | 64 |
| Figure 3.11: The maximum intrinsic fluorescence and average $\lambda_{\max}$ of insulin, $\alpha$ -crystallin (1:1 molar ratio) and 10% w/v dextran .....             | 66 |
| Figure 3.12: ANS binding and average maximal fluorescence to insulin, $\alpha$ -crystallin and 10% w/v dextran in the presence and absence of DTT .....               | 68 |
| Figure 3.13: The intrinsic tryptophan fluorescence spectra of $\alpha$ -lactalbumin, $\alpha$ -crystallin and 10% w/v dextran.....                                    | 71 |
| Figure 3.14: The maximum intrinsic fluorescence and the average $\lambda_{\max}$ of $\alpha$ -lactalbumin, $\alpha$ -crystallin and 10% w/v dextran .....             | 72 |
| Figure 3.15: ANS binding and average maximal fluorescence to $\alpha$ -lactalbumin, $\alpha$ -crystallin and 10% w/v dextran in the presence and absence of DTT ..... | 74 |
| Figure 3.16: The real-time $^1\text{H}$ 1D NMR of apo $\alpha$ -lactalbumin in the presence and absence of $\alpha$ -crystallin and 10% w/v dextran .....             | 80 |

Figure 3.17: Decay in the Tyr (3,5) resonance at 6.8 ppm of reduced  $\alpha$ -lactalbumin in the presence and absence of  $\alpha$ -crystallin and dextran.....81

Figure 3.18: The apparent first order rate constant of signal decay at 6.8 ppm of reduced  $\alpha$ -lactalbumin.....81

Figure 3.19: 1D  $^1\text{H}$  NMR of  $\alpha$ -lactalbumin in the presence and absence of 5% w/v dextran .....84

Figure 3.20: The aromatic region of a TOCSY spectrum of  $\alpha$ -lactalbumin in the presence and absence of 5% w/v dextran .....85

## **Chapter 4: The effect of $\alpha$ -crystallin on the fibril formation of target protein in the presence and absence of dextran**

Figure 4.1:  $\alpha$ -Lactalbumin amyloid formation in the presence and absence of different concentration of dextran.....99

Figure 4.2: Amyloid fibril formation of native  $\alpha_s$ -casein in presence and absence of different concentration of dextran.....100

Figure 4.3: Amyloid fibril formation of reduced  $\alpha_s$ -casein in the presence and absence of different concentration of dextran .....101

Figure 4.4: Amyloid fibril formation of reduced  $\alpha_s$ -casein in the presence and absence of  $\alpha$ -crystallin and 10% w/v dextran.....102

Figure 4.5: Negatively stained electron micrograph of reduced  $\alpha_s$ -casein in the presence and absence of 10% w/v dextran .....104

Figure 4.6:  $\kappa$ -casein amyloid formation in the presence and absence of  $\alpha$ -crystallin.....105

Figure 4.7:  $\kappa$ -casein amyloid formation in the presence and absence of  $\alpha$ -crystallin in a plate reader .....106

|   |     |
|---|-----|
| Figure 4.8: $\kappa$ -casein amyloid formation in the presence and absence of $\alpha$ -crystallin and 10% w/v dextran.....                                     | 107 |
| Figure 4.9: Negatively stained electron micrograph of reduced $\kappa$ -casein in the presence and absence of $\alpha$ -crystallin and 10% w/v dextran .....    | 110 |
| Figure 4.10: The intrinsic tryptophan fluorescence spectra of $\kappa$ -casein, $\alpha$ -crystallin and dextran .....  | 111 |
| Figure 4.11: A: The maximum intrinsic fluorescence and the average $\lambda_{\max}$ of $\kappa$ -casein, $\alpha$ -crystallin and 10% w/v dextran .....         | 112 |
| Figure 4.12: ANS binding and average maximal fluorescence of $\kappa$ -casein, $\alpha$ -crystallin and 10% w/v dextran in the presence and absence of DTT..... | 115 |
| Figure 4.13: Near-UV CD spectra of the native state, the reduced state and the dextran-induced species of $\kappa$ -casein.....                                 | 118 |
| Figure 4.14: The aromatic region of series 1D $^1\text{H}$ NMR spectra of reduced $\kappa$ -casein in the presence and absence of 10% w/v dextran .....         | 119 |
| Figure 4.15: First order decay plots of reduced $\kappa$ -casein in tyrosine (3,5) ring proton resonance with time .....  | 121 |
| Figure 4.16: $^1\text{H}$ 1D NMR spectra of $\kappa$ -casein in the presence and absence of 5% w/v dextran .....  | 123 |
| Figure 4.17: The aromatic region of a TOCSY spectrum of $\kappa$ -casein in the presence and absence of 5% w/v dextran .....                                    | 125 |
| Figure 4.18: HPLC standard curve.....   | 127 |
| Figure 4.19: Size exclusion HPLC traces for $\kappa$ -casein aggregates in the presence and absence of different concentration of dextran .....                 | 128 |
| Figure 4.20: HPLC standard curve.....   | 130 |

|   |     |
|---|-----|
| Figure 4.21: Size exclusion HPLC traces for $\kappa$ -casein aggregate in the presence and absence of dextran ..... | 131 |
|---|-----|

|   |     |
|---|-----|
| 4.22. Average molecular masses (kDa) aggregates of $\kappa$ -casein around the 7.5-6.70 minute retention time in the presence and absence of 10% w/v dextran with different incubation times..... | 132 |
|---|-----|

|   |     |
|---|-----|
| 4.23. Thioflavin T binding assay of $\kappa$ -casein in the presence and absence of dextran at different time ..... | 133 |
|---|-----|

## **Chapter 5: The effect of dextran on the rate of subunit exchange of $\alpha$ A-crystallin**

|  |     |
|--|-----|
| Figure 5.1: Elution profile for $\alpha$ -crystallin subunits on a G-75 Sephadex size-exclusion column ..... | 144 |
|--|-----|

|   |     |
|---|-----|
| Figure 5.2: SDS-PAGE gel of purified $\alpha$ A- and $\alpha$ B-crystallin from $\alpha$ -crystallin. | 145 |
|---|-----|

|  |     |
|--|-----|
| Figure 5.3: $\beta_L$ -Crystallin aggregation in the presence and absence of $\alpha$ -, $\alpha$ A- and $\alpha$ B-crystallin ..... | 146 |
|--|-----|

|  |     |
|--|-----|
| Figure 5.4: Ovotransferrin aggregation in the presence of $\alpha$ - or $\alpha$ A-crystallin and 10% w/v dextran at 37°C, 42°C, and 45°C..... | 148 |
|--|-----|

|  |     |
|--|-----|
| Figure 5.5: Effect of temperature on chaperone action of $\alpha$ A- and $\alpha$ -crystallin from light scattering profiles of reduced ovotransferrin at 37, 42 and 45°C..... | 150 |
|--|-----|

|  |     |
|--|-----|
| Figure 5.6: Elution profile for AIAS-labeled and LYI-labeled $\alpha$ A-crystallin on a Sephadex G-25 column ..... | 152 |
|--|-----|

|   |     |
|---|-----|
| Figure 5.7 Time dependent changes in the emission spectra of AIAS-labeled $\alpha$ A-crystallin due to subunit exchange ..... | 154 |
|---|-----|

|  |     |
|--|-----|
| Figure 5.8 Time-dependent changes in AIAS and LYI fluorescence during subunit exchange of labeled $\alpha$ A-crystallin in the absence of 10% w/v dextran at 37°C..... | 156 |
|--|-----|

Figure 5.9 Time-dependent changes in AIAS and LYI fluorescence during subunit exchange of labeled  $\alpha$ A-crystallin in the presence of 10% w/v dextran at 37°C ..... 157

Figure 5.10 Time-dependent changes in AIAS and LYI of labeled  $\alpha$ A-crystallin and ovotransferrin at 37°C ..... 159

Figure 5.11 Time-dependent changes in AIAS and LYI of labeled  $\alpha$ A-crystallin in the presence of ovotransferrin and 10% w/v dextran at 37°C ..... 160

Figure 5.12 Time-dependent changes in AIAS and LYI of labeled  $\alpha$ A-crystallin in the presence and absence of 10% w/v dextran at 42°C ..... 163

Figure 5.13 Time-dependent changes in AIAS and LYI fluorescence of labeled  $\alpha$ A-crystallin in the presence and absence of 10% w/v dextran at 45°C ..... 165

Figure 5.14: Temperature-dependent changes in the rate of  $\alpha$ A-crystallin subunit exchange in the presence and absence of 10% w/v dextran..... 168

Figure 5.15: Near-UV CD spectra of  $\alpha$ -crystallin at temperatures between 25°C and 48°C in the presence and absence of 10% w/v dextran..... 170

Figure 5.16: Decrease in the ellipticity of the CD at 264 nm of  $\alpha$ -crystallin in the presence and absence of 10% w/v dextran at different temperatures ..... 171

Figure 5.17: Intrinsic fluorescence of  $\alpha$ -crystallin at temperatures between 25°C and 48°C in the presence and absence of dextran ..... 173

Figure 5.18: Intrinsic fluorescence of L-tryptophan at temperatures between 25°C and 48°C in the presence and absence of dextran ..... 174

## **Chapter 6: The effect of $\alpha$ -crystallin on the heat-induced aggregation of $\beta$ -lactoglobulin**

Figure 6.1: Heat induced aggregation of  $\beta$ -lactoglobulin at different pH values 184

|  |     |
|--|-----|
| Figure 6.2: Heat induced aggregation of $\beta$ -lactoglobulin in the presence and absence of dextran and different pH values..... | 187 |
|--|-----|

|   |     |
|---|-----|
| Figure 6.3: Heat induced aggregation of $\beta$ -lactoglobulin in the presence and absence of $\alpha$ -crystallin at different pH values ..... | 189 |
|---|-----|

|  |     |
|--|-----|
| Figure 6.4: SDS-PAGE gels of pellet and supernatant of $\beta$ -lactoglobulin aggregation..... | 191 |
|--|-----|

|   |     |
|---|-----|
| Figures 6.5: Heat induced aggregation of $\beta$ -lactoglobulin in the presence and absence of $\alpha$ -crystallin and DTT at different pH values..... | 194 |
|---|-----|

## **Chapter 7: Nuclear Magnetic Resonance (NMR) spectroscopic and chaperone studies of dogfish $\alpha$ -crystallin**

|   |     |
|---|-----|
| Figure 7.1: $^1\text{H}$ 1D NMR of dogfish $\alpha$ -crystallin ..... | 204 |
|---|-----|

|  |     |
|--|-----|
| Figure 7.2: TOCSY and WET NOESY spectra of dogfish $\alpha$ -crystallin..... | 204 |
|--|-----|

|   |     |
|---|-----|
| Figure 7.3: A portion of the aliphatic region of a TOCSY spectrum of dogfish $\alpha$ -crystallin showing the proline residue assignments ..... | 206 |
|---|-----|

|   |     |
|---|-----|
| Figure 7.4: Deviation from random coil chemical shifts for the $^1\text{H}$ $\alpha$ -CH resonances of the C-terminal domain of dogfish $\alpha$ -crystallin..... | 210 |
|---|-----|

|  |     |
|--|-----|
| Figure 7.5: Insulin aggregation in the presence and absence of dogfish $\alpha$ -crystallin..... | 212 |
|--|-----|

|   |     |
|---|-----|
| Figure 7.6: $\beta$ -Crystallin aggregation in the presence and absence of different concentration of dogfish $\alpha$ -crystallin..... | 214 |
|---|-----|

|  |     |
|--|-----|
| Figure 7.7: Amino acid sequences in the C-terminal region of the A and B subunits of dogfish and bovine..... | 216 |
|--|-----|

# List of Tables

## **Chapter 4: The effect of $\alpha$ -crystallin on the fibril formation of target protein in the presence and absence of dextran**

|   |     |
|---|-----|
| Table 4.1: Summary of rate constants for reduced $\kappa$ -casein in thioflavinT binding assays ..... | 108 |
|---|-----|

## **Chapter 5: The effect of dextran on the rate of subunit exchange of $\alpha$ A-crystallin**

|  |     |
|--|-----|
| Table 5.1: Summary of rate constants for ovotransferrin aggregation in reduction assay ..... | 149 |
|--|-----|

|  |     |
|--|-----|
| Table 5.2: Summary of apparent rate constants for subunit exchange experiments of $\alpha$ A-crystallin under different conditions at 37°C ..... | 161 |
|--|-----|

|   |     |
|---|-----|
| Table 5.3: Summary of rate constants for subunit exchange experiments of AIAS and LYI-labeled $\alpha$ A-crystallin at 37, 42, and 45°C ..... | 166 |
|---|-----|

|   |     |
|---|-----|
| Table 5.4: Summary of time for complete exchange of labeled $\alpha$ A-crystallin in various temperatures ..... | 167 |
|---|-----|

## **Chapter 6: The effect of $\alpha$ -crystallin on the heat-induced aggregation of $\beta$ -lactoglobulin**

|   |     |
|---|-----|
| Table 6.1: Summary of rate constants for aggregation of $\beta$ -lactoglobulin at different pH values ..... | 185 |
|---|-----|

|   |     |
|---|-----|
| Table 6.2: Summary of rate constants for the aggregation of $\beta$ -lactoglobulin in the presence and absence of $\alpha$ -crystallin at different pH values ..... | 190 |
|---|-----|

|  |     |
|--|-----|
| Table 6.3: Summary of rate constants for the aggregation of reduced and heated $\beta$ -lactoglobulin at different pH values ..... | 194 |
|--|-----|

## **Chapter 7: Nuclear Magnetic Resonance (NMR) spectroscopic and chaperone studies of dogfish $\alpha$ -crystallin**

|  |     |
|--|-----|
| Table 7.1: $^1\text{H}$ chemical shifts of C-terminal residues of dogfish $\alpha\text{B}$ -crystallin.. | 207 |
|--|-----|

|   |     |
|---|-----|
| Table 7.2 Chemical shifts for $\alpha\text{-CH}$ resonances of dogfish $\alpha\text{B}$ -crystallin compared to random coil values of Wishart et al. (1995) ..... | 208 |
|---|-----|



## List of abbreviation used

|                       |   |
|-----------------------|---|
| 1D                    | one-dimensional   |
| $^1\text{H}$          | proton  |
| $\alpha$ T-crystallin | purified $\alpha$ -crystallin containing $\alpha$ A and $\alpha$ B subunits |
| AIAS                  | 4-acetamido-4'-((iodoacetyl) amino) stilbene-2, 2'-disulfonic acid          |
| ANS                   | 1-anilino-8-naphthalene sulfonic acid                                       |
| CD                    | Circular Dichroism  |
| DTT                   | dithiothreitol  |
| D <sub>2</sub> O      | deuterium oxide   |
| EDTA                  | ethylenediaminetetraacetic acid   |
| FRET                  | Fluorescence resonance energy transfer                                      |
| g                     | gram  |
| KDa                   | kilodalton  |
| L                     | litre   |
| LYI                   | Lucifer yellow iodoacetamide  |
| mg                    | milligram   |
| $\mu\text{L}$         | microlitre  |
| mL                    | milliliter  |
| mM                    | mili molar  |
| $\mu\text{M}$         | micro molar   |
| min                   | minute  |
| MW                    | molecular weight  |
| NMR                   | nuclear magnetic resonance  |
| NOESY                 | Nuclear Overhauser Effect Spectroscopy                                      |

|          |  |
|----------|--|
| ppm      | parts per million  |
| SDS-PAGE | sodium dodecyl sulphate polyacrylamide gel electrophoresis |
| sHsp     | small heat-shock proteins                                  |
| TEM      | transmission electron microscopy                           |
| ThT      | Thioflavin T   |
| TOCSY    | Total correlation spectroscopy                             |

# Abstract

$\alpha$ -Crystallin is the principal lens protein. It is a member of the small heat shock protein family (sHsp) and acts as a molecular chaperone by stabilizing proteins under stress conditions through the formation of a soluble sHsp target protein complex to prevent their aggregation.

Macromolecular crowding is ubiquitous in all types of cells and describes the normal conditions inside a cell. The concentration of macromolecules inside a cell is very high (up to 300 mg/mL) arising from species such as polysaccharides, proteins and nucleic acids and therefore it greatly promotes the self-assembly of proteins. Thus, there is a major difference between *in vivo* and *in vitro* conditions such as those used in most studies of protein behaviour and properties.

Appropriate destabilizing conditions (i.e. heating or reduction) cause damage and misfolding of proteins, which as a result expose previously buried hydrophobic regions. Hydrophobic interactions of nearby molecules cause self-association and aggregate formation. Aggregation of intermediately folded peptide or protein molecules also leads to the formation of amyloid fibrils, highly-ordered  $\beta$ -sheet structures associated with a number of neuro-degenerative disorders such as Alzheimer's, Parkinson's, and Creutzfeldt-Jakob diseases.

*In vitro*, much work has been published on the interactions of  $\alpha$ -crystallin with target proteins in dilute solutions. In order to better understand the chaperone activity of  $\alpha$ -crystallin under conditions more closely resembling the intracellular environment, its interaction with a range of destabilized proteins (ovotransferrin,  $\beta_L$ -crystallin, insulin,  $\alpha$ -lactalbumin,  $\alpha_s$ - and  $\kappa$ -casein) in the presence of dextran (68 kDa) have been examined using visible absorption spectroscopy, tryptophan fluorescence spectroscopy, ANS binding, TEM, HPLC and NMR spectroscopy studies.

In the presence of dextran, the rate and extent of aggregation of reduced ovotransferrin, insulin,  $\alpha$ -lactalbumin and  $\beta_L$ -crystallin was accelerated. Under these conditions,  $\alpha$ -crystallin was less effective in preventing aggregation and precipitation of target proteins. It is proposed that a kinetic competition exists between aggregation of target proteins and the chaperone action of  $\alpha$ -crystallin which supports the hypothesis that  $\alpha$ -crystallin interacts more effectively with slowly aggregating rather than rapidly aggregating target proteins.

Amyloid fibril formation by  $\alpha$ -lactalbumin,  $\alpha_s$ - and  $\kappa$ -casein was verified by a sigmoidal increase in Thioflavin T fluorescence over time.  $\alpha$ -Crystallin prevented amyloid formation in  $\alpha_s$ - and  $\kappa$ -casein. In the presence of dextran, the rate of amyloid formation by  $\alpha$ -lactalbumin,  $\alpha_s$ - and  $\kappa$ -casein was enhanced. Under these conditions,  $\alpha$ -crystallin was less effective in preventing amyloid formation of  $\kappa$ -casein and this was supported by TEM, CD, NMR spectroscopy and HPLC

studies.

Subunit exchange is an important feature of sHsp chaperone action. This study found that subunit exchange of  $\alpha$ A-crystallin increased with increasing temperature and decreased as a result of interaction with reduced ovotransferrin. It was further demonstrated that the presence of the dextran markedly reduced the rate of subunit exchange of  $\alpha$ A-crystallin and with increasing temperature, this effect was exacerbated. Moreover, in the presence of reduced ovotransferrin, dextran further slowed the subunit exchange of  $\alpha$ A-crystallin.

Aggregation of  $\beta$ -lactoglobulin occurs mainly via intermolecular disulphide bond exchange. Upon heating,  $\beta$ -lactoglobulin aggregated which increased with increasing pH. The presence of dextran or DTT led to more rapid aggregation and precipitation of  $\beta$ -lactoglobulin.  $\alpha$ -Crystallin prevented the aggregation of heat-stressed  $\beta$ -lactoglobulin and was a more efficient chaperone at higher pH values. In the presence of DTT, however,  $\alpha$ -crystallin was a less efficient chaperone due to faster aggregation of heated and reduced  $\beta$ -lactoglobulin.

In order to obtain further structural and functional information on the C-terminal extension,  $\alpha$ -crystallin from dogfish (*Squalus acanthias*) was studied to allow comparisons with bovine  $\alpha$ -crystallin to be made. Chaperone assays under heat and reduction stresses as well as NMR spectroscopy showed that the C-terminal extension of dogfish  $\alpha$ -crystallin was very flexible and had a similar

structure and function to that of bovine  $\alpha$ -crystallin. Its chaperone action under heat stress was found to be comparable to bovine  $\alpha$ -crystallin but it was a better chaperone under reduction stress.