Structural Aspects of $\beta$-Lactoglobulin during Self-assembly into Amyloid-like Fibrils

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Abstract

This study explores the structural characteristics of $\beta$-lactoglobulin ($\beta$-Lg) during its self-assembly into long amyloid-like fibrils on heating at low pH and low ionic strength. $\beta$-Lg (1%, w/v) was heated at 80°C, pH 2 and low ionic strength and the different processes occurring during self-assembly were characterized using a variety of techniques including circular dichroism spectroscopy, sodium dodecyl sulfate polyacrylamide gel electrophoresis, and mass spectrometry.

The results of this study indicate that fibril formation from $\beta$-Lg self-assembly consists of four processes: 1) protein unfolding; 2) heat- and acid-induced protein hydrolysis; 3) nuclei formation; and 4) growth of nuclei into mature fibrils by peptide self-assembly. It was found that the heat-induced unfolding of $\beta$-Lg and its acid hydrolysis promoted self-assembly by removing structural constraints and generating assembly-capable peptides. The peptides from the N-terminal region (1-53) of $\beta$-Lg were found to play an important role during nucleation and may form the core of the fibrils. The characterization of fibril composition strongly indicated the presence of disulfide bonding in fibrils and the native disulfide bond Cys66-Cys160 in $\beta$-Lg appeared to be conserved during fibril formation.

The substitution of amino acid residues in $\beta$-Lg variants A, B and C did not significantly affect the kinetics of different self-assembly processes. The fibrils from $\beta$-Lg A, B and C had similar morphology, but were slightly different in their peptide compositions. The latter may be explained on the basis of sites of genetic substitutions, in particular, the Asp64 of $\beta$-Lg A that is Gly in variants B and C. In comparison, glucosylation and lactosylation of $\beta$-Lg strongly inhibited fibril formation primarily by inhibition of peptide self-assembly due to the steric conformational
restrictions. The inhibitory effect of glycation varied with the type of sugar and the degree of glycation. Lactosylation produced a stronger effect than glucosylation, but glycation with either sugar did not appear to have any effect on the morphology of fibrils.

The modification of the aqueous phase composition by glycerol and sorbitol (0-50 % w/v) greatly decreased the rate of β-Lg self-assembly and the effect of glycerol and sorbitol on β-Lg self-assembly was concentration-dependent. Sorbitol inhibited the self-assembly by stabilizing β-Lg against unfolding and acid-hydrolysis, resulting in fewer fibril-forming peptides, whereas glycerol inhibited peptide self-assembly without affecting unfolding and acid-hydrolysis. Although, both polyols increased the viscosity of the solutions, viscosity did not affect the self-assembly of peptides, indicating that, under these conditions, the self-assembly was not diffusion-limited.

The effects of β-casein on β-Lg self-assembly were investigated by heating β-Lg-β-casein mixtures (molar ratios 1:0.0625 to 1:1). Heating under fibril-forming conditions resulted in acid hydrolysis of both proteins at approximately equal rate. β-Casein produced a small but consistent effect in inhibiting β-Lg self-assembly in heated β-Lg-β-casein mixtures. The transmission electron microscopy images of solutions showed irregular, coiled and ribbon-like structures co-existing with the β-Lg fibrils. These aggregates were absent in respective heated control samples of either protein indicating that β-Lg assembly-competent peptides have alternate competing pathway during self-assembly. The limited effect of β-casein on β-Lg self-assembly may be explained by the aggregation of β-casein peptides by a separate alternate pathway which competed with their interaction with β-Lg peptides.
Overall, the findings of this study have advanced our understanding of the mechanisms of self-assembly of globular proteins and provided insights into the ways to decouple self-assembly processes. This may help to design protocols for the control of globular protein self-assembly and extend the functionality and applications of protein fibrils.
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Contents

Abstract ........................................................................................................................ I

Acknowledgements .................................................................................................... V

List of Figures ...................................................................................................... XVII

List of Tables ...................................................................................................... XXXI

List of Publications .......................................................................................... XXXIII

1. Introduction ........................................................................................................... 1

2. Review of literature ............................................................................................. 7

   2.1. Amyloid fibrils .............................................................................................. 8

   2.1.1. Defining characteristics of amyloid fibrils ............................................. 11

   2.1.1.1. Characteristic cross β-structure ...................................................... 11

   2.1.1.2. Nucleation-dependent polymerization ........................................... 12

   2.1.2. Structural basis for self-assembly ....................................................... 13

   2.1.2.1. Role of amyloidogenic sequences ............................................... 13

   2.1.2.2. Role of protein structure ............................................................... 16

   2.1.3. Factors affecting the self-assembly of proteins ................................... 18

   2.1.3.1. Factors promoting protein self-assembly ...................................... 18

   2.1.3.2. Factors inhibiting protein self-assembly ....................................... 20

   2.2. β-Lactoglobulin .......................................................................................... 23

   2.2.1. Native structure ..................................................................................... 23

   2.2.2. Genetic variants of β-Lg ...................................................................... 27

   2.2.3. Heat-induced unfolding of β-Lg.......................................................... 29

   2.2.4. Heat-induced aggregation of β-Lg ...................................................... 31

   2.2.5. Factors affecting thermal aggregation of β-Lg .................................. 33

   2.2.5.1. Genetic variation ............................................................................ 34

   2.2.5.2. Effects of polyols ........................................................................... 35

   2.2.5.3. Glycation ....................................................................................... 36

   2.2.5.4. Chaperone-like properties of β-casein ........................................... 38
2.3. Self-assembly of β-Lg into amyloid-like fibrils ...................................... 42
   2.3.1. Conditions of β-Lg self-assembly .............................................. 42
   2.3.2. Mechanism of β-Lg self-assembly at low pH ............................ 44
   2.3.3. Heat-induced acid hydrolysis ...................................................... 46
   2.3.4. Characteristics of β-Lg fibrils ..................................................... 50
   2.3.5. Sequences present in fibrils ........................................................ 53
   2.3.6. Factors affecting kinetics of self-assembly ..................................... 54
      2.3.6.1. Temperature ........................................................................ 54
      2.3.6.2. pH and ionic strength .......................................................... 55
      2.3.6.3. Cations ................................................................................ 56
      2.3.6.4. Seeding ................................................................................ 56
      2.3.6.5. Shear .................................................................................. 57
   2.3.7. Stability of β-Lg fibrils .............................................................. 58
2.4. Objectives ................................................................................................ 59
   2.4.1. Experimental Approach ............................................................. 59
   2.4.2. Experimental techniques ............................................................... 60
3. Materials and Methods .................................................................................. 61
   3.1. Materials ............................................................................................. 61
      3.1.1. Water ......................................................................................... 61
      3.1.2. Whey protein isolate ................................................................. 61
      3.1.3. Trypsin ..................................................................................... 62
      3.1.4. β-Lg variants A, B and C ............................................................ 62
      3.1.5. Bovine β-casein ......................................................................... 62
      3.1.6. Chemicals .................................................................................. 62
   3.2. Instruments ............................................................................................ 65
      3.2.1. Centrifuges ............................................................................... 65
      3.2.2. Waterbath ................................................................................ 66
      3.2.3. Spectrofluorometer .................................................................... 66
      3.2.4. Spectrophotometer .................................................................... 66
      3.2.5. pH meter .................................................................................. 66
      3.2.6. Circular dichroism spectrometer ............................................... 67
      3.2.7. High performance liquid chromatography system ..................... 67
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.8.</td>
<td>Mass spectrometers</td>
<td>67</td>
</tr>
<tr>
<td>3.2.9.</td>
<td>Transmission electron microscope</td>
<td>67</td>
</tr>
<tr>
<td>3.2.10.</td>
<td>Other instruments</td>
<td>67</td>
</tr>
<tr>
<td>3.2.11.</td>
<td>General consumables</td>
<td>68</td>
</tr>
<tr>
<td>3.3.</td>
<td>Methods</td>
<td>68</td>
</tr>
<tr>
<td>3.3.1.</td>
<td>Isolation of β-lactoglobulin</td>
<td>68</td>
</tr>
<tr>
<td>3.3.2.</td>
<td>Preparation of fibrils</td>
<td>69</td>
</tr>
<tr>
<td>3.3.3.</td>
<td>Thioflavin T assay for detection of fibrils</td>
<td>69</td>
</tr>
<tr>
<td>3.3.4.</td>
<td>Separation of fibrils using ultracentrifugation</td>
<td>74</td>
</tr>
<tr>
<td>3.3.5.</td>
<td>Polyacrylamide gel electrophoresis (PAGE)</td>
<td>76</td>
</tr>
<tr>
<td>3.3.5.1.</td>
<td>Native non-denaturing PAGE</td>
<td>78</td>
</tr>
<tr>
<td>3.3.5.2.</td>
<td>Glycine SDS-PAGE</td>
<td>79</td>
</tr>
<tr>
<td>3.3.5.3.</td>
<td>Tricine SDS-PAGE</td>
<td>80</td>
</tr>
<tr>
<td>3.3.5.4.</td>
<td>Running, staining and imaging of gels</td>
<td>81</td>
</tr>
<tr>
<td>3.3.5.5.</td>
<td>2-Dimensional nonreducing-reducing (2D NR-R) SDS-PAGE</td>
<td>82</td>
</tr>
<tr>
<td>3.3.5.6.</td>
<td>Densitometry</td>
<td>82</td>
</tr>
<tr>
<td>3.3.6.</td>
<td>CD spectroscopy</td>
<td>83</td>
</tr>
<tr>
<td>3.3.6.1.</td>
<td>Introduction</td>
<td>83</td>
</tr>
<tr>
<td>3.3.6.2.</td>
<td>Sample preparation</td>
<td>85</td>
</tr>
<tr>
<td>3.3.6.3.</td>
<td>Recording scans</td>
<td>86</td>
</tr>
<tr>
<td>3.3.7.</td>
<td>Mass Spectrometry</td>
<td>88</td>
</tr>
<tr>
<td>3.3.7.1.</td>
<td>Introduction</td>
<td>88</td>
</tr>
<tr>
<td>3.3.7.2.</td>
<td>Determination of molecular weights</td>
<td>91</td>
</tr>
<tr>
<td>3.3.7.3.</td>
<td>Sequence characterization by MALDI-TOF MS/MS</td>
<td>92</td>
</tr>
<tr>
<td>3.3.7.4.</td>
<td>Characterization of sequences in fibrils by ESI-MS/MS</td>
<td>93</td>
</tr>
<tr>
<td>3.3.8.</td>
<td>Transmission electron microscopy</td>
<td>95</td>
</tr>
<tr>
<td>3.4.</td>
<td>Software packages</td>
<td>96</td>
</tr>
</tbody>
</table>

4.1. Abstract ................................................................................................... 97
4.2. Introduction ............................................................................................. 99
4.3. Materials and Methods .......................................................................... 101
4.4. Results ................................................................................................... 102
  4.4.1. Characterization of β-Lg isolated from WPI ...................................... 102
  4.4.2. Self-assembly of β-Lg ..................................................................... 105
  4.4.3. Hydrolysis of β-Lg in heated samples ........................................... 106
  4.4.4. Structural transitions in the lag phase ........................................... 108
    4.4.4.1. Hydrolysis of β-Lg ................................................................. 108
    4.4.4.2. Unfolding of β-Lg during heating ........................................... 110
  4.4.5. Characterization of fibril composition ........................................... 115
    4.4.5.1. Composition of fibrils after different heating times ... 115
    4.4.5.2. Disulfide bonding in fibrils ................................................... 120
    4.4.5.3. Characterization of sequences of fibril peptides .......... 122
4.5. Discussion ............................................................................................. 138

5. Self-assembly from β-Lg A, B and C .................................................. 147

5.1. Abstract ................................................................................................... 147
5.2. Introduction ........................................................................................... 149
5.3. Materials and Methods .......................................................................... 150
5.4. Results ................................................................................................... 151
  5.4.1. Characterization of β-Lg variants ............................................. 151
  5.4.2. Unfolding of β-Lg ................................................................. 153
  5.4.3. SDS-PAGE of heated samples ................................................... 156
  5.4.4. Self-assembly of β-Lg A, B and C ........................................... 159
  5.4.5. Morphology of fibrils ................................................................. 160
  5.4.6. Composition of fibrils ................................................................. 162
5.5. Discussion ............................................................................................. 163
## Contents

8. **Interactions of β-Lactoglobulin and β-casein during self-assembly** 233  
   8.1. Abstract .................................................................................................. 233  
   8.2. Introduction ........................................................................................... 235  
   8.3. Materials and Methods ........................................................................ 237  
        8.3.1. Preparation of β-casein .............................................................. 237  
        8.3.2. Preparation of samples for self-assembly ................................. 238  
   8.4. Results ................................................................................................... 240  
        8.4.1. Self-assembly from β-Lg in the presence of β-casein ............... 240  
        8.4.2. Heat-induced acid hydrolysis of β-Lg and β-casein ............. 241  
        8.4.3. Composition of aggregates in the heated solutions .............. 250  
        8.4.4. Morphology of aggregates ...................................................... 251  
   8.5. Discussion ............................................................................................. 253  

9. **Overall discussion and avenues for future work** ......................... 263  
   9.1. Summary ............................................................................................... 263  
        9.1.1. Unfolding of β-Lg ...................................................................... 264  
        9.1.2. Heat-induced acid hydrolysis .................................................... 264  
        9.1.3. Self-assembly ............................................................................. 265  
        9.1.4. Fibril composition and amyloidogenic sequences ................ 267  
        9.1.5. Morphology of fibrils ............................................................... 268  
   9.2. Overall discussion ................................................................................. 271  
   9.3. Applications ........................................................................................... 281  
   9.4. Avenues for Further Work .................................................................... 283  

10. **References** ............................................................................................ 287  

11. **Annexures** ............................................................................................ 322  

12. **DRC 16 forms: Statement of contribution to doctoral thesis**  
        containing publications ............................................................................. 349
List of Figures

Figure 1.1 Overview of following thesis chapters. ................................................................. 6

Figure 2.1 Schematic representation of characteristic X-ray fibre diffraction arising from the cross-β structure of fibrils. The distance between two β-strands is approximately 4.8 Å, while between the anti-parallel arrangements of β-strands is 9.6 Å. The distance between two β-sheets is 10-11 Å. Adapted from Serpell (2000). ........................................... 12

Figure 2.2 Free energy landscape of protein aggregation. The area in the pink region represents different transitional states during aggregation. Adapted from Hartl et al. (2009). ............................................................ 17

Figure 2.3 Aggregation pathways for misfolded proteins. N, I and U represent native, intermediate and unfolded polypeptide conformation states of the protein. Adapted from Dobson (2003a). ........................................... 18

Figure 2.4 Sequence of β-Lg A (Variant B shows presence of glycine instead of aspartic acid at residue 64 and alanine instead of valine at residue 118) adapted from the Protein Data Bank© (PDB ID 1BSO) (Qin et al., 1998a). Dotted lines indicate disulfide bonding. Solid arrows indicate β-strands; waveforms indicate helices and arches indicate loops. ................................................................................................................. 24

Figure 2.5 pH-dependent reversible association behavior of β-Lg. Adapted from Cheison et al. (2011). ........................................................................................................ 27

Figure 2.6 Proposed mechanism of acid hydrolysis of proteins. Adapted from Blackburn et al. (1954). ........................................................................................................ 47
List of Figures

**Figure 2.7** Characteristics of $\beta$-Lg fibrils (A) wide angle X-ray diffraction pattern of $\beta$-Lg fibrils, adapted from Bromley et al. (2005); (B) TEM and (C) AFM image of $\beta$-Lg fibrils, adapted from Adamcik et al. (2010); (D) Distribution of proto-filaments in $\beta$-Lg fibrils, numbers indicate number of protofilaments, adapted from Lara et al. (2011)........ 52

**Figure 2.8** Comparison of sequences found in $\beta$-Lg fibrils reported by (i) Akkermans et al. (2008b) and (ii) Hettiarachchi et al. (2012). The numbers on the top show the locations of aspartic acid residues in the sequence, based on the sequence of $\beta$-Lg A. (A) and (B) show the peptides from $\beta$-Lg A and B. ................................................................. 54

**Figure 3.1** (A) Structure of Thioflavin T molecule and (B) orientation of ThT molecules bound to fibrils. The ThT molecules align themselves in the channels formed by the ordered arrangement of side-chain residues in cross-$\beta$ sheets of fibrils. Adapted from Krebs et al. (2005). ..................................................................................................... 71

**Figure 3.2** Residual Thioflavin T intensities of supernatants obtained by ultracentrifugation at different speeds for 30 and 60 minutes (20 °C). $\beta$-Lg 1% (w/v) was heated at 80 °C at pH 2 for 12 h. Sample intensities were corrected by subtracting the intensity of the blank containing ThT solution without any sample. ................................................. 75

**Figure 3.3** Components of circularly polarized light (A) without absorption and (B) after absorption by chiral component. Adapted from Kelly et al. (2005). ............................................................................................................. 84

**Figure 3.4** Schematic representation of mass spectrometer. ................................................. 89

XVIII
Figure 4.1 Native PAGE pattern of $\beta$-Lg isolated from WPI by salt precipitation................................................................. 103

Figure 4.2 SDS-PAGE (with or without reducing agent) patterns of $\beta$-Lg isolated from WPI by salt precipitation method. I represents interface of stacking and resolving gel. For description of bands 1 to 4, see text.................................................................................... 104

Figure 4.3 Deconvoluted MS spectrum of the isolated $\beta$-Lg. ..................... 105

Figure 4.4 Thioflavin T (ThT) fluorescence intensity (empty circles) at 486 nm for 1% $\beta$-Lg at pH 2 heated at 80 °C. Error bars show standard deviations for triplicate measurements for three separate samples and the solid line show fit of Equation 3.1. Filled circles represent normalized intensity of the SDS-PAGE band corresponding to intact monomer. Error bars show standard deviations from three separate samples while the solid line shows fit of Equation 3.8......................... 106

Figure 4.5 Reduced SDS-PAGE of $\beta$-Lg heated at 80 °C and pH 2 for different times. M$_0$, Molecular mass marker in kDa; 0, unheated sample, numbers above the lanes indicate the heating times in hours. .............. 107

Figure 4.6 Reduced SDS-PAGE of $\beta$-Lg (1% w/v) heated at 80 °C and pH 2 during the lag phase. M$_0$, Molecular mass marker (kDa); 0, unheated sample; Numbers above the lanes indicate the heating time in minutes. For description of bands A-E, M and N, see text. .............. 110

Figure 4.7 CD spectra of $\beta$-Lg at pH 2 and 80 °C. (A) Near-UV scans, (10 mg/mL). (B) Far-UV spectra (0.01 mg/mL). Conditions for data collection were: path length 10 mm, temperature 80 °C and scans were averaged at 2-minute intervals each. ............................................ 113
List of Figures

**Figure 4.8** Relative change in ellipticity at (A) 293 nm representing the loss of tertiary structure, and (B) 208 nm representing change in secondary structure, calculated from data shown in Figure 4.7. Solid lines are for visual reference. ................................................................. 114

**Figure 4.9** High-tension (HT) curves of circular dichroism scans shown in Figure 4.7 (B) showing high absorption by the sample between 180 and 200 nm. ................................................................................ 115

**Figure 4.10** Thioflavin T fluorescence intensities at 486 nm in heated β-Lg (1%) samples (filled circles) and supernatants in (empty circles) obtained after centrifugation at 2.4 x10^5 g for 60 minutes. The pellets were used for SDS-PAGE analysis shown in Figure 4.12. Solid line shows the fit of Equation 3.1................................................................. 116

**Figure 4.11** Comparison of reduced SDS-PAGE profiles of centrifuged samples at different heating times. M₀, Molecular mass marker, weights in kDa; 0, unheated sample; U, heated and uncentrifuged; S, supernatant; P, pellet. Numbers above the lanes indicate heating times in hours. Uncentrifuged sample and supernatants were diluted 1:10 with the PAGE sample buffer. Surface-washed pellets were suspended in the loading buffer without dilution. For description of bands M, N and A to E see text ................................................................. 117

**Figure 4.12** SDS-PAGE profiles of pellets obtained after centrifugation of heated samples (1% β-Lg) at different stages of self-assembly. Pellets were obtained by ultracentrifugation and were complete-washed (see Chapter 3 for details). The washed pellets were suspended in PAGE loading buffer with 4% SDS and 100 mM DTT
and allowed to dissolve for 7 days 20 °C. Samples were run on tricine SDS-PAGE gels prepared in house. M₀: Molecular weight marker (weights in kDa); U: unheated; numbers above the lanes indicate heating times in h. For description of bands A to E, M and N see text.

- **Figure 4.13** SDS-PAGE comparison of peptide bands in fibrils under reducing and non-reducing conditions. Complete-washed pellets obtained after ultracentrifugation for 12 h. M₀, Molecular mass marker (weights in kDa); 0, unheated sample; U, heated and uncentrifuged sample; Pᵣ, complete-washed pellet suspended in reducing PAGE buffer; Pᵦ, complete-washed pellet suspended in non-reducing PAGE buffer. For description of bands C, D and E, see text.

- **Figure 4.14** 2D SDS-PAGE of the pellet obtained from a sample heated for 12 h. Complete-washed pellet suspended in buffer containing 4% SDS and run under non-reducing conditions in 1D. The target gel lane was excised, reduced and run in the 2nd dimension. Pᵣ: reduced sample obtained from the same sample.

- **Figure 4.15** Typical MS/MS spectra of showing ions with assigned masses for peptide SLAMAASDILLDAQSMAPLR (with oxidation of methionine) from band C in Table 4.2 with a cut-off ion score of 100 (expect value 6.3x10⁻⁷).

- **Figure 4.16** Schematic representation of regions found in peptides from in-gel digestion ESI-MS/MS and MALDI-TOF MS/MS. The dark blocks in the sequence show location of aspartic acid residues indicating potential sites of acid hydrolysis of β-Lg. Sequence of β-Lg variant
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.17</td>
<td>Schematic illustration of how disulfide-bonded peptides come to be present in fibrils. Red colored regions indicate the N-terminal region (1-53) of the β-Lg sequence.</td>
</tr>
<tr>
<td>5.1</td>
<td>Native non-denaturing PAGE of β-Lg A, B and C.</td>
</tr>
<tr>
<td>5.2</td>
<td>De-convoluted MS spectra of β-Lg variants. (A) β-Lg A, (B) β-Lg B and (C) β-Lg C.</td>
</tr>
<tr>
<td>5.3</td>
<td>Spectra of native β-Lg A, B and C at pH 2. (A) NUV spectra (1 mg/mL) and (B) FUV spectra (0.01 mg/mL).</td>
</tr>
<tr>
<td>5.4</td>
<td>Unfolding of the β-Lg A, B and C upon heating at 80 °C. The NUV scans in (A), (C) and (D) show effect of heating on the tertiary structure, while FUV scans in (B), (D) and (E) show the effect of heating on the secondary structure of β-Lg A, B and C respectively. The concentration of β-Lg was 1 mg/mL for the NUV scans, and 0.01 mg/mL for the FUV scans.</td>
</tr>
<tr>
<td>5.5</td>
<td>Relative losses of ellipticities at (A) 293 nm and (B) 208 nm calculated from Equations 3.11 and 3.12. Solid lines are for visual reference.</td>
</tr>
<tr>
<td>5.6</td>
<td>Reducing tricine SDS-PAGE profiles of control and heated samples after heating for 12 and 24 h at 80 °C. M₀ Molecular weight marker; molecular weights are in kDa. For description of band Q, see text.</td>
</tr>
<tr>
<td>5.7</td>
<td>Residual intensities of β-Lg band at different heating times. Each value represents an average of two separate experiments. Solid lines</td>
</tr>
</tbody>
</table>
indicate fit of Equation 3.8. Thick solid lines represent $\beta$-Lg A; dots, $\beta$-Lg B; and dashes, $\beta$-Lg C. ................................................................. 158

**Figure 5.8** ThT fluorescence intensities at 486 nm in heated samples of $\beta$-Lg A, B and C (1% w/v) at 80 °C and pH 2. Error bars represents standard error of measurements from two experiments with two replicates in each. Solid lines indicate fit to Equation 3.1. Thick solid lines represent $\beta$-Lg A; dots, $\beta$-Lg B; and dashes, $\beta$-Lg C. ........................... 159

**Figure 5.9** TEM images of fibrils from $\beta$-Lg A, B and C after 24 h at 80 °C....... 161

**Figure 5.10** Reducing tricine SDS-PAGE of fibrils formed after heating at 80 °C for 12 h and separated by ultracentrifugation. $M_0$, Molecular weight marker; S, supernatant; P pellet containing fibrils. Molecular weights in kDa; for descriptions of (i), bands A to E and R, see text. ... 162

**Figure 6.1** De-convoluted MS spectra of glucosylated $\beta$-Lg (A) 8 h, (B) 16 h and (C) 36 h. Numbers in the figure represent the number of glucose residues attached on the $\beta$-Lg variant A......................................................... 178

**Figure 6.2** De-convoluted MS spectra of lactosylated $\beta$-Lg (A) 3 days, (B) 5 days and (C) 7 days. Numbers in the figure represent the number of lactose residues attached on the $\beta$-Lg variant A......................................................... 179

**Figure 6.3** De-convoluted MS spectra of glucosylated (A) LowGlu $\beta$-Lg and (B) HighGlu $\beta$-Lg. Peak labels show the number of attached glucose residues........................................................................................................... 181

**Figure 6.4** De-convoluted MS spectra of lactosylated (A) LowLac $\beta$-Lg and (B) HighLac $\beta$-Lg. Peak labels show the number of attached lactose residues........................................................................................................... 182
List of Figures

**Figure 6.5** Comparison of CD spectra of β-Lg glycated with glucose and lactose (A) NUV (10 mg/mL β-Lg) and (B) FUV (0.01 mg/mL β-Lg) region. ................................................................. 184

**Figure 6.6** CD spectra of LowGlu β-Lg in the (A) NUV region (10 mg/mL β-Lg) and (B) in the FUV region (0.01 mg/mL β-Lg). The numbers in the figures indicate heating time in minutes. ........................................... 185

**Figure 6.7** Relative change in ellipticity at (A) 293 nm in the NUV region and (B) 208 nm in the FUV region. Solid lines are for visual reference. For details of x and y-axes see Section 3.3.6.3, Chapter 3.......................... 186

**Figure 6.8** Tricine SDS-PAGE profiles of control and glycated samples after heating for 12 h at 80 °C, at pH 2. Mo, Molecular weight markers in kDa; C, heated control. ................................................................. 187

**Figure 6.9** The normalized band intensities of the un-hydrolyzed β-Lg band at different times in glycated samples. Each value is an average of quantifications from two sets of independent experiments. The solid lines represent fits to the first-order exponential decay model of Equation 3.8. ................................................................. 188

**Figure 6.10** The normalized band intensities of the un-hydrolyzed β-Lg band at different times in the controls with no sugar added. Each value is an average of quantifications from two sets of independent experiments. The solid lines represent fits the first-order exponential decay model in Equation 3.8. ................................................................. 190

**Figure 6.11** (A) ThT fluorescence intensity of control and glycated β-Lg (1% w/v) during heating at pH 2 and 80 °C. Each data point is an average of triplicates from two independent experiments, and error bars are
standard deviations. Solid lines indicate the fit to Equation 3.1. (B) Normalized parameters of self-assembly calculated from Equations 3.2 and 3.4. The kinetic parameters of individual samples were normalized by those of control sample. ................................................ 192

**Figure 6.12** Self-assembly from β-Lg (1% w/v) without sugars used as control during glycation and treated similarly to the glycated samples. Error bars represent standard error of triplicate measurements in samples. Each value represents an average of triplicate values. Solid lines indicate fits to Equation 3.1................................................................... 194

**Figure 6.13** Tricine SDS-PAGE profiles of centrifugally separated fibrils made by heating glycated β-Lg at 80 °C for 24 h. M₀, Molecular weight markers in kDa; C, control fibrils made from the same heat treatment........................................................................................................ 195

**Figure 6.14** Residual ThT intensities in supernatants obtained for upon ultra centrifugation. Heated β-Lg solutions obtained after heating at 80°C for 24 h were ultracentrifuged. The pellets obtained after ultracentrifugation were used for SDS-PAGE (Figure 6.13). .............. 196

**Figure 6.15** TEM images of fibrils from glycated β-Lg after heating for 24 h at 80 °C. (A) LowGlu β-Lg; (B) HighGlu β-Lg................................................................. 197

**Figure 6.16** TEM images of fibrils from glycated β-Lg after heating for 24 h at 80 °C. (A) LowLac β-Lg; (B) HighLac β-Lg................................................................. 198

**Figure 6.17** Proposed mechanism of inhibition of self-assembly of glycated β-Lg by the steric effect of the sugar adduct. ......................................................... 201

**Figure 7.1** Thioflavin T (ThT) fluorescence intensity at 486 nm for β-Lg (1%, w/v) heated at 80 °C and pH 2 in presence of different levels (%,
List of Figures

w/v) of (A) glycerol and (B) sorbitol. Solid lines indicate the fit using Equation 3.1. Error bars represent the standard errors of measurements from two separate experiments with three replicates in each................................................................. 210

Figure 7.2 Normalized ThT fluorescence intensities β-Lg sample containing fibrils diluted using different levels of polyols (0-50% w/v, used for self-assembly studies). The ThT fluorescence intensity in the undiluted sample was 920 fluorescence units. The intensity of the sample diluted using water (pH 2) was used for normalization. A value close to 1 indicates that the fluorescence intensity in samples diluted with water or polyols were similar................................. 213

Figure 7.3 Reduced SDS-PAGE of samples heated at 80 °C for 12 h in the presence of different concentrations of (A) glycerol or (B) sorbitol. (M₀) Molecular weight marker in kDa; (U) unheated sample. Numbers above the lanes indicate polyol concentrations (% w/v)........ 214

Figure 7.4 Normalized β-Lg band intensities from SDS-PAGE gels of samples containing different concentrations of (A) glycerol and (B) sorbitol. Values are the average of two independent experiments. Solid lines indicate fit to Equation 3.8................................................................. 215

Figure 7.5 Effect of glycerol (A) and sorbitol (B) on the normalized kinetic parameters of hydrolysis and self-assembly.............................................. 217

Figure 7.6 CD spectra of β-Lg at 20 °C in presence of different levels of (A) and (C) glycerol and (B) and (D) sorbitol. For the NUV scans (A) and (B), the concentration of β-Lg was 1 mg/mL, while that for FUV

XXVI
spectra (C) and (D) was 0.01mg/mL. The sample scans were corrected by subtracting the baseline scans and smoothed. .................. 220

**Figure 7.7** NUV (A, B) or FUV (C,D) circular dichroism spectra of β-Lg during heating at 80 °C and pH 2 with 30% glycerol (A,C) or 30% sorbitol (B,D). Numbers indicate the heating time in minutes.............................. 221

**Figure 7.8** Relative loss of ellipticity at 293 nm in β-Lg solutions during heating at 80 °C with 0-50% glycerol (A) or sorbitol (B) calculated from Equations 3.13 and 3.14. For details of the notations of Y axis see Section 3.3.6.3 in Chapter 3. The same symbols representing glycerol concentrations in (A) apply for sorbitol concentrations in (B). Solid lines are a visual guide only. .................................................. 222

**Figure 7.9** Normalized ellipticities in the samples at 293 nm at the end of 10 minutes at 80 °C. Absolute ellipticities in the samples with different levels of polyols ($\theta_{\text{polyol}}$) were normalized by the ellipticity of the control sample without any polyols ($\theta_{\text{control}}$). Values >1 indicates a higher residual signal in the sample and hence the stabilized state of β-Lg. Solids lines are a visual guide. .......................................................... 223

**Figure 7.10** High tension curves for FUV scans of β-Lg in presence of 30% w/v of (A) glycerol and (B) sorbitol during heating at 80 °C. The data in A and B represent the high tension (HT) values for the same scans shown in Figure 7.7 C and D. Solid lines are a guide and are not fitted with any mathematical model..................................................... 223

**Figure 7.11** Transmission electron microscopy images of β-Lg fibrils separated from heated solutions. (A) Without any polyol; (B) With 20%
glycerol; and (C) With 20% sorbitol. Samples heated at 80 °C, pH 2 and 24 h. ................................................................. 225

Figure 7.12 Transmission electron microscopy images of β-Lg fibrils separated from β-Lg samples heated at 80 °C, pH 2 and 24 h in the presence of (A) 50% (w/v) glycerol and (B) 50% (w/v) sorbitol at 80 °C, pH 2 and 24 h. ................................................................................................ 226

Figure 7.13 Schematic illustration of how β-Lg fibril assembly is affected by sorbitol (slowing unfolding) and glycerol (slowing assembly of peptides). ............................................................................................... 228

Figure 7.14 Viscosity of polyol solutions at 80 °C at a shear rate of 1 s\(^{-1}\). Polyol solutions were made in Milli Q water without pH adjustment. Measurements were carried out on an AR-G2 rheometer (TA Instruments, New Castle, DE) using a double gap measuring system equilibrated at 80 °C for two minutes prior to measurements.............. 230

Figure 8.1 ThT fluorescence intensities at 486 nm in samples of heated β-Lg-β-casein mixtures. The control sample contained β-Lg alone. Error bars show the standard errors of measurements from two separate experiments with two replicates for each data point. Solid lines indicate fit of Equation 3.1........................................................................................................ 240

Figure 8.2 Reduced SDS-PAGE of β-Lg and β-casein mixtures heated at 80 °C for (A) 12 h and (B) 24 h. M0 molecular weight marker (kDa); C, control. Lanes marked 1 to 5 represent β-Lg and β-casein mixtures at different molar ratios. Lane marked 1 represents a molar ratio 1:0.0625; 2, 1:0.125; 3, 1:0.250; 4, 1:0.50; 5, 1:1. ............................................. 242
Figure 8.3 Reduced SDS-PAGE of β-casein controls heated at 80 °C for (A) 12 h and (B) 24 h. M₀ molecular weight marker (kDa); Lanes marked 1 to 5 represent the same β-casein concentrations as used in β-Lg-β-casein mixtures of Figure 8.4.

Figure 8.4 Peptide compositions of heated solutions after 12 h at 80 °C. M₀ molecular weight marker (kDa); C, control β-Lg (without β-casein); 1, β-Lg and β-casein mixture (molar ratio 1:1) and 2, control β-casein (without β-Lg) at same concentration as in 1. For description of band F to J, see text. All samples analyzed under reducing conditions.

Figure 8.5 Residual SDS-PAGE monomeric band intensities of (A) β-Lg and (B) β-casein in heated β-Lg-β-casein mixtures. Symbols in (B) represent the same β-casein concentrations as used in (A). Values are an average of two separate estimations. Solid lines indicate fit of Equation 3.8.

Figure 8.6 Composition of aggregates formed from the heated and ultracentrifuged samples containing β-Lg (1%, w/v) with (0.65% w/v, molar ratio 1:0.5) or without β-casein. Samples were heated at 80 °C for 12 h. M₀ represents molecular weight markers (kDa); H, heated solution; S, supernatant and P, Pellet. Supernatants were diluted 1:10 in reducing PAGE sample buffer, while the pellets were suspended in 1.2 ml PAGE sample buffer without further dilution.

Figure 8.7 TEM images of β-Lg samples heated (A) without, and (B to D) with β-casein at molar ratios of 1:0.5 (B and C) or 1:1 (D and E). Samples
List of Figures

heated at 80 °C for 24 h selected for TEM analysis. For description of white arrows see text................................................................. 252

Figure 8.8 TEM images of β-casein samples heated at 80 °C for 24 h without β-Lg. The β-casein concentration was equivalent to that in mixed solutions at molar ratio of 1:0.5......................................................... 253

Figure 8.9 Charge distribution on β-casein polypeptide at pH (A) 6.7 and (B) 2.6. Adapted with permission from Moitzi et al. (2008), Copyright 2008 American Chemical Society......................................................... 254

Figure 8.10 Overview of the hydrophobicity (pH 7) of β-casein peptides G to J of Figure 8.4 calculated using the method of Kyte et al. (1982). At pH 2, the hydrophobicity of the peptides is likely to be higher due to protonation of carboxylic groups of Asp and Glu (Kuhn et al., 1995). Bands G (A), I (D) and J (E) contain phosphate groups bound to serine residues. ................................................................. 257

Figure 8.11 Proposed mechanism for the effect of β-casein on β-Lg self-assembly. (i), (ii) and (iii) represent aggregation pathways. .......... 259

Figure 9.1 Mechanism of β-Lg self-assembly at 80 °C, pH 2 at low concentrations (<3% w/v). ......................................................... 266

Figure 9.2 Strategies to decouple acid hydrolysis from self-assembly by modifying (A) glycation of β-Lg or (B) composition of aqueous solution by glycerol. ......................................................... 277
List of Tables

Table 2.1 Human diseases associated with amyloid fibrils .............................................. 9
Table 2.2 Sites of differences in β-Lg A, B and C. ............................................................... 28
Table 2.3 Non-native conditions promoting fibril formation from β-Lg. ......................... 43
Table 2.4 Overview of experimental techniques used in this study. ............................... 60
Table 3.1 List of chemicals and reagents used and their vendors. ................................. 63
Table 3.2 Composition of native and SDS-PAGE gels. .................................................. 79
Table 3.3 Composition of resolving and stacking gels for tricine SDS-PAGE analysis. ......................................................................................................................... 81
Table 4.1 Proximate composition of extracted β-Lg ......................................................... 103
Table 4.2 Peptides from β-Lg hydrolysis present in fibrils analyzed by MALDI-TOF MS/MS. .................................................................................................................. 123
Table 4.3 Peptide sequences of peptide bands A to E (Figure 4.12) in the pellet (12 h) analyzed by ESI-MS/MS after extraction following in-gel digestion with trypsin. ................................................................................................................. 125
Table 4.4 MS/MS data from Mascot search showing masses of the assigned ion peaks shown in Figure 4.16. .................................................................................. 131
Table 4.5 Peptides obtained by in-gel digestion of unheated β-Lg followed by ESI-MS/MS. β-Lg loaded on to SDS-PAGE gel (resolving gel concentration 20%). After running, the β-Lg band was excised and processed along with the peptide bands A-E which have been characterized in Table 4.3. ................................................................. 133
Table 4.6 Possible sequences of peptides present in SDS-PAGE bands of fibrils. 144
Table 5.1 Rate constants of monomer hydrolysis calculated from Equation 3.8. ... 158
List of Figures

**Table 5.2** Kinetic parameters of self-assembly calculated from Equations 3.2 to 3.4......................................................................................................... 160

**Table 5.3** Possible sequences present in the peptide bands of fibrils of β-Lg A, B and C, shown in Figure 5.10. ............................................................. 165

**Table 6.1** Rate constants of acid hydrolysis of glycated β-Lg calculated from data in Figure 6.9 and Equation 3.8 ...................................................... 189

**Table 6.2** Rate constants of acid hydrolysis of β-Lg used as glycation control calculated from the data in Figure 6.10 and Equation 3.8 ............... 190

**Table 6.3** Kinetic parameters describing β-Lg self-assembly calculated using Equations 3.2 to 3.4.............................................................................. 193

**Table 6.4** Kinetic parameters describing self-assembly calculated from Equations 3.1-3.4 for β-Lg samples used as controls during glycation experiments. The ThT fluorescence intensities are shown in Figure 6.12 above. ............................................................................................ 194

**Table 7.1** Kinetic parameters from Equation (3.2-3.4) showing effect of glycerol and sorbitol on β-Lg self-assembly. ................................................................. 211

**Table 7.2** Fit parameters from Equation 3.8 describing the kinetics of acid hydrolysis of β-Lg during heating under fibril forming conditions. ..... 216

**Table 8.1** Composition of β-Lg-β-casein mixtures ................................................................. 239

**Table 8.2** Kinetic parameters of self-assembly calculated from Equations 3.2-3.4......................................................................................................... 241

**Table 8.3** Predicted sequences of β-casein peptides shown in Figure 8.6 .......... 246

**Table 8.4** Rate constants of acid hydrolysis of β-Lg and β-casein in heated β-Lg-β-casein mixtures during heating at 80 °C................................. 249

**Table 9.1** Summary of the results from Chapters 5-8 ......................................................... 269

XXXII
List of Publications


