Determination of thermally-induced conformational slides in β-lactoglobulin prior to irreversible thermal denaturation

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Introduction

β-lactoglobulin (β-Lg) is the major globular protein in milk and the dominant functional agent in whey and its derivatives ingredients. It is generally recognized that β-Lg undergoes conformational changes above temperatures of 60 °C and unfolds irreversibly after 70 °C. The conformational changes that happen during this process have critical implications in β-Lg functional properties, thus affecting both technological quality of milk (i.e. cheese production) and whey ingredients, which use are currently widespread in food formulations. In this study thermal effects in β-Lg have been investigated through an innovative approach, combining on-line spectroscopy techniques, aiming at achieving a more detailed characterization of these events below and above the melting temperature.

Methods

β-Lg (10μM) in phosphate buffer pH 7 (25 mM)

Differential scanning Calorimetry

MicroCal VP-DSC, Malvern

- Scanning from 20 to 110 ºC

Circular Dichroism

Chirascan plus CD, Applied Photophysics

- Scanning from 20 to 90 and back to 20 ºC

Steady-state fluorescence spectroscopy and static light scattering

PTi fluorescence RTC 2000

- Scanning from 20 to 50, 60, 70, 90 and back to 20 ºC

Results

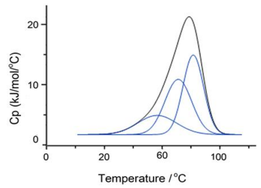


Fig. 1. DSC profile of B-Lg and its deconvolution in three Gaussian curves

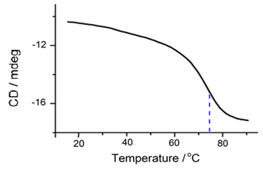


Fig. 2. CD profile of B-Lg with indication of the transition middle point

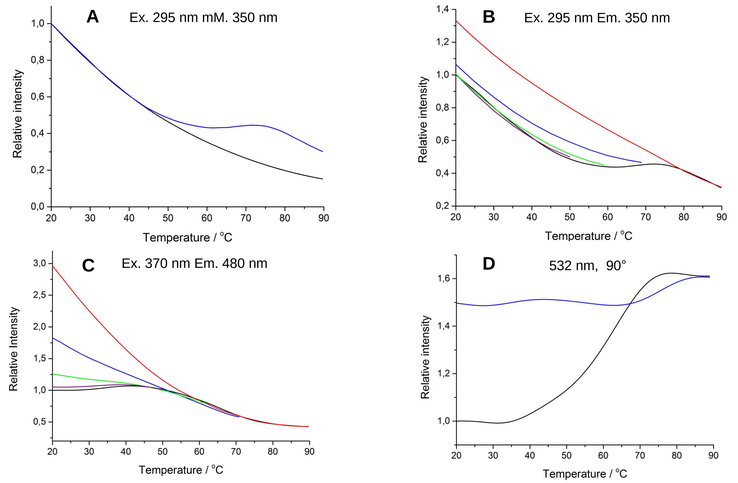
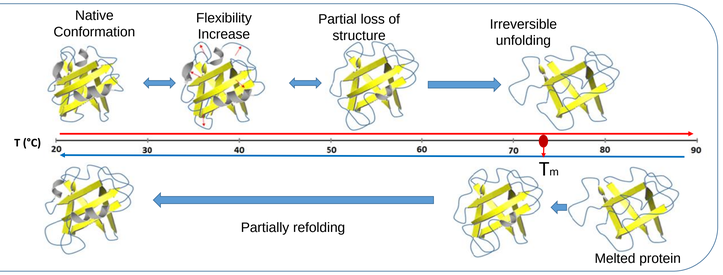


Fig. 3. A: Free tryptophan (black line) and β-Lg (blue line); fluorescence profiles during heating **B**: β-Lg fluorescence profiles during heating (black line) and cooling (colored lines); **C**: β-Lg + ANS fluorescence profiles during heating (black line) and cooling (colored lines); **D**: SLS profile of β-Lg during heating (black line) and cooling (blue line)

- **Free tryptophan and β-Lg present different fluorescence profiles**
- **Conformational changes in β-Lg start at 45 °C and are reversible until 60 °C**
- **ANS affinity decreases at temperatures above 45 °C and is enhanced after refolding of denatured protein**
- **SLS shows a molecular radius increase during heating, and a small decrease during cooling, indicating a partial refolding**

- **DSC indicates multiple transitions between 60 and 80 °C**
- **CD shows secondary structure transition with a middle point at 74 °C**

- **Proposed molecular model for β-Lg thermal response**



Conclusions

- **β-Lg thermal behaviour is complex and possibly explained by a multi-step denaturation model.**
- **Reversible conformational changes occur between 35 °C and 60 ºC, irreversible and progressive changes occur further on.**
- **The disclosure of this complex behaviour, particularly the conformational changes below the melting point, bring new insights regarding the technological properties and industrial processing of β-Lg.**

References

1. C. Bhattacharjee and K. P. Das, *Eur. J. Biochem.*, vol. 267, no. 13, pp. 3957–3964, 2000
2. P. Busti, S. Scarpeci, C. Gatti, and N. Delorenzi, *Food Res. Int.*, vol. 35, no. 9, pp. 871–877, 2002
3. Z. Zhang, V. Arrighi, L. Campbell, J. Lonchamp and S. Euston, *Foodhyd*, 52, pp 95-105, 2016

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