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**Effect of bound ligands and κ -casein
on the denaturation of β -lactoglobulin**

**A THESIS PRESENTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN FOOD TECHNOLOGY AT MASSEY UNIVERSITY**

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2000

ABSTRACT

The objective of this study was to gain greater understanding of the behaviour of bovine β -lactoglobulin (β -lg) during thermal denaturation. The first part of the study was focused on gaining more knowledge of the structural changes in β -lg during heat-induced and urea-induced denaturation and the effect of ligands and the genetic variants of β -lg. The second part of the study explored the mechanism of the heat-induced interaction of β -lg with κ -casein (κ -CN) and the effects of genetic variants.

The reversible early steps during thermal denaturation of β -lg are not readily separated from the later irreversible steps which involve sulphhydryl-disulphide interchange reactions. However, it should be possible to examine the behaviour of β -lg in the early steps without reactive thiols either by using a porcine β -lg, that does not have cysteine residue equivalent to Cys-121 of bovine β -lg, by blocking the free thiol or by using solvent denaturation.

Heating to 80 °C irreversibly altered near- and far-UV circular dichroism (CD) spectra, and 1,8-anilinonaphthalene sulphonate and retinol binding fluorescence spectra for bovine β -lg, but not for porcine β -lg which unfolded reversibly. Also the examination of the induced CD spectral changes of retinol and *cis*-parinaric acid (PnA) on binding to bovine β -lg B upon heating revealed that bovine β -lg lost the ability to bind retinol and PnA in a chiral environment. In contrast, porcine β -lg did not show significantly induced CD bands upon mixing with retinol, but PnA/ β -lg mixtures showed induced CD bands of low intensity. In addition to the lack of a free thiol group in porcine β -lg, the sequence differences between bovine β -lg and porcine β -lg are also likely to affect the behaviour of these β -lgs during heat treatment and the binding of ligands. Although palmitic acid does not show any absorbance in CD spectra, it enhanced the stability of the bovine β -lg and porcine β -lg mixture.

The urea-induced unfolding of bovine β -lg at neutral pH (6.7) revealed that the stability of the genetic variants followed the order, β -lg B < β -lg A < β -lg C, as observed during thermal denaturation and tryptic hydrolysis. The stabilisation effect was also

observed by adding retinol, retinoic acid and palmitic acid during urea denaturation of β -lg, and by retinol, retinyl acetate and PnA during ammonium sulphate denaturation of β -lg. Blocking the sulphhydryl group of β -lg destabilised the native protein against urea denaturation through the introduction of a bulky group to the compact structure of β -lg. This result, together with the results for porcine β -lg, confirms that the sulphhydryl group plays an important role in the unfolding of bovine β -lg.

In the second part of the study, an attempt was made to investigate the effect of bovine κ -CN on the established heat-induced unfolding and aggregation pathway of bovine β -lg, by adding κ -CN A or κ -CN B to native or pre-heated β -lg A, B or C and heating the mixture. The CD band intensity of the mixture of β -lg and κ -CN at 270 nm, an index of significant alteration to the disulphide bond dihedral angle, indicated increasing structural changes involving disulphide bonds during heat treatment.

The rates of loss of β -lg and the distributions of intermediate products were determined using alkaline- and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). During reaction with β -lg, some monomeric κ -CN was found by SDS-PAGE, probably as a result of disulphide bond interchanges between κ -CN and β -lg, and two-dimensional PAGE also revealed disulphide-bonded β -lg/ κ -CN aggregates. In the presence of κ -CN, the loss of monomeric β -lg increased and less non-native monomer and dimer were observed compared to β -lg alone and κ -CN reacted more rapidly with β -lg that had been unfolded by prior heat treatment than with native β -lg. This suggested that β -lg probably denatured (unfolded) independently and either simultaneously or consequently and contained higher sulphhydryl reactivity than native β -lg, which lead to the reactions with κ -CN via disulphide bond interchange. It is possible that the equilibrium between native β -lg and denatured β -lg shifted rapidly, because κ -CN preferred to interact with denatured (unfolded) β -lg that has higher sulphhydryl reactivity than native β -lg.

The kinetics of the interaction between β -lg and κ -CN were evaluated from the heat-induced loss of alkaline-monomeric β -lg at 80 °C. The interaction between β -lg and κ -CN could not be described by any reaction order between 1.0 and 2.0. The slopes of the plots changed at about 7.5-10 min heating time and this corresponded to intensity

changes in the alkaline-monomeric, non-native monomeric and dimeric β -lg bands, which increased during the first 10 min of heating and then slightly decreased or remained relatively constant for the rest of the heating.

The loss of native β -lg in β -lg/ κ -CN mixtures during heating at 80 °C was shown to be significantly influenced by the genotypes of both β -lg and κ -CN. The κ -CN B variant showed considerably higher reactivity than κ -CN A, while the β -lg B variant was the most reactive. The greatest loss of native β -lg was observed from the β -lg B/ κ -CN B mixture.

ACKNOWLEDGMENTS

I would like to thank my great supervisors, Prof. Harjinder Singh and Dr. Lawrie Creamer for their guidance and assistance in all aspects of this course. I have learned not only this lovely subject (β -lactoglobulin) but also the attitude of real scientist from them. Thanks for always there for me and your encouragement during last three years. I never could thank enough to you.

I am thankful to Dr. Gavin Manderson who helped me to set up and run my experiment during my first few weeks at NZDRI. Thanks to Dr. John Lucey for teaching me about SEC-MALLS. I also thank Dr. Don Otter for helping me to run mass spectrometry, Dr. Paul Harris for introducing me isoelectric focusing technique and Dr. Skelte Anema for giving me useful tips about SigmaPlot^{to the}. Grateful thanks go to Dr. Palatasa Havea for sharing knowledge of 2D-PAGE as well as his friendship and valuable advice throughout this course. I also thank the members of the Food Science Section (NZDRI) for their help and friendship during course of this project (Marlene, Michelle, Robyn, Carmen, Michael, Christina and Nicola). Thanks guys!

I thank for the financial support by the Foundation of Research, Science and Technology (Contract number DRI 403). I also like to thank Dr. Jeremy Hill (Section manager, Food Science Section, NZDRI) for the opportunity to conduct research at NZDRI.

I would like to express my genuine gratitude to my parents and father-in-law in Seoul for their support and love. Finally, I have to thank to my lovely and wonderful husband, Dr. Sang-dong Yoo, for his love and patience.

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