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**THE EFFECT OF HEAT ON THE STRUCTURE AND
AGGREGATION BEHAVIOUR OF BOVINE
 β -LACTOGLOBULINS A, B AND C.**

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of
Philosophy in Biochemistry at Massey University.

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Errata.

- 1) The citation Elofsson *et al.* (1996b) should read Elofsson (1996b).
- 2) The errors shown for the ΔG_{app} values in Chapters 5 and 7 were calculated incorrectly and are smaller than the true errors.

Abstract.

The bovine milk protein β -lactoglobulin (BLG) possesses a thiol group which becomes solvent exposed at elevated temperatures, leading to the formation of disulphide-linked milk protein aggregates. This phenomenon is of interest to the dairy industry because milk is heat-treated in many modern processes. This study is concerned with how the structure of BLG is altered during and as a consequence of heat treatment and how aggregates are formed. Bovine BLG exhibits genetic polymorphism and the A, B and C variants, present in New Zealand milks, differ in their susceptibilities to heat-induced structural change and aggregate formation, and their response to heat treatment is examined in the present study.

This study used the following techniques: near and far UV CD, intrinsic protein fluorescence, hydrophobic probe fluorescence, thiol group solvent-exposure and both native-PAGE and SDS-PAGE.

Spectroscopic and thiol exposure results suggest that the tertiary structure of BLG is altered during and as a consequence of heat treatment and that the amount of β -sheet in this protein does not alter appreciably as a consequence of heat treatment. PAGE results indicate that BLG forms a mixture of non-covalently-linked and disulphide-linked aggregates during heating, and that disulphide-linked dimers in particular are associated into larger non-covalently-linked aggregates. These non-covalently-linked aggregates are intermediates and large disulphide-linked aggregates are the end product of the BLG aggregation pathway. β -Lactoglobulin A forms aggregates, particularly large disulphide-linked aggregates, more slowly than BLGs B and C. Spectroscopic and thiol availability results suggest that the "intrinsic thermostability" of BLG C is appreciably greater than that of BLG B, which is slightly greater than that of BLG A. Furthermore, the extent of "irreversible structural change" in molecules of BLG C which occurs as a consequence of heat treatment is less than that in molecules of BLG A, which is less than that in molecules of BLG B. In the case of BLGs A and B this reflects the slower rate at which aggregates of BLG A form compared to that of BLG B. The present study has advanced the understanding of the BLG aggregation mechanism and how the A, B and C variants differ in their response to heat treatment.

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LIST OF ABBREVIATIONS.

ANS -	1-anilinonaphthalene-8-sulphonate
BLG -	β -lactoglobulin
BLG A/B -	a 1:1 mixture of BLGs A and B
BSA -	bovine serum albumin
CSA -	(+)-10-camphorsulphonic acid
DEAE -	diethylaminoethyl cellulose
DLS -	dynamic light scattering
DSC -	differential scanning calorimetry
DTNB -	5,5'-dithio-bis(2-nitrobenzoic acid)
ΔC_p -	change in heat capacity
ΔH° -	change in enthalpy
far UV CD -	far ultra-violet circular dichroism
FPLC -	fast protein liquid chromatography
Gdn-HCl -	guanidine hydrochloride
HPLC -	high performance liquid chromatography
I_{ANS} -	ANS fluorescence emission intensity
I_{Trp} -	tryptophan fluorescence emission intensity
K -	equilibrium constant
NATA -	N-acetyltryptophanamide
near UV CD -	near ultra-violet circular dichroism
NEM -	N-ethylmaleimide
NMR -	nuclear magnetic resonance
ODNB -	5-(octyldithio)-2-nitrobenzoic acid
PAGE -	polyacrylamide gel electrophoresis
PCS -	photon correlation spectroscopy
PMT -	photomultiplier tube
Q -	fluorescence quantum yield
RET -	radiationless (or resonance) energy transfer
S -	Svedberg unit (measure of sedimentation velocity)
SDS -	sodium dodecyl sulphate
SDS-PAGE -	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SMUF -	simulated milk ultrafiltrate
TCA -	trichloroacetic acid
TEMED -	N,N,N',N'-tetramethylethylenediamine
T_m -	midpoint temperature
T_{max} -	denaturation temperature (DSC)
TNB -	thionitrobenzoate
3D -	three dimensional