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Backbone Dynamics of Bovine β -Lactoglobulin by ^{15}N NMR Spectroscopy

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In Memory of Clinton John Reeve

Abstract

Bovine β -lactoglobulin (β -Lg) is a small 162 residue protein of unknown function from the whey component of milk, constituting ~50 % by dry mass. The protein is of great interest to the dairy industry due, in part, to its role in the fouling of dairy plants during heat treatment, and the significant operational costs this incurs. The structure of this protein is an eight stranded β -barrel with one long and two short flanking α helices. It is dimeric at neutral pH but dissociates at $\text{pH} < 3$.

In New Zealand herds there are three genetic variants, with variants A and B of bovine β -Lg predominating, while the C variant occurs at low levels in Jersey cows. However, despite the structural similarities of the three variants, milks containing one of A, B or C behaves differently when subjected to thermal processing. A greater understanding of factors that differentiate these protein variants is therefore important. In this study, ^{15}N nuclear magnetic (NMR) spectroscopy methods have been used to study the backbone dynamics of β -Lg A and B, at one temperature, and the hitherto unstudied C variant, at three temperatures. For follow-up functional studies a mutant protein, a covalently linked Ala34Cys dimer, was produced.

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Glossary of Abbreviations

Å	Ångstrom (10^{-10} m)
Aa	Amino acid
AEC	Anion exchange chromatography
α -La	α -Lactalbumin
Amp	Ampicillin
Bis-tris	1,3-Bis(tris(hydroxymethyl)methylamino)propane
β -Lg	β -Lactoglobulin
BME	β -Mercaptoethanol
Bp	Base-pair
C	Carbon
°C	Degrees Celsius
CPMG	Carr-Purcell-Meiboom-Gill
Da	Dalton
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DsbC	Disulfide bond isomerase C
EDTA	Ethylene diamine tetra-acetic acid
EtBr	Ethidium bromide
EtOH	Ethanol
FID	Free induction decay
g	Gram
$\times g$	Multiples of gravitational force
GER	Germany
H	Hydrogen
<i>HindIII</i>	DNA restriction endonuclease sourced from <i>Haemophilus influenza</i>
HMH	6-Hydroxy-6-methyl-3-heptanone
HSQC	Hetero-nuclear single quantum correlation
I	Italy
IEC	Ion exchange chromatography
IPTG	Isopropyl- β -D- thiogalactopyranoside
K	Kelvin
Kan	Kanamycin
Kb	Kilo bases
kDa	Kilo-Dalton
<i>KpnI</i>	DNA restriction endonuclease sourced from <i>Klebsiella pneumonia</i>
LB	Luria Bertani media

m	Metre
mAU	Milli absorbance units
MCS	Multiple cloning site
MCS1	Multiple cloning site one
MCS2	Multiple cloning site two
μg	Micro gram
MHz	Mega hertz
mL	Milli litre
μL	Micro litre
mM	Milli molar (mmol L ⁻¹)
mol	Mole
ms	Millisecond
N	Nitrogen
<i>NcoI</i>	DNA restriction endonuclease sourced from <i>Gordonia rubripertincta</i>
<i>NdeI</i>	DNA restriction endonuclease sourced from <i>Neisseria denitrificans</i>
ng	Nanograms
nm	Nanometers
NMR	Nuclear Magnetic Resonance spectroscopy
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
ns	Nanoseconds
NZ	New Zealand
1D	One-dimensional
OD ₆₀₀	Optical density (at a wavelength of 600 nanometres)
Pa	Pascal (= 10 ⁻⁵ bar, 145.05 × 10 ⁻⁶ psi)
PCR	Polymerase chain reaction
pH	Negative decadal logarithm of proton concentration
pKa	Acid dissociation constant, as negative decadal logarithm
ppm	Parts per million
ps	Picoseconds
R_1	Longitudinal (or spin-lattice) relaxation rate
R_2	Transverse (or spin-spin) relaxation rate
RBP	Retinol binding protein
RBS	Ribosome binding site
RCI	Random coil index
R_{ex}	Exchange induced relaxation rate
S^2	Squared order parameter
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC	Size-exclusion chromatography
ss-NOE	Steady state-nuclear Overhauser effect
TAE	Tris-acetate-EDTA buffer
τ_e	Effective correlation time
Temp	Temperature
Tet	Tetracycline

τ_m	Molecular correlation time
TOCSY	Total correlation spectroscopy
2D	Two-dimensional
3D	Three-dimensional
USA	United States of America
UV	Ultraviolet light
V	Volts
v/v	Volume per volume
w/v	Weight per volume

Abbreviations of Nucleic Acids

One Letter Code	Base Represented
A	Adenine
T	Thymine
C	Cytosine
G	Guanine
U	Uracil

Abbreviations of Amino Acids

Amino Acid	3-Letter Code	1-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

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