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EXPRESSION AND CHARACTERISATION
OF THE N-TERMINAL HALF
OF HUMAN LACTOFERRIN

This thesis is submitted to Massey University as partial fulfilment of the requirements
for the degree of Doctor of Philosophy in Biochemistry.

Catherine Louise Day

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Day

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ABSTRACT

Lactoferrin is an 80 kDa iron binding protein which is found in human milk and other exocrine solutions. Each molecule contains two metal binding sites which each bind a single iron atom with high affinity. The properties of the two sites are slightly different. In an attempt to more fully understand the nature of these differences a construct for the high level expression of the N-terminal half of human lactoferrin (Lf_N) has been constructed and protein expressed from this construct has been purified and characterised. Characterisation of the recombinant protein has shown that the signal peptide is correctly removed from Lf_N and that an N-linked carbohydrate moiety is added to Lf_N . Lf_N has been shown to bind one iron atom and the spectral properties are very similar to those of Fe_2Lf . The most significant difference between hLf and Lf_N is in the pH stability of iron binding. Iron is released from Lf_N 2 pH units higher than from hLf.

In an attempt to understand the bases for this difference a structural analysis of Lf_N was initiated. Using deglycosylated protein high quality crystals of both iron free and iron saturated Lf_N have been grown. The structures of both $FeLf_N$ and $ApoLf_N$ have both been solved by molecular replacement using the coordinates from the N-lobe of Fe_2Lf as the starting model.

The structure of $FeLf_N$ has been refined using data between 8.0 and 2.0 Å. The current model has good geometry and is believed to accurately represent the structure of $FeLf_N$. The structure of $FeLf_N$ provides the highest resolution and most accurate structure of a member of the transferrin family. Analysis of the structure has shown that the folding pattern and the environment of the iron atom in $FeLf_N$ are very similar to the N-lobe of Fe_2Lf although several differences exist. Most of the differences seen are due to the absence of the C-lobe and the rearrangement of residues 315 - 327. The altered conformation of residues 315 - 327 and the changes in the solvent accessibility to other residues are believed to be responsible for the different iron binding and release properties of Lf_N .

Although the structure of $ApoLf_N$ is not complete analysis of this structure has shown that unlike the N-lobe of intact apo hLf the domains are closed in $ApoLf_N$. The structure of $ApoLf_N$ is very similar to that of $FeLf_N$ even though the crystal packing is quite different. In addition although the protein was believed to be iron free there is some density in the iron site which is unaccounted for at present. This study continues.

Several mutants of Lf_N have also been created. These mutants have shown that the carbohydrate groups attached to lactoferrin probably have a role in folding and secretion of lactoferrin by BHK cells. Several mutants involving changes to residues involved in metal and anion binding have also been created. These mutants have helped us begin to define the changes responsible for preventing iron binding in the C-lobe of melanotransferrin. In addition the role of arginine 121 has been investigated however further analysis of these mutants is required before the structural changes responsible for the different properties can be defined.

ABBREVIATIONS

A ₂₈₀	Absorbance at 280 nm
A ₄₀₆	Absorbance at 406 nm
A ₄₅₄	Absorbance at 454 nm
ApoLf	Iron free native lactoferrin
ApoLf _N	Iron free Lf _N
ATP	adenosine triphosphate
BG	background
BHK	baby hamster kidney
bLf	bovine lactoferrin
bp	base pair
BRL	Bethesda research laboratories
BSA	bovine serum albumin
cDNA	complementary DNA
cfu	colony forming units
CIF	colony inhibitory factor
CML	chronic myeloid leukaemia
CM-sephadex	carboxymethyl sephadex
cpm	counts per minute
C-terminal	carboxy terminal
Cu ₂ Lf	copper saturated native lactoferrin
CuLf _N	copper saturated Lf _N
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DEPC	diethylpyrocarbonate
DG	deglycosylated
DGLf _N	deglycosylated Lf _N
dGTP	deoxyguanosine triphosphate
DHFR	dihydrofolate reductase
DMEM	Dulbeco's modification of Eagle's medium
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DTT	dithiothreitol
dUTP	deoxyuridine triphosphate
EDTA	ethylene diamine tetra-acetate
EEO	electroendosmosis
ELISA	enzyme linked immunoabsorbant assay

ESR	electron spin resonance
F' s	structure factors
F _{calc}	calculated structure factors
F _{obs}	observed structure factors
F12	Hams-F12 medium
FCS	foetal calf serum
FeLf _N	iron saturated Lf _N
Fe ₂ Lf	iron saturated native lactoferrin
gDNA	genomic DNA
GF/A	glass fibre/A
GM	granulocyte macrophage
GM-CSF	granulocyte macrophage colony stimulating factor
HBS	HEPES buffered saline
HBV	hepatitis b virus
HEPES	N-2-hydroxethyl piperazine-N'-2-ethane sulfonic acid
hGH	human growth hormone
hLf	human lactoferrin
hTf	human transferrin
I	intensities
IPA	isopropanol
IPTG	isopropyl β-D-thiogalactopyranoside
kb	kilobase
kDa	kilodalton
LB	Luria-Bertani
Lf	lactoferrin
Lf _N	the amino terminal half of human lactoferrin
Lf _{C50}	50 kDa carboxy terminal fragment from lactoferrin
Lf _{N30}	30 kDa amino terminal fragment from lactoferrin
LMP	low melting point
MEM	minimal essential medium
MES	2(N-morpholino) ethane sulfonic acid
MIR	multiple isomorphous replacement
MPD	2-methyl-2,3-pentanediol
MR	molecular replacement
MT-1	metallothionein-1
mtx	methotrexate
N-terminal	amino terminal
NTA	nitrilotriacetate
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline

PCR	polymerase chain reaction
pdb	protein data bank
PEG	polyethylene glycol
pfu	plaque forming units
PNGase	peptide:N-glycosidase
RIA	radio immunoassay
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal ribonucleic acid]
SDS	sodium dodecylsulphate
SDS-PAGE	sodium dodecylsulphate polyacrylamide gel electrophoresis
SSC	standard saline citrate
sTf	serum transferrin
sT _N	the amino terminal half of serum transferrin
sT _{N35}	35 kDa fragment from the amino terminal half of human lactoferrin
SV-40	simian virus 40
TA	Tris acetate
TAE	Tris acetate EDTA
TCA	trichloroacetic acid
TE	10 mM Tris, 1 mM EDTA
Tris	Tris-(hydroxymethyl) aminomethane
U-DNA	uracil containing DNA
UV	ultraviolet
X-gal	5-bromo-4-chlor-3-indoyl β-D-galactopyranoside

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