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Cloning and sequencing of the cDNA for bovine lactoferrin

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

Dedication

This thesis is dedicated to my parents
Patricia and David Sidwell.

Thankyou for all your love and support.

Abstract

Bovine lactoferrin isolated from colostrum was partially sequenced by tryptic mapping and automated peptide sequencing. Homogeneous lactoferrin was used to raise polyclonal antibodies in rabbits. Specific anti-lactoferrin antibodies were isolated from the total rabbit gamma-globulin fraction by affinity chromatography on bovine lactoferrin Sepharose. These antibodies were used to quantify lactoferrin in various solutions (by electroimmuno-diffusion assay) and to demonstrate the *de novo* synthesis of lactoferrin in involuting bovine mammary tissue.

RNA was isolated from mammary tissue biopsies that were synthesizing lactoferrin. The presence of lactoferrin messenger RNA was verified by northern blot analysis. Complementary DNA (cDNA) was prepared from RNA samples and ligated into either the bacteriophage vector λgt11 or the plasmid vector pGEM-2. Recombinant clones with cDNA inserts coding for bovine lactoferrin were identified by hybridisation to radiolabelled human lactoferrin cDNA. Several clones were isolated and characterised by restriction map analysis and DNA sequencing. The overlapping nucleotide sequence from these clones encoded most of the mature protein sequence for bovine lactoferrin.

Nucleotide sequence encoding the 5' end of the lactoferrin messenger RNA was isolated by enzymatic amplification of homopolymeric-tailed first strand cDNA. Specific oligonucleotide primers were used to direct the synthesis of lactoferrin-specific sequences by the polymerase chain reaction (PCR). Double-stranded products were produced by the inclusion of an oligo-nucleotide that would prime DNA synthesis from the homopolymeric tract on the 3' end of the first strand cDNA. The nucleotide sequence of the PCR products overlapped the 5'-most sequence of the cDNA clones and extended to encode the initiation codon for bovine lactoferrin.

The combined nucleotide sequence of the cDNA and PCR clones overlapped to encode the entire coding region for bovine lactoferrin and included 5' and 3' untranslated flanking sequences. The deduced amino acid sequence of the mature protein concurred with the amino acid sequence of the tryptic peptides prepared from bovine colostrum lactoferrin.

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Chapter one: Introduction and literature review

Chapter two: Isolation and partial characterisation of bovine lactoferrin and studies on its biosynthesis in mammary tissue

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Abbreviations

ADP	adenosine diphosphate
AHSG	α_2-HS-glycoprotein
AMV	avian myleoblastosis virus
ATP	adenosine triphosphate
BAPNA	Nα-benzoyl-DL-arginine-p-nitroanilide
Bas	basophil
BFU-E	blast forming unit-erythroid
bp	base pair
cDNA	complementary DNA
CDI	carbonyldiimidazole
CFU	colony forming unit
CFU-GEMM	multipotential colony forming unit
CMC	1-cyclohexyl-3-(2-morpholinyl-(4)-ethyl)carbodiimide metho-*p*-toluene sulphonate
CM-Sephadex	carboxylmethyl-Sephadex
CP	ceruloplasmin
cpm	counts per minute
C-terminal	carboxyl terminal
CTP	cytosine triphosphate
dH_2O	deionised water
ddH_2O	deionised, distilled water
DEPC	diethylpyrocarbonate
DMF	dimethylformamide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DOC	sodium deoxycholate
$d(pC)_{20}$	deoxycytosine twenty-mer oligonucleotide
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
Fe-lactoferrin	iron-loaded lactoferrin
γG	gamma-globulin
GM	granulocyte-macrophage
GM-CSF	GM colony stimulating factor
GTP	guanosine triphosphate
HPLC	high performance liquid chromatography
kb	kilobase pairs

K_d	dissociation constant
kDa	kilodalton
IEP	isoelectric point
IM	intramuscular (injection)
IPTG	β-D-isopropyl-thiogalactopyranoside
LB	Luria-Bertani broth
Lf	lactoferrin
Meg	megakaryocyte
M-MLV	Moloney murine leukemia virus
mRNA	messenger RNA
MW	molecular weight
NLS	n-lauryl sarcosine
N-terminal	amino terminal
NTA	nitrilotriacetate
O_2^-	superoxide anion
·OH	hydroxyl radical
oligo (dT)-cellulose	oligo (deoxythymidine)-cellulose
p97	melanotransferrin (oncofetal 97 kDa protein)
PB	phosphate buffer
PBL	peripheral blood lymphocytes
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDB	phage dilution buffer
PHA	phytohemagglutinin
pO_2	partial pressure of oxygen
Poly A^+ RNA	RNA having a polyadenylate tract at its 3' end
Poly A^- RNA	RNA lacking a polyadenylate tract at its 3' end
POPOP	1,4 di-[2,-(5-phenyloxazoyl)] benzene
PPO	2,5-diphenyloxazole
RES	reticuloendotheial system
RME	receptor-mediated endocytosis
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal RNA
RP-HPLC	reverse phase-HPLC
rpm	revolutions per minute
SAM	S-adenosyl-L-methionine
SC	subcutaneous (injection)

sdH_2O	sterile, distilled water
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SM	suspension medium
SSC	sodium chloride and sodium citrate solution
TAE	tris·acetate buffer containing EDTA
TBE	tris·borate buffer containing EDTA
TCA	trichloroacetic acid
TdT	terminal deoxynucleotidyl transferase
TE	tris·HCl buffer containing EDTA
Tf	transferrin
TFA	trifluroacetic acid
TfR	transferrin receptor
T-lymphocyte	thymus derived lymphocyte
Tris	tris-(hydroxymethyl) aminomethane
TTP	thymidine triphosphate
TX-100	triton X100
UV	ultraviolet
(v/v)	volume:volume ratio
(w/v)	weight:volume ratio
X-Gal	5-bromo-4-chloro-3-indolyl-β-galactopyranoside