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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 \textit{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 \textlambda gtl1 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 oligonucleotide 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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Abbreviations

ADP  adenosine diphosphate
AHSG  \(\alpha_2\)-HS-glycoprotein
AMV  avian myeloblastosis virus
ATP  adenosine triphosphate
BAPNA  N\(\alpha\)-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-morpholiny-(4-ethyl)carbodiimide
      metho-p-toluene sulphonate
CM-Sephadex  carboxymethyl-Sephadex
CP  ceruloplasmin
cpm  counts per minute
C-terminal  carboxyl terminal
CTP  cytosine triphosphate
dH\(_2\)O  deionised water
ddH\(_2\)O  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
\(\gamma\)G  gamma-globulin
GM  granulocyte-macrophage
GM-CSF  GM colony stimulating factor
GTP  guanosine triphosphate
HPLC  high performance liquid chromatography
kb  kilobase pairs
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_d</td>
<td>dissociation constant</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>IEP</td>
<td>isoelectric point</td>
</tr>
<tr>
<td>IM</td>
<td>intramuscular (injection)</td>
</tr>
<tr>
<td>IPTG</td>
<td>β-D-isopropyl-thiogalactopyranoside</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertani broth</td>
</tr>
<tr>
<td>Lf</td>
<td>lactoferrin</td>
</tr>
<tr>
<td>Meg</td>
<td>megakaryocyte</td>
</tr>
<tr>
<td>M-MLV</td>
<td>Moloney murine leukemia virus</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>NLS</td>
<td>n-lauryl sarcosine</td>
</tr>
<tr>
<td>N-terminal</td>
<td>amino terminal</td>
</tr>
<tr>
<td>NTA</td>
<td>nitrilotriacetate</td>
</tr>
<tr>
<td>O_2^-</td>
<td>superoxide anion</td>
</tr>
<tr>
<td>·OH</td>
<td>hydroxyl radical</td>
</tr>
<tr>
<td>Oligo (dT)-cellulose</td>
<td>oligo (deoxythymidine)-cellulose</td>
</tr>
<tr>
<td>p97</td>
<td>melanotransferrin (oncofetal 97 kDa protein)</td>
</tr>
<tr>
<td>PB</td>
<td>phosphate buffer</td>
</tr>
<tr>
<td>PBL</td>
<td>peripheral blood lymphocytes</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PDB</td>
<td>phage dilution buffer</td>
</tr>
<tr>
<td>PHA</td>
<td>phytohemagglutinin</td>
</tr>
<tr>
<td>P02</td>
<td>partial pressure of oxygen</td>
</tr>
<tr>
<td>Poly A+ RNA</td>
<td>RNA having a polyadenylate tract at its 3' end</td>
</tr>
<tr>
<td>Poly A- RNA</td>
<td>RNA lacking a polyadenylate tract at its 3' end</td>
</tr>
<tr>
<td>POPOP</td>
<td>1,4 di-[2,-(5-phenyloxazoyl)] benzene</td>
</tr>
<tr>
<td>PPO</td>
<td>2,5-diphenyloxazole</td>
</tr>
<tr>
<td>RES</td>
<td>reticuloendothelial system</td>
</tr>
<tr>
<td>RME</td>
<td>receptor-mediated endocytosis</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>ribonuclease</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal RNA</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>reverse phase-HPLC</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosyl-L-methionine</td>
</tr>
<tr>
<td>SC</td>
<td>subcutaneous (injection)</td>
</tr>
</tbody>
</table>
sdH2O: 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: trifluoroacetic 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