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Isolation and Characterisation of the 5' Region of the Bovine Lactoferrin Gene

Heather B. Bain

1995

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

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Dedication

This thesis is dedicated to the memory of my grandfathers,

Claud Charles Brown and John Morris Bain.

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Abstract

Lactoferrin is an iron-binding protein found in a variety of mammalian secretions and polymorphonuclear leucocytes. Many biological roles have been proposed for lactoferrin however despite extensive research, the precise function(s) of this protein remain unclear. Lactoferrin appears to be regulated in a highly specific manner, dependent upon the developmental stage, the tissue and the species being investigated. RNA analyses have suggested that transcriptional regulation may be a significant factor controlling the expression of bovine lactoferrin within the mammary gland. A detailed knowledge of the molecular mechanisms and factors involved in the transcriptional regulation of the bovine lactoferrin gene will provide insights into the biological role of this protein.

It was with this primary aim in mind that an unamplified bovine genomic library was screened for the presence of bovine lactoferrin 5' regulatory sequences. This resulted in the isolation of a clone which was characterised by detailed restriction mapping and found to contain sequences complementarity to bovine lactoferrin exon I and exon II sequences. Analysis of this clone indicated that is contained ~10 kb of DNA 5' to the transcriptional start site of bovine lactoferrin. A ~2.85 kb fragment which hybridised to an exon I probe was isolated and subjected to dideoxy sequencing. ~2.5 kb of DNA 5' to the lactoferrin coding sequence was identified within this fragment. A computer sequence homology search indicated that several putative consensus binding sites for transcription factors may be located within this DNA sequence. Fragments prepared from this subclone by PCR were subcloned into reporter gene expression constructs. The transcriptional activity of one of these constructs was investigated using COS cells and transient reporter gene expression studies. This construct, containing 565 bp of 5' bovine lactoferrin sequence, exhibited promoter activity.

The successful isolation of this promoter will enable further investigations directed towards elucidating the transcriptional regulation of the bovine lactoferrin gene to be performed.

Abbreviations

amp	ampicillin
AP-1	Activator Protein 1
AP-2	Activator Protein 2
bLf	Bovine lactoferrin
bp	base pair
BSA	Bovine serum albumin
CAT	Chloramphenicol acetyl transferase
CMV	cytomegalovirus
COUP-TF	Chicken Ovalbumin Upstream Promoter-Transcription Factor
Da	Dalton
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DMEM	Dulbecco's modification of Eagle's medium
DMSO	dimethyl sulphoxide
DNase	deoxyribonuclease
BRL	Bethesda research laboratories
dTTP	deoxythymine triphosphate
EEO	electroendosomosis
EGF	Epidermal Growth Factor
ELISA	enzyme-linked immunoabsorbant assay
ERE	Estrogen response element
FBS	Fetal Bovine Serum
GCG	Genetics Computing Group
GF/A	glass fibre/A
hGH	human growth hormone
HTf	human transferrin
IPTG	isopropyl β-D-thiogalactopyranoside
IRE	iron-responsive element
kb	kilobase
kDa	kilo Dalton
λ	Bacteriophage lambda
NF-Il 6	Nuclear factor-interleukin 6
nt	nucleotide
Oct-1	Octamer transcription factor 1

PBS	Phosphate buffered saline
PEG 8000	polyethylene glycol 8000
pfu	plaque forming units
RES	reticuloendothelial system
RNase	ribonuclease
SDS	sodium dodecylsulphate
SSC	standard saline citrate
TAE	Tris acetate EDTA
TBE	Tris borate EDTA
TE	tris (10 mM) EDTA (1mM) pH 8.0
UTR	untranslated region
UV	ultraviolet
XGal	5-bromo-4-chloro-3-indolyl β-D-galactopyranoside

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