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Identification of Mitogenic Factors in Bovine Whey

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Doctor of Philosophy

by

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**FIGURE TITLES HAVE BEEN ABBREVIATED*

ABBREVIATIONS

α_2-M	alpha two-macroglobulin
$A_{214\ nm}$	absorbance at 214 nm
$A_{280\ nm}$	absorbance at 280 nm
A431	human epidermoid carcinoma cell line
AG2804	simian virus 40-transformed human lung fibroblasts
BALB/c 3T3	mouse BALB/c 3T3 embryo fibroblasts
BHK-21	baby hamster kidney fibroblasts
BSA	bovine serum albumin
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DMEM	Dulbecco's modified minimal essential Eagles Medium
DNA	deoxyribonucleic acid
EGF	epidermal growth factor
EGFR	EGF receptor
FBS	fetal bovine serum
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FPLC	fast protein liquid chromatography
h	hour
HB-EGF	heparin binding growth factor
HBSS	HEPES-buffered saline
HER/ erbB	human EGF receptor/ EGF receptor
HFBA	heptafluorobutyric acid

HPLC	high pressure liquid chromatography
HSPG	heparan sulphate proteoglycans
IEC-6	rat small intestinal epithelial cells
IGF	insulin-like growth factor
IgG	immunoglobulin G
L6	rat L6 skeletal muscle myoblasts
LAP	latency associated peptide
LTBP	latent TGF-β binding protein
MDCK	canine kidney epithelial cells
min	minute
Mr	molecular weight
Mv1Lu	mink lung epithelial cells
PAGE	polyacrylamide gel electrophoresis
PDGF	platelet-derived growth factor
PDGF-Rα	platelet derived growth factor receptor alpha
PDGF-Rβ	platelet derived growth factor receptor beta
RRA	radioreceptor assay
SDS	sodium dodecyl sulphate
SPARC	secreted protein, acidic and rich in cysteine
TFA	trifluoroacetic acid
TGF-β	transforming growth factor-beta
TGF-α	transforming growth factor alpha
Tris	tris (hydroxymethyl) aminomethane

This list excludes nomenclature of amino acids, chemical elements and SI units.

ABSTRACT

Bovine milk contains factors that can support the growth of cells in culture. However, milk growth factors are at very low concentrations and not easily purified and investigated. Cation-exchange chromatography of cheese whey was shown by Francis *et al.* (1995) to produce a mixture of whey proteins with enriched growth factor activity (termed whey extract). In the current thesis, growth factors and binding proteins of bovine milk that have not been extensively investigated are done so using whey extract as the starting material.

Initially, whey extract was shown to contain more than one type of growth factor and to support the *in-vitro* growth of mesodermal-derived cells (such as BALB/c 3T3 cells) but inhibit epithelial cell growth. Gel-filtration experiments showed that mitogens and epithelial cell growth inhibitors present in whey extract are attached to high molecular weight whey proteins and are released from such associations under acid conditions.

Platelet-derived growth factor (PDGF) was identified and measured in acid gel-filtration fractions of whey extract. There was only a small amount of PDGF found in whey extract and this was predominantly the PDGF-BB isoform. Importantly, PDGF did not account for the majority of the BALB/c 3T3 bioactivity of whey extract. PDGF was associated with a high molecular weight whey protein that conferred latency on this factor and was released from this association under acid conditions. The PDGF binding protein was not identified, but appeared not to be related to known PDGF binding proteins.

A small amount of fibroblast growth factor (FGF)-1 and FGF-2 was detected in bovine whey extract and is the first report of FGF in bovine milk. However, when FGF

was removed from whey extract there was no significant effect on its BALB/c 3T3 cell bioactivity.

The epithelial cell growth inhibitory activity found in whey extract was identified as transforming growth factor-beta (TGF-β). The bulk of TGF-β in bovine whey and whey extract was latent and could be activated by acid. Over 85% of this activity shown to be TGF-β2. An 80 kDa latent TGF-β complex present in whey extract was identified and is the first description of this size complex in a biological fluid. TGF-β was a significant contributor to the BALB/C 3T3 bioactivity of acid treated whey extract. However, neither TGF-β or any other growth factor known to be present in bovine milk could account for all the BALB/C 3T3 bioactivity of whey extract. Epidermal growth factor (EGF) has previously not been purified from bovine milk. In the current thesis, an EGF-like molecule was identified in bovine whey extract and may be a major contributor to the BALB/c 3T3 bioactivity of whey extract. Heparin affinity chromatography showed that the EGF-activity of whey extract was a betacellulin-like molecule.

The results of this thesis show bovine whey extract contain small amounts of active growth factors and large amounts of latent mitogens that can be activated by acid treatment. It is also concluded that in addition to the known levels of IGF, the major growth factors in bovine whey are TGF-β and to a lesser extent PDGF. FGF appears to be a minor bovine whey growth factor. Preliminary results show an EGF-like molecule may be a major BALB/c 3T3 cell mitogen of whey extract.

STATEMENT

This thesis contains no material that has been accepted for the award of any other degree or diploma at any university or other tertiary institution, and to the best of my knowledge, it contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I give consent to this copy of my thesis when deposited in the University library, being available for loan and photocopying.

Mary-Louise Rogers

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PUBLICATIONS FROM THESIS

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Patent

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