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**Isolation and Partial Characterisation  
of a Calcium-dependent Lectin-like  
Protein from the Flat Oyster,  
*Ostrea chilensis***

A thesis presented in partial fulfilment of the requirements for  
the degree of Doctor of Philosophy in Veterinary Pathology  
at Massey University, Palmerston North, New Zealand.

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## Abstract

The (Chilean) flat oyster, *Ostrea chilensis*, is native to New Zealand and the west coast of South America. It is a commercially important species in New Zealand because of its exquisite taste that attracts premium prices.

This thesis describes the first isolation and partial characterisation of an oyster haemolymph calcium-dependent carbohydrate-binding protein. This protein 'chiletin' was originally isolated from oyster haemolymph by binding to the agarose-galactan matrix of a Sepharose column. Chiletin was predominantly composed of a 24 kilodalton (kDa) band when examined with one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis under non-reducing conditions and a 12 kDa band with reduction of disulphide bonds. The N-terminal sequence of the 24 kDa band was determined to be 'IAGPGWEKYN'. This sequence was not homologous to any known protein. Examination of isolated chiletin with two-dimensional protein analysis gel electrophoresis revealed the presence of three (~12 kDa) subunits ranging in isoelectric point from 5.2 to 6.0.

The 24 kDa protein was used to immunise rabbits and a separate antiserum was also raised in rabbits using a synthetic peptide (identical to that above) coupled to keyhole limpet haemocyanin. These antisera were used to confirm the size of the chiletin subunits with Western blots and to examine the elution of chiletin in oyster haemolymph with size exclusion chromatography in phosphate buffered saline (PBS) and 8 M urea. There were four or five different sized conformational aggregates of chiletin present in oyster haemolymph under physiological conditions (PBS). The use of 8 M urea produced two separate aggregates.

A major characteristic of lectins is the ability to agglutinate sheep red blood cells and both whole oyster haemolymph and isolated chiletin had this property. Chiletin was identified by immunohistochemistry to be present in a number of tissues. Staining intensity was most consistent in the auricular myocardial cells, followed by the digestive gland epithelium. Chiletin was not induced in haemolymph in response to temperature (30°C) stress or injection of turpentine into the adductor muscle.

There have been few immunological studies performed with *O. chilensis*. The results of the project contribute to what is known about comparative immunology. Greater

understanding of how oysters respond to stress and deal with pathogens will ultimately be of benefit to the aquaculture industry.

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## Glossary/Abbreviations

<i>A. papillata</i>	<i>Aaptos papillata</i>	sponge
<i>A. fulica</i>	<i>Achatina fulica</i>	snail
<i>A. crassispina</i>	<i>Anthocardaris crassispina</i>	sea urchin
<i>A. pectinifera</i>	<i>Asterina pectinifera</i>	starfish
<i>B. glabrata</i>	<i>Biomphalaria glabrata</i>	snail
<i>B. exitiosus</i>	<i>Bonamia exitiosus</i> sp. nov	Haplosporidian parasite
<i>B. ostreae</i>	<i>Bonamia ostreae</i>	Haplosporidian parasite
<i>B. leachii</i>	<i>Botrylloides leachii</i>	tunicate (ascidian, urochordate)
<i>B. schlosseri</i>	<i>Botryllus schlosseri</i>	tunicate (ascidian, urochordate)
<i>B. belcheri</i>	<i>Branchiostoma belcheri</i>	amphioxus (cephalochordates)
<i>C. hortensis</i>	<i>Cepaea hortensis</i>	garden snail
<i>C. picta</i>	<i>Clavelina picta</i>	colonial tunicate (urochordate)
<i>C. gigas</i>	<i>Crassostrea gigas</i>	Pacific oyster
<i>C. virginica</i>	<i>Crassostrea virginica</i>	American/eastern oyster
<i>C. grayanus</i>	<i>Crenomytilus grayanus</i>	sea mussel
<i>D. candidum</i>	<i>Didemnum candidum</i>	tunicate (ascidian, urochordate)
<i>D. ternatanum</i>	<i>Didemnum ternatanum</i>	tunicate (ascidian, urochordate)
<i>D. busckii</i>	<i>Drosophila busckii</i>	fruit fly
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>	fruit fly
<i>E. coli</i>	<i>Escherichia coli</i>	bacterium
<i>H. roretzi</i>	<i>Halocynthia roretzi</i>	solitary tunicate
<i>H. nelsoni</i>	<i>Haplosporidium nelsoni</i>	protozoan parasite
<i>L. polyphemus</i>	<i>Limulus polyphemus</i>	American horseshoe crab
<i>L. anguillarum</i>	<i>Listonella anguillarum</i>	bacterium
<i>L. stagnalis</i>	<i>Lymnaea stagnalis</i>	snail
<i>M. edulis</i>	<i>Mytilus edulis</i>	blue mussel
<i>O. chilensis</i>	<i>Ostrea (=Tiostrea) chilensis</i>	(Chilean) flat oyster
<i>O. edulis</i>	<i>Ostrea edulis</i>	European flat oyster
<i>P. mamillata</i>	<i>Phallusia mamillata</i>	tunicate (ascidian, urochordate)
<i>P. martensii</i>	<i>Pinctada fucata martensii</i>	Japanese (akoya) pearl oyster
<i>P. marinus</i>	<i>Perkinsus marinus</i>	protozoan parasite
(n/a)	<i>Petromyzon marinus</i>	lamprey

<i>P. maxima</i>	<i>Pinctada maxima</i>	giant hatchery-reared pearl oyster
<i>P. corneus</i>	<i>Planorbarius corneus</i>	freshwater snail
<i>P. platessa</i>	<i>Pleuronectes platessa</i>	plaice
<i>P. papatasi</i>	<i>Phlebotomus papatasi</i>	sandfly
<i>P. misakiensis</i>	<i>Polyandrocarpa misakiensis</i>	budding tunicate
<i>P. stolonifera</i>	<i>Pyura stolonifera</i>	tunicate (ascidian, urochordate)
<i>T. tridentatus</i>	<i>Tachypleus tridentatus</i>	Japanese horseshoe crab
<i>T. gondii</i>	<i>Toxoplasma gondii</i>	sporozoan parasite
<i>S. peregrine</i>	<i>Sarcophaga peregrine</i>	flesh fly
<i>S. mansoni</i>	<i>Schistosoma mansoni</i>	metazoan parasite
<i>S. exigua</i>	<i>Spodoptera exigua</i>	beet armyworm
<i>S. clava</i>	<i>Styela clava</i>	tunicate (ascidian, urochordate)
<i>S. plicata</i>	<i>Styela plicata</i>	solitary tunicate
<i>V. anguillarum</i>	<i>Vibrio anguillarum</i>	bacterium
<i>V. splendidus</i>	<i>Vibrio splendidus</i>	bacterium

Å	Ångström: one hundred-millionth ( $10^{-8}$ ) of a centimetre
achantininH	sialic acid binding lectin of snails ( <i>Achatina fulica</i> )
ACTH	adrenocorticotropin
AMP	antimicrobial peptides
ANK antiserum	the rabbit antiserum against ANKNGAYIHI synthetic peptide
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
APP	acute phase protein
AU	auricle
BCA	bicinchoninic acid
BSA	bovine serum albumin
C	control group
C(number)	complement or reversed phased column
°C	degrees centigrade
CaCl <sub>2</sub>	calcium chloride
cDNA	complementary deoxyribonucleic acid
CE	cation exchange
CEC	cation exchange column
CL	chemiluminescence

cm	centimetre(s)
CRD	carbohydrate recognition domain
CRP	C-reactive protein
CTLDcp	C-type lectin domain-containing proteins
d	day(s)
Da	dalton
DAB	diaminobenzidine/3,3'-diaminobenzidine peroxidase substrate and urea hydrogen peroxide (Sigma fast™ 3,3'-diaminobenzidine tablet sets, SIGMA, St. Louis, MO, USA)
DGE	digestive gland epithelia
dH <sub>2</sub> O	distilled water
DMSO	dimethyl sulfoxide
DSC	desalting column
DTT	dithiothreitol
echinoidin	lectin of the sea urchin, <i>A. crassispina</i>
EDTA	ethylenediaminetetraacetic acid
e.g.	exempli gratia (= for example)
18K-LAF	<i>Limulus</i> 18 kDa agglutination-aggregation factor
ELISA	enzyme-linked immunosorbent assay
etc.	et cetera (= and the rest)
Factor-C, -B, -G	glycoproteins that are intracellular serine-protease zymogens from horseshoe crab haemocytes/ <i>Limulus</i> clotting factor
FMRFamide	a tetrapeptide amide: Phe-Met-Arg-Phe-NH <sub>2</sub> phenylalanyl-methionyl-arginyl-phenylalanine amide
g	gramme
<i>g</i>	centrifugal force
G	gauge
Gal-lectin	Gal/GalNAc lectin of <i>Entamoeba histolytica</i>
GBL	glucose-binding lectin
GBP	galactose-binding protein
GHR-P63	rat liver anti-protease
gigalins	lectin of Pacific oyster, <i>C. gigas</i>
GoαRaIg	goat anti-rabbit immunoglobulin labelled with peroxidase
GPC	Macrosphere GPC size exclusion column
HA	haemagglutination

HCl	hydrogen chloride/hydrochloric acid
HI	haemagglutination inhibition
HIC	hydrophobic interaction column
HPLC	high performance liquid chromatography
HOCl	hypochlorous acid
hr(s)	hour(s)
H&E stain	hematoxylin and eosin stain
HSP	heat shock protein
IAG antiserum	rabbit antiserum raised against IAGPGWEKYN synthetic peptide
i.e.	id est (= that is)
IEF	isoelectric focusing
Ig	immunoglobulins
IL	interleukin
kDa	kilodalton
KLH	keyhole limpet haemocyanin
l	litre
LBP	lipopolysaccharide (LPS)-binding protein
limulin	lectin of horseshoe crab
LPS	lipopolysaccharide
M	molar
MASPs	mannose-binding lectin-associated serine proteases
MBP/MBL	mannose-binding protein/lectin
MES	(3S,4S)-4 $\beta$ -D-glucopyranosyloxy-3-methyloctanoic acid
mg	milligramme
mGDF	molluscan growth and differentiation factor
min(s)	minute(s)
ml	millilitre
mM	milimolar
mm	millimetre(s)
modiolin	lectin of the horse mussel, <i>Modiolus modiolus</i>
MPa	mega pascal
MSX	multinucleated spore unknown
MT	methallothionein
MW	molecular weight

NaCl	sodium chloride
NADPH	$\beta$ -nicotinamide adenine dinucleotide phosphate
NaN <sub>3</sub>	sodium azide
NaOH	sodium hydroxide
Na <sub>2</sub> SO <sub>4</sub>	sodium sulphate
nm	nanometre
NO	nitric oxide
O antigen	an antigen that occurs in the body of a Gram-negative bacterial cell also called somatic antigen
O1 antigen	<i>Vibrio cholerae</i> (Gram-negative bacillus) is differentiated by the lipopolysaccharide in the outer membrane; strains of <i>V. cholerae</i> that produce cholera belong to serogroup O1 or O139. <i>V. cholerae</i> O1 is divided into two biotypes: classical and El Tor. The A, B and C factors differentiate O1 antigens.
OH	oyster haemolymph
1D	one-dimensional
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	phosphorylethanolamine
PEG	polyethylene glycol
PG	prostaglandin (E <sub>2</sub> : dinoprostone, F <sub>2<math>\alpha</math></sub> : dinoprost)
PGN	(bacterial) peptidoglycan
pH	the negative logarithm of hydrogen ion concentration expressed in molarity
pI	isoelectric point
PMSF	phenylmethyl sulphonyl fluoride
ppt	parts per thousand
psi(g)	pounds per square inch (gauge)
RBC	red blood cell
ROIs	reactive oxygen intermediates
RNIs	reactive nitrogen intermediates
RPC	reversed phase column
RT-PCR	reverse transcriptase-polymerase chain reaction
SAA	serum amyloid A component

SAP	serum amyloid P component
SCPs	small cardioactive peptides
SD	sub-lethal dose of turpentine trial group
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SDX	Superdex size exclusion column
sec(s)	second(s)
SE	standard error
SEC	size exclusion column
SI	the South Island control group
ST	sub-lethal temperature trial group
SYPRO Ruby	SYPRO <sup>®</sup> Ruby protein gel stain (Molecular Probes)
T3	3,5,3'-triiodothyronine
T4	thyroxine
TBS	tris buffered saline
TCRP 1-3	<i>Tachypleus</i> C-reactive protein 1-3
TES	temperature stress group
TFA	trifluoroacetic acid
TGF	transforming growth factor
3'AURE	AUUUA reiterations in 3' untranslated regions (AU-rich elements)
TLs-5	tachylectin 5A and 5B
TNF	tumour necrosis factor
TS	turpentine stress group
TSH	thyroid stimulating hormone (thyrotropin)
TTA	<i>Tachypleus tridentatus</i> agglutinin
2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
V	volt(s)
vs.	versus
W	watt(s)
zymosan	inflammatory agent
µg	microgramme
µl	microlitre
µm	micrometer
%	percent/ per cent/ percentage

Units in the thesis are written according to the *Journal of Invertebrate Pathology*.