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

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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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<th>Species/Abbreviation</th>
<th>Category</th>
</tr>
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<tbody>
<tr>
<td>A. papillata</td>
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<td>A. fulica</td>
<td>Achatina fulica</td>
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<td>A. crassispina</td>
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<td>Clavelina picta</td>
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<td>C. gigas</td>
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<td>Ostrea (=Tiostrea) chilensis</td>
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<td>--------------------------------------------------</td>
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<td><em>P. maxima</em></td>
<td>giant hatchery-reared pearl oyster</td>
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<tr>
<td><em>P. corneus</em></td>
<td>freshwater snail</td>
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<tr>
<td><em>P. platessa</em></td>
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<td><em>P. stolonifera</em></td>
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<td><em>T. tridentatus</em></td>
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<td><em>T. gondii</em></td>
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<td><em>S. exigua</em></td>
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<tr>
<td><em>V. splendidus</em></td>
<td>bacterium</td>
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</table>

Å Ångström: one hundred-millionth \((10^{-8})\) of a centimetre
achantininH sialic acid binding lectin of snails (Achatina fulica)
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 bicinchnonic acid
BSA bovine serum albumin
C control group
C(number) complement or reversed phased column
°C degrees centigrade
CaCl2 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
dH₂O  distilled water
DMSO  dimethyl sulfoxide
DSC  desalting column
DTT  dithiothreitol
echinoidin  lectin of the sea urchin, *A. crassispina*
EDTA  ethylenediaminetetraacetic acid
e.g.  exempli gratia (= for example)
18K-LAF  *Limulus* 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/*Limulus* clotting factor
FMRFamide  a tetrapeptide amide: Phe-Met-Arg-Phe-NH₂ phenylalanyl-methionyl-arginyl-phenylalanine amide
g  gramme
G  gauge
Gal-lectin  Gal/GalNAc lectin of *Entamoeba histolytica*
GBL  glucose-binding lectin
GBP  galactose-binding protein
GHR-P63  rat liver anti-protease
gigalins  lectin of Pacific oyster, *C. gigas*
GoaRaIg  goat anti-rabbit immunoglobulin labelled with peroxidase
GPC  Macrosphere GPC size exclusion column
HA  haemagglutination
<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Description</th>
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<tbody>
<tr>
<td>HCl</td>
<td>hydrogen chloride/hydrochloric acid</td>
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<tr>
<td>HI</td>
<td>haemagglutination inhibition</td>
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<tr>
<td>HIC</td>
<td>hydrophobic interaction column</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<td>HOCl</td>
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<td>i.e.</td>
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<td>IEF</td>
<td>isoelectric focusing</td>
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<td>Ig</td>
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<td>kDa</td>
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<td>l</td>
<td>litre</td>
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<tr>
<td>LBP</td>
<td>lipopolysaccharide (LPS)-binding protein</td>
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<td>MASP</td>
<td>mannose-binding lectin-associated serine proteases</td>
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<td>MES</td>
<td>(3S,4S)-4β-D-glucopyranosyloxy-3-methyloctanoic acid</td>
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<td>mGDF</td>
<td>molluscan growth and differentiation factor</td>
</tr>
<tr>
<td>min(s)</td>
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</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
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<td>mM</td>
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<td>mm</td>
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<tr>
<td>modiolin</td>
<td>lectin of the horse mussel, <em>Modiolus modiolus</em></td>
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<tr>
<td>MPA</td>
<td>mega pascal</td>
</tr>
<tr>
<td>MSX</td>
<td>multinucleated spore unknown</td>
</tr>
<tr>
<td>MT</td>
<td>methallothionein</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
</tbody>
</table>
NaCl  sodium chloride
NADPH  β-nicotinamide adenine dinucleotide phosphate
NaN₃  sodium azide
NaOH  sodium hydroxide
Na₂SO₄  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  *Vibrio cholerae* (Gram-negative bacillus) is differentiated by the
lipopolysaccharide in the outer membrane; strains of *V. cholerae*
that produce cholera belong to serogroup O1 or O139. *V. cholerae*
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 (E₂: dinoprostone, F₂α: 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® Ruby protein gel stain (Molecular Probes)
T3  3,5,3'-triiodothyronine
T4  thyroxine
TBS  tris buffered saline
TCRP 1-3  Tachypleus 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  Tachypleus tridentatus agglutinin
2D-PAGE  two-dimensional polyacrylamide gel electrophoresis
V  volt(s)
vs.  versus
W  watt(s)
zymosan  inflammatory agent
µg  microgramme
µl  microlitre
µm  micrometre
%  percent/ per cent/ percentage

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