PURIFICATION AND CHARACTERIZATION OF A LECTIN FROM TAMARILLO FRUITS (*CYPHOMANDRA BETACEA*)

by

Chanxing Xu

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

1991

Massey University Library. Thesis Copyright Form

Title of thesis: Purification and characterization of a lectin from tamarillo fruits (Cyphomandra betacea)

(1) (a) ✓ I give permission for my thesis to be made available to readers in the Massey University Library under conditions determined by the Librarian.

(b) I do not wish my thesis to be made available to readers without my written consent for _____ months.

(2) (a) ✓ I agree that my thesis, or a copy, may be sent to another institution under conditions determined by the Librarian.

(b) I do not wish my thesis, or a copy, to be sent to another institution without my written consent for _____ months.

(3) (a) ✓ I agree that my thesis may be copied for Library use.

(b) I do not wish my thesis to be copied for Library use for _____ months.

Signed [signature]

Date _____

The copyright of this thesis belongs to the author. Readers must sign their name in the space below to show that they recognise this. They are asked to add their permanent address.

NAME AND ADDRESS	DATE

Lectins specific in their binding to oligomers of β1,4 linked N-acetylglucosamine were identified in the fruits of *Cyphomandra* species of the family Solanaceae. Thus, *Cyphomandra* species can be considered as a new source of lectins for basic and applied studies.

New lectins (designated as CBL1 and CBL2) were identified from tamarillo fruits (*Cyphomandra betacea*). CBL1 was purified. Biochemical characterization, subcellular localization and molecular sequence analysis for this new lectin were made. CBL2, which was immunologically unrelated to CBL1, was not further characaterized.

CBL1 could be readily purified using affinity and ion exchange chromatography. CBL1 comprised two subunits joined by nonconvalant interactions. Subunit size was 25 kDa. N,N,'N",N""-tetraacetylchitotetraose was the most effective carbohydrate for inhibition of CBL1 induced agglutination of rabbit erythrocytes. CBL1 consists of abundant residues of Cys (16 %), Gly (14 %), Glx (13 %), Ser (11 %), Pro (9 %) and Asx (7 %), and to a lesser extent, hydroxyproline residues.

CBL1 was found to be an abundant, extremely stable and developmentally regulated protein. It was found predominantly in cell walls of fruit tissues using immunofluorescence techniques. CBL1 could play a defence role in seed development.

Despite the general resemblance of chemical composition and carbohydrate specificities, no cross-reaction among solanaceous lectins in double immunodiffusion tests performed

in gels containing their carbohydrate ligands was demonstrated, suggesting they may not have similar epitopes.

Four tryptic peptides and the N-terminal fragment of CBL1 were sequenced, which showed some homologies with the Gramineae lectins. Since CBL1 and the Gramineae lectins shared similar properties such as amino acid composition and sugar specificities, it is suggested that CBL1, a solanaceous lectin, might be evolutionarily related to the Gramineae lectins.

Two cDNA clones were isolated with anti-CBL1 serum, and sequenced. One of them (X200), which reacted weakly with anti-CBL1 serum, was 96 % identical with a bacterial gene *ilv*C encoding acetohydroxy acid isomeroreductase. The peptide encoded by this cDNA could have some similar epitopes to CBL1, which resulted in its isolation. Another clone (X208), which showed stronger reaction with anti-CBL1 serum, was found to contain putative peptide sequences which did not show homology with CBL1 peptide sequences. This clone could be derived from one domain of CBL1's coding region, while the peptide sequences could be confined to another domain. Complexity in immunoscreening the clone encoding CBL1 is discussed, and future work on the isolation of cDNA clone encoding this interesting lectin is suggested.

ACKNOWLEDGEMENTS

I am grateful to my chief supervisor Dr Pak-Lam Yu and co-supervisor Dr David Fountain for their encouragement, supervision, and support.

The project and other preliminary projects was supported by a Vice Chancellor Fellowship and Massey University Research Funds from November, 1986 to December 1990.

I thank Dr Chris Moore for performing amino acid composition analysis and Dr Mervyen Birtles for his assistance in the immunocytochemical work. I am also grateful to my former co-supervisor Prof Barry Scott for permitting the use of facility in the Molecular Genetics Unit and other help.

Thanks also go to the following people for their help and friendship:
Prof R Earle, Prof Ian Maddox, Prof Edward Baker, Dr Brian Mansfield, Heather Baker, Prof Bob Chong, Dr Clive Cornford, Dr C O'Kelly, Dr Sue longford, Elizabeth NicKless, Dr Derek Knighton, Dr Ian Andrew, Dr David Greenwood, Professor Kelvin Moriarty.

Wil Canbourn, Bob Hodge, Fengfeng Xu, Cryn Russell, Tania Naga, Chungming Huang, Nick Ellison, Sirinda Yunchalard and technical and secretarial staff of the Biotechnology Department.

Pinthita Mungkarndee, Sridar Susarlar, Hong Chen, Qingnong Tang, Sunthorn Kanchanatawee, Jian Sun, Guoqiang Xing, Zhengzhong Xu, Zhang Lin and Jimei Zhu.

I thank Yu Yang for discussion, encouragement, patience, understanding and support during my Ph.D work. Special thanks go to my father, mother and sisters for their unending love and support. Without their support, this Ph.D research which started on October 15, 1988 and finished on December 31, 1990 would not have been completed.

LIST OF PUBLICATIONS AND ABSTRACTS

Xu, C., Moore, C.H., Fountain, D.W. and Yu, P.-L. (1991) Purification and characterization of a new lectin from tamarillo fruit (*Cyphomandra betacea*). In Preparation.

Xu, C., Birtles, M., Fountain, D.W. and Yu, P.-L. (1991) Immunocytolocalization of tamarillo lectin and its immunological relationship to other solanaceous lectins. In preparation.

Xu, C., Moore, C.H., Fountain, D.W. and Yu, P.-L. (1990) Purification and characterization of a novel lectin from tamarillo fruit (*Cyphomandra betacea*). Combined meeting of Institute of Chemistry, NZ Biochemical Society and NZ Society of Plant Physiology, 20-23 August 1990, Victoria University, Wellington.

Yu, P.-L., Xu, C. and Fountain, D.W. (1991) Purification and characterization of a novel lectin from tamarillos by chromatography. 1991 Conference of NZ Biotechnology Society, 21-22 February 1991, Palmerston North.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 5

Chapter 6

LIST OF TABLES

ABBREVIATIONS

BCIP	5-bromo-4-chloro-3-indolyl phosphate
Bisacrylamide	N,N'-Methylene-bis-acrylamide
BpB	Bromophenol blue
BSA	Bovine serum albumin
CBL1	tamarillo lectin (*Cyphomandra betacea*), subuint size 25 kDa
CBL2	tamarillo lectin (*Cyphomandra betacea*), subuint size larger than 50 kDa
cDNA	complementary DNA
Con A	concanavalin A
DEAE	diethylaminoethyl
DEPC	diethylpyrocarbonate
DMSO	dimethyl sulphoxide
dNTPs	2'-Deoxyribonucleoside 5'-triphosphates
dCTP	2'-Deoxycytidine 5'-triphosphate
DSA	*Datura* seed lectin (thorn apple lectin, TAL)
ds DNA	double stranded DNA
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
Fuc	fucose
Gal	galactose
GalNAc	N-acetylgalactosamine
GlcNAc	N-acetylglucosamine
Glu	glucose
Hepes	N-2-hydroxy ethyl piperazine-N'-2-ethane sulphonic acid
HPLC	High-pressure liquid chromatography
IEF	isoeletric focusing
IPTG	isopropylthio-β-D-galactoside
LEL	tomato lectin (*Lycopersicon esculentum*)
LB	Luria broth
Man	mannose
NBT	nitro blue tetrazolium chloride
NeuNAc	sialic acid
PBS	phosphate-buffered saline
PBSB	phosphate buffered saline containing bovine serum albumin
pfu	plaque forming unit
PHA	phytohemagglutinin
SDS	sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophresis
SM	Phage buffer supplemented with 0.1 % gelatin

STE	Tris Cl buffered NaCl/ethylenediaminetetraacetic acid
STL	lectin of potato tuber (*Solanum tuberosum*)
TAL	thorn apple lectin (*Datura* seed lectin, DSA)
TBE	Tris-borate/EDTA electrophoresis buffer
TCA	trichloroacetic acid
TE	Tris buffered ethylenediaminetetraacetic acid
TEMED	N,N,N′,N,-tetramethylethylene diamine
TFA	Trifluoroacetic acid
TNT	Tris-Cl containing NaCl and Tween-20
TPCK	N-tosyl-L-phenylalanine chloromethyl ketone
Tris	Tris(hydroxymethyl)aminomethane
TTBS	Tris-Cl/tween-20 and NaCl buffer
WGA	Wheat germ agglutinin
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside