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

by

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for the degree of Doctor of Philosophy in Biotechnology at
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Title of thesis: Purification and characterization of a lectin from tamarillo fruits (Solanum betaceum)

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Lectins specific in their binding to oligomers of \( \beta 1,4 \) 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 characterized.

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 \textit{ilvC} 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.
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LIST OF PUBLICATIONS AND ABSTRACTS


TABLE OF CONTENTS

ABSTRACT .......................................................................................................................... i
ACKNOWLEDGEMENTS ..................................................................................................... iii
LIST OF PUBLICATIONS ...................................................................................................... iv
TABLE OF CONTENTS ........................................................................................................ v
LIST OF FIGURES ............................................................................................................... x
LIST OF TABLES .................................................................................................................. xii
ABBREVIATIONS ................................................................................................................. xiii

CHAPTER 1: LITERATURE REVIEW. .................................................................................... 1

1.1 Historical background of lectin research ................................................................. 1
   1.1.1 Definition of lectin .................................................................................................. 8

1.2 General overview of plant lectins ............................................................................. 10
   1.2.1 Introduction ........................................................................................................... 10
   1.2.2 Classification ......................................................................................................... 10
   1.2.3 Chemical and structural properties ....................................................................... 14
       1.2.3.1 Introduction ....................................................................................................... 14
       1.2.3.2 N-acetyl glucosamine specific lectins .............................................................. 14
           1.2.3.2.1 Wheat germ agglutinin (WGA) ................................................................. 16
           1.2.3.3 N-acetyl galactosamine and galactose specific lectins ................................ 18
               1.2.3.3.1 Glycine max lectin (Soybean agglutinin) .............................................. 19
               1.2.3.3.2 Phaseolus vulgaris lectin (PHA) ............................................................ 20
       1.2.3.4 Mannose and glucose specific lectins .............................................................. 23
           1.2.3.4.1 Concanavalin A (Con A) ........................................................................... 26
           1.2.3.5 L-Fucose and sialic acid specific lectins ...................................................... 27
       1.2.3.6 Summary .......................................................................................................... 28
   1.2.4 Possible in vivo functions and applications of plant lectins ................................ 28
       1.2.4.1 Possible in vivo functions of plant lectins ....................................................... 28
       1.2.4.2 Applications of plant lectins ............................................................................ 30

1.3 Lectins from the Solanaceae family ........................................................................... 31
1.3.1 Chemical composition and structure ................... 32
1.3.2 Carbohydrate specificities ............................. 35
1.3.3 Distribution, localization, and possible function of solanaceous lectins .......... 36

1.4 Aim of this study ........................................... 37

CHAPTER 2: MATERIALS AND METHODS ............................................ 38

2.1 Enzymes and fine chemicals .................................... 38

2.2 Vectors, bacteria strains, media and growth conditions ................. 39

2.3 Protein biochemical methods .................................... 41

2.3.1 Isolation of lectins from tamarillo fruits ............... 41
2.3.1.1 Chitin affinity chromatography ....................... 41
     2.3.1.1.1 Preparation of chitin affinity column ........... 42

     2.3.1.1.2 Preparation of chitin hydrolysate solution .......... 42

     2.3.1.2 Gel filtration chromatography ...................... 43

2.3.2 Hemagglutination and carbohydrate inhibition assay ..................... 43

2.3.3 Mitogenic study ............................................. 44

2.3.4 Protein concentration assay ................................ 45

2.3.5 Carbohydrate assay ......................................... 45

2.3.6 Polyacrylamide gel electrophoresis (PAGE) ............... 46
     2.3.6.1 Coomassie blue (R250) staining .................... 47
     2.3.6.2 Silver staining ................................... 47

2.3.7 Isoelectric focusing ...................................... 48

2.3.8 Determination of protein size by gel filtration ............ 49

2.3.9 Amino acid analysis ...................................... 49

2.3.10 Tryptic digestion ......................................... 49

2.3.11 HPLC separation of peptides .............................. 50
2.3.12 Peptide sequencing ........................................... 50

2.4 Immunological methods ............................................. 50
  2.4.1 Antiserum preparation ........................................ 50
  2.4.2 Double immunological diffusion test
    (Ouchterlony test) ............................................. 51
  2.4.3 Western blotting ............................................ 51
  2.4.4 Immunocytochemical localization ............................ 52

2.5 Molecular biological methods ..................................... 54
  2.5.1 Poly(A') RNA preparation ..................................... 54
    2.5.1.1 Preparation of the frozen tamarillo fruits ... 54
    2.5.1.2 Total RNA isolation .................................... 54
    2.5.1.3 Poly(A') RNA isolation ............................... 55
  2.5.2 In vitro translation ......................................... 56
  2.5.3 RNA quantitation ............................................ 56
  2.5.4 Siliconization of glassware and
    plastic materials ............................................... 56
  2.5.5 cDNA library construction ................................... 57
    2.5.5.1 cDNA synthesis ........................................ 58
    2.5.5.2 Incorporation assay and calculation .................... 59
    2.5.5.3 Alkaline gel analysis .................................. 60
    2.5.5.4 Methylation of cDNA ................................... 60
    2.5.5.5 Linker ligation ....................................... 61
    2.5.5.6 Digestion with EcoRI ................................... 61
    2.5.5.7 Removal of undigested linkers ......................... 61
    2.5.5.8 Ligation of cDNA with lambda gt11 arms and
      in vitro package of ligated lambda gt11 ................... 62
  2.5.6 Amplification of cDNA library ............................... 63
  2.5.7 Immunological screening of cDNA library .................... 64
    2.5.7.1 Screening ............................................ 64
    2.5.7.2 Identification and purification of positive
      plaques .................................................... 65
  2.5.8 Lambda phage DNA preparation ................................ 65
  2.5.9 Restriction enzyme digestion ................................ 66
  2.5.10 Plasmid isolation methods .................................. 67
    2.5.10.1 Alkaline lysis method ................................ 67
CHAPTER 3: PURIFICATION AND CHARACTERIZATION OF TAMARILLO LECTINS

3.1 Introduction ........................................... 75

3.2 Results and discussion ................................ 75
  3.2.1 Lectin screen ...................................... 75
  3.2.2 Isolation of tamarillo lectins ...................... 76
  3.2.3 Molecular size of CBL1 ................................ 81
  3.2.4 Isoelectric points .................................. 83
  3.2.5 Carbohydrate specificity ............................ 85
  3.2.6 Stability of CBL1 .................................. 86
  3.2.7 Chemical composition ................................ 88
  3.2.8 Mitogenic activity .................................. 90

3.3 Summary .................................................... 90

CHAPTER 4: IMMUNOLOGICAL RELATIONSHIPS AMONG SOLANACEOUS LECTINS

4.1 Introduction ............................................. 92

4.2 Results and discussion ................................ 93
CHAPTER 5: SUBCELLULAR LOCALIZATION OF CBL1 IN TAMARILLO FRUITS

5.1 Introduction

5.2 Results and discussion

5.3 Summary

CHAPTER 6: SEQUENCE ANALYSIS OF TAMARILLO LECTIN (CBL1)

6.1 Introduction

6.2 Results and discussion

6.2.1 Peptide sequences

6.2.2 cDNA library construction

6.2.3 cDNA library screening

6.2.4 DNA sequencing and sequence analysis

6.3 Summary

CHAPTER 7: GENERAL CONCLUSIONS AND SUMMARY

BIBLIOGRAPHY

APPENDIX: CLASSIFICATION OF PLANT LECTINS

CORRECTIONS
LIST OF FIGURES

Chapter 1

Fig.1-1 Classification of pyranose of lectin-reactive monosaccharides.
Fig.1-2 Homologies among the deduced amino acid sequences of win1 and win2 and protein sequences of hevein, chitinase, wheat germ agglutinin, rice lectin and nettle lectin.
Fig.1-3 α-Carbon backbone drawing of the WGA protomer.
Fig.1-4 Schematic illustration of the disposition of the primary and secondary binding locations on the WGA dimer.
Fig.1-5 Schematic representation of the tetrameric structure of the five isolectins from Phaseolus vulgaris.
Fig.1-6 Complete sequences of soybean agglutinin, fava bean lectin, lentil lectin, pea lectin, sainfoin seed lectin, phytohaemagglutinin, and concanavalin A.
Fig.1-7 Schematic representation of Con A tetramer.
Fig.1-8 Hypothetical model of the structure of potato lectin.

Chapter 2

Fig.2-1 λgt11 map.
Fig.2-2 A diagram of cDNA synthesis.
Fig.2-3 A diagram of ExoIII nuclease digestion of DNA.

Chapter 3

Fig.3-1 Chitin affinity chromatography of lectins from tamarillo fruits.
Fig.3-2 Cation-exchange chromatography of tamarillo lectins on a S-Sepharose column.
Fig.3-3 SDS-PAGE of tamarillo lectin samples from fractions of the S-Sepharose chromatography.
Fig.3-4 Gel filtration chromatography of lectins from tamarillo fruits.
Fig.3-5 SDS-PAGE analysis of CBL1.
Fig.3-6 Molecular size determination of CBL1 by gel filtration chromatography.
Fig.3-7 Isoelectric focusing of tamarillo lectins

Chapter 4

Fig.4-1 Double immunodiffusion test I.
Fig.4-2 Double immunodiffusion test II.
Chapter 5

Fig. 5-1 Western blotting of purified CBL1 and tamarillo extract with anti-CBL1 serum.
Fig. 5-2 Immunocytochemical localization of CBL1 using an immunofluorescent technique.
Fig. 5-3 SDS-PAGE analysis of tamarillo juice.

Chapter 6

Fig. 6-1 Partial separation of a tryptic digest of valyl-CBL1 by HPLC.
Fig. 6-2 Homologies of CBL1 peptide sequences with WGA.
Fig. 6-3 SDS-PAGE analysis of in vitro translation products of poly(A') RNAs.
Fig. 6-4 Size distribution of the first strand of cDNA and ds cDNA (double stranded cDNA).
Fig. 6-5 Dot blot analysis of CBL1 on nitrocellulose filters.
Fig. 6-6 Immunological screening and identification of recombinant phages X208 and X200 on nitrocellulose filters.
Fig. 6-7 EcoR1 digestion of the recombinant phages X208 and X200.
Fig. 6-8 ExoIII digestion of cDNA insert of the recombinant phage X200.
Fig. 6-9 Nucleotide sequence of cDNA insert of recombinant phage X200 and its comparison with that of a bacterial gene ilvC.
Fig. 6-10 Nucleotide sequences and inferred amino acid sequences of cDNA insert of the recombinant phage X208.
LIST OF TABLES

Chapter 1
Table 1-1 Major uses of lectin.
Table 1-2 Binding constants (Kᵢ) of interaction of solanaceous lectins with β(1,4)-linked oligomers of N-acetylglucosamine.

Chapter 2
Table 2-1 Bacterial strains and vectors.

Chapter 3
Table 3-1 Purification of tamarillo lectins.
Table 3-2 The minimum concentration of sugars required for complete inhibition of 8 haemagglutination units.
Table 3-3 The effect of high temperature, pH and EDTA on CBL1 haemagglutination activity.
Table 3-4 Amino acid composition of CBL1 and other known lectins from Solanaceae.

Chapter 6
Table 6-1 Amino acid composition of CBL1 and WGA.
Table 6-2 Amino acid composition of CBL1 and two putative peptides (pepl and pep2) encoded by X208 cDNA insert.
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), subunit size 25 kDa
CBL2 tamarillo lectin (Cyphomandra betacea), subunit size larger than 50 kDa
cDNA complementary DNA
Con A concanavalin A
DEAE diethylaminoethyl
DEPC diethylpyrocarbonate
DEAE diethylaminoethyl
dNTPs 2' -Deoxyribonucleoside 5'-triphosphates
dCTP 2' -Deoxyctydine 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-acetylgalcosamine
Glu glucose
Hepes N-2-hydroxy ethyl piperazine-N'-2-ethane sulphonic acid
HPLC High-pressure liquid chromatography
IEF isoelectric 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 electrophoresis
SM Phage buffer supplemented with 0.1 % gelatin
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>STE</td>
<td>Tris Cl buffered NaCl/ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>STL</td>
<td>lectin of potato tuber (<em>Solanum tuberosum</em>)</td>
</tr>
<tr>
<td>TAL</td>
<td>thorn apple lectin (Datura seed lectin, DSA)</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-borate/EDTA electrophoresis buffer</td>
</tr>
<tr>
<td>TCA</td>
<td>trichloroacetic acid</td>
</tr>
<tr>
<td>TE</td>
<td>Tris buffered ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>TEMED</td>
<td>N,N,N',N'-tetramethylethylene diamine</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TNT</td>
<td>Tris-Cl containing NaCl and Tween-20</td>
</tr>
<tr>
<td>TPCK</td>
<td>N-tosyl-L-phenylalanine chloromethyl ketone</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>TTBS</td>
<td>Tris-Cl/tween-20 and NaCl buffer</td>
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<td>WGA</td>
<td>Wheat germ agglutinin</td>
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