Expression of the cDNA Encoding the *Pterocarpus angolensis* (Mukwa Tree)-Seed Lectin in *Escherichia coli* and Site-Directed Mutagenesis of the Sugar-Binding Specificity Loop

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ABSTRACT

The mannose/glucose specific lectin from *Pterocarpus angolensis* (mukwa tree) seeds was expressed in *Escherichia coli* using the pBAD expression system. The expression vector pBAD*Myc*HisA was digested with *Nco*I and filled-in with T4 DNA polymerase in order to introduce an initiator ATG codon preceding the polymerase chain reaction-amplified cDNA encoding the mature mukwa seed lectin. The recombinant plasmid was used to transform the expression cell line *E. coli* TOP10 cells.

The cDNA clone, Muk151QII28, encoding the wild type mukwa seed lectin, was used as the template for oligonucleotide-directed mutagenesis of the sugar binding specificity. The first approach involved removing the part of the mukwa seed lectin sugar-specificity loop (loop D) that interacts with the sugar, and replacing it with the corresponding region of either the *Ulex europaeus* II lectin (UEA II) or the *Erythrina corallodendron* lectin (ECorL). In the second approach, two other mutants, predicted from X-ray crystallography to change the mukwa seed lectin sugar specificity from α-mannose/glucose to β-mannose/glucose, were generated. The DNA region carrying the mutations was then sub-cloned into the pBAD*Myc*HisA-wild type mukwa seed lectin recombinant in which the corresponding DNA region had been excised. The four mutants were expressed in *E. coli* TOP10 cells. The mutant lectins were assayed for cross-reactivity with antiserum directed against the native mukwa seed lectin in order to determine if the antiserum could be used in Western blotting. Hen egg white glycoproteins and glycoproteins of high variability isolated from porcine and bovine plasma were then blotted onto nitrocellulose and used to determine if the mutant lectins were capable of recognizing any carbohydrate moieties on glycoproteins.

Maximum expression of both the wild type and the mutant lectins was obtained after induction with 0.2 % L-arabinose in cultures grown overnight. The presence or absence of a protease inhibitor cocktail did not seem to improve the yield. Up to 7.7 mg/500 ml culture of the expressed wild type lectin could be isolated from the extract by affinity chromatography on mannose-Sepharose. The purified lectin has a specific absorbance of OD_{280nm} 1 mg/ml = 1.3 and shows an absorbance ratio of OD_{280nm}/ OD_{250nm} ≈3, the same as for the native lectin isolated from mukwa seeds. The expressed lectin has a slightly lower molecular mass than the native lectin but the two are essentially indistinguishable by Western blot analysis with anti-mukwa seed lectin polyclonal antibodies, haemagglutinating activity and both are inhibited by methyl-α-D-mannopyranoside.

The mutant lectins cross-reacted with antiserum directed against the native mukwa seed lectin and all of them were capable of binding some carbohydrate moieties as shown by Western blotting. However, the wild type lectin showed a higher affinity for the carbohydrate moieties on the glycoproteins compared to the mutant lectins. The mutants, except for the UEA II specificity loop mutant, were successfully purified on an anti-mukwa seed lectin IgG-Sepharose column and used in agglutination assays. None of the mutants was capable of agglutinating any of the different animal erythrocytes tested showing that other factors apart from loop D determine sugar specificity in legume lectins.

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“I can do all things through Christ who strengthens me.”
Phillipians 4[13].

To the two men in my life, Weddington and Tirivashe, who many a time, had to bear with an absentee wife and mother respectively.

CONTENTS

ABBREVIATIONS

A	amperes
α	alpha
AS	ammonium sulphate
ATP	adenosine triphosphate
β	beta
BCA	bicinchoninic acid
BCIP	5-bromo-4-chloro-3-indoyl phosphate
bp	base pair
BSA	bovine serum albumin
°C	degrees Celsius
$CaCl_2$	calcium chloride
CBD	carbohydrate-binding domain
cDNA	complementary deoxyribonucleic acid
cm	centimetre
CNBr	cyanogen bromide
CTP	cytidine triphosphate
d	deoxy
dd	dideoxy
DEAE	diethylaminoethyl
DMF	dimethylformamide
DNA	deoxyribonucleic acid
dNTP	deoxy nucleoside triphosphate
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
ER	endoplasmic reticulum
Fuc	fucose
g	gram
Gal	α-D-galactose
GalNAc	N-acetyl-D-galactosamine
Glc	α-D-glucose
GlcNAc	N-acetyl-D-glucosamine
GTP	guanosine triphosphate
HCl	hydrochloric acid
hr	hour
IgG	immunoglobulin G
ITP	inosine triphosphate
kb	kilobase
kDa	kilodalton
KOH	potassium hydroxide
L	litre
LB	Luria-Bertani
Man	α-D-mannose
m	milli
ml	millilitre
mm	millimetre
M	molar
$MgCl_2$	magnesium chloride

min	minute
MM	molecular mass
$MnCl_2.4H_2O$	manganese chloride
mRNA	messenger ribonucleic acid
Na_2CO_3	sodium carbonate
$NaHCO_3$	sodium hydrogen carbonate
NaCl	sodium chloride
$NaIO_4$	sodium periodate
NaN_3	sodium azide
NaOH	sodium hydroxide
NBT	nitroblue tetrazolium
NEB	New England Biolabs
ng	nanogram
nm	nanometre
NMR	nuclear magnetic resonance
NeuNAc	N-acetyl-D-neuramic acid
OH	hydroxyl group
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethyleneglycol
pmol	picomol
PMSF	phenylmethylsulfonylfluoride
RIP	ribosome inactivating protein
rRNA	ribosomal ribonucleic acid
rpm	revolutions per minute
sec	second
SDS	sodium dodecyl sulphate
TB	tris borate
TBE	tris-borate-ethylenediamine tetraacetic acid
TEA	triethylamine
TEMED	N,N,N´N´-tetramethylethylenediamine
tRNA	transfer ribonucleic acid
TTP	thymidine triphosphate
µF	micro Farads (units of capacitance)
µl	microlitre
µg	microgram
U.S.E.	unique site elimination
V	volts
v/v	volume for volume
W	watts
w/v	weight for volume
x g	centrifugal force (gravity)

TABLES

FIGURES

APPENDICES