MANNOSE-BINDING LECTIN FROM SEEDS OF *ARTOCARPUS INTEGER*

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CONTENTS

ABBREVIATIONS

The abbreviations used in this thesis are those recommended in the Instructions To Authors of the Biochemical Journal (1997), with the following additions:-

A_{280}	- Absorbance readings at 280 nm
α	- Heavy chain of IgA
AP	- Alkaline phosphatase
B cells	- Cell lineage responsible for immunoglobulin production
C1	- Complement 1
Con A	- Concanavalin A
cpm	- Radioactive counts per minute
2D	- Two dimensional
DTT	- Dithiothreitol
DVS	- Divinylsulfone
FCS	- Foetal calf serum
γ	- Heavy chain of IgG
Gal	- D-galactopyranose
GalNAc	- N-acetylgalactosamine
GlcNAc	- N-acetylglucosamine
HEPES	- N-2 hydroxyethylpiperazine-N'-2-ethane-sulfonic acid
Hr	- Hour
HRP	- Horseradish peroxidase
Ig	- Immunoglobulin
kDa	- Kilodalton
Man	- D-mannopyranose
M_r	- Relative molecular mass
μ	- Heavy chain of IgM
NeuNAc	- N-acetylneuraminic acid
NHS	- Normal human serum

PBMC	- Peripheral blood mononuclear cells
PBS	- Phosphate buffered saline
PHA	- Phytohemagglutinin
SDS	- Sodium dodecyl sulphate
TBS	- Tris buffered saline
T cells	- Thymus derived lymphocytes
Tris	- Tris(hydroxymethyl)methylamine
Vh	- Volt-hours
WGA	- Wheat germ agglutinin

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ABSTRAK

Lektin pengikat manosa, yang dikenali sebagai lektin M champedak, telah diasingkan dari ekstrak mentah biji cempedak (*Artocarpus integer*) dengan menggunakan kromatografi keafinan. Apabila kromatografi penurasan gel dilakukan, lektin ini dielusikan dalam satu puncak yang bersamaan dengan berat molekul 64 kDa. Electroforesis gel SDS-poliakrilamida menunjukkan bahawa lektin pengikat manosa ini terdiri daripada beberapa polipeptida 16.8 kDa. Sesetengah daripada polipeptida ini wujud sebagai dimer melalui ikatan disulfida.

Apabila diuji dengan semua jenis iso (isotype) imunoglobulin, lektin M champedak ini menunjukkan interaksi selektif yang kuat dengan IgE dan IgM manusia, dan interaksi lemah dengan IgA2. Interaksi pengikatan lektin M ini adalah tidak bersandar kepada ion-ion logam. Lektin tersebut juga didapati berinteraksi dengan horseradish peroksidase, ovalbumin, tiroglobulin (khinzir), α_1-asid glikoprotein, transferin dan α_1-antitripsin (manusia) seperti diperlihatkan melalui esei pengikat HRP. Ia mengikat lebih kuat pada ligan Manα1-3Man berbanding dengan Manα1-2Man dan Manα1-6Man. Profil elusi kromatografi keafinan lektin M dengan pelbagai jenis glikoprotein dengan struktur karbohidrat yang diketahui menunjukkan bahawa pengikatan lektin mengutamakan oligosakarida jenis kompleks biantenari dan sesetengah oligosakarida jenis kandungan tinggi manosa.

Interaksi di antara lektin M dengan protein-protein serum manusia telah dikaji dengan menggunakan elektroforesis 2-dimensi dan blot afiniti lektin M. Lektin M didapati berinteraksi dengan beberapa protein yang mengandungi rantai glikan

pengikat-N seperti α_1-asid glikoprotein, transferin dan α_1-antitripsin, IgM dan IgA dalam bentuk natif dan juga dengan rantai berat-γ IgG dalam keadaan terturun.

Kajian yang dilakukan untuk melihat kesan lektin M ke atas limfosit tikus telah menunjukkan yang ia merangsang proliferasi sel T dengan kepekatan optimum 2.5 μg/ml bila dikultur selama 3 hari. Lektin M dipercayai adalah mitogen yang khusus kepada sel T kerana ia tidak mengaruh sintesis DNA apabila dikultur dengan sel-sel limpa tikus *nude* (iaitu strain tikus tanpa timus). Ia juga tidak berupaya untuk mengaruh sel B rehat untuk membeza kepada sel plasma yang merembeskan imunoglobulin.

ABSTRACT

A mannose-binding lectin, termed champedak lectin-M, was isolated from the crude extract of the seeds of champedak (*Artocarpus integer*) by affinity chromatography. On gel filtration chromatography, the lectin eluted in a single peak at elution volumes corresponding to 64 kDa. SDS-PAGE showed the mannose-binding lectin to be composed of 16.8 kDa polypeptides with some of the polypeptides being disulphide-linked to give dimers.

When tested with all isotypes of immunoglobulins, champedak lectin-M demonstrated a selective strong interaction with human IgE and IgM, and a weak interaction with IgA2. The binding interactions of lectin-M were metal ion independent. The lectin was also shown to interact with horseradish peroxidase, ovalbumin, porcine thyroglobulin, human α_1-acid glycoprotein, transferrin and α_1-antitrypsin as observed in HRP-binding assay. It demonstrated a binding preference to Manα1-3Man ligands in comparison to Manα1-6Man or Manα1-2Man. The elution profile of various glycoproteins with defined carbohydrate structure on lectin M-affinity chromatography have demonstrated that lectin M preferentially bind to biantennary complex-type and certain high-mannose-type oligosaccharides.

The interaction of lectin M with human serum proteins was studied using 2D-electrophoresis and lectin M-affinoblotting. Interestingly, lectin M interacted with quite a number of N-linked glycan chain containing proteins such as haptoglobin, α_1-acid glycoprotein, transferrin, α_1-antitrypsin, IgM, and IgA in their native conformation as well as the γ-heavy chain of IgG under reducing condition.

Investigation on the effect of lectin M on murine lymphocytes revealed that it stimulated the proliferation of murine T cells with an optimal concentration of 2.5 μg/ml in a 3 days culture. Lectin M is believed to be a T-cell mitogen as it does not induce significant DNA-synthesis when cultured with spleen cells from nude mouse. It was also incapable of inducing the resting B cells to differentiate into an immunoglobulin secreting plasma cells.