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Comparison of the amino acid sequences of lectins from the seeds of *Dioclea reflexa* and *Canavalia ensiformis*

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ABSTRACT

The amino acid sequence of a glucose/mannose specific lectin from the seeds of *Dioclea reflexa* (Dioclea *reflexa* agglutinin II, DRA-II) was determined by sequential Edman analyses of the intact subunit, and of the peptides derived from the protein by enzymatic digestion with trypsin. This sequence was found to be very similar to those of the lectins from other Dioclea species, *Dioclea grandiflora* and *Dioclea lehmanii, and* also the lectin from *Canavalia ensiformis* (Con A). Comparison of these amino acid sequences showed that a high degree of homology exists among these proteins.

Keywords: *Dioclea reflexa,* Leguminosaea, Dioclea seeds, lectins, amino acids.

INTRODUCTION

Lectins are proteins or glycoproteins of non-immunoglobin nature, capable of specific recognition and reversible binding to carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized glycosyl ligands (D'adamo, 1999).These lectins bind to sugar moieties in cell walls or membranes and thereby change the physiology of the membrane to cause agglutination, mitosis or other biochemical changes in the cell.

Lectins are a group of intensely investigated proteins because of their many exploitable biological activities. These include: antiproliferative, antitumor, immuno-modulatory (Wang et al., 2000; Yu et al., 2001, Singh-Bains et al., 2005), antifungal (Ye et al., 2001), antiviral (Ooi et al., 2000), and anti-insecticidal (Machuka & Oladapo, 2000) activities. They have been isolated from a diverse number of organisms encompassing plants, animals and microorganisms. (Knibbs et al., 1991; Pusztai, 1991; Wang et al., 1996; Inamori & Saito, 1999). Plant lectins, which are multivalent cell-agglutinating proteins, are widely used as powerful tools in cell-surface and complex carbohydrate research, as well as for the purification and characterization of glycoconjugates (Sharon and Lis, 1990; Goldstein and Poretz, 1986) due to their unique ability to bind specific sugars or sugar containing macromolecules (Goldstein and Hayes, 1978). Concanavalin A (Con A), the lectin from the seeds of *Canavalia ensiformis* is one of the most investigated lectins (Cunningham et al.,1975; Terada et al., 1992) and belongs to the general group of Mannose/Glucose binding lectins (Kristiansen, 1974). Previous studies on the genus Dioclea have also shown that the lectins from seeds of *Dioclea grandiflora* (Ainouz et al., 1987; Perez et al.,1991) and *Dioclea lehmanii* (Richardson et al., 1984) are glucose/mannose specific and are very similar in properties and amino acid sequences to the

well known Concanavalin A (Con A). Comparison of the amino acid sequences of lectins from these *Dioclea* species (*Dioclea reflexa, Dioclea grandiflora* and *Dioclea lehmanii*) and that of lectins from *Canavalia ensiformis* (Con A) has shown that a high degree of homology exists among these proteins. In addition, these lectins exhibit a circular permutation in their amino acid sequences when compared with those of lectins from seeds of other tribes of Leguminosae. This behaviour has been attributed to a transpeptidation event during post-translational processing of a precursor protein (Bowles et al., 1986).

In this paper, we report the amino acid sequence of DRA-II, one of the two lectins found in the seed of *Dioclea reflexa*, and further compare the sequence with those of lectins from two other *Dioclea* species and *Canavalia ensiformis.*

MATERIALS AND METHODS

Materials

Potassium dihydrogen orthophosphate, Glycine, Glutaraldehyde, Ammonium persulphate, N,N,N^1,N^1-tetramethyl ethylene-diamine (TEMED), Trypsin, different monosaccharide sugars and cellobiose are all products of BDH Chemical Limited, Poole, England. All other reagents were of analytical grade and were purchased from Sigma Chemical Company, St.Louis, Mo, USA.

The seeds of *Dioclea reflexa* were collected from Ile-Ife in Nigeria and authenticated by the Department of Botany of Obafemi Awolowo University, Ile-Ife, Nigeria.

Human blood cells from the ABO system were obtained from healthy donors in the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife.

Preparation of Phosphate buffered saline (PBS), pH 7.2

KH_2PO_4 (6.8 g) and NaCl (11.70 g) were mixed and dissolved in 1.5 litre of distilled water. The mixture was adjusted to pH 7.2 with 1M KOH and the volume was made up to 2 litres with distilled water.

Preparation of crude seed extracts

Mature seeds were removed from their husks and ground to a fine powdery form. One gram of seed powder was suspended in 10ml of PBS, pH 7.2 and the mixture stirred overnight at 4 °C after which it was centrifuged at 5000 rpm for 15 min. The supernatant which constitutes the lectin preparation (crude extract) was collected, filtered and stored at -20 °C until used.

Affinity chromatography

Sephadex G-150 (15 g) was swollen in distilled water for three days (sodium azide was added to prevent fungal growth). This was packed into a column (30 x 2.5cm) and equilibrated with PBS, pH 7.2. Ten millilitres of the crude extract was layered on the column, and after washing the sample into the gel bed, the column was connected to a buffer reservoir containing PBS. Fractions of 3 – 4 ml were collected until the absorbance at 280 nm was zero. The column was then washed with an additional 200 ml of the buffer, after which 0.2 M Glucose in PBS was applied to elute any bound lectin.

Fractions in the protein peaks were assayed for heamagglutinating activity as described by Bing et al. (1967). In a U-shaped microtitre plate arranged in rows of wells, 1:2 dilutions of the sample (each fraction) in PBS buffer were mixed with 50µl of 2% suspension of erythrocytes (the erythrocytes of human blood group A,B and O were fixed with 1% glutaraldehyde). The plate was left undisturbed for 30 min to 1 hr at room temperature in order to allow for agglutination of the erythrocytes to take place. The titre of the lectin was expressed as the reciprocal of the highest dilution showing visible agglutination of erythrocytes. The fractions eluted with glucose-buffer solution were pooled and dialysed exhaustively against PBS before heamagglutination assay was carried out. This bound lectin peak was labeled DRA–II.

The protein peaks containing heamagglutinating activity were pooled and dialysed against PBS, pH 7.2 at 4 °C. The unbound lectin peak (labeled DRA–I) was purified further by concentrating the pooled fractions and rechromatographing on the Sephadex G-150 column with PBS, pH 7.2.

Amino acid analysis

The amino acid composition of the native protein, DRA-II, was obtained after

hydrolysis in vacuo at 110 °C in 6N HCl for 24 hrs. The concentration of free amino acids were determined on a Hewlett-Packard Amino Quant Series II Amino Acid Analyser by precolumn derivatization technique employing both ortho-phthaldehyde (OPA) and 9-fluorenylmethylchloroformate (FMOC) chemistry reaction according to the manufacturer's protocol. Detection was performed by measuring UV absorbance at 338 and 262 nm respectively.

N-terminal group analysis

The N-terminal group of the native protein was determined by the automated Edman degradation method using the Hewlett Packard G 100 A protein sequencer system. The released amino acids were estimated after derivatization with phenylisothiocyanate.

Digestion of the protein with trypsin

One milligram of protein was digested with enzyme (ratio of enzyme to protein 1:25) according to the method of Aiken et al. (1989). This generated tryptic peptides. The peptides were purified by reverse-phase HPLC (μBond pack C 18 column) using a 50min linear gradient of 0% - 80% acetonitrile containing 0.05% trifluoroacetic acid. Elution was performed at room temperature at a flow rate of 1ml/min.

Sequence determination

Sequence determination of purified peptides and the intact protein was carried out using the adsorptive biphasic column technology using the HPG 1000A Protein sequencer (Hewlett-Packard, Palo Alto, CA) .The peptides are numbered on the basis of progressive peptide sequencing starting from the N-terminal end.

Sequence analysis

The amino acid sequence of DRA II was compared and aligned with those of lectins from two other species of Dioclea and the lectin from *Canavalia ensiformis* using the hierarchical clustering procedure of Corpet (1988).

RESULTS AND DISCUSSION

The isolation and purification of the lectins in the saline extract of the seeds of *Dioclea reflexa* are as summarized in the legends to Figure 1. Two lectins, DRA-I, which flows out of the column, and DRA-II which is adsorbed on the gel matrix and elutes upon the addition of glucose to the eluting buffer were obtained. DRA-I was found to be specific for N-Acetylglucosamine while DRA-II showed specificity for D-Mannose/D-Glucose as determined by Hapten-Inhibition studies using different monosaccharides (Goldstein and Hayes, 1978).

The purity of the proteins was established by both non-denaturing and denaturing polyacrylamide gel electrophoresis on 7.5% acrylamide gels (figure not shown).

Dioclea reflexa resembles *Dolichos lab lab* (Mo et al., 1990) in containing two different kinds of lectins each with its own sugar specificity. Our preliminary observation (Adeyemi et al., 1994) and work suggests that DRA-I which preferentially binds N-Acetylglucosamine, is structurally a different protein from DRA-II.

In this report, DRA-II is the lectin of interest because it belongs to the group of lectins that include Concanavalin A, lentil lectin as well as lectins from the seeds of many papilionaceae plants for which Mannose and Glucose are the inhibitory sugars. Its amino acid sequence has been deduced from sequencing of the intact protein and also of the peptides arising from the degradation of the intact protein with trypsin (Figure 2 & 3).

The similarities in the amino acid composition of lectins from three species of the genus *Dioclea* is summarized in Table 1, and at the level of the primary structure of the proteins in Figure 4. This figure also contains the comparative data from Con A.

Analysis of the data using the hierarchical clustering procedure of Corpet (1988), suggests that the degree of homology between DRA-II and the lectins from *D. lehmanii, D. grandiflora* and Con A is, respectively 86.6%, 94% and 75.5%. This degree of homology, buttressed by the invariance of the residues which form the binding site for carbohydrate, viz.: Tyr^{12}, $Asn^{!4}$,Asp^{36},Leu^{89},Tyr^{100},Asp^{208}, and Arg^{228}, suggests that DRA-II must also be subject to the same post-translational circular permutation in its amino acid sequence as is found in Con A.

It should be observed that although there is an equally complete conversion of

residues whose side chains serve as ligands in Con A for Mn^{2+} (Glu^{8}, Asp^{19}, and His^{29}) on the one hand, and for Ca^{2+} (Asp^{10}, Tyr^{12}, Asn^{14} and Asp^{19}) on the other hand, we do not have any evidence so far that DRA-II requires these metal ions for sugar binding.

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Table 1: Amino acid composition of DRA-II, *D. lehmanii* and *D. grandiflora*.

Amino Acid	*D*RA-II Sequence	*D. lehmanii* Sequence	*D. grandiflora* Sequence
ASX	34	33	34
GLX	11	9	11
SER	29	29	33
HIS	4	5	5
GLY	17	17	18
THR	20	22	19
ALA	16	18	17
ARG	9	9	6
TYR	8	7	7
CYS	0	1	0
VAL	17	17	17
MET	1	1	1
PHE	11	11	12
ILE	15	15	16
LEU	18	17	18
LYS	12	9	10
PRO	10	12	11
TRP	4	4	4

(Sources: Perez et al., 1991; Richardson et al., 1984)

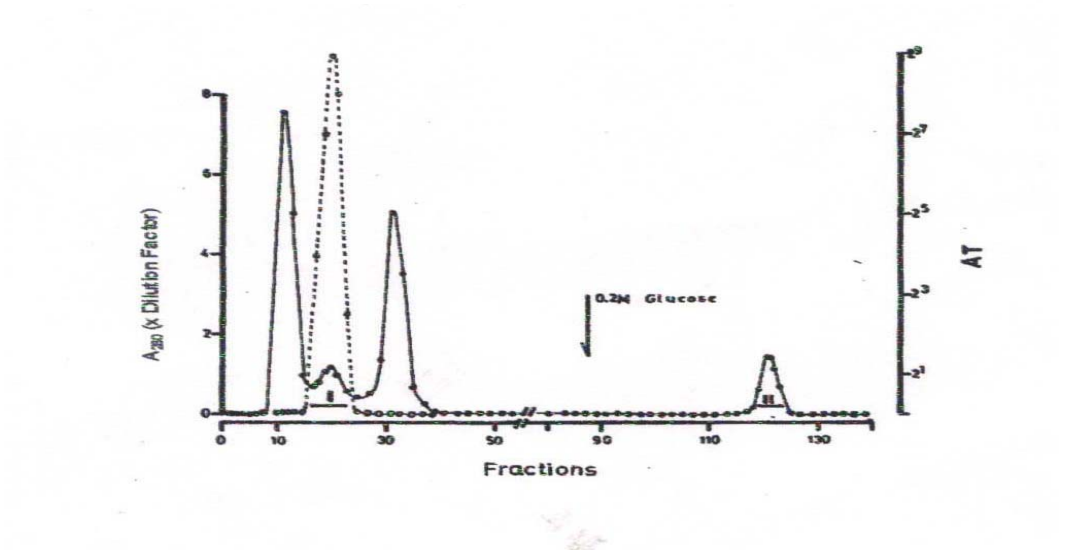

Figure 1: Affinity chromatography of *Dioclea reflexa* seed extract on Sephadex G-150 column. Eluent was PBS, pH 7.2. Protein bound to the column was eluted with 0.2 M Glucose in PBS. Column size: 30 x 2.5cm; Fraction size: 4.0ml; Flow rare: 16ml/hr.

0 : A_{280} x dilution factor

● : Agglutination Titre (AT)

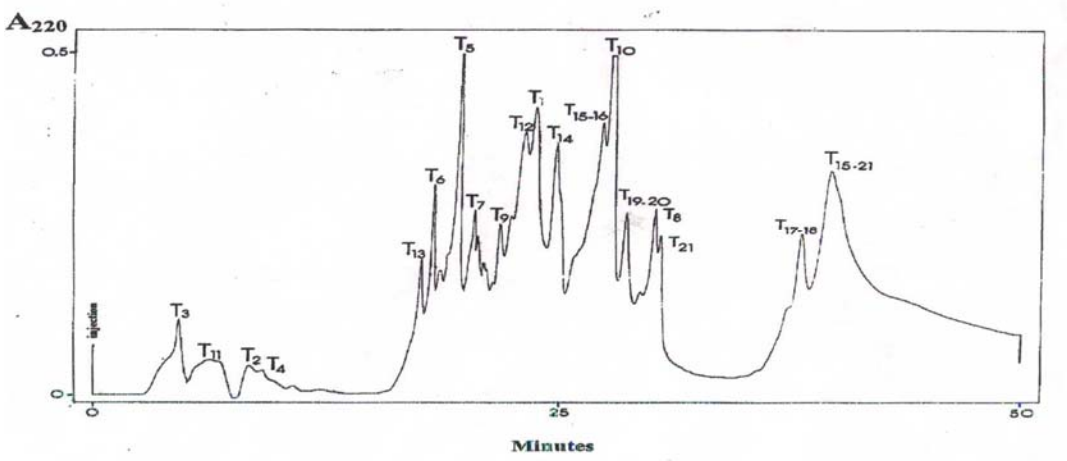

Figure 2: Reverse phase HPLC separation of the tryptic digest of DRA-II. The peptides have been numbered on the basis of progressive sequence starting from the N-terminal end.

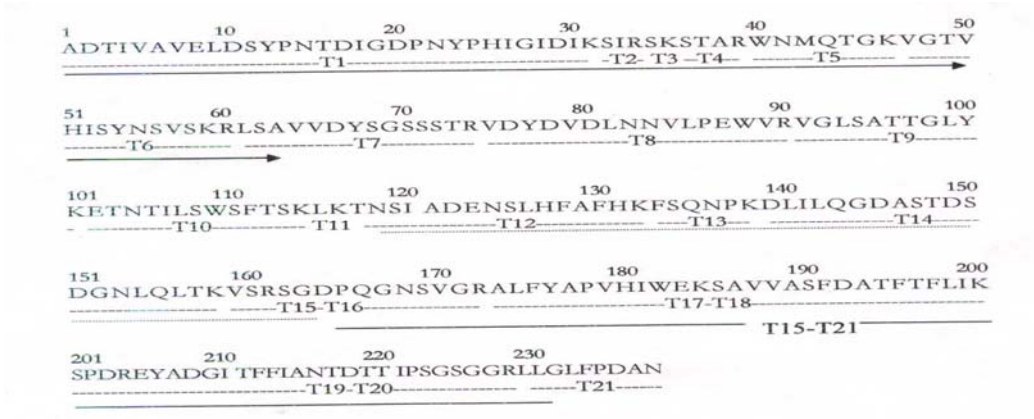

Figure 3: Amino Acid sequence of *Dioclea reflexa* Agglutinin DRA-II. The tryptic peptides (-----) are indicated as are also the non-enzymatically derived 44- residue peptide fragment (—) the structure of which conveniently provided an overlap for the tryptic peptides T12, T13 and T14. The N-terminal sequence is also derived from the intact protein (→).

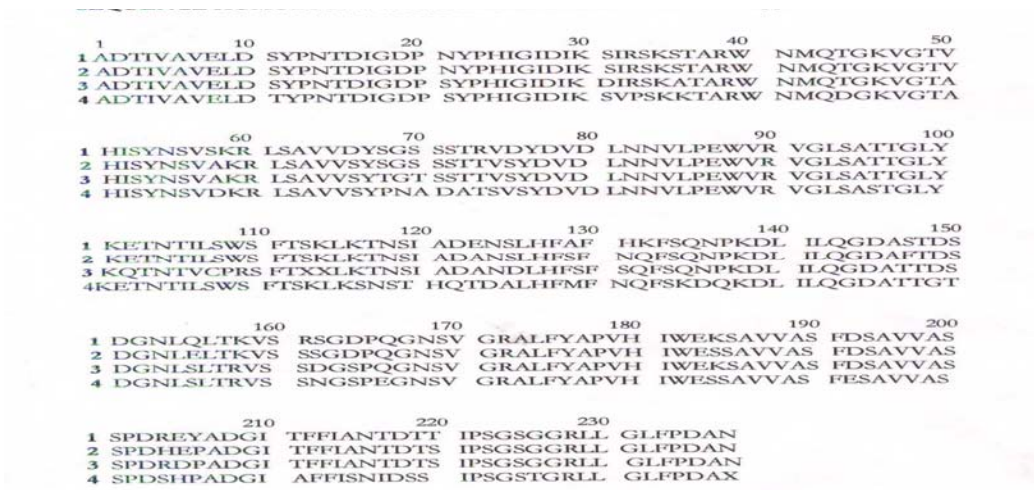

Figure 4: Comparison of the Amino Acid sequence of Lectins from **1**) *Dioclea reflexa* (DRA-II), **2**) *Dioclea grandiflora*.**3**) *Dioclea lehmanni* and **4**) *Concanavalia ensiformis*. (Sequence homology among lectins from *Dioclea spp.* and Con A.).

REFERENCES

Adeyemi A, Togun RA, Ogunbiyi OA, Aboderin A. 1994. Some observations on the Heamagglutinins from the Seeds of *Dioclea reflexa*. *Nig. J. Biochem.,* **9**: 26-31.

Aiken A, Geisow MJ, Findlay JBC, Holmes C, Yarwood A. 1989. In *Peptide Preparation and Characterisation in Protein Sequencing: A Practical Approach,* Findlay JBC, Geisow HJ (eds). IRL Press NY, 43-68.

Ainouz IL, Moreira RA, Campos FDAP, Richardson M, Begbie R, Stewart JC, Watt B, Putztai A. 1987. The Isolation and amino acid sequence of the β– and γ- subunits of the lectin from the seeds of *Dioclea grandiflora. Phytochem.,* **26**: 1435-1441.

Bing DH, Weyand JGM, Stavitsky AB. 1967. Heamagglutination with aldehyde fixed erythrocytes for assay of antigens and antibodies. *Proc. Soc. Exp. Biol. Med.,* **124**: 1166 – 1170.

Bowles DJ, Marcus SE, Pappin DJC, Findlay JBC, Eliopoulos, Maycox PR, Burgess J. 1986. Posttranslational processing of Concanavalin A precursors in Jack bean cotyledons. *J.Cell.Biol.,* **102**(4): 1284-1297.

Corpet F. 1988. Multiple sequence alignment with hierarchical clustering. *Nucl.Acids Res.,***16**: 10881–10890.

Cunningham BA, Wang JL, Waxdal MJ, Edelman GM. 1975. The Covalent and 3-Dimensinal structure of Concanavalin A-II - Amino acid sequence of cyanogen bromide fragment F3. *J.Biol.Chem.,* **250**(4): 1503–1512.

D'Adamo PJ. 1999. Lectins as mitogens. *Auto-immunity Inflammation and the Agglutinins,* 12-18.

Goldstein IJ, Hayes CE. 1978. The Lectins: Carbohydrate-binding proteins of plants and animals. *Adv. Carbohydr. Chem.Biochem.,* **35**: 127-340.

Goldstein IJ, Poretz RD. 1986. In *The Lectins: Properties, Functions and Applications in Biology and Medicine,* Liener IE, Sharon N, Goldstein IJ (eds). Academic Press NY; 33-247.

Inamori K, Saito T. 1999. A newly identified horseshoe crab lectin with specificity for blood group A antigen recognizes specific O-antigens of bacterial lipopolysaccharides. *J. Biol. Chem.,* **274**: 3272-3278.

Knibbs RN, Goldstein IJ, Patcliffe RM, Shibuya N. 1991. Characterization of the carbohydrate binding specificity of the leukoagglutinating lectin from *Maakia amurensis.*Comparison with other sialic acid-specific lectins. *J. Biol. Chem.,* **266**: 83–88.

Kristiansen T. 1974. Group-specific separation of glycoproteins. *Methods In Enzymology* (New York), **34**: 331-341.

Machuka JS, Oladapo G. 2000. The African yam bean seed lectin affects the development of the cowpea weevil but does not affect the development of larvae of the legume pod borer. *Phytochemistry,* **49**: 667–673.

Mo H, Lin Z, Sun C. 1990. Lectins, Biology, Biochemistry. *Clin. Biochem.,* **7**: 35.

Ooi LS, Ng TB, Geng Y, Ooi VE. 2000. Lectins from bulbs of the Chinese daffodil *Narcissus tazetta* (family Amaryllidaceae). *Biochem. Cell Biol*., **78**: 463 – 468

Perez G, Perez C, Sousa-Cavada B, Moreira R, Richardson M. 1991. Comparison of the amino acid sequences of the lectins from seeds of *Dioclea lehmanii* and *Canavalia maritima. Phytochem.,* **30**(8): 2619–2621.

Pusztai AP. 1991. *Plant lectins, vol.1. Chemistry and pharmacology of natural products.* Cambridge University Press: Cambridge.

Richardson M, Campos FDAP, Moreira RA, Ainouz Il, Begbie B, Watt WB, Pusztai A. 1984. The complete amino acid sequence of the major alpha subunit of the lectin from the seeds of Dioclea grandiflora. *Eur. J. Biochem.,* **144**: 101–111.

Sharon N, Lis H. 1990. Legume lectins: a large family of homologous proteins *FASEB. J*., **4**: 3198-3208

Singh-Bains J, Singh J, Kamboj SS, Nijar KK, Agrewale JN, Kumar V, Kumar A, Saxena AK 2005. Mitogenic and anti-proliferative activity of a lectin from the tubers of Voodoo lily (*Sauromatum venosum). Biochem. Biophys. Acta,* **1723:** 163–174.

Terada S, Fujimura S, Noda K, Kimoto E. 1992. Separation of dimethylaminoazobenzene-thiohydantoin derivatives by a reversed-phase high-performance liquid chromatography. *Fukuoka Univ. Sci. Rep.* **22**: 35-39.

Wang H, Gao J**,** Ng TB. 2000. A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus. Biochem. Biophysics Res. Commun.* **275**: 810–816.

Wang HX, Liu WK, Ng TB, Ooi VE, Chang ST. 1996. The immunomodulatory and antitumor activities of lectins from the mushroom *Tricholoma mongolicum*. *Immuno-pharmacology,* **31**: 205–211.

Yu LG, Milton JD, Fermig DG. 2001. Opposite effects on human colon cancer cell proliferation of two dietary Thomson-Friedenreich antigen-binding lectins. *J. Cell Physiol.,* **186**: 282–287.