Erythrocyte-binding studies on an acidic lectin from winged bean (*Psophocarpus tetragonolobus*)

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An acidic lectin (WBA II) was isolated to homogeneity from the crude seed extract of the winged bean (*Psophocarpus tetragonolobus*) by affinity chromatography on lactosylaminoethyl-Bio-Gel. Binding of WBA II to human erythrocytes of type-A, -B and -O blood groups showed the presence of 10^5 receptors/cell, with high association constants (10^6 – 10^8 M⁻¹). Competitive binding studies with blood-group-specific lectins reveal that WBA II binds to H- and T-antigenic determinants on human erythrocytes. Affinity-chromatographic studies using A-, B-, H- and T-antigenic determinants coupled to an insoluble matrix confirm the specificity of WBA II towards H- and T-antigenic determinants. Inhibition of the binding of WBA II by various sugars show that *N*-acetylgalactosamine and T-antigenic disaccharide (Thomsen–Friedenreich antigen, Gal β 1-3GalNAc) are the most potent mono- and di-saccharide inhibitors respectively. In addition, inhibition of the binding of WBA II to erythrocytes by dog intestine H-fucolipid prove that the lectin binds to H-antigenic determinant.

INTRODUCTION

Sugars, whether from membrane glycoproteins or glycolipids, constitute the essential elements of the cell-surface make-up, provide cells with recognition patterns and play a decisive role in the 'social life' of the cell (Goldstein & Hayes, 1978; Lis & Sharon, 1986a). Our recent appreciation of the role of cell-surface carbohydrates, at least in part, is due to a class of multivalent, carbohydrate-binding and cell-agglutinating proteins called lectins. Lectins provide some of the most discriminating probes for studying the expression of the cell-surface carbohydrates during development, differentiation and malignancy (Lis & Sharon, 1986b).

The winged bean (*Psophocarpus tetragonolobus*) is a tropical legume whose agronomic potential is marred by the presence of a large amount of cytotoxic components like haemagglutinins (Kortt & Caldwell, 1985). The seeds contain two lectins, WBA I and WBA II, with almost identical molecular masses, but very different isoelectric points and erythrocyte-binding properties (Kortt, 1984, 1985). The thermodynamic and kinetic analysis of saccharide binding to WBA I, the basic lectin (pI $\approx$ 9.5, M_r 58000) have already been reported by Khan *et al.* (1986). WBA II, the acidic lectin, is a glycoprotein (14%, w/w, carbohydrate) with an M_r of 54000 (pI $\approx$ 5.5) and consists of two identical subunits (Kortt, 1985). In the present study the binding of WBA II to human erythrocytes has been used to evaluate carbohydrate specificity, the number of receptors per cell and the association constants. On the basis of these studies, the most complementary structural determinant recognized by WBA II on human erythrocytes has been identified.

EXPERIMENTAL

Materials

Winged-bean seeds were purchased from the University of Agricultural Sciences, Hebbal, Bangalore. GalNAc, 1-*O*-methyl- α -galactopyranoside, 1-*O*-methyl- β -galactopyranoside, galactosamine, melibiose, galactose, thiodigalactoside, lactulose, LacNAc, BSA and the reagents for polyacrylamide-gel electrophoresis were obtained from Sigma Chemical Co. Lactose was obtained from BDH. Bio-Gel P-200 was obtained from Bio-Rad Laboratories. Na¹²⁵I (carrier-free iodine) was obtained from Bhabha Atomic Research Centre, Bombay, India. Sephadex G-25 (medium grade) and other reagents for isoelectric focusing were obtained from Pharmacia. Iodobeads and GF/C filters were purchased from Pierce Chemical Co. and Millipore respectively. Synsorb matrices were obtained from Chembiomed, University of Alberta, Edmonton, Alberta, Canada. All other chemicals used were of analytical grade.

Methods

Purification of WBA II. The defatted and dried winged-bean seed meal was extracted in PBS with continuous stirring for 12 h at 4 °C. The extract was centrifuged at 10000 *g* for 30 min, and the clear supernatant was decanted. The (NH₄)₂SO₄ fraction between 30 and 65% saturation was collected, redissolved in PBS and dialysed thoroughly against PBS. The clear extract after dialysis was applied to a 50 ml lactosyl-AE-Bio-Gel (P-200) column, prepared as described by Reinhold *et al.* (1977). The column was

Abbreviations used: WBA I, winged-bean (*Psophocarpus tetragonolobus*) agglutinin (basic lectin); WBA II, winged-bean agglutinin (acidic lectin); DBL, *Dolichos biflorus* (horse gram) lectin; GS I(B4), *Griffonia simplicifolia* lectin I isolectin B₄; LTL, *Lotus tetragonolobus* lectin; PNA, peanut (*Arachis hypogaea*) agglutinin; GalNAc, 2-acetamido-2-deoxygalactopyranose; GlcNAc, 2-acetamido-2-deoxyglucopyranose; Me α Gal, methyl α -D-galactopyranoside; Me β Gal, methyl β -D-galactopyranoside; L-Fuc, L-fucose; LacNAc, *N*-acetyl-lactosamine; T-antigenic disaccharide, Thomsen–Friedenreich antigen (Gal β 1-3GalNAc); PBS, phosphate-buffered saline (20 mM-sodium phosphate buffer, pH 7.4, containing 150 mM-NaCl); PBS-BSA, PBS containing 0.25% (w/v) bovine serum albumin (BSA); RBC(s), red blood cell(s); AE, aminoethyl.

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extensively washed with PBS until the A_{280} was less than 0.005. The bound protein was eluted with 0.2 M-galactose and dialysed thoroughly against PBS to remove galactose and freeze-dried to the desired concentration.

Preparation of other lectins. DBL was purified as described by Etzler & Kabat (1970), except that Synsorb-A was used as affinity matrix. A mixture of *Griffonia simplicifolia* isolectins was generously provided by Professor S. Basu, University of Notre Dame, Notre Dame, IN, U.S.A., and Professor I. J. Goldstein, University of Michigan, Ann Arbor, MI, U.S.A. LTL was a gift from Professor S. Basu. GS I-(B4) was isolated as described by Delmotte & Goldstein (1980). PNA was prepared as described by Mazumder & Surolia (1979).

Labelling of lectins. Lectins were iodinated by the method of Markwell (1983), with the use of Iodobeads. Typical iodination mixture contained 100–150 μg of lectin in 100–150 μl of PBS, a 50 mM concentration of monosaccharides specific for each lectin, two to three Iodobeads and 1 mCi of carrier-free iodine. Reactions were carried out for 10 min, and free iodine was separated from the proteins by chromatography on Sephadex G-25 (medium grade) column (1 cm $\times$ 30 cm). The radioactivity in the samples was counted on an LKB α -radiation counter.

Human RBCs. Human RBCs were washed at least five times in PBS-BSA. The cells were subsequently suspended in the same buffer to a final concentration of 1% (v/v). This suspension was diluted 1:20, and the number of cells was counted by using a haemocytometer under a standard microscope. The molar concentration [(no. of cells/litre)/Avagadro's Number] of the cells was calculated from these RBC counts. Some of the RBCs were suspended in 0.05 M-sodium acetate buffer (pH 5.5)/0.15 M-NaCl to a final concentration of 1% (v/v). To 1.0 ml of packed cell volume, 0.1 unit of *Clostridium perfringens* neuraminidase was added and incubated at 37 °C for 1 h. The cells were then washed five times, each in 75 vol. of PBS-BSA and finally resuspended in PBS-BSA to a final concentration of 1%.

Haemagglutination. The haemagglutinating activity of native and labelled lectins was tested by using human RBCs. Haemagglutination was performed by serial-dilution method in microtitre plates. The final concentration of RBCs in each well was kept constant at 2% (v/v), and the agglutination was scored visually.

Cell-binding studies. Binding studies were carried out in 1.5 ml Eppendorf tubes. The reaction mixture contained 7.5×10^7 cells/ml and the labelled lectin concentration varied from 2.0×10^{-8} M to 3.0×10^{-7} M in a final volume of 0.3 ml of PBS-BSA. The samples were incubated at 20 °C for 30 min. Cells were pelleted in a Beckman Microfuge for 20 s, the supernatant was removed, and the cells were further washed three times, each with 0.3 ml of PBS-BSA. The amount of bound lectin was determined on an LKB γ -radiation counter. Appropriate corrections were made for the non-specific binding to the tube. The number of receptors/cell was calculated from the ordinate intercepts of the plots of (molarity of the cells)/(bound-protein concentration) versus the inverse of the free-protein concentration

(Steck & Wallach, 1965). The inverse of the intercept yielded the total number of receptors. From the values of slope and the number of receptors the association constant was calculated as described by Steck & Wallach (1965).

Competitive binding studies with lectins. Various blood-group-specific lectins were incubated with RBCs in presence of labelled WBA II. The incubation mixture contained labelled lectin (1.65×10^{-7} M), various concentrations of blood-group-specific lectins (1.28×10^{-8} M– 2.57×10^{-6} M) and 7.5×10^7 RBCs/ml. The competitive inhibition was also tested at a range of 20–100-fold molar excess of the competing lectin over labelled WBA II. After 30 min of incubation the cells were pelleted down and washed with PBS-BSA. From the radioactivity remaining in the pellet, the association constant for the competing lectin was calculated as described by Kawaguchi *et al.* (1974) and as explained in the Results section. The percentage inhibition was calculated from the radioactivity in the pellet at different concentrations of the inhibiting lectin.

Competitive binding studies with different sugars. Inhibition studies with various sugars were carried out by pre-incubating different concentrations of each sugar (0.5–100 mM) with the lectin in 0.3 ml of the incubation mixture at 20 °C. Then native human O-blood-group RBCs were added and further incubated for 30 min. The resultant concentration of the lectin and erythrocytes in the incubation mixture was 2×10^{-7} M and 1.2×10^{-13} M respectively. The pellet was spun down and the amount of bound protein was determined by the amount of radioactivity retained in the pellet. Dog intestine H-fucolipid was used for the inhibition of WBA II binding to type-O-blood-group RBCs over the range 1.21×10^{-8} M– 1.03×10^{-6} M.

Affinity chromatography. The binding of WBA II to various blood-group determinants was tested using Synsorb matrices. Synsorb A, B, H (type 2) and T were packed in small columns of 5 ml bed volume. Labelled WBA II was mixed in a 1:10 ratio with the unlabelled WBA II and 500 μg of the mixture was applied to the Synsorb columns, which had been pre-equilibrated with PBS. The columns were then washed extensively with PBS until the radioactivity in the washings reached a basal value. This was generally achieved after washing the column with 10–15 bed volumes of PBS at a flow rate of 2–3 ml/h. The bound protein was eluted with 0.1 M-acetic acid. The eluted fractions were counted for radioactivity in an LKB γ -radiation counter.

Polyacrylamide-gel electrophoresis. Gel electrophoresis using 7.5% polyacrylamide gels was performed in the acidic and basic buffer systems of Davis (1964). The gels were stained with Coomassie Brilliant Blue R-250. Electrophoresis in the presence of SDS was carried out as described by Weber & Osborn (1969) in the presence, as well as in the absence, of 2-mercaptoethanol, 7.5% (w/v) polyacrylamide gels being employed.

Isoelectric focusing. Agarose-gel isoelectric focusing for 1500 V·h in Pharmalyte (pH 3–10) was carried out according to the directions of the manufacturer. Gels were stained in Coomassie Brilliant Blue R-250.

Fig. 1. Electrophoretic patterns of WBA II

(a) SDS/polyacrylamide-gel electrophoresis of 60 μ g of WBA II on 7.5 % (w/v) polyacrylamide gels at pH 7.2; (b) isoelectric focusing (pH range 3–10) on a Pharmalyte/agarose-gel plate, of 30 μ g of WBA II.

Synthesis of Gal β 1-3GalNAc and Gal β 1-3GlcNAc. Gal β 1-3GalNAc was synthesized by the method of Flowers & Shapiro (1965). Gal β 1-3GlcNAc was synthesized as described by Roseman & Ludwig (1954) and Flowers & Jeanloz (1963).

RESULTS

Biological activity of the lectins

Five lectins, namely WBA II, PNA, LTL, DBL and GS I(B4), were radioiodinated during the course of the work. The radiolabelling yielded 0.1–0.5 μ Ci/ μ g of the lectin. All the labelled lectins were tested for their haemagglutinating activity and compared with the corresponding activities of the unlabelled lectins. Labelling was done in the presence of specific sugars so as to retain their sugar-binding activity. Excess of label as well as the sugar were removed initially by chromatography on a Sephadex G-25 (medium grade) column and later by dialysis against PBS. All the lectins after radioiodination retained their biological activity between 80 and 95 % for at least 10 days. Appropriate corrections were made when calculating the concentrations of these lectins.

Purification of WBA II

The purified protein showed a single band in both acidic and basic buffer systems of Davis (1964) on

polyacrylamide-gel electrophoresis. It exhibited a single band in SDS/polyacrylamide-gel electrophoresis in the presence or absence of 2-mercaptoethanol. Isoelectric focusing showed the pI of this molecule to be 5.5 (Fig. 1), with two closely associated bands. These results are in agreement with those for the acidic lectin isolated by Kortt (1985). WBA II was first isolated on a lactobionate Bio-Gel P-150 affinity matrix by Pueppke (1979). Later Appukuttan & Basu (1981) reported the presence of the lectin by using an aminohexanoylgalactosamine–Sephacrose column. Since our observations show that WBA II preferentially binds to a lactosyl-AE-Bio-Gel (P-200) column, the unbound fraction was applied to an aminohexanoylgalactosamine–Sephacrose column to purify WBA I. This observation is consistent with (i) a previous report by Khan *et al.* (1986) that WBA I does not bind to lactose and (ii) the present study on sugar inhibition, which shows that lactose binds reasonably well to WBA II. If WBA II is first removed from the affinity matrix, as in the present case, aminohexanoylgalactosamine–Sephacrose could be employed as affinity matrix for the homogeneous purification of WBA I.

Estimation of the number of receptor sites

The binding of WBA II and other lectins to RBCs was plotted as described by Steck & Wallach (1965), as shown in Fig. 2. DBL, LTL and GS I(B4) showed monophasic plots (Fig. 2b), but the binding of WBA II showed two definite phases (Fig. 2a). This observation was consistent with all the three blood groups tested, namely A, B and O. The total number of receptors increased to 2.0×10^6 sites/cell on native O-blood-group RBCs upon desialylation. PNA does not show a biphasic binding pattern when tested with native or neuraminidase-treated RBCs. The numbers of receptors for different lectins were also estimated for the sake of comparison and are listed in Table 1. It may be noted that the number of receptor sites for GS I(B4) [$(3.75\text{--}4.1) \times 10^5$ sites/cell] obtained by us is in the range of values reported by Hayes & Goldstein (1975) (1.34×10^5 sites/cell) and Economidou *et al.* (1967) (8.3×10^5 sites/cell). Although PNA does not agglutinate untreated human RBCs, 9.2×10^3 receptors/cell were found for it on native O-blood-group RBCs. These values are two orders of magnitude less than the number of receptors on desialylated cells (1.35×10^6 receptors/cell). The total number of receptors/cell for WBA II on untreated RBCs of all the three human blood groups tested was of the order of 10^5 . After desialylation, although the biphasicity was retained, the total number of receptors was increased by an order of magnitude, as listed in Table 1.

Inhibition studies with various lectins

DBL, GS I(B4) and LTL lectins were used as competitive inhibitors for the binding of WBA II to the respective blood-group RBCs. DBL (specific for A blood group) and GS I(B4) (specific for B blood group) fail to inhibit the binding of WBA II to native human A- and B-blood-group RBCs respectively. LTL (O-blood-group-specific) inhibits by 25 % the binding of WBA II to native O-blood-group RBCs, at 50-fold molar excess over WBA II. A further increase in the LTL concentration did not produce any pronounced effect on the binding of WBA II. Binding of WBA II to desialylated cells was inhibited by PNA, a well-known anti-T lectin, up to 80 %

Fig. 2. Binding of radiolabelled lectin to human O-type RBCs

(a) Binding of WBA II to native (Δ) and desialylated ($\circ$) erythrocytes; (b) binding of LTL to native ($\square$) erythrocytes.

Table 1. Number of receptors and the association constants for various lectins obtained by cell-binding studies using radiolabelled lectins

The values for association constants of WBA II are for the major receptor sites. Abbreviation: n, native RBCs; ds, desialylated RBCs.

Lectin	Blood group	Number of receptors		Association constant (M^{-1}) 20 °C
		Major	Total	
WBA II	A (n)	3.44×10^5	5.0×10^5	$3.12(\pm 0.52) \times 10^7$
	A (ds)	4.76×10^5	5.1×10^6	$3.51(\pm 0.43) \times 10^7$
	B (n)	1.62×10^5	5.0×10^5	$2.28(\pm 0.29) \times 10^7$
	B (ds)	2.1×10^5	5.3×10^6	$2.12(\pm 0.38) \times 10^7$
	O (n)	5.2×10^5	6.6×10^5	$2.08(\pm 0.10) \times 10^7$
	O (ds)	7.4×10^5	2.0×10^6	$2.52(\pm 0.21) \times 10^7$
LTL	O (n)	—	1.6×10^5	$1.1(\pm 0.39) \times 10^7$
	O (ds)	—	5.0×10^5	$2.7(\pm 0.29) \times 10^7$
GS (IB4)	B (n)	—	3.8×10^5	$9.52(\pm 0.28) \times 10^6$
	B (ds)	—	4.1×10^5	$9.52(\pm 0.32) \times 10^6$
DBL	A (n)	—	4.0×10^5	$8.18(\pm 0.85) \times 10^6$
	A (ds)	—	4.3×10^5	$9.02(\pm 0.52) \times 10^6$
PNA	O (n)	—	9.2×10^3	$7.52(\pm 0.37) \times 10^8$
	O (ds)	—	1.35×10^6	$7.75(\pm 0.41) \times 10^8$

in the presence of 25-fold molar excess of the latter. Further increase in the concentration of PNA did not produce any more inhibition of the binding of WBA II to the cell surface.

The association constants for the binding of a competing lectin (K_i) were calculated by the method of Harris & Pestka (1973), and as described by Kawaguchi *et al.* (1974), by using the equation below:

$$\log \left(\frac{K_0[WBA]([R_0] - [WBA-R])}{[WBA-R]} \right) = m \log [I] + \log K_i$$

K_0 and K_i are the association constants of WBA II and the inhibiting lectin to RBC receptor, $[WBA]$, $[R_0]$,

$[WBA-R]$ and $[I]$ are the concentration of the labelled lectin, receptor, labelled lectin-receptor complex and the inhibiting lectin respectively. Since the above equation is in the form of $y = mx + c$, K_i could be calculated from the x intercept of the plots. The value of the slope, m , corresponds to the number of inhibitor molecules involved in displacing the radiolabelled WBA II from the cell-surface receptor.

The inhibition plots were monophasic (Fig. 3), and the K_i values for the inhibitory lectins cited in Fig. 3 are comparable with the values obtained by equilibrium measurements (Table 1).

LTL has 1.6×10^5 receptors/cell on O-type RBCs and is able to inhibit the binding of WBA II to these cells only

Fig. 3. Inhibition of the binding of WBA (II) to RBCs

Inhibition by PNA using desialylated (○—○) erythrocytes; inhibition by LTL using native (□—□) and desialylated (△—△) type O erythrocytes. The association constants for the inhibiting lectin (K_i) are calculated from these plots. K_i for PNA is $1.77 \times 10^9 \text{ M}^{-1}$. K_i values for LTL binding to native and desialylated cells are $7.24 \times 10^6 \text{ M}^{-1}$ and $5.75 \times 10^7 \text{ M}^{-1}$, respectively. K_o and the other symbols are defined in the text.

to the extent of 25 %. Binding studies using desialylated RBCs show that there is an enormous increase in the PNA receptors after desialylation (from 9.2×10^3 to 1.34×10^6 receptors/cell). The number of receptors increased after desialylation is $1.34 \times 10^6/\text{cell}$. The difference in the total number of receptors on desialylation for WBA II is also $1.34 \times 10^6/\text{cell}$ ($2.0 \times 10^6 - 6.6 \times 10^5 = 1.34 \times 10^6$). Thus the increase in the number of receptors for PNA after desialylation is identical with the increase observed in WBA II. Further PNA effectively inhibited the binding of WBA II to desialylated RBCs.

Inhibition with sugars

Since there are two kinds of receptors for WBA II on erythrocytes, the effect of the ligands is difficult to interpret. The concentrations of various mono- and disaccharides required to inhibit 50 % of the total binding of WBA II to RBCs (IC_{50}) are listed in Table 2. Inhibitory strengths of various sugars, in comparison with galactose, show that GalNAc and T-antigenic disaccharide are the best inhibitory mono- and disaccharides respectively. H-fucolipid inhibits the binding better than any other sugar tested.

Affinity chromatography

WBA II did not bind to Synsorb A and Synsorb B matrices, but was observed to bind to Synsorb H (type 2) and Synsorb T matrices. Almost all of WBA II loaded on the first two matrices was seen in the flow-through of the

Table 2. Concentration of mono- and di-saccharides required for 50 % of the inhibition (IC_{50}) of WBA II to human RBCs and the relative inhibitory potencies of the sugars when compared with galactose

Sugar	IC_{50} (M)	Relative inhibitory potency
Galactose	1.41×10^{-3}	1.0
Me α Gal	1.47×10^{-3}	0.95
Me β Gal	1.81×10^{-3}	0.78
GalNAc	7.15×10^{-4}	1.96
Galactosamine	6.67×10^{-3}	0.21
L-Fuc	No binding	
Lactose	1.39×10^{-3}	1.01
Thiodigalactoside	9.71×10^{-4}	1.44
Melibiose	6.71×10^{-4}	2.09
Lactulose	4.52×10^{-4}	3.1
LacNAc	4.39×10^{-4}	3.19
Gal β 1-3GlcNAc	2.71×10^{-4}	5.18
Gal β 1-3GalNAc	1.08×10^{-4}	12.95
H-fucolipid	1.58×10^{-7}	886

columns. Approx. 200 μg and 125 μg of binding was observed to Synsorb H (type 2) and Synsorb T matrices respectively on elution with 0.1 M-acetic acid.

Association constants for lectin-receptor interaction

The biphasicity of Steck & Wallach (1965) plots for WBA II binding to RBCs indicates that the lectin binds to two types of receptors on cell surface with differential affinity. From the slope of the first phase of these plots (Fig. 2a) the association constant was calculated for major receptors. Since an unequivocal approach to determine the association constants for the second phase is not available in the literature (Kawaguchi *et al.*, 1974), we have estimated the association constants for the high affinity of receptors from the slope of the first phase (Table 1) alone. The values of association constants for DBL, GS I(B4) and LTL are in the range of 10^7 – 10^8 , which is generally expected for lectin-cell-surface-receptor interaction (Lis & Sharon, 1986a). The association constants for major receptors of WBA II with different human blood groups and the association constants for other lectins are listed in Table 1.

DISCUSSION

The results reported here on the inhibition of WBA II by appropriate complementary ligands and lectins which recognize blood-group antigens demonstrate that the acidic lectin from winged bean recognizes H(O)- and T-antigenic determinants on the RBC surfaces.

Cell-binding studies

The number of receptors (10^5) on human RBCs estimated for WBA II is comparable with those in the earlier reports on other lectins (Steck & Wallach, 1965; Presant & Kornfield, 1972; Hoebeke *et al.*, 1978; Hayes & Goldstein, 1975). Upon desialylation the number of receptors increased by an order of magnitude. Excepting PNA, no other lectin showed such increase (Table 1). PNA, an anti-T lectin, showed the expected rise in the number of receptors on desialylated RBCs that is mainly

Table 3. Structures of A, B, H and T antigenic determinants

A	GalNAc α 1-3Gal β 1-3GlcNAc	GalNAc α 1-3Gal β 1-4GlcNAc
	2 L-Fuc α 1	2 L-Fuc α 1
B	Gal α 1-3Gal β 1-3GlcNAc	Gal α 1-3Gal β 1-4GlcNAc
	2 L-Fuc α 1	2 L-Fuc α 1
H	Gal β 3-GlcNAc	Gal β 1-4GlcNAc
	2 L-Fuc α 1	2 L-Fuc α 1
T	Gal β 1-3GalNAc	

due to the exposure of T-antigenic determinants otherwise masked by sialic acid residues.

Competitive binding studies with blood-group-specific lectins were aimed at the identification of the receptor(s) for WBA II on the cell surface. LTL, which specifically binds to type 2 chains of H-antigenic determinant (Pereira & Kabat, 1974), partially inhibited the binding of WBA II to type-O RBCs. Assuming that type 1 chains are equally abundant on type-O RBCs, WBA II has 3.2×10^5 potential receptors contributed by O-blood-group determinant. The remainder of the total receptors of WBA II are 3.4×10^5 receptors/cell ($6.6 \times 10^5 - 3.2 \times 10^5$; Table 1), which are likely to be common on A-, B- and O-blood-group RBCs. This number is in reasonable agreement with the number of receptors (5×10^5) found on type A and B RBCs. The number of receptor sites estimated individually for LTL and WBA II, in conjunction with the pattern of LTL inhibition of WBA II binding to RBCs, suggests that WBA II binds H-antigenic determinant. Inhibition of WBA II binding to type-O erythrocytes by dog intestine H-fucolipid, which has H-antigenic determinant attached to ceramide (Smith *et al.*, 1975), conclusively proves that WBA II binds to H-antigenic determinant on the RBC surface.

DBL and GS I(B4) do not inhibit the binding of WBA II to the A- and B-blood-group RBCs respectively, suggesting that WBA II does not bind to either of these determinants. This is probably due to the substitution of the C-3 hydroxy group of the terminal α -D-galactopyranose residue of the H(O)-blood-group determinant by α -N-acetyl-D-galactosamine or α -D-galactose units in the A- and B-blood-group determinants respectively (structures of blood-group determinants are shown in Table 3). The increase in the number of receptors for PNA and WBA II upon desialylation of RBCs is identical. Moreover, PNA effectively inhibits the binding of WBA II, suggesting that both of these lectins share a common receptor, that is, T-antigen. The monophasic inhibition patterns (Fig. 3) observed with PNA and LTL are not surprising in view of their exquisite specificities for the T-antigenic and type 2 chains of H-antigenic determinants respectively. The binding of WBA II Synsorb H (type 2) and T-antigen columns and non-binding to Synsorb A and B columns further support the above conclusions. Although WBA II binds to 1-O-methyl- α -D-galactopyranoside and α -N-acetyl-D-galactosamine, the non-binding of WBA II to A- and B-blood-group determinants appears surprising. However, it is

not altogether unexpected, as there are precedents for lectins which bind to monosaccharides reasonably well but do not bind to them when they occur as a part of an oligosaccharide (Khan *et al.*, 1986; Sastry *et al.*, 1986). These results, taken together, indicate that WBA II binds to Gal β 1-3GlcNAc or Gal β 1-4GlcNAc or their fucosylated derivatives substituted with α -L-fucose at the second position of D-galactose (Table 3). Apart from this it can also bind to T-antigen (Gal β 1-3GalNAc).

Sugar-inhibition studies

The above interpretations are supported by sugar-inhibition studies. Considering D-galactose as the minimum structural determinant required for the binding, 1-O-methyl- α -D-galactose and 1-O-methyl- β -D-galactose do not differ much in their inhibitory strengths compared with D-galactose. Of the monosaccharides tested, α -N-acetyl-D-galactosamine is the most potent inhibitor. The substituent on the second carbon of the sugar, the acetamido group, is probably involved in hydrogen-bonding and/or hydrophobic interactions. On the other hand, galactosamine with an amino group in the second position is a poor inhibitor. Thus the site corresponding to the second position of galactose accommodates bulkier substituents like acetamido group more favourably. The fact that L-fucose does not bind at all suggests that WBA II is not binding to the fucose termini *per se* on the blood-group-O determinant.

T-antigen appears to be the best ligand for the lectin among the mono- and di-saccharides tested. T-antigen is 2.5-fold more potent than Gal β 1-3GlcNAc which is the next potent disaccharide. It is pertinent to mention that PNA, the best studied T-antigen-binding lectin (Neurohr *et al.*, 1982), shows 11-fold higher binding for T-antigen over LacNAc (Gal β 1-4GlcNAc), which is its next potent inhibitor. In this sense WBA II does not exhibit the preference towards T-antigen as does PNA, although it is the most potent disaccharide inhibitor.

Apart from PNA, lectins from *Bauhinia purpurea* (camel's foot tree), *Maculra pomifera* (osage orange), *Sophora japonica* (Japanese pagoda tree) and *Artocarpus integrifolia* (jackfruit) have also been identified as T-antigen-binding lectins by precipitin assays (Wu, 1984) and by fluorescence methods (Sastry *et al.*, 1986). WBA II resembles the *B. purpurea* lectin in its sugar-binding properties. The earlier observation of Wu (1984) suggests that T-antigen is the best inhibitor for *B. purpurea* lectin. The order of affinity is:

T-antigen > Gal β 1-3GlcNAc > LacNAc > lactose.

Our results for inhibition by sugars also suggest an order of affinity for WBA II similar to that of the *B. purpurea* lectin.

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