The Purification, Composition and Specificity of Wheat-Germ Agglutinin

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1. The purification of wheat-germ agglutinin from commercial wheat germ is described. By ion-exchange chromatography three active proteins (isoelectins) were separated, one of which was examined in detail. 2. The amino acid composition is unusual, as 20% of residues are half-cystine and 21% are glycine. Unlike most lectins and contrary to previous reports, this protein is not a glycoprotein. 3. The efficiency of various saccharides as inhibitors of the agglutination reaction was investigated and from this the specificity of the binding site was inferred. Of monosaccharides, only derivatives of glucose with a 2-acetamido group and a free 3-hydroxyl group are effective inhibitors, and glycosides of either anomeric configuration are bound. Oligosaccharides are much more powerful inhibitors of agglutination than are monosaccharides. 4. It is proposed that the binding site consists of three or four subsites with differing specificities, in a cleft in the molecule resembling that proposed for hen's-egg-white lysozyme.

Wheat-germ agglutinin is a lectin, i.e. a protein that is capable of agglutinating erythrocytes and other types of cells (see review by Sharon & Lis, 1972). Because the agglutination reaction of many lectins is specifically inhibited by simple sugars, lectins are thought to act by binding to sugar residues on the surfaces of cells. Thus concanavalin A from jack bean is inhibited by α -D-manno- or gluco-pyranosides (Poretz & Goldstein, 1970), soya-bean agglutinin by 2-acetamido-2-deoxy- α - and - β -D-galactopyranosides (Lis *et al.*, 1970), and wheat-germ agglutinin by *N*-acetylglucosamine and its dimer *NN'*-diacetylchitobiose (Burger & Goldberg, 1967).

Recent work (see Sharon & Lis, 1972) has shown that lectins have a much greater capacity for agglutinating cells grown in tissue culture which have transformed spontaneously, or have been transformed by the action of carcinogenic chemicals or viruses, than they have for normal cells. Normal cells can, however, be made more susceptible to the action of lectins by treatment with proteolytic enzymes. After recent work in our laboratories (Sharon, 1966; Davies *et al.*, 1969) on the specificity of lysozymes that split polymers of *N*-acetylglucosamine and bind *N*-acetylglucosamine and its oligomers, we became interested in wheat-germ agglutinin, which also binds *N*-acetylglucosamine and its derivatives. We found that the established methods (Burger & Goldberg, 1967; Ozanne & Sambrook, 1971) for the purification of this lectin from wheat-germ lipase did not yield a pure product. As a first step, we developed an improved procedure for the purification of wheat-

germ agglutinin directly from commercial wheat germ and have isolated three closely related agglutinins, or isoelectins. While this work was in progress, Nagata & Burger (1972) published a procedure for the purification of wheat-germ agglutinin from wheat-germ lipase; they concluded from ultracentrifugation that the molecular weight of the protein was about 23 500. They did not, however, report the presence of isoelectins, and concluded, in contrast to ourselves, that wheat-germ agglutinin is a glycoprotein. A preliminary communication on this subject has been published (Allen & Neuberger, 1972).

Materials and Methods

Wheat germ was a gift of the Lord Rank Centre, High Wycombe, Bucks., U.K. DEAE-cellulose (Whatman grade DE 23) was obtained from H. Reeve Angel and Co., London E.C.4, U.K. SE (sulpho-ethyl)-Sephadex (C-50), QAE(quaternary amino-ethyl)-Sephadex (A-50) and Sephadex G-100 were from Pharmacia, Uppsala, Sweden. The standard amino acid mixture used for amino acid analysis was supplied by Beckman-Spinco, Palo Alto, Calif., U.S.A., and the other amino acids used as standards by BDH Chemicals Ltd., Poole, Dorset, U.K. Glucosamine, *N*-acetylglucosamine (which was recrystallized before use), *N*-acetylgalactosamine and *N*-acetylmannosamine were obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. 1,2-Diacetamido-1,2-dideoxy- β -D-glucopyranose (GlcNAc-NAC) and the glycoprotein linkage compound 2-acetamido-1-(L- β -aspartamido)-1,2-

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dideoxy- β -D-glucopyranose (GlcNAc-Asn) were gifts of Dr. B. M. Austen. *NN'*-Diacetylchitobiose (GlcNAc)₂, the triose (GlcNAc)₃, the tetraose (GlcNAc)₄ and the pentaose (GlcNAc)₅ were gifts of Dr. R. C. Davies. Methyl 2-acetamido-2-deoxy- α -D-glucopyranoside (methyl α -D-GlcNAc-furanoside) and benzyl 2-acetamido-2-deoxy- α -D-glucopyranoside (benzyl α -D-GlcNAc) were gifts of Mr. D. M. Morgan. Ethyl 2-deoxy-2-propionamido- α -D-glucopyranoside (ethyl α -D-GlcNProp) and the bacterial cell wall di- and tetra-saccharides, GlcNAc-MurNAc and (GlcNAc-MurNAc)₂, which contain alternate residues of *N*-acetylglucosamine and *N*-acetylmuramic acid, were gifts of Dr. B. M. Wilson. All the sugars mentioned above were prepared while the donors were working in the Department of Chemical Pathology, St. Mary's Hospital Medical School, London W.2, U.K.

Methyl 2-acetamido-2-deoxy- α - and - β -D-glucopyranosides (methyl α - and β -D-GlcNAc) were prepared by the method of Zilliken *et al.* (1955) and purified by chromatography on a column (60cm $\times$ 3cm) of Dowex 1 (OH⁻ form) eluted with deionized, distilled, CO₂-free water (Neuberger & Wilson, 1971). The same column was used to separate and purify the methyl ethers of methyl α -D-GlcNAc. We found that all three monomethyl ethers as well as the dimethyl ether, methyl 3,4-*O*-methyl- α -D-GlcNAc, were separated from each other. The elution volumes for the compounds were, in order of elution: methyl 3,4-*O*-methyl- α -D-GlcNAc, 340ml; methyl 4-*O*-methyl- α -D-GlcNAc, 430ml; methyl 3-*O*-methyl- α -D-GlcNAc, 515ml; methyl 6-*O*-methyl- α -D-GlcNAc, 630ml; methyl α -D-GlcNAc, 700ml.

Methyl 2-acetamido-2-deoxy-3-*O*-methyl- α -D-glucopyranoside (methyl 3-*O*-methyl- α -D-GlcNAc) was prepared by the method of Neuberger (1941). This involved reaction of methyl α -D-GlcNAc with benzaldehyde to block the 4- and 6-hydroxyl groups, followed by methylation with dimethyl sulphate to introduce a 3-*O*-methyl group. Hydrolysis of this product with 60% (v/v) acetic acid gave methyl 3-*O*-methyl- α -D-GlcNAc contaminated with about 5% methyl α -D-GlcNAc. Instead of purification by repeated crystallization the contaminant was removed by chromatography on a column of Dowex 1 (OH⁻ form) as reported above.

Methyl 2-acetamido-2-deoxy-4-*O*-methyl- α -D-glucopyranoside (methyl 4-*O*-methyl- α -D-GlcNAc) was prepared by the following procedure given by Jeanloz (1952) and Jeanloz & Gansser (1957). Methyl α -D-GlcNAc was treated with chlorotriphenylmethane in order to substitute the 6-hydroxyl group with a triphenylmethyl (trityl) group. The methyl 6-*O*-trityl- α -D-GlcNAc was methylated with methyl iodide and silver oxide and the trityl group removed by hydrolysis with 60% (v/v) acetic acid. Methyl 4-*O*-methyl- α -D-GlcNAc was separated from methyl

3-*O*-methyl- α -D-GlcNAc and methyl 3,4-*O*-methyl- α -D-GlcNAc by chromatography on a column of Dowex 1 (OH⁻ form) as described above. Jeanloz (1952) separated the three methyl ethers by chromatography on columns of alumina before removal of the trityl groups.

Methyl 2-acetamido-2-deoxy-6-*O*-methyl- α -D-glucopyranoside (methyl 6-*O*-methyl- α -D-GlcNAc) was prepared by the procedure of Jeanloz (1954) in which methyl 6-*O*-trityl- α -D-GlcNAc (see above) was treated with benzoyl chloride which resulted in the introduction of benzoyl groups at C-3 and C-4. The trityl group was selectively removed by hydrolysis with 60% (v/v) acetic acid and then a methyl group was introduced in the 6-hydroxyl position by the use of methyl iodide and silver oxide. The benzoyl groups were then removed by saponification. We found that the preparation of methyl 6-*O*-methyl- α -D-GlcNAc contained some methyl α -D-GlcNAc but this was removed by chromatography on Dowex 1 (OH⁻ form).

The procedure of Matsushima & Park (1963) was followed for the preparation of methyl $\alpha\beta$ -*N*-acetylmuramide (methyl $\alpha\beta$ -MurNAc). This involved synthesis of a 4,6-*O*-benzylidine derivative of methyl $\alpha\beta$ -GlcNAc and reaction of this compound with 2-chloro-DL-propionic acid. The benzylidine group was removed by hydrolysis with 60% (v/v) acetic acid and the methyl $\alpha\beta$ -MurNAc purified by recrystallization from ethanol.

All other products used were of BDH AnalaR grade or the highest purity available.

Assay of agglutination

Blood cells from New Zealand White rabbits were harvested by centrifugation, resuspended in a volume of Alsever's solution (Herbert, 1967) equal to the original volume of the blood, and stored at +4°C until required. A 1.5% suspension of trypsin-treated cells was prepared in phosphate-buffered saline (pH 7.4) (Herbert, 1967) in the manner described by Liener (1955). To assay agglutination, a serial dilution of the agglutinin was made in 100 μ l of phosphate-buffered saline and 200 μ l of a 1.5% suspension of trypsin-treated cells were added. The tubes were examined after 2h and the degree of agglutination was assessed on the serological scale (0 to ++++). The amount of agglutinin required to cause half-maximal (++) agglutination of the cells was taken as 1 unit. This assay is only semi-quantitative and should be regarded as being liable to an error of $\pm 20\%$. With this method an activity of 4000 units/mg was obtained for pure agglutinin fraction I; in comparison, the spectrophotometric method (Liener, 1955) gave an activity of 2800 units/mg.

To determine the 50%-inhibition values for various

sugars in this system, the method used by Lis *et al.* (1970) for soya-bean agglutinin was used, except that the degree of agglutination was determined on the visual serological scale rather than spectrophotometrically.

Results and Discussion

Purification of wheat-germ agglutinin

The purification scheme is shown in Table 1. Wheat germ (1000 g) was defatted with light petroleum (b.p. 40°–60°C) and dried in an air stream. The product (900 g) was stirred with 3 litres of water in the cold room over 18 h, centrifuged at 600g for 15 min and the supernatant was retained. The precipitate was resuspended in water and the procedure was repeated twice. $(\text{NH}_4)_2\text{SO}_4$ was added to the combined supernatants to 55% saturation at 4°C, the precipitate was allowed to settle overnight and then collected by centrifugation at 9000g for 20 min. The precipitate was dissolved in water and dialysed extensively against water. This resulted in a very heavy precipitate of inactive protein which was removed by centrifugation at 9000g for 20 min.

DEAE-cellulose column. The pH of the supernatant from the previous step was adjusted to 8.6 with 0.5 M-tris solution and applied to a column (30 cm × 3.5 cm) of DEAE-cellulose equilibrated with 0.05 M-Tris-HCl buffer (pH 8.6) and washed with the same buffer. Most of the pigment and 80% of the protein was retained by the column, but no agglutinin was retained. The column was washed until a 10 µl sample of eluate had no agglutinating activity.

First SE-Sephadex column. The material that was

not adsorbed on DEAE-cellulose was dialysed against a 0.1 M-sodium acetate buffer (pH 3.8) containing 0.1 M-NaCl. It was then applied, without concentration, to a column (30 cm × 3.5 cm) of SE-Sephadex which had been equilibrated with the same buffer. The column was washed with the same buffer until the E_{280} had fallen to a value below 0.1. About 50% of the protein that had been applied to the column was eluted. Agglutinin was eluted by 0.1 M-sodium acetate buffer (pH 3.8) which contained 0.5 M-NaCl; the active fractions were bulked, dialysed against water and freeze-dried.

Second SE-Sephadex column (Fig. 1). The active material from the previous column was dialysed against 0.1 M-sodium acetate buffer (pH 3.8) containing 0.1 M-NaCl and applied to a column (40 cm × 2.5 cm) of SE-Sephadex which had been equilibrated with the same buffer. The proteins were then eluted by the following system: first, a linear gradient from 0.1 M-sodium acetate buffer (pH 3.8) containing 0.1 M-NaCl (300 ml) to the same buffer containing 0.25 M-NaCl (300 ml), secondly, elution with 0.1 M-sodium acetate buffer (pH 3.8) containing 0.25 M-NaCl (400 ml), and finally, a linear gradient was applied from 0.1 M-sodium acetate (pH 3.8) containing 0.25 M-NaCl (150 ml) to the same buffer containing 0.5 M-NaCl (150 ml).

This system gave a better separation of the isolectins than did a linear gradient over the same range of concentration. Fractions (10 ml) were collected, and the tubes containing the three active fractions I, II and III were pooled separately, dialysed against water and freeze-dried.

QAE-Sephadex column. Fraction I from the SE-Sephadex column was applied to a column

Table 1. *Purification of wheat-germ agglutinin from wheat germ*

This procedure starts from 900 g of de-fatted wheat germ. Owing to the inaccuracy of the haemagglutination assay, the activities are liable to an error of ±20%. In the first four steps protein was determined by the method of Warburg & Christian (1941) and for later steps the weight of the total freeze-dried material is given. Other experimental details are given in the text.

Procedure	Total activity (agglutinating units)	Total protein content (g)	Specific activity (units/mg)	Yield (%)
Aqueous extraction	3 000 000	300	10	100
$(\text{NH}_4)_2\text{SO}_4$ precipitation, dialysis and centrifugation	1 500 000	40	40	50
DEAE-cellulose column	1 000 000	8	125	33
First SE-Sephadex column	1 000 000	1.0	1000	33
Second SE-Sephadex column				
Fraction I	400 000	0.16	2500	13
Fraction II	420 000	0.17	2500	14
Fraction III	220 000	0.22	1000	7
QAE-Sephadex column				
Fraction I	320 000	0.08	4000	11

Fig. 1. Elution profile of wheat-germ agglutinin isolectins from SE-Sephadex

The column (40 cm $\times$ 2.5 cm) was equilibrated with 0.1 M-sodium acetate buffer (pH 3.8) containing 0.1 M-NaCl, and the proteins were eluted by increasing the concentration of NaCl. This discontinuous system (see the text) gave a better separation of the isolectins than did a simple linear gradient. $\bullet$, E_{280} ; Δ , agglutinating activity (units/ml). The NaCl concentration gradient is also shown.

(40 cm $\times$ 1.9 cm) of QAE-Sephadex equilibrated with 0.05 M-2-amino-2-methyl-1,3-propanediol-HCl buffer (pH 9.4) and eluted with a linear gradient from this buffer (250 ml) to the same buffer containing 0.1 M-NaCl (250 ml). Fractions (10 ml) were collected. Inactive protein was eluted by 0.01–0.02 M-NaCl and the active protein was eluted as a symmetrical peak by 0.05 M-NaCl. The tubes containing the active fractions were bulked, dialysed against water and freeze-dried.

The present paper is concerned mainly with the first isolectin eluted from SE-Sephadex (fraction I in Fig. 1). This was found to be homogeneous by electrophoresis at pH 8.6 in 15% polyacrylamide gel, both in the presence and in the absence of 0.1% sodium dodecyl sulphate. In the latter system, by the use of protein markers, a molecular weight of 35000 ± 1000 was estimated for the protein. In the same system,

after pretreatment with 1% 2-mercaptoethanol, a molecular weight of 18000 ± 1000 was estimated for the protein. This increase in mobility could be regarded as being due to the separation of two polypeptide chains by reduction of their disulphide bonds. It could alternatively be explained by the presence of a single polypeptide chain which opens out after reduction (since one residue in five is half-cystine) and therefore binds a much greater quantity of sodium dodecyl sulphate. By the method of Andrews (1965) a molecular weight of 18500 ± 1000 was obtained by chromatography on Sephadex G-100 with 0.1 M-Tris buffer (pH 8.6) containing 0.2 M-NaCl. When fraction I was chromatographed under the same conditions on Bio-Gel P-100, an apparent molecular weight of under 10000 was obtained. Because we did not obtain a consistent value for the molecular weight of our preparation, we have taken the value of 23500

obtained by Nagata & Burger (1972) by ultracentrifugation as a basis for our calculations of amino acid and carbohydrate composition.

Amino acid composition

Amino acid analyses were performed on the 23 cm column of a Locarte Mini analyser. Samples of protein were hydrolysed for 24, 48 and 72 h in constant-boiling HCl at 110°C *in vacuo* and correction factors were obtained for the destruction of serine and threonine residues. Half-cystine values were obtained from the analysis of cysteic acid resulting from the performic acid oxidation (Hirs, 1956) and subsequent hydrolysis of the protein for 24 h in constant-boiling HCl. Tryptophan was determined on the analyser from samples which had been hydrolysed *in vacuo* in constant-boiling HCl at 110°C to which 5% thioglycollic acid had been added (Matsubara & Sasaki, 1969). Hen's-egg-white lysozyme was hydrolysed under identical conditions, and the recovery of tryptophan from this protein (81%) was used as a

factor for the calculation of the recovery of tryptophan in the hydrolysate of fraction I.

The amino acid composition of agglutinin fraction I (see Table 2) is remarkable for the very high content of glycine (21%) and half-cystine (20%) residues. The only other proteins with such a high content of cystine are the high-sulphur proteins of mammalian hair (Gillespie *et al.*, 1968) and the metallothioneins (Kägi & Vallee, 1961). Most of the lectins which have been purified so far contain little or no cysteine or cystine. An exception is the pokeweed mitogen, which is a lectin and a glycoprotein. It resembles wheat-germ agglutinin fraction I in containing about 20% half-cystine residues and a high proportion of glycine, aspartic acid and glutamic acid residues (Reisfeld *et al.*, 1967).

Carbohydrate analyses

No trace of glucosamine or galactosamine was found when the 24 h hydrolysates of fraction I were examined on the amino acid analyser. When a sample

Table 2. Amino acid analysis of wheat-germ agglutinin fraction I

The values are calculated as molar proportions assuming that the molecular weight is 23 500. The values for the nearest integer were derived from an average of the three hydrolyses, with the exception of threonine and serine which were extrapolated to zero time and half-cystine, methionine and tryptophan (see footnotes). For further details see the Results and Discussion section.

Time of hydrolysis ...	Amino acid content (mol/mol)			
	24h	48h	72h	Nearest integer
Asp	18.2	17.9	18.2	18
Thr	6.4	6.1	5.6	7
Ser	15.3	13.3	11.3	17
Glu	22.0	22.1	21.8	22
Pro	6.1	9.4	6.9	7
Gly	48.1	48.1	48.1	48
Ala	13.1	13.9	13.1	13
Cys	37.6	31.6	31.7	44*
Val	1.9	2.2	2.1	2
Met	1.0	0.5	2.4	2†
Ile	2.7	2.8	2.9	3
Leu	5.5	5.9	5.7	6
Tyr	7.4	9.3	8.8	9
Phe	3.7	3.7	3.9	4
His	1.7	1.5	2.2	2
Lys	8.0	7.7	8.5	8
Arg	5.0	5.1	5.0	5
Trp	2.8‡	—	—	3
Hexosamine	0	0	0	0
Total				220

* Determined separately as cysteic acid.

† Determined separately as methionine sulphone.

‡ Determined from a sample hydrolysed in the presence of thioglycollic acid.

of methyl β -D-GlcNAc was added to fraction I and hydrolysed for 24 h in constant-boiling HCl, a 50% yield of glucosamine was found on the amino acid analyser trace; a similar recovery could be assumed for any bound *N*-acetylhexosamine in the protein. We concluded that there could not be more than 0.05 mol of hexosamine per mol of lectin. A 1 mg sample of fraction I gave no reaction for neutral sugar with the phenol-H₂SO₄ reagent (Dubois *et al.*, 1956). With mannose as a standard at least 2.5 μ g of neutral sugar would have been detected. Therefore fraction I cannot contain more than 0.25% of neutral sugar. This would be equivalent to about 0.3 mol of mannose per mol of fraction I. Preliminary experiments on the other isolectins also indicated the absence of sugars.

It is obvious from a comparison of the amino acid compositions that Nagata & Burger (1972) have isolated a similar protein to our fraction I. We, however, could not find any carbohydrate in our preparation, whereas Nagata & Burger (1972) found 3–5 mol of glucose, 1–2 mol of xylose and 1–2 mol of hexosamine per mol of agglutinin. It is possible that Nagata & Burger (1972) had germ from a different strain of wheat, or different isolectins; but when we consider that the main sugar reported to be present is glucose, which is only rarely found in glycoproteins (Marshall & Neuberger, 1970), we think it possible that the carbohydrate found by Nagata & Burger (1972) was tightly bound by non-covalent forces. We therefore conclude that at least our wheat-germ agglutinin fraction I, like concanavalin A, but unlike most lectins, is not a glycoprotein.

Inhibition experiments and the specificity of the binding site

Our inhibition experiments (Table 3) confirm the initial observations of Burger & Goldberg (1967) that, of the sugars found in glycoproteins, only GlcNAc and (GlcNAc)₂ are inhibitors of wheat-germ agglutinin. It is obvious that, at least for glycosides, fraction I lacks anomeric specificity since both the α - and β -methyl pyranosides of GlcNAc are about three times as inhibitory as GlcNAc itself. The size of the aglycone does not seem to be of importance, since benzyl α -D-GlcNAc is at least as inhibitory as the methylglycoside. In view of the lack of specificity with regard to the aglycone, it is surprising that the glycoprotein linkage compound GlcNAc-Asn is not an inhibitor, particularly as Shier (1971) has shown that if the linkage compound is incorporated into a large synthetic peptide, it is a powerful inhibitor of wheat-germ agglutinin. It seems likely that the exclusion of the linkage compound from the binding site is due to its free amino and carboxyl groups rather than to the substitution of the glycosidic oxygen atom by a nitrogen atom, as GlcNAc-NAc

Table 3. *Inhibitory effect of various sugars on the agglutinating activity of wheat-germ agglutinin*

For abbreviations and conditions of assay see the Materials and Methods section. All compounds were pyranosides unless otherwise stated.

Compound	Concn. needed for 50% inhibition (mm)
Glc	(0% at 200 mm)
GalNAc	200
ManNAc	200
GlcN	(0% at 200 mm)
GlcNAc	30
Methyl α -D-GlcNAc	10
Methyl β -D-GlcNAc	10
Methyl 3-O-methyl- α -D-GlcNAc	200
Methyl 4-O-methyl- α -D-GlcNAc	10
Methyl 6-O-methyl- α -D-GlcNAc	10
Methyl α -D-GlcNAc-furanoside	10
Benzyl α -D-GlcNAc	5
Ethyl α -D-GlcNProp	10
Methyl α -D-MurNAc	(0% at 80 mm)
GlcNAc-Asn	(0% at 35 mm)
GlcNAc-NAc	10
(GlcNAc) ₂	0.05
(GlcNAc) ₃	0.01
(GlcNAc) ₄	0.01
(GlcNAc) ₅	0.005
GlcNAc-MurNAc	0.5
(GlcNAc-MurNAc) ₂	0.01

(which has this replacement without the addition of a charged group) is an inhibitor.

It is essential for binding that there should be an uncharged substituent, such as an amide group, in the 2-position (either an acetamido or a propionamido), as glucose and glucosamine are not inhibitors. The amide group should be in the *gluco* configuration, as ManNAc is a very weak inhibitor. Replacement of the 4- and 6-hydroxyl groups by methoxy groups has no appreciable effect on inhibitory activity, but substitution of the 3-hydroxyl group, either by replacement with a methoxy or a lactyl group (in methyl α -D-MurNAc) almost completely abolishes binding. Although substitution of the 4-hydroxyl group does not affect binding, the configuration of that hydroxyl group is important, as GalNAc is a weak inhibitor. It is noteworthy that methyl α -D-GlcNAc-furanoside is as inhibitory as the pyranoside, since binding of furanosides to concanavalin A (So & Goldstein, 1969) and to the lectin of *Lotus tetragonolobus* (Springer *et al.*, 1964) has been reported.

Since (GlcNAc)₂ is about 600 times, and (GlcNAc)₃

Table 4. *System of subsites proposed for the binding site of wheat-germ agglutinin*

It is suggested that derivatives of *N*-acetylglucosamine must have a free 3-hydroxyl group for binding to subsite C, but that this is not required for binding to subsite B. For further explanation see the text.

Subsite ...	A	B	C	D	Concn. of compound needed for 50% inhibition (mM)
			GlcNAc-Benz		5
			GlcNAc-Me		10
		GlcNAc-	GlcNAc		0.05
		GlcNAc-GlcNAc-	GlcNAc		0.01
		GlcNAc-MurNAc			0.5
		GlcNAc-MurNAc-GlcNAc-	MurNAc		0.01

about 3000 times more inhibitory than GlcNAc, the binding site must consist of two or three adjacent subsites. It should be noted that the bacterial cell-wall disaccharide (GlcNAc-MurNAc) is a weaker inhibitor than (GlcNAc)₂, but that the tetrasaccharides (GlcNAc)₄ and (GlcNAc-MurNAc)₂ are about equally inhibitory. This could be explained by the following system of adjacent subsites (Table 4) in a manner similar to that assumed for hen's-egg-white lysozyme (Blake *et al.*, 1967). Such an open arrangement of the binding site rather than a deep pocket in the lectin molecule might allow access of the lectin to internal GlcNAc residues.

Subsites A, B and C bind GlcNAc residues, and subsite D accommodates the aglycones of the glycosides. Monosaccharides are only bound in subsite C and they must be derivatives of GlcNAc with a free 3-hydroxyl group to be effectively bound. Disaccharides and trisaccharides are bound in subsites A, B and C.

For full binding of an oligosaccharide to take place, it is essential that in subsite C there is a derivative of GlcNAc with a free 3-hydroxyl group, but for binding to subsite B a free 3-hydroxyl group is not essential. Thus (GlcNAc)₂ is strongly bound in subsites B and C. GlcNAc-MurNAc cannot occupy subsites B and C, since in subsite C (where GlcNAc binds) substitution at the 3-hydroxyl position of the saccharide prevents binding. It is probably not bound in subsites C and D, since D is a very weak binding site, and the inhibitory activity of GlcNAc-MurNAc is 20 times greater than that of methyl β -D-GlcNAc. It is therefore most likely that GlcNAc-MurNAc binds in subsites A and B. The tetrasaccharide (GlcNAc-MurNAc)₂ will have a GlcNAc residue bound in subsite C and a MurNAc residue in subsite B where a free 3-hydroxyl group is not required for binding, and therefore it is as strongly bound as is (GlcNAc)₄.

It is interesting to compare the specificity of wheat-germ agglutinin with that of other lectins. Con-

canavalin A is specific for the α -anomer of glucose and free hydroxyl groups are required at C-3, C-4 and C-6. However, at C-2 either configuration and a number of substituents, including an acetamido group, are permitted (Poretz & Goldstein, 1970). In concanavalin A, there is probably an extended binding site, since disaccharides and trisaccharides are stronger inhibitors than monosaccharides (So & Goldstein, 1968). Soya-bean agglutinin is specific for *N*-acetylgalactosamine, both anomeric glycosides are bound, and an axial 4-hydroxyl group is essential for binding. Disaccharides are not bound more strongly than monosaccharides.

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