Chemical modification studies on a lectin from winged-bean [*Psophocarpus tetragonolobus* (L.) DC] tubers

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The effect of chemical modification on a D(+)-galactose-specific lectin isolated from winged-bean tubers was investigated to identify the type of amino acid involved in its haemagglutinating activity. Various anhydrides of dicarboxylic acids, such as acetic anhydride, succinic anhydride, maleic anhydride and citraconic anhydride, modified 57–68 % of the amino groups of the winged-bean tuber lectin. Treatment with *N*-acetylimidazole modified only 45 % of the total amino groups. Reductive methylation of free amino groups modified 57 % of the amino groups. Modification of the amino groups of the lectin by acetic anhydride and succinic anhydride did not lead to any significant change in the haemagglutinating activity (≥ 75 % active). However, citraconylation and maleylation of the lectin led to a significant decrease in the haemagglutinating activity (≤ 20 % active). Acetylation and succinylation (3-carboxypropionylation) of the lectin led to a decrease in the pI value of the native lectin from approx. 9.5 to approx. 4.5. Treatment of the lectin with *N*-bromosuccinimide led to the modification of two and four tryptophan residues per molecule in the absence and in the presence of 8 M-urea respectively. The immunological identity of all the modified lectin preparations showed no gross structural changes except the lectin modified with *N*-bromosuccinimide in the presence of urea at pH 4.0.

INTRODUCTION

Lectins are multivalent carbohydrate-binding proteins of non-immune origin (Goldstein *et al.*, 1980). They initiate a number of activities on cells, such as blood-group specificity, tumour-cell specific agglutination, mitogenic stimulation of lymphocytes etc. (Nowell, 1960; Novogrodosky & Katchalski, 1971; Nicolson & Blaustein, 1972; Goldstein & Hayes, 1978).

Specific amino acid residues are essential for maintaining the sugar-binding and haemagglutinating activities of lectins. Identification of these amino acid residues is a prerequisite in investigations of the structure–function relationships of lectins. Chemical modification studies of particular active-site amino acid side chains will alter their properties and bring about a change in the biological activity of a lectin if they contribute to it.

A basic lectin from the tubers of winged bean [*Psophocarpus tetragonolobus* (L.) DC] has previously been purified to homogeneity in this laboratory (Shet *et al.*, 1985). The purified lectin has an M_r of 29000 and approx. 45000 as determined by SDS/polyacrylamide-gel electrophoresis and gel filtration respectively, with a sedimentation coefficient of 3.0 S. It is a glycoprotein containing 6 % total sugar, composed of mannose, fucose and xylose in the proportions 7:2:1. The isoelectric point of the purified lectin was found to be approx. 9.5. After acid hydrolysis the basic lectin was found to have contained aspartic acid, glutamic acid, lysine, arginine and methionine in amounts corresponding to 25, 16, ten, four and one residues per subunit (M_r , 29000) of the protein. In order to develop this lectin as a sensitive carbohydrate reagent in cell-surface studies and in the isolation and characterization of complex carbohydrates, we have undertaken the chemical modification study.

The present paper describes the involvement of different amino acid residues in its biological activity and the requirement of tryptophan and tyrosine residues in its sugar-binding property.

EXPERIMENTAL

Materials

N-Acetylimidazole, citraconic anhydride, 2,4,6-trinitrobenzenesulphonic acid, *N*-bromosuccinimide, bovine serum albumin and M_r markers for gel filtration were obtained from Sigma Chemical Co. Succinic anhydride and cyclohexane-1,2-dione were the products of Aldrich Chemical Co. Acetic anhydride, *N*-ethylmaleimide and other chemicals were purchased from BDH Chemicals. Pharmalyte was obtained from Pharmacia. All other reagents used were of analytical grade of highest purity.

Preparation of winged bean tuber lectin

The lectin was prepared from the tubers of the winged bean [*Psophocarpus tetragonolobus* (L.) DC] as described by Shet *et al.* (1985).

Protein determination

Protein content was estimated by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as standard.

Isoelectric focusing

Gel electrofocusing of the native and modified [acetylated and succinylated (3-carboxypropionylated)] lectin preparations was carried out according to the instructions of the manufacturer (Pharmacia), with a

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Pharmalyte pH gradient 3–10, and their approximate pI values were determined.

Haemagglutinating activity

The haemagglutinating activity of native and modified lectin samples was assayed by the serial 2-fold dilution method (Liener & Hill, 1953) with 0.15 M-NaCl in microtitre plates with trypsinized rabbit erythrocytes. The end point of the assay was visually estimated after 1 h, and the lowest dilution showing haemagglutination was used to calculate the specific activity of the lectin. Relative haemagglutinating activity of the chemically modified lectin samples is expressed as a percentage of the specific activity of the native lectin.

Gel filtration

The M_r values of the native and modified lectin preparations were determined on a Bio-Gel P-100 column (1.5 cm $\times$ 98 cm) at a flow rate of 12.5 ml/h. M_r markers used were bovine serum albumin, ovalbumin, chymotrypsinogen A, myoglobin and ribonuclease.

Immunodiffusion

The native and modified lectin samples were tested for immunological similarity by double immunodiffusion in 1% agar in 0.15 M-NaCl/50 mM-sodium phosphate buffer, pH 7.2, containing 0.02% NaN_3 . Lactose was added to inhibit the interaction of galactose residues of serum glycoproteins or agar with the lectin.

Chemical modification of the lectin

Acetylation. Acetylation of the lectin was carried out by the method of Gunther *et al.* (1973). Acetyl-lectin was prepared by dissolving 5 mg of the lectin/ml in a saturated solution of sodium acetate, pH 8.5, in the cold (4 °C). To this was added 25 μ l of acetic anhydride/ml four times over a period of 1 h. The pH was maintained at 8.5. After this treatment, the sample was dialysed exhaustively against distilled water in the cold. The extent of amino group modification was measured by using 2,4,6-trinitrobenzenesulphonic acid as described by Habeeb (1967).

Succinylation. Succinylation of the lectin was also carried out by the method of Gunther *et al.* (1973). The lectin (5 mg/ml) dissolved in a saturated solution of sodium acetate, pH 8.5, was stirred in an ice bath for 1 h with simultaneous addition of solid succinic anhydride (1 mg/ml). After the addition, the solution was dialysed extensively and freeze-dried. The freeze-dried sample was once again subjected to a second succinylation as described above, and the extent of modification was determined by the 2,4,6-trinitrobenzenesulphonic acid method of Habeeb (1967).

Maleylation. Maleylation of the lectin was carried out as described by Butler & Hartley (1972). To 4 mg of the lectin in 1 ml of 0.2 M-sodium phosphate buffer, pH 9.0, was added 25 μ l of 1 M-maleic anhydride in predistilled dioxan. The contents were stirred continuously at room temperature for 6 h and then dialysed against deionized water in the cold.

Citraconylation. The amino groups of the lectin were reversibly blocked by citraconic anhydride by the method of Dixon & Perham (1968). Three 6 μ l portions

of citraconic anhydride were added with constant stirring to 6 mg of lectin in 2 ml of 0.1 M-Tris/HCl buffer, pH 8.0, at 25 °C over a period of 30 min. The pH was maintained between 8 and 9. After 1 h, the contents were dialysed exhaustively against 5 mM-Tris/HCl buffer, pH 8.6, in the cold. The reaction was reversed by leaving the citraconylated lectin overnight at 4 °C at pH 3.0.

Reductive methylation. The reductive methylation of the lectin was carried out as described by Means & Feeney (1968). To the lectin (5 mg) in 2 ml of 0.2 M-sodium borate buffer, pH 9.0, at 4 °C, was added 0.5 mg of NaBH_4 followed by six portions (5 μ l each) of 3.5% formaldehyde at 10 min intervals. The contents were extensively dialysed against deionized water.

Modification of arginine residues. The arginine residues of the lectin were modified by cyclohexane-1,2-dione by the method of Patthy & Smith (1975). The lectin (2 mg) in 0.2 M-sodium borate buffer, pH 9.0, was treated with 50 mM reagent under N_2 atmosphere at 37 °C for 4 h in the dark. The reaction was terminated by the addition of 1 ml of 5% (v/v) acetic acid. The mixture was dialysed against 1% (v/v) acetic acid and then with water in the cold.

Modification of tyrosine residues. The tyrosine residues were modified by using the procedure of Riordan *et al.* (1965). To the lectin (4 mg/ml) in 20 mM-Veronal buffer, pH 7.5, was added a 200-fold molar excess of *N*-acetylimidazole, and this was kept for 1 h at room temperature. The excess reagents were removed by dialysis against distilled water. The number of tyrosine residues modified was determined spectrophotometrically by the decrease in absorbance at 278 nm by using a molar absorption coefficient of $1160 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Riordan & Vallee, 1967b).

Modification of tryptophan residues. The modification of tryptophan residues was carried out with *N*-bromosuccinimide and was monitored spectrophotometrically (Spande & Witkop, 1967) both in the presence and in the absence of 8 M-urea in 0.1 M-sodium acetate buffer, pH 4.0, and 0.1 M-sodium citrate buffer, pH 6.0, respectively. Similar steps were followed at pH 6.0 by preincubation of the lectin with 0.5 M-galactose and 0.5 M-glucose. In both cases, after the reaction the samples were dialysed extensively to remove urea and sugar before measurements of activity etc. were made. The number of oxidized tryptophan residues per molecule of lectin was calculated by the decrease in the absorbance at 280 nm, with the use of the empirical factor 1.31, which corrects for the absorption at 280 nm of the oxidation products of tryptophan, oxindole (Patchornick *et al.*, 1958; Spande & Witkop, 1967).

Treatment with *N*-ethylmaleimide. This was carried out by the method of Riordan & Vallee (1967a). To the lectin solution (2 mg/ml) in 0.05 M-sodium phosphate buffer, pH 7.0, was added 5 mg of *N*-ethylmaleimide/ml and the mixture was incubated at room temperature for 12 h. It was then dialysed exhaustively against 0.15 M-NaCl/50 mM-sodium phosphate buffer, pH 7.2, in the cold.

Periodate oxidation. The lectin solution was previously dialysed against 50 mM-sodium acetate buffer,

Table 1. Effect of amino group modification of winged-bean tuber lectin

For experimental details see the text. Abbreviations: N.D., not distinct; n.d., not determined.

Chemical treatment	Type of residues modified	No. of residues modified	$\lambda_{\max}$ (nm)
None (native protein)	—	—	280
Acetic anhydride	Lys ϵ -amino, <i>N</i> -terminal α -amino, Tyr hydroxy	20	281
Succinic anhydride	Lys ϵ -amino, <i>N</i> -terminal α -amino, Tyr hydroxy	23	276
Maleic anhydride	Lys ϵ -amino, <i>N</i> -terminal α -amino, Tyr hydroxy	23	N.D.
Citraconic anhydride	Lys ϵ -amino, <i>N</i> -terminal α -amino	24	—
Decitraconylation	Lys ϵ -amino, <i>N</i> -terminal α -amino	1	—
Reductive methylation	Lys ϵ -amino, <i>N</i> -terminal α -amino, Tyr hydroxy	20	—
Cyclohexane-1,2-dione	Arg guanidine	n.d.	—
<i>N</i> -Acetylimidazole	Tyr hydroxy	6	281
	Lys ϵ -amino	16	—
<i>N</i> -Bromosuccinimide			
In the absence of urea	Trp indole ring	2	281
In the presence of urea	Trp indole ring	4	281

Table 2. Effect of chemical modification on haemagglutinating activity and immunological property of lectin

Haemagglutinating activity is expressed as a percentage of that given by native, unmodified lectin under similar conditions. Assay was carried out using trypsinized rabbit erythrocytes. Immunological identities of the modified samples were determined using antibodies raised against purified winged-bean tuber lectin.

Treatment of lectin	Haemagglutinating activity (%)	Immunological property
Acetylation	89	Unchanged
Succinylation	78	Unchanged
Maleylation	20	Unchanged
Citraconylation	20	Unchanged
Decitraconylation	95	Unchanged
Reductive methylation	45	Unchanged
Acetylation with <i>N</i> -acetylimidazole	30	Unchanged
Deacetylation	95	Unchanged
Cyclohexane-1,2-dione	100	Unchanged
<i>N</i> -Bromosuccinimide		
In the absence of urea	10	Unchanged
In the presence of urea	0	Changed
<i>N</i> -Ethylmaleimide	95	Unchanged

pH 3.95, at 4 °C. To 5 ml of the chilled lectin solution was added 1 ml of 0.1 M-sodium metaperiodate in 50 mM-sodium acetate buffer, pH 3.95, in the cold, and the reaction was carried out in the dark for 24 h in the cold. After incubation, the reaction was stopped by

adding 0.1 ml of ethylene glycol, and the product was dialysed extensively against 0.15 M-NaCl/50 mM-sodium phosphate buffer, pH 7.2, in the cold.

RESULTS

The effect of chemical modification on haemagglutinating activity, the extent of modification of the different amino acid residues and immunological properties of the native and modified lectin preparations are summarized in Tables 1 and 2. From these data it is evident that during acetylation and succinylation of the lectin 20 and 23 amino groups respectively were found to be modified out of the total 35, including the terminal α -amino group of the lectin subunit of M_r 29000. The native lectin was found to contain 35 amino groups, four arginine and four tryptophan residues per molecule of lectin. Various anhydrides of dicarboxylic acids, such as acetic anhydride, succinic anhydride, maleic anhydride and citraconic anhydride, modified 57–68 % of the amino groups, but *N*-acetylimidazole modified only 45 % of the total amino groups. The modification of the amino groups of the lectin by acetic anhydride and succinic anhydride did not lead to any significant change in the haemagglutinating activity (≥ 75 % active). However, citraconylation and maleylation of the lectin led to a significant decrease in the haemagglutinating activity (≤ 20 % active). Reductive methylation of free amino groups modified 57 % of them and the modified lectin retained approx. 45 % of the lectin activity. Decitraconylation at pH 3.0 restored approx. 90 % of the original lectin activity.

The elution profiles of acetylated, succinylated, maleylated and citraconylated lectins on gel filtration on Bio-Gel P-100 are shown in Fig. 1. It shows that there is considerable change in the elution volume (V_e) of the

Fig. 1. Gel-filtration pattern of native and modified lectin samples on Bio-Gel P-100

The column (98 cm $\times$ 1.5 cm) was equilibrated with 0.5 M-NaCl/50 mM-phosphate buffer, pH 6.0. The flow rate was 12.5 ml/h and the fraction volume was 3 ml. Markers: a, bovine serum albumin (M_r 68000), b, ovalbumin (M_r 45000); c, pepsin (M_r 35000); d, α -chymotrypsinogen (M_r 25000), e, ribonuclease (M_r 14000). $\circ$, Native lectin; $\bullet$, succinylated lectin; $\blacktriangle$, acetylated lectin; $\triangle$, citraconylated lectin; $\bullet$, maleylated lectin.

modified lectin samples as compared with the native lectin. The ratio of V_e/V_0 has decreased from 1.50 to 1.385 for the modified lectin preparations. These modified lectins are highly negatively charged and have an M_r of approx. 58000. The absorption spectra of the modified lectin samples did not change significantly except for the succinylated lectin (results not shown).

Figs. 2(a), 2(b) and 2(c) show the isoelectric focusing pattern of native, acetylated and succinylated lectin samples respectively. The native lectin is focused in a sharp band with an approximate pI value of 9.5. However, both acetylated and succinylated lectins were focused as diffused bands with an approximate pI value of 4.5. From the above observation it is clear that both modified lectins are acidic lectins, in contrast with the native lectin, which is strongly basic.

Fig. 2. Isoelectric focusing pattern of native and modified lectin samples

This was carried out on polyacrylamide-gel tubes with Pharmalyte (pH 3.0–10.0 gradient). The gels were stained with Coomassie Brilliant Blue R-250 for proteins. (a) Native lectin; (b) acetylated lectin; (c) succinylated lectin.

The guanidino groups of arginine are specifically modified by cyclohexane-1,2-dione, and the resulting complex can be stabilized by borate ions. The haemagglutinating activity remains unchanged (Table 2) even after modification of the arginine residues.

Modification of the tryptophan residues of the winged-bean tuber lectin by *N*-bromosuccinimide in the presence and in the absence of urea leads to the modification of four and two residues respectively, as presented in Table 3. The u.v.-absorption spectrum of the native lectin in 0.1 M-citrate buffer, pH 6.0, showed a

Table 3. Oxidation of winged-bean tuber lectin with *N*-bromosuccinimide

The lectin (1 mg/ml) in both 0.1 M-citrate buffer, pH 6.0, and 0.1 M-acetate buffer, pH 4.0, containing 8 M-urea was treated with the stepwise addition of 0.01 M-*N*-bromosuccinimide. The haemagglutinating activities and immunological properties of the individual fractions were determined after dialysis against 0.15 M-NaCl/50 mM-sodium phosphate buffer, pH 7.2, at 4 °C. The number of tryptophan residues modified was determined by the decrease in absorbance at 280 nm by using the factor 1.31. Abbreviation: NBS, *N*-bromosuccinimide.

Molar ratio (mol of NBS/mol of lectin)	Trp residues oxidized (mol/mol of lectin)		Haemagglutinating activity (%)		Immunological property	
	In the absence of urea	In the presence of urea	In the absence of urea	In the presence of urea	In the absence of urea	In the presence of urea
Control (0)	—	—	100	100	Unchanged	Unchanged
2.5	0.52	0.78	60	20	Unchanged	Changed
5.0	0.64	1.59	40	10	Unchanged	Changed
10.0	0.85	2.26	20	2	Unchanged	Changed
15.0	1.56	3.68	5	0	Unchanged	Changed
20.0	2.20	4.02	0	0	Unchanged	Changed

Fig. 3. Absorption spectra of native and *N*-bromosuccinimide-oxidized winged-bean tuber lectin

Oxidation of the lectin (1 mg/ml) in 0.1 M-citrate buffer, pH 6.0, was carried out with stepwise addition of 10 mM-*N*-bromosuccinimide. Spectrum a, native lectin; spectra b, c, d and e, lectin oxidized with 5-fold, 10-fold, 15-fold and 20-fold molar excess of *N*-bromosuccinimide respectively.

maximum at 280 nm and a minimum at 250 nm, with a remarkable shoulder in the range of 288–292 nm, as shown in Fig. 3. Increasing amounts of *N*-bromosuccinimide added to the lectin led to a decrease in the absorption at 280 nm with a simultaneous increase of absorption at 250 nm, showing that tryptophan residues become oxidized to their oxindole derivatives.

The haemagglutinating activity of the modified lectin samples decrease with an increase in the oxidation of tryptophan residues compared with the native lectin. The addition of a 20-fold molar excess of *N*-bromosuccinimide to the lectin at pH 6.0 leads to nearly complete oxidation of two tryptophan residues with a concomitant loss of 90–95% haemagglutinating activity. Similar results were obtained when the lectin in 0.1 M-acetate buffer containing 8 M-urea at pH 4.0 was treated with *N*-bromosuccinimide, leading to a complete loss of lectin activity and almost all the tryptophan residues being oxidized.

The protective effect of sugars against *N*-bromosuccinimide modification of the lectin was determined in 0.1 M-citrate buffer, pH 6.0, in the presence of 0.5 M-D-galactose and 0.5 M-D-glucose, and the results are presented in Table 4. In the presence of D-galactose the lectin retained almost its full lectin activity, and only one tryptophan residue was found to be modified or oxidized. On the other hand, under similar conditions in the presence of D-glucose there was complete loss of lectin activity, although the total number of tryptophan residues modified was similar to that in the absence of the sugar. From this observation it is clear that D-galactose behaves as a protective specific inhibitor of this lectin and that D-glucose is a non-specific sugar.

Modification of the winged-bean tuber lectin with *N*-

Table 4. Effect of sugars on oxidation of winged-bean tuber lectin with *N*-bromosuccinimide

The lectin (1 mg/ml) in 0.1 M-citrate buffer, pH 6.0, was incubated with 0.5 M-D-galactose and 0.5 M-D-glucose for 1 h. The stepwise addition of *N*-bromosuccinimide was carried out as described in the text. The number of tryptophan residues oxidized and the haemagglutinating activity were determined as described in the text. Abbreviations: NBS, *N*-bromosuccinimide.

Molar ratio (mol of NBS/mol of lectin)	Trp residues oxidized		Haemagglutinating activity (%)	
	0.5 M- Gal	0.5 M- Glc	0.5 M- Gal	0.5 M- Glc
Control (0)	—	—	100	100
2.5	0	0.61	100	65
5.0	0.12	0.68	100	55
10.0	0.18	0.94	95	15
15.0	0.34	1.70	95	10
20.0	0.69	2.16	92	0

acetylimidazole resulted in the acetylation of tyrosine residues of the lectin, as presented in Table 1. The 200-fold molar excess of the reagent modified six tyrosine and 16 amino groups of the lectin. However, the modified lectin sample exhibited a 30% decrease in haemagglutinating activity.

Treatment of the lectin with *N*-ethylmaleimide or oxidation of the lectin by sodium metaperiodate did not lead to any change in haemagglutinating activity. Thus it is evident that the free thiol groups and the carbohydrate moiety of the lectin may not be involved in the activity of the lectin molecule.

Samples of the modified lectin preparations were tested separately for cross-reactivity against anti-(tuber lectin) antibody by immunodiffusion. All the lectin solutions showed a positive cross-reactivity with the antiserum to the whole lectin. However, the immunological cross-reactivity of *N*-bromosuccinimide-treated lectin samples at pH 6.0 remained unchanged, whereas at pH 4.0 in the presence of 8 M-urea significant changes were observed.

DISCUSSION

Chemical modification of the winged-bean tuber lectin with various amino acid-modifying agents shows that 45–70% of the amino groups can be modified. However, there is no loss of haemagglutinating activity as such. It is evident that only 57–68% of the total amino groups in the lectin are freely reactive with acid anhydrides, suggesting that the remainder are internal or may be hindered in some way from reacting; under the conditions used, all the available amino groups should be modified. Acetic anhydride is not specific for lysine residues only since it can also modify tyrosine residues, whereas succinic anhydride and citraconic anhydride preferentially react with lysine residues (Klotz, 1967; Dixon & Perham, 1968) and they were used to modify these residues of the lectin. Maleylation and citraconylation of the lectin resulted in almost complete loss of haemagglutinating activity. Similar observations of impairment

of the haemagglutinating activity have been reported in the case of pea (Trowbridge, 1973), *Ricinus* (Khan & Surolia, 1982), jack-fruit (Appukuttan & Basu, 1985) and peanut (Nonnenmacher & Brossmer, 1981) lectins. Acylation of the amino groups by dicarboxylic acid anhydrides results in the replacement of a positive charge by a negative charge, leading to drastic changes in the electrostatic interactions and frequently to dissociation of oligomeric proteins (Klapper & Klotz, 1972; Gunther *et al.*, 1973). The change in the net charge of the modified lectin has been confirmed by isoelectric focusing. Similar variation in the pI value of modified lectins has been observed for wheat-germ agglutinin by Monsigny *et al.* (1979) and for other modified proteins (Riordan & Vallee, 1972; Atassi & Habeeb, 1972).

The M_r (≥ 45000) of this lectin obtained by gel filtration is much lower than that obtained by other methods for the dimeric form. This discrepancy may be due to interaction between the gel matrices and the lectin, which is a glycoprotein, since glycoproteins are known to show anomalous behaviour with gels (Nicolson *et al.*, 1974; Olsnes *et al.*, 1974; Young & Jackson, 1984). However, the M_r obtained by gel filtration for the modified lectin preparations is equal to the M_r of the dimeric form of the native lectin. During chemical modification there is every possibility for change in the conformation or in the state of aggregation. Retardation of the native lectin through the gel matrix may be abolished by chemical modification by introducing charges on it (Means & Feeney, 1971).

N-Bromosuccinimide has been shown to modify both tryptophan and tyrosine side chains in proteins (Viswanatha & Lawson, 1961). However, the modified tyrosine residues have not been determined in the present study. The u.v.-absorption spectrum of this lectin in the presence of *N*-bromosuccinimide showed a decrease in the absorbance at 280 nm, indicating that the tryptophan residues might be converted into their oxindole derivatives (Spande & Witkop, 1967). At pH 6.0 in the absence of urea only two tryptophan residues were oxidized, and in the presence of 8 M-urea the remaining two residues were also modified. Unfolding of the lectin molecule in the presence of 8 M-urea rendered all the tryptophan residues of the lectin exposed, making them more susceptible to oxidation with *N*-bromosuccinimide. From this it is clear that only two tryptophan residues are exposed at the surface of the molecule, and the remaining two may be buried inside the native molecule. The exact role of urea is that it reacts with *N*-bromosuccinimide to give *N*-bromo-urea, which is more specific for the oxidation of tryptophan residues than is *N*-bromosuccinimide alone (Funatsu *et al.*, 1964; Khan *et al.*, 1981). Modification of the lectin with *N*-bromosuccinimide in the presence of D-galactose leads to protection of the inactivation of the lectin, which further confirms the carbohydrate specificity of this lectin.

The immunological study of the modified lectins has suggested that all the antigenic determinant of the native lectin was apparently retained in the chemically modified lectin, and the modified amino acid residues are not essential for the antigenic action. Further, the immunological identity indicated that no gross conformational change occurred in the modified lectins, except the *N*-bromosuccinimide-treated lectin at pH 4.0. This supports the conclusion that the loss of lectin activity during modification is due to damage of specific amino acid

residues involved in the sugar-binding site of the lectin (Ashford *et al.*, 1981; Khan & Surolia, 1982).

The present study with winged-bean tuber lectin has revealed that tryptophan and tyrosine residues have been found to be involved in the activity of the lectin. The involvement of other amino acid residues and groups in maintaining the conformation of the lectin could be further investigated by c.d. analysis.

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