Chemical modification studies on *Abrus* agglutinin

Involvement of tryptophan residues in sugar binding

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The galactose-binding lectin from the seeds of the jequirity plant (*Abrus precatorius*) was subjected to various chemical modifications in order to detect the amino acid residues involved in its binding activity. Modification of lysine, tyrosine, arginine, histidine, glutamic acid and aspartic acid residues did not affect the carbohydrate-binding activity of the agglutinin. However, modification of tryptophan residues carried out in native and denaturing conditions with *N*-bromosuccinimide and 2-hydroxy-5-nitrobenzyl bromide led to a complete loss of its carbohydrate-binding activity. Under denaturing conditions 30 tryptophan residues/molecule were modified by both reagents, whereas only 16 and 18 residues/molecule were available for modification by *N*-bromosuccinimide and 2-hydroxy-5-nitrobenzyl bromide respectively under native conditions. The relative loss in haemagglutinating activity after the modification of tryptophan residues indicates that two residues/molecule are required for the carbohydrate-binding activity of the agglutinin. A partial protection was observed in the presence of saturating concentrations of lactose (0.15M). The decrease in fluorescence intensity of *Abrus* agglutinin on modification of tryptophan residues is linear in the absence of lactose and shows a biphasic pattern in the presence of lactose, indicating that tryptophan residues go from a similar to a different molecular environment on saccharide binding. The secondary structure of the protein remains practically unchanged upon modification of tryptophan residues, as indicated by c.d. and immunodiffusion studies, confirming that the loss in activity is due to modification only.

Lectins are multivalent carbohydrate-binding haemagglutinating proteins. The binding of lectins to cell-surface carbohydrates initiates several interesting activities on cells. These include blood-group-specific haemagglutinating activity, mitogenic activity on lymphocytes, tumour-cell-specific agglutinating activity and insulin-like activity on adipocytes (Novogrodosky & Katchalski, 1971; Moscona, 1971; Cuatrecasas & Tell, 1973; Lis & Sharon, 1973; Goldstein & Hayes, 1978). *Abrus precatorius* lectin isolated from seeds of the jequirity plant is a non-toxic but mitogenic and haemagglutinating protein (Olsnes *et al.*, 1974; Wei *et al.*, 1975). *Abrus* agglutinin is a non-specific haemagglutinin that binds specifically to α - and β -D-galactopyranosides. It has M_r 130000 and consists of two pairs of non-identical subunits, A and B, bridged by disulphide bonds (Kaufman &

McPherson, 1975; Olsnes, 1978). It has two binding sites located in the B subunits (Olsnes *et al.*, 1974).

The *Abrus* agglutinin has the property of binding galactopyranosyl residues, and this is responsible for most of its activity. Studies directed towards understanding the relationship between its structure and function would be facilitated by the study of the chemical nature of its combining site. Chemical modification of proteins serve as a tool to identify the amino acid residues involved in their binding activities. A detailed study of the chemical modifications of various amino acid side chains of *Abrus* agglutinin was therefore carried out.

The work presented below is a summary of the effects of various modifying agents on the structural, biological and immunological properties of

Abrus agglutinin. These studies indicate that two tryptophan residues/molecule are required for the carbohydrate-binding activity of this agglutinin.

Materials and methods

Agglutinin

Agglutinin was prepared from seeds of the jequirity plant. A mixture of toxin and agglutinin was obtained by affinity chromatography on cross-linked arabinogalactan as described by Mazumdar & Surolia (1978, 1979). The toxin was separated from agglutinin by gel filtration on a Sephadex G-100 column. The agglutinin was found to be pure by polyacrylamide-disc-gel electrophoresis in the presence and in the absence of sodium dodecyl sulphate.

Antiserum

Rabbits were immunized by injecting a 1:1 (v/v) mixture of Freund's complete adjuvant with the protein solution (0.5 mg/ml) (Olsnes *et al.*, 1975).

Protein determination

Bradford's (1976) method of dye binding and trichloroacetic acid precipitation (Layne, 1975) were used for determining the protein.

Chemicals

All the reagents used were of analytical grade. Succinic anhydride, citraconic anhydride, *N*-acetylimidazole, cyclohexane-1,2-dione and 2-hydroxy-5-nitrobenzyl bromide were obtained from Pierce Chemical Co. Diethyl pyrocarbonate (ethoxyformic anhydride) and *N*-bromo-succinimide were obtained from Sigma Chemical Co. Sephadex G-100 and G-25 were from Pharmacia, and *p*-nitrophenylglyoxal was synthesized by the method of Steinbach & Becker (1954).

Immunodiffusion

Immunodiffusion plates were made from 1% agarose in 20 mM-sodium phosphate buffer, pH 7.4, containing 0.15 M-NaCl, 0.2% NaN₃ and 1% (w/v) lactose (Olsnes *et al.*, 1975).

Measurement of radioactivity

Radioactivity was measured in an LKB (Wallac) 1217 Rackbeta liquid-scintillation counter, with the aid of scintillation fluid made up by dissolving 800 mg of diphenyloxazole and 20 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 200 ml of toluene and 100 ml of Triton X-100.

Haemagglutination test

Haemagglutination tests were performed by the serial dilution technique in microtitre plates with 4% (v/v) rabbit erythrocytes in 0.15 M-

NaCl/20 mM-sodium phosphate buffer, pH 7.3. Titres for native as well as modified lectin were scored after incubation at 25°C for 2 h. Minimal titre concentration required for the modified protein was compared with that for the native protein, and the percentage loss of activity was calculated.

Binding to Sepharose 6B

Native or modified protein (1 mg/ml) was loaded on a Sepharose 6B column (10 cm × 1 cm) equilibrated with 0.1 M-NaCl/20 mM-sodium phosphate buffer, pH 7.4 and their binding to Sepharose was measured as described (Surolia *et al.*, 1978). Relative binding of the modified protein to the column was calculated by comparison with the binding of the native protein.

Gel filtration on Sephadex G-200

Gel filtration was carried out on a Sephadex G-200 column (35 cm × 1.5 cm) at a flow rate of 10 ml/h. Fractions of volume 1 ml were collected, and the amount of protein in these fractions was determined. The column was calibrated with immunoglobulin G (*M_r* 180 000), peanut agglutinin (*M_r* 112 000), *Abrus* toxin (*M_r* 65 000) and *Abrus* agglutinin (*M_r* 130 000).

Removal of the modifying reagents

The modified protein samples were freed of excess reagent either by gel filtration on a Sephadex G-25 column equilibrated with 20 mM-sodium phosphate buffer, pH 7.4, containing 0.1 M-NaCl and/or by dialysis against 20 mM-sodium phosphate buffer, pH 7.4, containing 0.1 M-NaCl, unless otherwise specified.

Modification of amino groups of lysine residues

The amino groups were modified by reaction with succinic anhydride and citraconic anhydride by the methods described by Habeeb *et al.* (1958) and Dixon & Perham (1968) respectively. In each case a 5 mg/ml protein solution in saturated NaHCO₃ solution, pH 8.0, was treated with 400-fold molar excess of the reagent at 4°C for 1 h. The decitraconylation was carried out by leaving the citraconylated protein overnight at 4°C at pH 3.0. The percentage modification in each of these reactions was determined by determination of unmodified amino groups with 2,4,6-trinitrobenzenesulphonic acid as described by Habeeb (1967).

Modification of hydroxy groups of tyrosine residues

The amino groups of the protein were reversibly blocked by citraconic anhydride as described by Khan & Surolia (1982), and then 5 mg of the protein was treated with a 300-fold molar excess of *N*-acetylimidazole in 200 mM-sodium phosphate buffer, pH 7.2, at 5°C for 1 h (Riordan *et al.*, 1965).

The protein was de-*O*-acetylated with hydroxylamine, and then decitraconylation was carried out. The number of tyrosine groups modified was determined by the method of Riordan *et al.* (1965).

Modification of histidine residues, arginine residues and carboxy groups

Modification of histidine residues was carried out by the method of Melchior & Fahrney (1970). In a typical experiment diethyl pyrocarbonate was added in 3-fold molar excess over the histidine content to a 1 mg/ml protein solution in 20 mM-sodium phosphate buffer, pH 7.2, at 25°C. The extent of modification was determined as described by Anderson & Ebner (1979).

Arginine residues of the protein were modified by reaction with *p*-nitrophenylglyoxal by the method of Bryan *et al.* (1981). To a 2 mg/ml protein solution in 0.1 M-sodium pyrophosphate buffer, pH 9.0, was added 2.5 ml of 10% (v/v) *p*-nitrophenylglyoxal, and the mixture was incubated at 30°C for 30 min. The extent of modification was determined as described by Bryan *et al.* (1981).

Carboxy groups were modified as described by Hassing & Goldstein (1971). The reaction mixture contained 7.5 mg of protein in 1.5 ml of water along with 700 mg of urea and 200 mg of glycine ethyl ester containing 1.0 μ Ci of [¹⁴C]glycine ethyl ester. The mixture was freed from excess reagent by gel filtration as described above.

Modification of tryptophan residues

Modification of tryptophan residues was done with *N*-bromosuccinimide (Spande & Witkop, 1967) and 2-hydroxy-5-nitrobenzyl bromide (Barman & Koshland, 1967). An aqueous solution of *N*-bromosuccinimide (10 mg) was added in 10 μ l portions to a solution of protein (1 mg/ml) in 0.1 M-sodium acetate buffer, pH 4, at 25°C. The number of tryptophan residues oxidized was calculated as described by Spande & Witkop (1967). After the removal of excess of reagent the protein was tested for its activity.

Modification with 2-hydroxy-5-nitrobenzyl bromide was done by the procedure of Barman & Koshland (1967). In a typical experiment, to a 2 mg/ml protein solution in 0.1 M-sodium acetate buffer, pH 3.5, was added 5 mg of 2-hydroxy-5-nitrobenzyl bromide in 0.1 ml of dry acetone at room temperature. The modified protein was removed from the excess of reagent on a Sephadex G-25 column equilibrated with 0.1 M-sodium acetate buffer, pH 3.5. The number of tryptophan residues modified was determined as described by Barman & Koshland (1967). For modification of all the tryptophan residues in the protein the reaction mentioned above was carried out in the presence of 8 M-urea.

Protection by lactose

Restricted 10 μ l portions of *N*-bromosuccinimide were added to protein samples of 1 mg/ml concentration in the presence and in the absence of 0.15 M-lactose. The haemagglutination test was performed to estimate the loss in activity in both the cases after the samples had been desalted and dialysed against 0.1 M-NaCl/20 mM-sodium phosphate buffer, pH 7.4.

Fluorescence emission measurements

Fluorescence measurements were carried out on a Perkin-Elmer MPF 44A spectrofluorimeter at 25°C. The samples were excited at 280 nm or 300 nm, and the emission spectra were recorded above 300 nm.

Circular dichroism

C.d. spectra of native protein and modified protein were recorded on a Jasco J20 spectropolarimeter. The protein concentration was 1 mg/ml in the 250–350 nm scanning range and 0.1 mg/ml in the 250–200 nm scanning range in 5 mm-path-length cells.

Results

Modification of amino groups in lysine residues

Succinylation resulted in 71% modification and citraconylation resulted in 90% modification of the amino groups. Decitraconylation led to the release of 98% of the modified amino groups. There was no change in haemagglutination or Sepharose-binding activity of the modified proteins when compared with that of native protein, indicating that amino groups of lysine residues are not involved in the saccharide-binding activity of this protein. These results are summarized in Table 1.

Modification of hydroxy groups of tyrosine residues

When all the amino groups of lysine residues were blocked by citraconylation, treatment with *N*-acetylimidazole resulted in the modification of ten tyrosine residues in the undenatured protein molecule. Reaction in urea resulted in the modification of 35 tyrosine residues/molecule (Table 1). Haemagglutination and binding to Sepharose 6B remained unaltered when compared with the native protein even after the modification of almost all the tyrosine residues in the native protein (Table 1).

Modification of histidine residues, arginine residues and carboxy groups

Eleven histidine residues/molecule were modified in the native protein on treatment with diethyl pyrocarbonate. Similarly 60 arginine residues/mo-

Table 1. *Effects of modifying agents on Abrus agglutinin*

Various reagents used to modify groups are tabulated. The reaction procedures were followed as described in the Materials and methods section. Modification of lysine residues was measured by the trinitrobenzenesulphonic acid method. Results of immunochemical and haemagglutination activities are with respect to the native protein. Extent of binding to Sepharose 6B is expressed as percentage retention by the column when compared with that of the native agglutinin..

Reagent	Residue modified	Percentage modification or no. of residues/molecule modified	Immunochemical properties	Haemagglutination activity	Relative binding to Sepharose 6B (%)
Succinic anhydride	Lysine	71%	Unchanged	Unchanged	94
Citraconic anhydride	Lysine	90%	Unchanged	Unchanged	96
<i>N</i> -Acetylimidazole	Tyrosine				
With urea		35	Unchanged	Unchanged	97
Without urea		10	Unchanged	Unchanged	
<i>p</i> -Nitrophenylglyoxal	Arginine	60	Unchanged	Unchanged	97
Diethyl pyrocarbonate	Histidine	11	Unchanged	Unchanged	95
[¹⁴ C]Glycine ethyl ester	Aspartic acid and glutamic acid	100%	Unchanged	Unchanged	92

Table 2. *Effects of modification of tryptophan residues on Abrus agglutinin*

Tryptophan residues of the protein were modified by treatment with *N*-bromosuccinimide and 2-hydroxy-5-nitrobenzyl bromide, and the number of groups/molecule modified was determined as described in the Materials and methods section. The effect of modification on various properties of the agglutinin was compared with the properties of the native protein.

Reagent	Residue modified	No. of residues/molecule modified	Immunochemical properties	Haemagglutination activity	Relative binding to Sepharose 6B
<i>N</i> -Bromosuccinimide	Tryptophan				
With urea		30	Unchanged	No agglutination	No binding
Without urea		16	Unchanged	No agglutination	No binding
2-Hydroxy-5-nitrobenzyl bromide	Tryptophan				
With urea		30	Unchanged	No agglutination	No binding
Without urea		18	Unchanged	No agglutination	No binding

lecule were modified with *p*-nitrophenylglyoxal in undenatured agglutinin. Radioactivity measurements on the incorporation of [¹⁴C]glycine ethyl reveal that all of the carboxy groups were modified. All the above three modifications did not affect the haemagglutination or binding to Sepharose, indicating that these residues are not involved in the saccharide-binding activity of the *Abrus* lectin (Table 1).

Modification of tryptophan residues

Native protein showed that 16 tryptophan residues/molecule were available for oxidation by *N*-bromosuccinimide. *N*-Bromosuccinimide oxidation in the presence of 8M-urea revealed that 30 tryptophan residues/molecule had been oxidized (Table 2).

As revealed by spectrophotometric monitoring, controlled addition of 10mM-*N*-bromosuccinimide

in restricted 10 µl portions resulted in the sequential modification of different tryptophan residues. The samples withdrawn with a definite number of residues/molecule modified and desalted by passage through a Sephadex G-25 column were tested for activity by assay of haemagglutination and binding to Sepharose.

Gel filtration on Sephadex G-200 showed no change in the elution profile of modified protein samples as compared with that of native protein.

Modification of a single tryptophan residue/molecule led to a loss in activity up to 45% in the haemagglutination assay. Modification of a second residue/molecule incurred 75% loss in activity. The data are summarized in Fig. 1. The results shown in Fig. 1 indicate that modification of a few more surface tryptophan residues/molecule of the native protein resulted in a complete loss of activity.

The number of tryptophan residues/molecule involved in carbohydrate binding was determined by a plot of percentage activity remaining versus number of tryptophan residues/molecule oxidized

Fig. 1. Effect of the modification of tryptophan residues on the haemagglutinating activity of *Abrus agglutinin*. Percentage activity remaining after modification when compared with the activity of native protein is plotted against the number of tryptophan residues/molecule oxidized. Modification of two residues/molecule incurred 75% loss in the activity. Extrapolation of the plot to the ordinate shows that two residues/molecule are required for binding.

(Fig. 1). Extrapolation of this graph on the ordinate indicates that two residues/molecule are involved in binding.

There was a marked decrease in the fluorescence emission as the tryptophan residues/molecule are sequentially modified. On excitation at 300nm there was 65% and 80% loss in fluorescence emission when 9 and 16 residues/molecule were modified respectively (Table 3).

The fluorescence quenching was linear when plotted against the number of tryptophan residues oxidized in the absence of lactose (Fig. 2). However, in the presence of lactose, oxidation by *N*-bromosuccinimide as well as loss in fluorescence was biphasic.

C.d. spectra of the protein was characterized by a negative absorption maximum at 221 nm in the far-u.v. region (200–250nm) and two negative absorption maxima at 286 and 293nm in the chromophoric region (250–350nm). These plots (Fig. 3a) show no change in the secondary-structural region (200–250nm). Nevertheless, there was a decrease in molar ellipticity in the chromophoric region as the tryptophan residues were oxidized (Fig. 3b).

Protection by lactose

When the same amount of *N*-bromosuccinimide was added to protein samples (1mg/ml) in the presence and in the absence of 0.15M-lactose, there was a distinct change in the loss in haemagglutination activity. For example, loss in activity was almost 75% with the addition of 0.2 μ mol of *N*-bromosuccinimide in the absence of lactose, but in the presence of lactose for the same concentration of the reagent the loss was found to be 30%. These findings summarized in Table 3 suggest a partial protection by lactose against modification.

Table 3. Effect of the presence of lactose during the modification of tryptophan residues of *Abrus agglutinin* on fluorescence and absorbance and the requirement of *N*-bromosuccinimide

Changes in *N*-bromosuccinimide concentration used and fluorescence and the absorbance observed in the presence and in the absence of lactose during the sequential modification of tryptophan residues are tabulated. Percentage decrease in absorbance or fluorescence is a relative decrease when compared with the property of the native protein.

No. of residues/molecule modified	Amount of <i>N</i> -bromosuccinimide added (μ mol)		Decrease in absorbance (%)		Decrease in fluorescence (%)	
	Lactose present	Lactose absent	Lactose present	Lactose absent	Lactose present	Lactose absent
2	0.30	0.20	16	14	12	12
5	0.60	0.50	33	35	28	38
7	0.80	—	48	—	39	—
9	1.30	—	65	—	65	—
10	—	0.80	—	65	—	68
16	2.30	1.20	100	100	82	82

Fig. 2. Effect of the modification of tryptophan residues on the fluorescence of *Abrus agglutinin*

Decrease in the relative fluorescence pattern of the tryptophan emission with the modification of tryptophan residues is shown. The inset shows a linear decrease in the absence of lactose in the fluorescence intensity with the increase in the number of tryptophan residues/molecule oxidized. The emission maximum is observed at 334 nm for excitation at 300 nm. Curve *a*, native protein;

Ouchterlony double diffusion

The perfect fusion of precipitin lines in immunodiffusion suggests a complete cross-reactivity between the native and the modified protein samples, confirming that there was no change in the secondary structure of the modified proteins (Fig. 5).

Discussion

Chemical modification of the lysine, tyrosine, histidine, arginine, aspartic acid and glutamic acid residues of *Abrus agglutinin* does not affect its haemagglutinating and carbohydrate-binding activities. This rules out the possibility of these residues being involved in these activities of the agglutinin.

Modification of tryptophan residue by *N*-bromosuccinimide and 2-hydroxy-5-nitrobenzyl bromide led to a complete loss in the haemagglutinating and saccharide-binding activities of the agglutinin. *N*-Bromosuccinimide can attack both tryptophan and tyrosine residues in the pH range 3.5–4.0. In the present study, under the experimental conditions described, this reagent modifies only tryptophan residues. This is borne out by the absorption measurements, which clearly reveal that tyrosine residues were not modified because the isosbestic point at 263 nm remains un-

curve *b*, two residues/molecule modified; curve *c*, five residues/molecule modified; curve *d*, seven residues/molecule modified; curve *e*, ten residues/molecule modified; curve *f*, 16 residues/molecule modified.

Fig. 3. Effect of the modification of tryptophan residues on the circular dichroism of *Abrus agglutinin*. C.d. spectra of the native (—) and the modified (-----) agglutinin were plotted. (a) Far-u.v. region (200–250 nm). (b) Near-u.v. region (250–315 nm): curve *a*, native protein; curve *b*, three residues/molecule modified; curve *c*, six residues/molecule modified; curve *d*, 14 residues/molecule modified.

Fig. 4. Effect of the modification of tryptophan residues on the absorbance of *Abrus* agglutinin

The decrease in absorbance with the modification of tryptophan residue observed in the native protein at 280 nm is plotted: curve *a*, native protein; curve *b*, two residues/molecule modified; curve *c*, five residues/molecule modified; curve *d*, ten residues/molecule modified; curve *e*, 16 residues/molecule modified.

changed even after all the surface tryptophan residues had been modified (Fig. 4) (Spande & Witkop, 1967; Lowe & Whitworth, 1974).

The fluorescence emission intensity is almost totally quenched after oxidation of all the tryptophan residues exposed on the surface of the agglutinin. This is to be considered in conjunction with the fact that the fluorescence emission maximum of the agglutinin is at 334 nm (Fig. 2), which is typical of tryptophan emission in proteins, indicating that the fluorescence is solely due to exposed tryptophan residues in this protein (Kelly & von Hippel, 1976; Edelhoch & Chen, 1980; Herrmann & Behnke, 1980). The decrease in negative maxima at 286 and 293 nm in the c.d. spectrum, which are presumably contributed by tryptophan residues (Jirgensons, 1973), on modification of tryptophan residues also substantiates the above contention (Fig. 3*b*).

The decreases in the fluorescence intensity and absorbance of *Abrus* agglutinin show a linearity with respect to the number of tryptophan residues modified, indicating that all the residues/molecule modified were similarly exposed on the surface of the agglutinin. But in the presence of saturating concentration of lactose (0.15M) the decreases in the fluorescence intensity and absorbance of the agglutinin were biphasic (Fig. 2), indicating that the tryptophan residues were no longer in similar molecular environments on binding of lactose.

The fact that larger amounts of *N*-bromosuccin-

Fig. 5. Immunodiffusion test for various modifications on *Abrus* agglutinin

In each case the centre well contains anti-(*Abrus* agglutinin) serum. (a) 1, Tyrosine residues modified; 3, carboxy groups modified; 5, tryptophan residues modified by *N*-bromosuccinimide; 7, lysine residues modification by citraconylation; 2, 4, 6 and 8, native agglutinin. (b) 1, Arginine residues modified; 3, tryptophan residues modified by 2-hydroxy-5-nitrobenzyl bromide; 5, lysine residues modified by succinylation; 7, histidine residues modified; 2, 4, 6 and 8, native agglutinin.

imide were required to obtain the same extent of modification (Table 3) in the presence of lactose indicates that the saccharide binding decreased the accessibility of tryptophan residues in *Abrus* agglutinin. This also suggests that these residues are involved in the combining site of the agglutinin. A decrease in the rate constant of tryptophan fluorescence quenching by the I^- ion in the presence of lactose also substantiates our suggestion with regard to the presence of tryptophan in the binding region of this agglutinin (Herrmann & Behnke, 1980).

This loss in the haemagglutinating and saccharide-binding activities of the agglutinin on modification of the tryptophan residues could be due to a change in either the secondary structure or the state of aggregation of the protein. The former possibility is overruled, as the c.d. spectrum of the protein in the far-u.v. region (200–250 nm) remains practically unchanged even when all the exposed tryptophan residues were modified, when compared with the native lectin (Fig. 3a). The elution profile of the modified agglutinin on Sephadex G-200 shows a striking resemblance to that of the native protein, confirming that the state of aggregation of the native protein and the modified protein is the same. The perfect fusion of the precipitation bands in immunodiffusion studies of the modified and the native agglutinin also indicates that there is no change in the secondary structure, the state of aggregation and the immunogenic sites on modification (Fig. 5).

Evidence for the presence of tryptophan residues in the saccharide-binding site of other lectins has been reported. Hassing & Goldstein (1972) observed that *N*-bromosuccinimide-mediated oxidation of 1.5–3.0 tryptophan residues/molecule abrogated the haemagglutinating activity of concanavalin A. Photo-oxidation of tryptophan residues led to a dramatic decrease in the carbohydrate-binding activity of *Pisum sativum* lectin (Cermakova *et al.*, 1976). Privat *et al.* (1976) implicated a tryptophan residue in each binding site of wheat-germ agglutinin, as *N*-bromosuccinimide oxidation led to a loss in the haemagglutinating activity. Since more of *N*-bromosuccinimide was required to achieve the same degree of modification in the presence of *NN'*-triacylchitotriose, partial protection by the ligand was discernible for wheat-germ agglutinin, similar to that observed in our present study. In potato lectin both tyrosine and tryptophan residues were implicated in the carbohydrate-binding activity by a study on the modification of these residues and retention of haemagglutinating activity by protection with *NN'*-triacylchitotriose bound to protein (Ashford *et al.*, 1981). A preponderance of tryptophan, tyrosine, glutamic acid and aspartic

acid residues in the binding site of various lectins (Goldstein & Hayes, 1978) might subserve a structural motif for hydrogen-bonding with the hydroxy and acetamido groups of the sugar in the combining site of the lectin.

In conclusion, this present study overall strongly suggests that tryptophan residues are essential for the saccharide-binding activity of the *Abrus* agglutinin. The number of binding sites in the protein as described by Khan *et al.* (1981) by fluorescence polarization, quenching and equilibrium dialysis (Olsnes *et al.*, 1974) is two/molecule, and hence in each binding site there may be one tryptophan residue that is indispensable for its sugar-binding activity.

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