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SARACIN: A LECTIN FROM *SARACA INDICA* SEED INTEGUMENT RECOGNIZES COMPLEX CARBOHYDRATES

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Key Word Index—*Saraca indica*; seeds; saracin; lectin; complex carbohydrates; dehydration; Leguminosae.

Abstract—A lectin isolated from *Saraca indica* seed integument was purified by affinity chromatography on porcine thyroglobulin Sepharose followed by Sephadex G-50 and shown to be homogeneous by PAGE. It showed a single band on SDS–PAGE in the absence and presence of 2-mercaptoethanol corresponding to a M_r of $\simeq 12\,000$, thus indicating it to be a monomer. The lectin agglutinated erythrocytes of human A, B, O and AB blood group, animal erythrocytes as well as Ehrlich ascites cells. It is a thermostable glycoprotein containing 11.6% carbohydrates and large proportions of acidic amino acids. In haemagglutination–inhibition assays, among the tested glycoproteins, porcine thyroglobulin having the NeuAc α(2-6)/(2-3)D-Galβ(1-4)D-GlcNAc sequence was found to be the most potent; however, its asialo counterpart was non-inhibitory. The lectin is present solely in the seed integument even in the immature stage. During maturation of the seed the lectin activity declined and was completely absent when totally matured and dried. Studies *in vitro* showed that on incubation at 37° the seed gradually lost its lectin activity which was completely absent after 62 days with 88.5% loss of water. Similar studies on scraped seed integument revealed that the lectin activity was lost in 22 days with 87.5% dehydration.

INTRODUCTION

Lectins are abundant in nature and are found in the seeds of many plants [1–3]. However, despite the wealth of information available on the properties of isolated plant lectins, their physiological function(s) is not well understood. The localization of lectin(s) in plants is an essential prerequisite in the evaluation of any function of a lectin. The distribution of lectin within seeds differs among the various plant families and of these the members of the Leguminosae have been studied the most extensively [1–4]. The appearance of lectins in the seeds of almost all legumes occurs during the late stages of maturation of the seeds prior to their dehydration and most are localized in the cotyledons [2, 5–7]. Apart from the cotyledons, appreciable amounts of lectin have been found in the embryo [8–10] and in addition small amounts have been detected in the seed coat [5, 7, 9, 10]. Moreover, studies on the development of *Griffonia simplicifolia*, lentil (Leguminosae) [5] and *Ricinus communis* (Euphorbeaceae) seeds [10, 11] revealed that although there was no lectin activity in the immature seed it was present in the ripe mature seeds. Until the present study no lectin has been shown to be present exclusively in the seed coat and in the immature seed.

In this communication, we describe the purification and some properties of an integument lectin from *Saraca indica* L. seeds and compare its carbohydrate specificity with other legume lectins studied earlier. The cause of abolition of lectin activity in seed integument is also discussed.

RESULTS

Purification of S. indica lectin

The *S. indica* integument extract, on porcine-thyroglobulin-Sepharose CC with elution with 0.01 M glycine–HCl buffer (pH 3.5) followed by immediate neutralization, yielded the desired lectin (Fig. 1). Table 1 summarizes the purification scheme for *S. indica* lectin. The affinity matrix retained 0.95 mg of protein per ml of gel and the yield was 3.9 mg. The overall purification was 182 fold.

Homogeneity

On PAGE at pH 8.9, the lectin gave a single band (Fig. 2a) indicating it to be homogeneous. The homogen-

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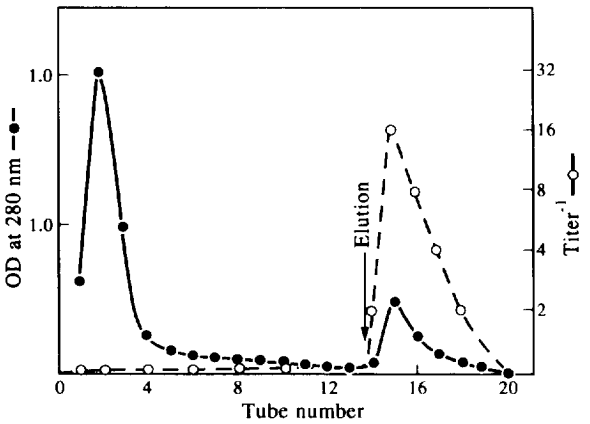

Fig. 1. Purification of *S. indica* lectin on a porcine thyroglobulin Sepharose column. The decolourized extract (2.0 ml) was applied to a column (1 × 5.2 cm) at 4° and after washing out the unretarded proteins with 10 mM Tris–HCl buffer, pH 8.0 (3 ml fractions), the column-retarded protein was eluted with 10 mM glycine–HCl buffer, pH 3.5 (fraction size 3 ml; arrow indicates the elution of the column).

Table 1. Purification of saracin

Fraction	Titre*	Protein (mg ml^{-1})	Sp. activity†	Purification (fold)
Saline extract	16	5.9	2.71	1
Decolourized with Pb-acetate	16	5.0	3.2	1.18
Affinity column (retarded)	32	0.08	400	147.60
Sephadex G-50	32	0.065	492	182

*Haemagglutination results with normal human B erythrocytes.

†Sp. activity is expressed as titre ml^{-1}. mg^{-1} protein.

eity was also confirmed by gel filtration when a single peak was obtained.

Haemagglutination assays

The haemagglutination pattern of normal and enzyme-treated human and animal erythrocytes by purified *S. indica* lectin (saracin) is shown in Table 2. The lectin agglutinated human A, B, O and AB erythrocytes almost equally well, indicating that the lectin was a blood group non-specific one. It agglutinated chicken, goat, rabbit and duck erythrocytes but only weakly agglutinated guinea-pig and rat erythrocytes.

M_r

On SDS–PAGE, with or without addition of 2-mercaptoethanol, the lectin showed a single band (Fig. 2b) corresponding to M_r 12000 thus showing it to be a monomer.

Carbohydrate and amino acid analyses

Saracin was shown to contain 11.3% neutral carbohydrates as determined by phenol–H_2SO_4 reaction and this corresponded well to the result obtained by GC analysis, i.e. neutral sugar 10.3% and amino sugar 1.3%. The glycan moiety of saracin was found to be composed of arabinose (3.43 g/100 g saracin), mannose (4.66), galactose (0.98), glucose (1.23) and 2-acetamido-2-deoxy-glucose (1.32). The amino acid analysis of saracin revealed a preponderance of acidic amino acids with little methionine (Table 3).

Physicochemical properties

The saracin induced agglutination of erythrocytes was not remarkably affected by variation of the pH of the medium or by the presence of divalent cations. The lectin retained its activity throughout pH range (3–10) tested. However, a slightly increased activity was observed at pH 8 (pH 3&4, titre 2^4; pH 5&7, titre 2^5; pH 8, titre 2^6) but this was subsequently decreased at pH 9 (2^4) and 10 (2^3).

The agglutination activity of saracin was not affected by temperature and remained constant (titre 2^4) up to 55°. Thereafter, its activity gradually decreased with further increase in temperature but still persisted slightly at 95°.

Interaction with Ehrlich ascites cells

Saracin agglutinated EAC cells, but with less potency compared to human erythrocytes. The minimum concentration of the lectin required to agglutinate EAC was 8.8 μg ml^{-1} while that required for human erythrocytes was 1.25 μg ml^{-1}. The agglutination was inhibited by glycoproteins, i.e. porcine thyroglobulin (2.5 mg ml^{-1}), fetuin (5 mg ml^{-1}) and asialofetuin (5 mg ml^{-1}) suggesting that the interaction is carbohydrate specific.

Haemagglutination inhibition assays

No simple carbohydrate, i.e. mono- and di-saccharides, free NeuAc, NeuGc, NeuAcα-D-Galβ(1-4) D-Glc, D-Galβ(1-4) D-GlcNAc and TF-disaccharide, D-Galβ(1-3)D-GalNAc inhibited saracin induced haemagglutination. However, certain sialoglycoproteins were inhibitory as shown in Table 4. Amongst them, porcine thyroglubulin was found to be most potent (0.078 mg ml^{-1}), whereas its asialo counterpart did not inhibit at all. Also, bovine thyroglobulin, γ-globulin, α_2-macroglobulin and blood group 'O' substance were found to be good inhibitors, while bovine, porcine and equine mucins, pig and horse erythrocyte mucoids, fetuin and asialofetuin were moderate inhibitors. Asialo derivatives of these glycoproteins except fetuin were non-inhibitors. Glycophorin and antithrombin III were weak inhibitors.

Variation of lectin activity with maturation

Haemagglutination by extracts of *S. indica* seed integument and cotyledon when examined separately, showed

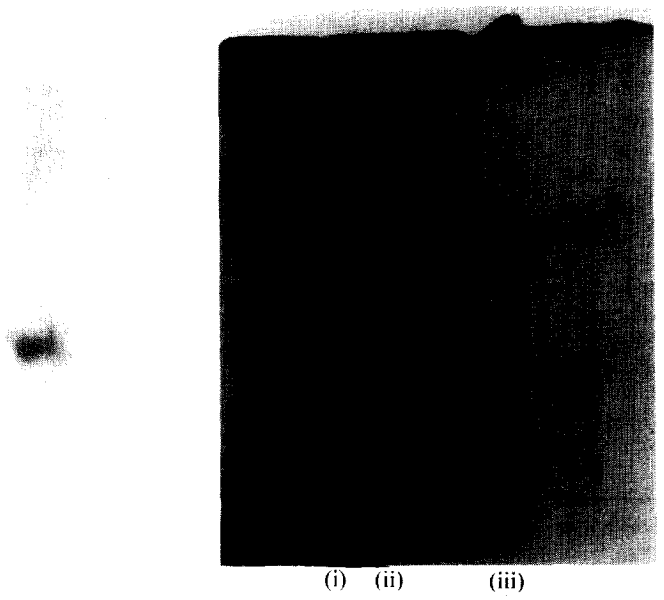

Fig. 2. Polyacrylamide slab gel (10%) electrophoresis of saracin (100 μg). (a) At pH 8.9, (b) in the presence of 0.1% SDS (i) with and (ii) without addition of 0.1% 2-mercaptoethanol, and (iii) protein markers. The gels were run at 30 mA for 3 hr.

Table 2. Haemagglutination pattern of different erythrocytes by saracin

Erythrocytes	Titre*		
	Untreated	Pronase treated	Neuraminidase treated
Human A	8	16	16
B	32	32	16
O	16	32	16
AB	16	16	16
Chicken	16	32	16
Goat	16	32	nd
Duck	16	32	nd
Rabbit	16	8	8
Rat	8	4	4

* At 80 μg ml^{-1} protein concentration.

Table 3. Amino acid composition of saracin

Amino acid	g·100 g^{-1}	Residue/mol*
Asp	6.25	5
Thr	5.65	5
Ser	7.18	7
Glu	12.36	9
Ala	12.02	11
Gly	15.25	12
Pro	4.08	4
Val	7.46	6
Meth	0.41	—
Ile	3.17	3
Leu	3.45	3
Tyr	0.97	1
Phe	1.815	1
His	0.57	1
Lys	5.65	4
Arg	1.48	1
Trp	4.125	3

* Nearest integer.

that the lectin was present solely in the integument and not in the cotyledon. The extract of immature seed coat showed high haemagglutination activity compared to that of mature seeds. The decrease of lectin activity with increasing seed weight is shown in Fig. 3.

Role of water in the integument lectin

The role of water present in the integument in relation to lectin activity was studied *in vitro*. It was found that the haemagglutination activity was abolished when the viable seed lost 88.5% of water. However, the lectin retained its original haemagglutinating activity (titre 16) even at 73% dehydration of the seed (not shown). Figure 4 (curve a) shows the decrease in specific activity with increasing number of days while Fig. 4 (curve a') shows the increase in percent dehydration with number of days. The results show that complete loss of lectin activity occurred after 62 days from the initial. By contrast, when similar experiments were performed with the

Table 4. Haemagglutination–inhibition of saracin by glycoproteins

Inhibitors	Minimum inhibitory concentration ($mg\,ml^{-1}$)*
Fetuin	1.25
Asialofetuin	1.25
Thyroglobulin (Porcine)	0.078
Thyroglobulin (Bovine)	0.625
Submaxillary mucin (Porcine)	2.5
Submaxillary mucin (Bovine)	1.25
Submaxillary mucin (Equine)	1.25
Erythrocyte mucoid (pig)	1.25
Erythrocyte mucoid (Horse)	1.25
Glycophorin (Human)	2.5
γ-Globulin	0.625
α_2-Macroglobulin	0.625
Blood group O-substance	0.625
Antithrombin III	5.0
Peptone A	5.0
α_1 Acid glycoprotein	0.31

* Required for complete inhibition of two haemagglutinating doses of lectin against human normal erythrocytes. D-Gal, D-Glc, D-Man, D-GlcNAc, D-GalNAc, α-Me-D-Gal, β-Me-D-Gal, α-Me-D-Glc, α-Me-D-Man, lactose, melibiose, *N*-acetyl-neuraminic acid were noninhibitors up to 200 mM. LacNAc, neuraminyl lactose was noninhibitor up to 100 mM.

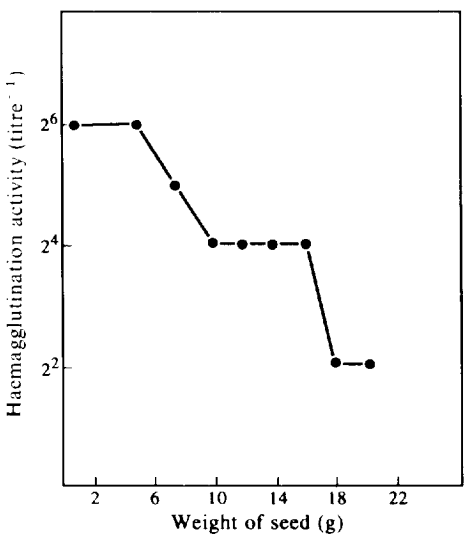

Fig. 3. Variation of lectin activity with maturation of seeds.

scraped seed coats, the complete loss of activity occurred only after 22 days from the initial [Fig. 4 (curve b)]. Nevertheless, the loss of 87.5% water in the scraped seed coat caused the complete loss of lectin activity [Fig. 4 (curve b')].

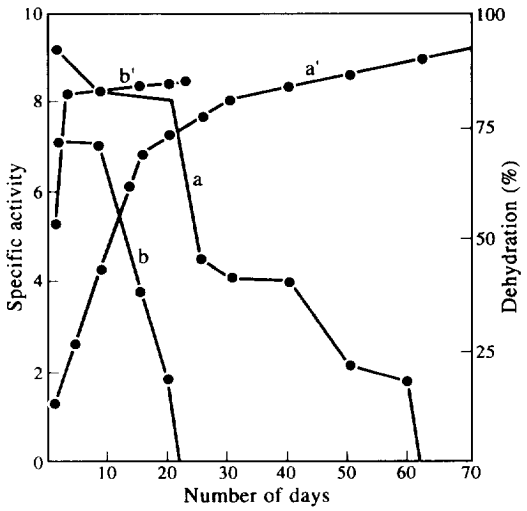

Fig. 4. Role of water on the activity of integument lectin. Relation of specific activity (a) and percentage dehydration (a') with number of days of intact seed integument. That for scraped integument is b and b', respectively.

DISCUSSION

The demonstration of haemagglutinating protein in the integument of *S. indica* seed represents the first demonstration of the occurrence of lectin in the developing seed of a legume. By using a combination of haemagglutination and immunodiffusion against antiserum to the seed lectin, the developmental study of lentil [4] and *Phaseolus vulgaris* [7] lectins suggested the presence of such proteins only in the ripening mature seeds. In *Dolichos biflorus* plant the lectin appeared abruptly only 26 days after flowering with the maximum level attained by the 28th day [2]. All the lectins studied so far were found to be present in the cell cytoplasm of the cotyledons. By using haemagglutination assays, the distribution of lectins in the seeds of dicotyledonous plants, i.e. *Maclura pomifera* and *Datura stramonium* has also been determined. The results showed that *M. pomifera* lectin started to accumulate during early seed development and attained a maximum at 15 weeks with complete maturity of the seed [12]. In dissected matured seeds, no lectin was observed to be present in the seed coat. In the remaining part of the seed the highest amount was found in the epicotyl. Similarly, an elevation in haemagglutinating activity was observed in *D. stramonium* lectin [10, 13] with the growth of seeds. By contrast, *S. indica* lectin is extractable solely from the integument and not from the cotyledon. The disappearance of the lectin in mature dried seeds is probably due to loss of water. Hydration, in addition to other factors is perhaps the most significant aspect for maintaining the three-dimensional conformation of the lectin(s) and also helps to release the lectins from the seeds, as has been demonstrated for soybean lectin [14]. Studies on *S. indica* lectin *in vitro* also confirm this phenomenon.

The integument extract of *S. indica*, on CC on a thyroglobulin-Sepharose 4B gel followed by Sephadex G-50, yielded an electrophoretically homogeneous lectin which we named saracin. It is a blood group non-specific lectin, active between pH 3 and 8, thermally stable and independent of any divalent cation.

It is a glycoprotein containing 11.6% of carbohydrates. It contains a trace amount of methionine and no cysteine. Saracin interacted with EAC cells. The strong interaction of EAC cells with plant seed lectins, such as those from *R. communis*, *P. vulgaris*, *G. simplicifolia* I, *A. integrifolia* and *A. lakoocha* is well established [15–19] and weaker agglutination has been observed with lectins from wheatgerm, lentil, pea, broad bean, soybean and potato [18]. Saracin agglutinated tumour cells presumably due to binding with the trisaccharide, NeuAcα(2-6)D-Galβ(1-4)D-GlcNAc, unit of the complex carbohydrate of the tumour cell surface glycoprotein [20].

Haemagglutination–inhibition studies on saracin with glycoproteins gave an insight into the specificity and size of the combining site. No simple mono- and oligo-saccharides including neuraminyllactose [NeuAcα(2,6)/(2,3)-D-Gal-β-(1-4)-D-Glc] were inhibitors whereas the glycoproteins could inhibit the haemagglutination indicating the specificity of the lectin towards the complex carbohydrate chains of the glycoproteins used. Porcine thyroglobulin was found to be the most potent inhibitor among the glycoproteins tested and contains at least two *N*-linked biantennary complex type oligosaccharide chains [21]. Likewise, the mucins were found to be good inhibitors. Porcine submaxillary mucin contains an *O*-linked oligosaccharide unit having an NeuAcα (2-6)-D-Gal β(1-3) D-GalNAc sequence [22] and the same unit, is also present in bovine submaxillary mucin [23] and hog submaxillary mucin [24]. Moreover, bovine submaxillary mucin contains a large number of NeuAcα(2-6) GalNAcα-units compared to porcine submaxillary mucin. This facilitated the inhibitory potency of the former than the later as shown in Table 4. Besides the mucins, fetuin also moderately inhibited saracin induced inhibition. Fetuin contains three *O*-linked and three *N*-linked oligosaccharide chains [25] of which the former are of two types, i.e. NeuAcα(2-3) D-Galβ-(1-3) D-GalNAc and NeuAcα(2-3)D-Galβ(1-3)[NeuAcα(2-6)]D-GalNAc. The second one is a complex type triantennary carbohydrate chain with a three NeuAc [α(2-6)/(2-3)]D-Gal β-(1-4)-D-GlcNAc sequence, [α, 2,6 2,3] linkage being almost equal [26]. It also contains another triantennary structure which differs from the previous one with the subterminal Gal linked 1,3 to the GlcNAc unit with substitution at the C-6 hydroxyl group by NeuAc [27]. The inhibitory effect of asialofetuin could be explained by the fact that α 2,3-linked NeuAc is less susceptible to acid hydrolysis compared to α 2,6-linked. In fact, fetuin after desialylation survived 5% NeuAc. By contrast, in thyroglobulin the NeuAcα (2-6)/(2-3)D-Galβ(1-4)D-GlcNAc sequence, NeuAc is mostly α 2,6-linked with a small amount α 2,3-linked [28]. Acid hydrolysis leads to the complete removal of α (2-6)-linked NeuAc making this glycoprotein a noninhibitor. Antithrombin III contains an alkali labile O-chain [29] and was found to be a weak inhibitor. Although NeuAc was noninhibitory, it in conjunction with *N*-acetyllactosylamine or with TF disaccharide was responsible for binding. Further, the noninhibitory effect of neuraminyllactose conclusively establishes that an acetamido group at C-2 of the reducing sugar in the trisaccharide must be necessary for binding. By contrast, the lectin from elderberry bark (*Sambucus nigra*) [30] and *Bordetella pertussis* toxin [28] have a binding specificity for terminal NeuAcα(2-6)D-Gal sequences irrespective of further sugar substitution on the galactose moiety. Thus, it appears that the combining site of saracin is complementary to the NeuAcα 2,6/2,3 Gal 1,4 GlcNAc sequence.

However, from these studies, the exact carbohydrate specificity of *S. indica* lectin could not be deduced. Similar type of results had been obtained for other lectins of different origins. The lectins from the pathogenic fungi *Trichophyton rubrum* [31], *Athelia rolfsii*, *Rhizoctonia crocorum* and *Rhizoctonia solani* [32], the tunicate *Amaroucium stellatum* [33] and estuarine crab *Scylla serrata* haemolymph [34] similarly recognized the complex carbohydrates of various macromoleules.

EXPERIMENTAL

Materials. Saraca indica seeds from pods were collected from the garden of our institute during March–April of the year. Seed integuments were obtained by scraping the seeds. Fetuin, porcine thyroglobulin, bovine and porcine submaxillary mucin, *N*-acetylneuraminyl lactose, *N*-acetyllactosamine, *N*-acetyl neuraminic acid, acrylamide, *N,N'*-methylenebisacrylamide, ammonium persulphate, *N,N',N'',N'''* tetra ethylmethylenediamide, pronase P, neuraminidase VIII (from *Clostridium perfringens*) and protein markers were purchased from Sigma, U.S.A. Mucoids from bovine, pig and horse erythrocytes were kindly supplied by Prof. G. Uhlenbruck, Medical University Clinic, Cologne, Germany. Equine submandibular gland mucin was the generous gift of Prof. R. Schauer, Biochemisches Institute, Kiel, Germany. All other chemicals and reagents used were of highest analytical grade and were supplied by local agencies.

Cells. Human blood from healthy donors was obtained from Ashok Laboratory, Jadavpur, Calcutta and was collected in citrate–dextrose soln. Rabbit, mouse and guinea-pig blood were collected by vein puncture. Duck blood was collected by sacrificing the animal and the blood from sheep, goat and chicken were procured from the slaughter house. The erythrocytes were washed with normal physiological saline (0.85%) and were treated with pronase P and neuraminidase as described [29]. Finally, a 2% (w/v) cell suspension was prepared in saline.

Ehrlich ascites carcinoma (EAC) cells were collected from Swiss albino mice as reported earlier [18].

Purification of S. indica *lectin.* Integuments (10.2 g) of mature seeds (wt. 8–10 g) were extracted with saline

(100 ml) and the extract was decolourized with a pinch of lead acetate by stirring. The soln (2.0 ml, 5.0 mg ml^{-1}), after passage through millipore filter (0.22 μm, Millipore), was applied to a porcine thyroglobulin conjugated Sepharose-4B column (1 × 5.2 cm) [prepared according to the method described in the Pharmacia booklet] pre-equilibrated with 0.01 M Tris–HCl buffer, pH 8.0. The column was washed with the same buffer to remove unretarded protein until the absorbance was less than 0.02, following which elution of the retarded protein was accomplished with 0.01 M glycine–HCl buffer, pH 3.5. Each fr. was neutralized immediately with satd $NaHCO_3$ soln. The active frs were pooled, mixed, concd by lyophilization and subsequently applied to a column of Sephadex G-50 (0.5 × 36 cm) pre-equilibrated with 0.01 M PBS, pH 7.0. The active frs were mixed, concd and preserved at −20° till use.

Homogeneity. The homogeneity of the purified lectin, saracin, was tested by electrophoresis using 10% polyacrylamide gel at pH 8.9 in Tris–glycine buffer [35]. The gel was stained with 0.5% Coomassie Brilliant Blue and destained with 7% HoAc in MeOH. The homogeneity was further checked in gel permeation chromatography on Sephadex G-50.

Haemagglutination and haemagglutination–inhibition assays. Haemagglutination assays were performed in 96-well polystyrene "U" plates (Flow Laboratories, U.K.) according to Chatterjee *et al.* [36]. To two-fold serially diluted lectin (25 μl) soln in saline was added in equal vol. of a 2% suspension of human and animal erythrocytes, and after incubation for 1 hr at room temp. the agglutination was observed with the naked eye. The titre was expressed as the reciprocal of the highest dilution of the lectin showing macroscopic agglutination. The haemagglutination–inhibition test was carried out as described earlier [36]. Briefly, serially, two-fold diluted carbohydrate inhibitors (25 μl) were mixed with an equal vol. of lectin soln. After keeping the plate for 1 hr at room temp., 25 μl of 2% human red cell suspension was added to each well and further incubated for 1 hr. The minimum conc. of sugar soln showing inhibition was recorded.

M_r *determination.* The M_r of saracin was determined by SDS–PAGE carried out according to Laemmli [37] with and without addition of 0.1% 2-mercaptoethanol. Protein markers used were ovalbumin (46 000), carbonic anhydrase (29 000), trypsin-inhibitor (20 000), α-lactalbumin (14 000) and aprotinin (6500). From the calibration curve (mobility vs. log M_r of known proteins) the M_r of purified lectin was determined.

Analytical procedure. The protein content of different lectin preparations was measured by the method of Lowry *et al.* [38] using BSA as the standard. The total neutral sugar in saracin was estimated by the phenol–H_2SO_4 method [39] with D-glucose as the standard. The individual neutral and amino sugars were identified and quantitated by GC analysis [40]. The amino acid analysis of the lectin was performed after hydrolysis of the sample (0.25 mg) with 6 M HCl at 110° for 22 hr (Pharmacia LKB ALPHA PLUS amino acid analyser). Tryptophan was determined spectrophotometrically [41].

Physico-chemical properties. Saracin induced haemagglutination was tested (i) in the pH range 3–6 (in 10 mM citrate–phosphate buffer) and 7–11 (in 10 mM Tris–HCl buffer), (ii) in the presence of divalent cations Ca^{2+}, Mg^{2+}, Mn^{2+} (30–100 mM) and (iii) at temps from 10° to 90°, at intervals of 10° for a 30 min incubation. At each specified temp., the heated soln was cooled to room temp. and then assayed.

Interaction with Ehrlich ascites carcinoma cells. The interaction of saracin with EAC was observed by an agglutination reaction analogous to haemagglutination except that instead of erythrocytes, EAC (1.5×10^6 cells ml^{-1}) was used and the specificity of the reaction was determined by agglutination–inhibition tests [19] using known carbohydrates.

Variation of lectin activity with maturation of seed. Variation of lectin activity with maturation was measured using seeds of increasing wt. Fresh seeds of *S. indica* of different wts ranging from 0.05 to 20 g were divided into a number of groups according to their wts. Integuments of each group were scraped off and extracted (10% w/v) with normal saline. The haemagglutinating property of each extract was tested with normal human erythrocytes.

Role of water in the integument lectin. To examine the role of water in lectin activity in the seed integument, a number of intact seeds of variable wts (13–16 g) and scraped integument (3.9 g) was incubated separately at constant temp. (37°) for a few days. Among the incubated seeds, one or two were taken at a certain interval of time, scraped and extracted with saline. Haemagglutinating activity and protein content were measured separately at each time. The scraped integument was treated likewise.

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REFERENCES

1. Liener, I. E. (1976) *Ann. Rev. Plant. Physiol.* **27**, 291.
2. Talbot, C. F. and Etzler, M. E. (1978) *Plant Physiol.* **61**, 847.
3. Su, L. C., Pueppke, S. G. and Friedman, H. G. (1980) *Biochim. Biophys. Acta* **629**, 292.
4. Howard, I. K., Sage, H. J. and Horton, C. B. (1972) *Arch. Biochem. Biophys.* **149**, 323.
5. Clarke, A. E., Knox, R. B. and Jermyn, M. A. (1975) *J. Cell Sci.* **19**, 157.
6. Pueppke, S. G., Bauer, W. D., Keegstra, K. and Ferguson, A. L. (1978) *Plant Physiol.* **61**, 779.
7. Mialonier, G., Prival, J. P., Monsigny, M., Kahlen, G. and Durand, R. (1973) *Physiol. Veg.* **11**, 519.
8. Rouge, P. (1974) *C. R. Hebd. Seances Acad. Sci. Ser.* D278, 449.
9. Rouge, P. (1975) *C. R. Hebd. Seances Acad. Sci. Ser.* D280, 2105.

10. Eisler, M. and Protheim, L. (1926) *Z. Immunitaets forsch.* **47**, 59.
11. Youle, R. J. and Huang, A. H. C. (1976) *Plant Physiol.* **58**, 703.
12. Jones, J. M., Cawley, L. P. and Teresa, G. W. (1967) *J. Immunol.* **98**, 364.
13. Kilpatrick, D. C., Yeoman, M. M. and Gould, A. R. (1979) *Biochem. J.* **184**, 215.
14. Foutain, D. W., Foard, D. E., Replogle, W. D. and Yang, W. K. (1977) *Science* **197**, 1185.
15. Tomita, M., Osawa, T., Sakurai, Y. and Ukita, T. (1970) *J. Cancer* **6**, 283.
16. Nachbar, M. S., Oppenheim, J. D. and Aull, A. (1976) *Biochim. Biophys. Acta* **419**, 512.
17. Eckhardt, A. E., Malone, B. N. and Goldstein, I. J. (1982) *Cancer Res.* **42**, 2977.
18. Ahmed, H., Chatterjee, B. P. and Debnath, A. K. (1988) *J. Biosci.* **13**, 419.
19. Chatterjee, B. P., Ahmed, H. and Chowdhury, S. (1988) *Carbohydr. Res.* **180**, 97.
20. Eckhardt, A. E. and Goldstein, I. J. (1983) *Biochemistry* **22**, 5290.
21. Louis, F. J., Nicole, V. and Claudiene, M. (1990) *Biochem. Biophys. Res. Commun.* **166**, 937.
22. Carlson, D. M. (1968) *J. Biol. Chem.* **243**, 616.
23. Gottschalk, A. and Graham, E. R. B. (1959) *Biochim. Biophys. Acta* **34**, 380.
24. Halbeck, H. V., Dorland, L., Haverkamp, J., Veldink, G. A., Vliegenthart, J. F. G., Fournet, B., Ricart, G., Montreuil, J., Gathmann, W. D. and Aminoff, D. (1981) *Eur. J. Biochem.* **118**, 487.
25. Nilsson, B., Norden, N. E. and Svensson, S. (1979) *J. Biol. Chem.* **254**, 4545.
26. Cumming, D. A., Hellerqvist, C. G., Harris-Brandts, M., Michnick, S. W., Carver, J. P. and Bendiak, B. (1989) *Biochemistry* **28**, 6500.
27. Takasaki, S. and Kobata, A. (1986) *Biochemistry* **25**, 5709.
28. Heerze, L. D. and Armstrong, G. D. (1990) *Biochem. Biophysics Res. Commun.* **172**, 1224.
29. Chatterjee, B. P., Vaith, P., Chatterjee, S., Karduck, D. and Uhlenbruck, G. (1979) *Int. J. Biochem.* **10**, 321.
30. Shibuya, N., Goldstein, I. J., Braekaert, W. F., NsimbaLubaki, M., Peeters, B. and Peumans, W. J. (1987) *J. Biol. Chem.* **262**, 1569.
31. Mitra, A., Sikdar, S., Chatterjee, B. P. and Guha, A. (1992) Abstract, p-51, 14th International Lectin Meeting, 24–30 May, Darjeeling, India.
32. Vranken, An M., Van Damme, Els J. M., Allen, A. K. and Peumans, W. J. (1987) *FEBS Letters* **216**, 67.
33. Vasta, G. R., Warr, G. W. and Marchalonis, J. J. (1982) *Comp. Biochem. Physiol.* **73B**, 887.
34. Chattopadhyay, T. and Chatterjee, B. P. (1993) *Biochem. Arch.* **9**, 65.
35. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404.
36. Ahmed, H. and Chatterjee, B. P. (1988) *Carbohydr. Res.* **117**, 173.
37. Laemmli, U.K. (1970) *Nature* (London) **227**, 680.
38. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
39. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Anal. Chem.* **28**, 350.
40. Ray, S. and Chatterjee, B. P. (1989) *Carbohydr. Res.* **191**, 305.
41. Spies, J. R. and Chambers, D. C. (1948) *Anal. Chem.* **20**, 30.