Interactions of soya-bean agglutinin with purified glycoconjugates and soya-bean seed components

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A radioaffinity assay for lectin binding to receptors was developed and characterized by using the interactions between soya-bean agglutinin and four glycoconjugates, namely thyroglobulin, galactomannan, fetuin and asialofetuin. On application of the assay to soya-bean extracts a wide range of seed components were found to have the capacity to interact with soya-bean agglutinin. These included both trichloroacetic acid-soluble and trichloroacetic acid-insoluble glycoconjugates and two classes of particulate matter distinguished by their differential solubility in Triton X-100.

An approach into understanding the functions of seed lectins is to analyse seed components that can interact with the lectin and may represent 'receptors' of physiological significance. Several techniques are available for identifying lectin-binding components, but each is limited if applied to an unknown population of glycoconjugates such as in a seed extract.

One such general technique is to overlay cell components after their separation by t.l.c. or sodium dodecyl sulphate/polyacrylamide-gel electrophoresis with a ^{125}I - or fluorescein isothiocyanate-labelled probe (West & McMahon, 1977; West *et al.*, 1978; Magnani *et al.*, 1982; Basha & Roberts, 1982; Bowles *et al.*, 1982, 1983). However, effectiveness is dependent on the relative abundance of the binding component and some idea as to an appropriate separation system, especially if the components are not glycoproteins or glycolipids. Similarly, immobilized lectin systems have been used with great success for characterization of glycoproteins from a wide variety of cell types, but their use in identification of potential 'receptors' within systems from which the lectin was isolated has been limited (Gansera *et al.*, 1979; Breuer & Siu, 1981; Bowles & Marcus, 1981; Ray & Lerner, 1982; Miller & Bowles, 1982; Marcus *et al.*, 1984).

Alternative strategies have involved indirect

inhibition studies, for example where 'receptor' activity has been measured as an ability to inhibit binding between lectin and immobilized ligand (Cooper *et al.*, 1983), lectin-induced haemagglutination (Drake & Rosen, 1982) or lectin-glycoconjugate precipitation (Carter & Sharon, 1977; Etzler, 1977). Several of these have included use of PEG to induce conditions in which lectin-glycoprotein complexes are more rapidly precipitated than are either lectin or glycoprotein alone.

The present paper describes a radioaffinity assay with PEG in which the interaction between a ^{125}I -lectin and binding component(s) in a cell extract can be measured directly. By using the assay it has been possible to distinguish several components of soya-bean seeds that are able to interact with SBA and to study changes in their concentrations during germination.

Materials and methods

Materials

Soya-bean seeds (*Glycine max* var. Williams) were provided by the U.S. Intsoy Program 1982, with a germination efficiency of 100%. SBA was prepared by affinity chromatography as described by Allen & Neuberger (1975). [^{125}I]iodide was purchased from Amersham International, and all other chemicals were from Sigma Chemical Co. or BDH Chemicals.

Turbidity assay

The assay mixture contained: 10 mM buffer (Hepes/NaOH, pH 8.0, or Mes/HCl, pH 5.0), 200 mM sugar (D-glucose or D-galactose), PEG (indicated concentrations) and 200 μg of glyco-

Abbreviations used: PBS, phosphate-buffered saline (145 mM NaCl / 10 mM KH_2PO_4 / K_2HPO_4 buffer, pH 7.4); PEG, poly(ethylene glycol) 6000; SBA, soya-bean agglutinin; HU, haemagglutination units (1 unit is the lowest weight of lectin to haemagglutinate positively in a final volume of 50 μl).

conjugate [thyroglobulin (bovine type 1) or galactomannan (guar gum)]. The reaction was started by the addition of 200 μg of SBA, bringing the final volume of the assay mixture to 3.0 ml, and after 20 min the absorbance at 405 nm was read.

Iodination of SBA

A 40 nmol portion of SBA in PBS containing 200 mM-D-galactose was iodinated with 1 mCi of ^{125}I in the presence of 40 nmol of KI and 400 nmol of chloramine-T. The reaction was stopped after 10 min by the addition of 800 nmol of sodium metabisulphite. Free ^{125}I iodine was separated by using a Bio-Gel P6 column. The fractions containing protein were applied to a 2 ml column of *N*-6-aminohexanoyl- β -D-galactosamine-Sepharose (Allen & Neuberger, 1975). ^{125}I -SBA was eluted with 0.2 M-galactose and dialysed against PBS before use. The specific radioactivity was 1.3×10^7 c.p.m./nmol of SBA. The haemagglutination activity of ^{125}I -SBA was 2133 HU/ μg compared with 2560 HU/ μg for unlabelled SBA (determined with a 2% suspension of trypsin-treated rabbit erythrocytes).

Radioaffinity assay

The incubation volume of 200 μl contained ^{125}I -SBA (1.2 μg , 6×10^5 c.p.m.), glycoconjugate or seed extract and 50 mM buffer (sodium acetate, pH 4.0; Mes/HCl, pH 5.0 and 6.0; Hepes/NaOH, pH 7.0 and 8.0; Tris/HCl, pH 9.0), 100 mM sugar (D-galactose or D-glucose) and 12% (w/v) PEG. For assays where inhibition by unlabelled SBA was investigated, glycoconjugates or cell extracts were mixed first with unlabelled SBA in the appropriate buffer. After 15 min at room temperature ^{125}I -SBA was added and, after mixing, PEG was added. After a further 5 min the incubation was transferred on to a 4 ml cushion of 15% (w/v) PEG (in 10 mM buffer of appropriate pH, containing 145 mM-NaCl and 200 mM-D-galactose or -D-glucose) in 5 ml polyallomer centrifuge tubes. Bound material and unbound material were then separated by ultracentrifugation (SW50.1 rotor, Beckman L8 centrifuge) at 200 000 *g* for 40 min at 4°C, and radioactivity in the pellet was measured.

Germination of seeds

Soya-bean seeds were germinated and grown on moist filter paper (25°C/12 h light–20°C/12 h dark). Germination, as defined by radicle emergence, had occurred under these conditions within 24 h. By 120 h post-imbibition, radicles measured 8–10 cm.

Preparation of seed extracts

Cotyledons or seed coats (defined as seed coat proper + endosperm) were homogenized (1 g fresh wt./10 ml of PBS) in a pestle and mortar and

sonicated at 4°C (Dawe sonicator 3×10 s, with a microprobe). Homogenates were centrifuged at 1000 *g* for 5 min at room temperature. The debris-free extract was further centrifuged at 200 000 *g* for 1 h at 4°C to give soluble and particulate fractions. The pellet was washed four times through sonication-centrifugation cycles (once with PBS containing 200 mM-D-galactose, three times with PBS) before suspension in a volume of PBS equivalent to the soluble fraction. Both fractions were then assayed for ^{125}I -SBA binding, protein content and haemagglutination activity.

A portion of the washed particulate fraction was made 1% (v/v) with respect to Triton X-100, sonicated and centrifuged at 200 000 *g* for 1 h at 4°C. Particulate matter that had not been solubilized by the detergent and had resedimented was washed with PBS (three times) before final suspension in a volume of PBS equivalent to that of the soluble fraction and assayed for ^{125}I -SBA binding and protein content. Triton X-100 at 1% (v/v) was found to inhibit the radioaffinity assay (results not shown); this made it necessary to remove the detergent by wash cycles with PBS, before assay. As a control for effectiveness of the wash procedure, a suspension of washed detergent-treated cotyledon pellet, with no binding activity towards ^{125}I -SBA, was added to an incubation containing thyroglobulin: extents of ^{125}I -SBA binding to thyroglobulin were unaffected (results not shown).

Seed coat and cotyledon soluble fractions were treated with 10% (w/v) trichloroacetic acid (final concn.) for 20 min at 4°C, and precipitates were removed by centrifugation (12 000 *g* for 3 min). The acid-soluble material was dialysed extensively against water at 4°C before the non-diffusible material was assayed for binding activity towards ^{125}I -SBA. Protein was assayed by the Coomassie Blue dye method (Sedmak & Grossberg, 1977).

Results

Characterization of assay by using defined glycoconjugates

An assay based on the ability of lectin-glycoconjugate complexes to increase turbidity was used initially to investigate the effect of PEG on the reaction. Two different glycoconjugates were studied, galactomannan and thyroglobulin, both shown previously by other workers to be co-precipitated with SBA or to inhibit lectin-induced haemagglutination respectively (Hammarstrom *et al.*, 1977; Bowles *et al.*, 1979). Results shown in Fig. 1 indicate that addition of PEG to a critical concentration, dependent on the nature of the glycoconjugate, induced the specific precipitation of lectin-glycoconjugate complexes. Incubation of

Fig. 1. *Effect of poly(ethylene glycol) concentration on the interaction between SBA and glycoconjugates*

Incubations were set up as described in the Materials and methods section and the turbidity was measured by absorbance changes at 405 nm. In addition to 200 μ g of SBA and increasing concentrations of poly(ethylene glycol), the constituents of each assay were as follows: ●, 10 mM-Hepes/NaOH buffer, pH 8.0, 200 mM-glucose, 200 μ g of galactomannan; ○, 10 mM-Hepes/NaOH buffer, pH 8.0, 200 mM-galactose, 200 μ g of galactomannan; ■, 10 mM-Mes/HCl buffer, pH 5.0, 200 mM-glucose, 200 μ g of thyroglobulin; □, 10 mM-Mes/HCl buffer, pH 5.0, 200 mM-galactose, 200 μ g of thyroglobulin; ▲, 10 mM-Mes/HCl buffer, pH 5.0, 200 mM-glucose; △, 10 mM-Hepes/NaOH buffer, pH 8.0, 200 mM-glucose.

SBA alone (Fig. 1) or glycoconjugates alone (results not shown) did not cause any substantial increase in turbidity even in high PEG concentrations. Since complex-formation, as measured by turbidity, could be inhibited by D-galactose (Fig. 1), the reaction between SBA and either galactomannan or thyroglobulin most probably involved the carbohydrate-binding sites of the lectin. In the presence of 10% (w/v) PEG, increasing amounts of either SBA or glycoconjugate gave increasing absorbance readings up to a maximal value, implying that neither excess lectin nor ligand caused inhibition of turbidity.

A radioaffinity assay based on the use of PEG was developed and was characterized by using thyroglobulin, galactomannan, fetuin and asialo-fetuin, as shown in Figs. 2 and 3. For each glycoconjugate the assay could be shown to be concentration- and pH-dependent and competitive with unlabelled SBA. By using the concentration curves in Figs. 2(b), 2(d), 3(b) and 3(d), K_d values were calculated by using a computer analysis (Chaplin, 1984) based on the direct linear plot (Eisenthal & Cornish-Bowden, 1974). Although there were large differences in the weight of glycoconjugates used in the assay, the amounts of

Fig. 2. *Characterization of the radioaffinity assay with ^{125}I -SBA: thyroglobulin and galactomannan*
Incubations were set up as described in the Materials and methods section. Results are expressed as the percentage bound of the total ^{125}I -SBA available ($1.2 \mu\text{g}$, $6 \times 10^5 \text{ c.p.m.}$). (a) Effect of pH on the binding of ^{125}I -SBA to thyroglobulin (100 μg). ●, Assayed in 200 mM-glucose; ○, assayed in 200 mM-galactose; ⊙, thyroglobulin treated with 10% (w/v) trichloroacetic acid, trichloroacetic acid-soluble material dialysed and used in the assay. (b) Effect of thyroglobulin concentration on the binding of ^{125}I -SBA at pH 5.0. ▲, Assayed in 200 mM-glucose; △, assayed in 200 mM-galactose. (c) Effect of pH on the binding of ^{125}I -SBA to galactomannan (0.05 μg). ●, Assayed in 200 mM-glucose; ○, assayed in 200 mM-galactose; ⊙, galactomannan treated with 10% (w/v) trichloroacetic acid, trichloroacetic acid-soluble material dialysed and used in the assay. (d) Effect of galactomannan concentration on the binding of ^{125}I -SBA at pH 8.0. ▼, Assayed in 200 mM-glucose; ▽, assayed in 200 mM-galactose. (e) Effect of increasing concentrations of unlabelled SBA on the binding of ^{125}I -SBA to glycoconjugates in 200 mM-glucose. ■, Thyroglobulin (20 μg , pH 5.0); □, galactomannan (0.1 μg , pH 8.0).

bindable galactose residues were less disparate (Table 1). Both galactomannan and asialofetuin had similar K_d values when expressed as molar contained-galactose, whereas the binding affinity

for fetuin was much less, as the galactose residues were less accessible for ^{125}I -SBA binding. The high binding affinity (low K_d) for thyroglobulin per molar contained-galactose was probably due

Fig. 3. Characterization of the radioaffinity assay with ^{125}I -SBA: fetuin and asialofetuin

Incubations were set up as described in the Materials and methods section. Results are expressed as the percentage bound of the total ^{125}I -SBA available (1.2 μg , 6×10^5 c.p.m.). (a) Effect of pH on the binding of ^{125}I -SBA to fetuin (100 μg). ●, Assayed in 200 mM-glucose; ○, assayed in 200 mM-galactose. (b) Effect of fetuin concentration on the binding of ^{125}I -SBA at pH 5.0. ▲, Assayed in 200 mM-glucose; △, assayed in 200 mM-galactose. (c) Effect of pH on the binding of asialofetuin (10 μg). ●, Assayed in 200 mM-glucose; ○, assayed in 200 mM-galactose. (d) Effect of asialofetuin concentration on the binding of ^{125}I -SBA at pH 5.0. ▼, Assayed in 200 mM-glucose; ▽, assayed in 200 mM-galactose. (e) Effect of increasing concentrations of unlabelled SBA on the binding of ^{125}I -SBA to glycoconjugates in 200 mM-glucose. ■, Fetuin (250 μg) at pH 5.0; □, asialofetuin (7.5 μg) at pH 5.0.

to the relatively low solubility of the large glycoprotein, which encouraged its precipitation.

The different glycoconjugates showed a variation in pH-dependence for interaction with ^{125}I -SBA. Thyroglobulin and fetuin showed optimal binding at pH 5.0, whereas asialofetuin and neutral

galactomannan showed a broader pH profile, in which binding only diminished at pH 4.0, at which SBA is known to lose its metal ions (Jaffe *et al.*, 1977; Novakova *et al.*, 1981). Binding between SBA and the glycoconjugates was completely inhibited by appropriate amounts of galactose or *N*-acetylgalactosamine.

Table 1. Comparison of dissociation constants (K_d) for ^{125}I -SBA binding to glycoconjugates, with the use of the radioaffinity assay

Binding constants were calculated from the concentration curves shown in Figs. 2 and 3 by using a direct linear method (Eisenthal & Cornish-Bowden, 1974) and analysed as described by Chaplin (1984). Data for M_r values and amounts of galactose + *N*-acetylgalactosamine (Gal + GalNAc) per polymer were taken from the references indicated: thyroglobulin (Spiro & Bhoyroo, 1984); fetuin and asialofetuin (Graham, 1972); galactomannan (Dea & Morrison, 1975).

Glycoconjugate	M_r	Gal + GalNAc (residues/molecule)	Gal + GalNAc per polymer (%)	K_d (molar polymer)	K_d (molar contained-galactose)
Thyroglobulin (bovine type 1)	670000	47 Gal (11 accessible)	1.2	9.6×10^{-8}	4.5×10^{-6}
Fetuin	49000	14 Gal, 2 GalNAc	5.5	2.8×10^{-5}	4.5×10^{-4}
Asialofetuin	45000	14 Gal, 2 GalNAc (14 Gal accessible)	5.9	6.7×10^{-7}	1.1×10^{-5}
Galactomannan (guar gum)	250000	541 Gal (541 Gal accessible)	39.3	2.9×10^{-8}	1.6×10^{-5}

Solubility in trichloroacetic acid was used as a method for broadly dividing the glycoconjugates into two types: galactomannan, a polysaccharide that retained binding activity towards ^{125}I -SBA after treatment with trichloroacetic acid (Fig. 2c), and thyroglobulin, a glycoprotein that was precipitated with the acid and had no binding activity, and was retained by the supernatant after dialysis (Fig. 2a). Excess unlabelled SBA competed with ^{125}I -SBA binding (Figs. 2e and 3e). If high concentrations of unlabelled SBA were incubated alone with ^{125}I -SBA, no increase in precipitation of lectin above background values (<10%) occurred at any pH between 4.0 and 9.0 (results not shown).

Application of radioaffinity assay to soya-bean seed components

The radioaffinity assay was applied to seed extracts to determine the nature and extent of cellular components able to interact with SBA. Two parts of the seed were studied at each time point during development: the seed coat, defined as the seed coat proper + residual endosperm (Carlson, 1973), shown in preliminary studies to have particularly high specific binding activity to the lectin, and the cotyledons, a storage tissue derived from the embryo and known to contain the bulk of the endogenous SBA (Pueppke *et al.*, 1978).

Fig. 4 shows pH curves of binding between ^{125}I -SBA and soluble components in the seed coat from 4h to 120h development. At 4h, binding was high across the pH range. High degrees of binding were maintained up to 48h, but from 72h onwards a binding component(s) unaffected by trichloroacetic acid treatment and measurable in the pH range 6–9 decreased to low concentrations. The pattern of ^{125}I -SBA binding to soluble components in the cotyledons was very different. As shown in

Fig. 5, two classes of glycoconjugate, on the basis of trichloroacetic acid-solubility, were able to interact with SBA throughout development. Binding to trichloroacetic acid-soluble material was optimal from pH 4.0 to pH 6.0, whereas trichloroacetic acid-precipitable components bound the lectin across the pH range. All binding was inhibited to background values by galactose (200mM) or *N*-acetylgalactosamine (20mM).

The presence of endogenous lectin in the soluble fractions was examined by haemagglutination assay. In extracts prepared from 4h-soaked seed coats low lectin activity (3.0 HU/ μg of protein) inhibited by *N*-acetylgalactosamine was found, which diminished over 120h to 0.5 HU/ μg . The high lectin activity (30 HU/ μg) found in cotyledon extracts remained unchanged during 0–120h of development. Since pure SBA exhibited a titre of 2560 HU/ μg , the amount of SBA in the cotyledon extract can be estimated to be approx. 0.05 $\mu\text{g}/\mu\text{l}$ and in the seed-coat extracts to be approx. 0.00012 $\mu\text{g}/\mu\text{l}$. Amounts of unlabelled SBA in the radioaffinity assays could therefore be in the region of 0.5 μg and unlikely to exceed 1 μg . To confirm that these quantities of unlabelled SBA would have little effect on the amount of ^{125}I -SBA binding, the endogenous lectin was removed from the extracts by passage through *N*-6-aminohexanoyl- β -D-galactosamine-Sepharose. Radioaffinity assays were then carried out on the lectin-depleted extracts, and also after addition of increasing amounts of unlabelled SBA. Results for two seed development times are shown in Table 2. Removal of endogenous SBA by passage through the affinity matrix did not significantly alter extents of ^{125}I -SBA binding to components in either the cotyledons or seed coats. As shown also for the interaction between ^{125}I -SBA and purified glycoconjugates, an excess of unlabelled SBA was required to decrease ^{125}I -SBA binding.

Fig. 4. Interactions between ^{125}I -SBA and soluble components of seed-coat extracts prepared from soya beans during germination and early development

Soluble fractions were prepared from seed-coat tissue as described in the Materials and methods section and portions were assayed directly (○). Alternatively portions were treated with 10% (w/v) trichloroacetic acid (final concn.) and the trichloroacetic acid-soluble material was assayed after dialysis (●). Each assay contained ^{125}I -SBA (1.2 μg , 6×10^5 c.p.m.), 5 μl of soluble fraction (equivalent to 0.9 μg of protein before trichloroacetic acid treatment), 12% (w/v) poly(ethylene glycol) and 200 mM-glucose at the pH stated. Results for each time point of development (a–f) are expressed as the percentage bound of the total ^{125}I -SBA available.

Table 2. Effect of unlabelled SBA on the binding of ^{125}I -SBA to glycoconjugates in soluble fractions of seed coats and cotyledons

Portions of soluble fractions prepared from 4h- and 96h-imbibed seeds were applied to *N*-6-aminohexanoyl- β -D-galactosamine–Sepharose. Effluents were assayed either directly for ^{125}I -SBA binding or after addition of increasing amounts of unlabelled SBA. Radioaffinity assays were carried out at pH 5.0 in 200 mM-glucose with 1.2 μg of ^{125}I -SBA. Results are expressed as percentages of the binding of ^{125}I -SBA that occurred in the absence of unlabelled SBA.

¹²⁵ I-SBA binding to soluble components in seed extracts (%)							
Development (h post-imbibition)	Pre-affinity matrix	Unlabelled SBA present in the assay . . .	Post-affinity matrix				
			0	1 μg	5 μg	10 μg	50 μg
Seed coat							
4	108		100	105	101	95	61
96	116		100	78	73	57	27
Cotyledon							
4	92		100	98	99	69	65
96	102		100	93	86	79	67

Fig. 5. Interactions between ^{125}I -SBA and soluble components of cotyledon extracts prepared from soya beans during germination and early development

Soluble fractions were prepared from cotyledon tissue as described in the Materials and methods section and samples were assayed directly (○). Alternatively samples were treated with 10% (w/v) trichloroacetic acid (final concn.) and the trichloroacetic acid-soluble material was assayed after dialysis (●). Each assay contained ^{125}I -SBA (1.2 μg , 6×10^5 c.p.m.), 10 μl of soluble fraction (equivalent to 46 μg of protein before trichloroacetic acid treatment), 12% (w/v) poly(ethylene glycol) and 200 mM-glucose at the pH stated. Results for each time point of development (a-f) are expressed as the percentage bound of the total ^{125}I -SBA available.

Binding of ^{125}I -SBA to particulate fractions was also studied: the fractions were washed with D-galactose followed by three cycles of PBS before assaying. Small amounts of endogenous lectin were released specifically by 200 mM-D-galactose from cotyledon particulate matter at all developmental stages, whereas none could be found in equivalent extracts from seed coats. By three subsequent wash cycles with PBS, no residual lectin activity could be detected in any of the particulate suspensions. Preliminary findings indicated that binding of ^{125}I -SBA to particulate matter of seed coats or cotyledons occurred optimally at pH 5.0. Results shown in Table 3 compare the binding of ^{125}I -lectin to particulate fractions prepared at different developmental stages. ^{125}I -SBA binding to seed-coat components was high and unaffected by treatment with detergent. In contrast, when the particulate matter in the cotyledons was treated

with Triton X-100, its binding capacity for ^{125}I -SBA was abolished. These results indicate that SBA interacts with a detergent-insoluble material in the seed coat, such as sedimentable cell-wall polymers, whereas in the cotyledons the binding components for the lectin are detergent-soluble and most probably are membrane glycoproteins and/or glycolipids. When 200 mM-galactose or 20 mM-N-acetylgalactosamine was included in the assays, binding of ^{125}I -lectin was inhibited to background values for all fractions except for 120 h cotyledon components, with which some binding (19 μg of SBA/ml) occurred even in the presence of inhibitory sugar.

Discussion

A radioaffinity assay has been developed in which direct binding between a lectin and glycoconjugate can be measured. The glycoconjugate

Table 3. *Effect of detergent treatment on the binding capacity of soya-bean particulate fractions to ^{125}I -SBA*
 Particulate fractions were prepared from seed coats or cotyledons as described in the Materials and methods section. The fractions were washed with 200 mM-galactose followed by three wash cycles with PBS. One portion of the final suspension was assayed directly for ^{125}I -SBA binding at pH 5.0 in 200 mM-glucose. Another portion was made 1% (v/v) (final concentration) with respect to Triton X-100, and after re-centrifugation the detergent-insoluble material was further washed three times with PBS before being assayed for ^{125}I -SBA binding. Activities are expressed per ml of original extract. Background amounts of sedimentable ^{125}I -SBA in the absence of seed extract were less than 10% of the total ^{125}I -SBA available: this background has been subtracted from the binding activities given.

Particulate fractions

Development (h post-imbibition)	Pre-detergent treatment		Post-detergent treatment	
	Protein ($\mu\text{g/ml}$)	^{125}I -SBA bound ($\mu\text{g/ml}$)	Protein ($\mu\text{g/ml}$)	^{125}I -SBA bound ($\mu\text{g/ml}$)
Seed coat				
4	8	22	3	21
72	8	11	3	8
120	5	8	3	7
Cotyledon				
4	90	12	8	0
72	160	19	8	0
120	280	47	10	0

can be a purified glycoprotein/polysaccharide or an undefined constituent of a cell extract. In the present study, the assay was characterized and then applied to the cell system in which SBA is the endogenous lectin.

PEG has been used previously in studies on lectins to enhance the specific precipitation of lectin-glycoconjugate complexes. However, competitive inhibition of complex-formation was generally used as an indirect measure of 'receptors' in a cell extract (Carter & Sharon, 1977; Drake & Rosen, 1982; Cooper *et al.*, 1983). PEG has also been used in studies involving antigen-antibody precipitations, including radioimmunoassays for lectins (Harrington *et al.*, 1971; Desbuquois & Aurbach, 1971; Kida & Muller-Eberhard, 1975; Etzler & Borrebaeck, 1980). By using the turbidity assay for measuring lectin-ligand interactions (Van Wauwe *et al.*, 1973; Podder *et al.*, 1974) it has been possible to demonstrate that precipitation of SBA-glycoconjugate complexes could be induced by PEG under conditions in which neither lectin nor glycoconjugate alone had significant effect on turbidity. A limitation of the turbidity assay is that, owing to its low sensitivity, large amounts of lectin and glycoconjugates are required. Further, its use is also limited to a study of interactions between purified components, since the background of non-specific precipitation in cell extracts is potentially high. To compensate for these limitations, a radioaffinity assay based on the use of PEG was developed.

The interaction between ^{125}I -SBA and purified glycoconjugates showed that the lectin exhibits different pH optima of binding with different ligands: binding towards thyroglobulin and fetuin

is optimal at pH 5.0, whereas that towards galactomannan and asialofetuin occurs from pH 5.0 to pH 8.0. These optima may reflect the influence of charge in any interactions between SBA of pI 6.1 and charged glycoproteins such as thyroglobulin (pI 4.9) or fetuin (pI 3.3) or a neutral polysaccharide such as guar-gum galactomannan. When the radioaffinity assay was applied to soya beans, the seed coat and cotyledons were each extracted to prepare subcellular fractions composed of soluble components, namely cytoplasmic, luminal and those released from non-covalent attachment to cell walls and membranes, and particulate matter, namely membranes, cell walls and polymers insoluble in the extraction media. Trichloroacetic acid treatment was used to divide the soluble components broadly into predominantly glycoproteins (insoluble) and polysaccharides (soluble). Detergent treatment (Triton X-100) was used to distinguish between membrane components and cell-wall polymers in the particulate fractions.

A variety of soya-bean components were found to have the potential to interact with ^{125}I -SBA. In the seed coat, trichloroacetic acid-soluble components that decreased in amounts after germination constituted the principal class of glycoconjugates able to bind to the lectin. Soya beans contain galactomannans located in the endosperm, which in soya-bean seeds is restricted to a layer of cells closely associated with the seed coat (Whistler & Saarnio, 1957; Aspinall & Whyte, 1964; Dey, 1978). It is possible that the decrease in ^{125}I -SBA binding to the trichloroacetic acid-soluble glycoconjugates is correlated with hydrolysis of the galactomannan, since the polysaccharide is known to act as a primary storage reserve, that is rapidly

degraded after emergence of the radicle (McCleary & Matheson, 1974; Halmer *et al.*, 1978; Bewley & Halmer, 1980–1981; Leung & Bewley, 1983). Detergent-insoluble particulate matter in the seed coat is most probably also polysaccharide, related either to cell-wall fragments or to galactomannan storage reserves.

In the cotyledons, several components have the capacity to interact with SBA, including trichloroacetic acid-soluble and trichloroacetic acid-insoluble glycoconjugates and membrane components. Since SBA is located principally in the cotyledons of dry seeds (Pueppke *et al.*, 1978) and remains at high concentrations at least up to 120h development, it is possible that the lectin-binding components in the cotyledons could represent 'receptors' of physiological significance. However, since cell and tissue compartmentalization has been disrupted through homogenization, the radioaffinity assay can only provide a measure of the cell components potentially able to interact with SBA *in vivo*. Whether the interactions are of physiological significance depends on the subcellular localization of the lectin and its potential 'receptors' during development and their availability for binding to one another within an intact system.

These results on soyabean and studies on the interactions between spore coat polysaccharides and the discoidins (Barondes, 1981; Cooper *et al.*, 1983; Barondes *et al.*, 1983) emphasize the potential importance of cell components other than glycoproteins as endogenous 'receptors' to lectins of eukaryotic cells.

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