IMMUNOLOGICAL EVIDENCE FOR STRUCTURAL SIMILARITY AMONG LECTINS FROM SPECIES OF THE SOLANACEAE

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1. Introduction

Lectins appear to be a heterogenous group of proteins displaying wide differences in molecular size, sub-unit composition and carbohydrate content [1]. Little is known about the primary structure of lectins, but amino acid sequence data recently reported indicate a large measure of structural homology between several lectins from leguminous plants [2–5].

Primary sequence analysis is highly time-consuming however, and a more rapid, if less precise, means of investigating structural homology is required. We have used immunological cross-reactivity to study structural relationships of lectins from the family Solanaceae. The serological evidence presented shows that all of the Solanaceous lectins studied so far are related, and that the degree of similarity is consistent with the phylogenetical relationships of these genera. Moreover, lectins from all of the seeds had antigenic properties in common which were distinct from lectins occurring in other tissues of the same plants.

2. Materials and methods

2.1. *Plants*

Tomato (*Lycopersicon esculentum*) and green sweet pepper (*Capsicum annuum*) fruits and potato (*Solanum tuberosum*) tubers were purchased from a local greengrocer. Potato fruits were obtained from the Scottish Plant Breeding Station, Roslin, Midlothian, U.K. Seeds of *Datura stramonium* and *Nicandra physaloides* were purchased from Thomson and Morgan Ltd., Ipswich, U.K. Fruits of *D. stramonium* and *N. physaloides* were obtained from plants grown from seeds in a Botany Department greenhouse.

2.2. *Preparation of soluble homogenates*

Tissues were homogenised using a pestle and mortar in 10 vol. of 50 mM sodium phosphate, pH 7.2, containing 0.15 M NaCl (PBS) and left overnight at 4°C. The homogenate was first strained through Miracloth, then centrifuged at 40 000 × g for 1 h at 4°C. The supernatant was diluted or concentrated by ultrafiltration, as described, before use.

2.3. *Preparation of immunoglobulin*

Antiserum to the *Datura* lectin was prepared as previously described [6]. An immunoglobulin fraction was obtained from the antiserum by DEAE-cellulose fractionation [7] followed by precipitation by adding ammonium sulphate to 42% saturation. The precipitate obtained was collected by centrifugation (30 000 × *g*; 30 min), redissolved in PBS, and dialysed overnight in PBS. The volume was adjusted to that of the original volume of serum used.

2.4. *Immunofluorescent staining of sections*

Sections (20–30 μm) were cut from tissue protected with Tissuetek (Miles Lab.) using a cryostat. Sections were fixed in 10% formaldehyde in PBS for a minimum of 30 min at 22°C. After washing with a large excess of PBS for 10 min, the sections were treated either with an immunoglobulin fraction prepared from anti (*Datura* lectin) serum, or an immunoglobulin fraction prepared in an identical way from non-immune rabbit serum. After incubation for 15 min (22°C) the sections were washed as before in PBS and then incubated for 15 min at 22°C with Rhodamine-conjugated goat anti-(rabbit immunoglobulin G) solution (1 vol. of conjugate from Miles Laboratories with 20 vol. of PBS). Finally the sections were washed with PBS and examined under green light (546 nm)

with a Vickers Photoplan fluorescence microscope fitted with a 590 nm barrier filter.

2.5. *Assays*

Protein was measured by the method of Lowry et al. [8] with bovine serum albumin (Sigma type V) as standard. Lectin activity was estimated using glutaraldehyde-fixed human (group O) erythrocytes [6].

3. Results

3.1. *Double diffusion analysis*

The soluble protein fractions of the following tissues were prepared and the specific lectin activity of each (in parentheses, expressed as units $\times 10^{-3}$/mg protein) determined: *Datura* seeds (4.0), tomato seed (0.1), the juice inside tomato fruits (10.0), the juice inside potato fruits (5.1), potato seeds (0.27), potato tubers (10.1), *Capsicum* seeds (0.34) and *Nacandra* seeds (0.02). The pericarps of both tomato and potato fruits also possessed appreciable activities but as the specific activities of the juices within the pericarps were higher, these were used in the experiments described. No lectin activity was found in homogenates prepared from the pericarp of *Datura* or *Capsicum*, at a concentration of 1 mg/ml protein.

The antiserum to *Datura* lectin was placed in the centre well of double diffusion plates and tested with soluble protein extracts from other Solanaceous sources. The findings are summarised in table 1. Extracts of tomato fruit juice, tomato seeds, potato fruit juice, potato seeds, potato tubers, *Capsicum* seeds and *Nicandra* seeds all cross-reacted with a corresponding extract from *Datura* seeds or purified *Datura* lectin. Moreover, all seed extracts (except *Datura*) formed lines of identity with each other when placed in adjacent wells in any combination, but each exhibited spur formation with soluble proteins from either potato tubers, potato fruit juice or tomato fruit juice. Similarly, extracts from tomato juice, potato juice and potato tubers formed lines of identity with each other, but formed spurs with any of the seed extracts.

None of the extracts formed precipitation lines when non-immune rabbit serum was used instead of anti (*Datura* lectin) serum. In contrast, PHA from *Phaseolus vulgaris* (Wellcome) gave two lines of precipitation with both immune and non-immune serum.

3.2. *Precipitation curves*

The ability of anti (*Datura* lectin) immunoglobulin to complex with *Datura* lectin as a function of immunoglobulin concentration is illustrated in fig.1. The immunoglobulin preparation was also highly active towards the lectins from tomato fruits and potato tubers. However, no precipitation of *Capsicum* seed lectin or *Nicandra* seed lectin was obtained, over the range of immunoglobulin concentrations used.

It was necessary to use a purified immunoglobulin fraction for these experiments since non-immune rabbit serum caused marked inhibition of *Datura* lectin activity. A purified immunoglobulin fraction from non-immune serum had no detectable effect on *Datura* lectin activity.

3.3. *Saccharide inhibition*

The lectins under study were inhibited to different

Table 1
Double diffusion analysis of solanaceous lectins

	Datura seed	Tomato seed	Potato seed	*Nicandra* seed	*Capsicum* seed	Tomato fruit juice	Potato fruit juice	Potato tuber
Datura seed	–	cr	cr	cr	cr	cr	cr	cr
Tomato seed	cr	–	id	id	id	cr	cr	cr
Potato seed	cr	id	–	id	id	cr	cr	cr
Nicandra seed	cr	id	id	–	id	cr	cr	cr
Capsicum seed	cr	id	id	id	–	cr	cr	cr
Tomato fruit juice	cr	cr	cr	cr	cr	–	id	id
Potato fruit juice	cr	cr	cr	cr	cr	id	–	id
Potato tuber	cr	cr	cr	cr	cr	id	id	–

Double diffusion was carried out in agarose (1% in PBS) with anti (*Datura* lectin) serum in centre wells. For each combination of adjacent homogenates, cross-reaction (cr) or formation of a line of identity (id) is indicated

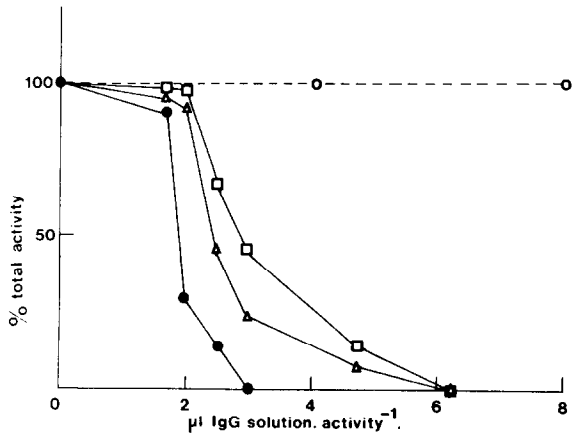

Fig.1. Precipitation of lectins with anti-(*Datura* lectin) immunoglobulin. Lectin preparations were prepared from soluble homogenates by precipitation with ammonium sulphate added to 50% saturation, redissolving in PBS and diluting with PBS to give the same titre for each preparation. Each preparation (100 μl) was mixed with varying volumes of immunoglobulin prepared from anti-(*Datura* lectin) serum, and the volume made up to 200 μl with PBS. After overnight incubation at 4°C, 250 μl of 4.2% polyethylene glycol in 10 mM sodium borate buffer, pH 8.4, was added. After a further hour at 4°C, the precipitates were collected by centrifugation and the supernatants assayed for residual lectin activity. The origins of the lectin preparations were *Datura* seeds (●), tomato fruit juice (△), potato tubers (□), and *Capsicum* or *Nicandra* seeds (○). For further details, see section 2.

extents by *N*,*N*′-diacetylchitobiose or a mixture of oligomers of *N*-acetylglucosamine (table 2). The *Datura* lectin, the tomato fruit juice lectin and the lectins from potato fruit juice and tubers were all strongly inhibited by *N*-acetylglucosamine oligomers. The lectins from tomato seed and potato seed were also inhibited, but less markedly so. The lectins from *Capsicum* and *Nicandra* were not inhibited at all under those conditions.

None of the lectin activities examined were inhibited by the following monosaccharides at a final concentration of 33 mM: glucose, mannose, galactose, fucose, *N*-acetylglucosamine, *N*-acetylgalactosamine.

3.4. *Immunofluorescent staining of sections*

Sections were prepared from stems of *Datura*, tomato, potato, *Capsicum* and *Nicandra*. In every case, the sections exhibited intense fluorescent staining when treated with anti (*Datura* lectin) immunoglobulin solution followed by Rhodamine-conjugated anti-immunoglobulin. (Corresponding sections treated with immunoglobulin prepared from non-immune serum showed only weak, non-specific fluorescence and some autofluorescence associated with chloroplasts.) The specific fluorescence was mainly localised in the cytoplasm, and was particularly associated with the limiting membranes of the cells (fig.2).

4. Discussion

The seeds of *Datura stramonium*, the tubers of the potato, and the juice within the fruit of the tomato are the tissues from which lectin activity can most readily be extracted from these species. These three lectins have all been at least partially characterised [9–12] and their immunological cross-reactivity has been briefly noted elsewhere [12].

Table 2
Inhibition of lectin activity by *N*-acetylglucosamine oligomers

Source of homogenate	*N*,*N*′-diacetylchitobiose	Mixed GlcNAc oligomers
Datura seed	25	<1
Tomato seed	50	25
Potato seed	50	50
Nicandra seed	100	100
Capsicum seed	100	100
Tomato fruit juice	50	<1
Potato fruit juice	25	<1
Potato tuber	50	<1

Lectin activities from the sources mentioned were assayed in the presence of *N*,*N*′-diacetylchitobiose (Sigma) at a final concentration of 3.5 mM, or a mixture of oligomers of *N*-acetylglucosamine prepared from chitin and described elsewhere [9]. Results are expressed as a percentage of the appropriate control to which no sugar was added

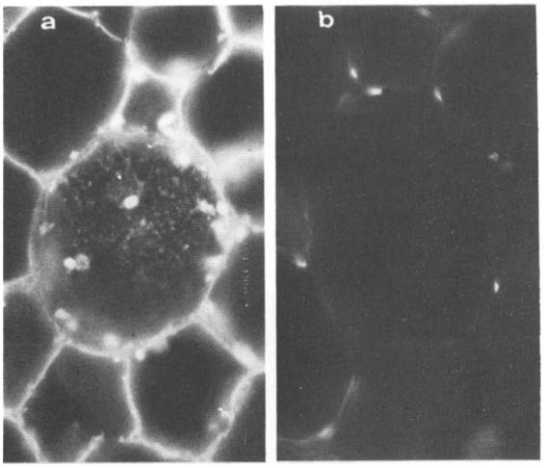

Fig.2. Immunofluorescent staining of *Nicandra* stem sections. (a) *Nicandra* stem sections treated with specific immunoglobulin from anti-(*Datura* lectin) serum followed by Rhodamine-conjugated antiimmunoglobulin. (b) Control using immunoglobulin from non-immune serum. For further details, see section 2. Fluorescence in chloroplasts was partly due to autofluorescence of chlorophyll. Magnification ~×430.

Using an antiserum to the lectin from *Datura stramonium* seeds, we have shown that lectin-containing extracts from the seeds of tomato, *Capsicum* and *Nicandra*, as well as potato tissues other than the tubers, cross-react with the *Datura* lectin. The quantitative precipitation curves suggest that the previously described lectins from *Datura*, potato and tomato are very closely related indeed, as only twice as much immunoglobulin was required to remove the potato and tomato lectins from solution as that for the *Datura* lectin. *Capsicum* and *Nicandra* lectins, however, were unaffected over the range of immunoglobulin concentrations used.

The double-diffusion studies enable the lectins to be classified into two antigenic groups. The lectins from the seeds of Solanaceous plants other than *Datura* are antigenically indistinguishable by the anti (*Datura* lectin) serum but are all antigenically distinct from the non-seed lectins. These antigenically similar seed lectins can, however, be further sub-divided within the group, and also from non-seed lectins on the basis of saccharide specificity. Non-seed lectins are readily inhibited by low concentrations of *N*-acetylglucosamine oligomers. The seed lectins of potato and tomato are also inhibited, but less readily, and the seed lectins of *Capsicum* and *Nicandra* are not affected at all. If the serological lines of identity between the seed lectins reflect structural homology, the differences in saccharide specificity may be taken as an indication of further divergence. By these criteria, the tomato and potato lectins appear to be more closely related to each other (and to *Datura*) than either is to *Nicandra* or *Capsicum*. Such a conclusion is consistent with the accepted taxonomic relationships of these genera [13].

Hankins and co-workers have used specific antisera to investigate structural similarities among lectins from leguminous seeds [14]. It is noteworthy that they were also able to divide the lectins studied into two groups on the basis of serological reactions. Lectins with a specificity for sugars with a galactose-type configuration cross-reacted with each other, but were antigenically unrelated to lectins with a specificity for mannose or glucose.

As lectins are generally isolated from seeds (and usually from leguminous plants), it is noteworthy that of the Solanaceous plant tissues we have examined, only *Datura stramonium* seed homogenate possessed a high lectin activity towards human erythrocytes. Homogenates prepared from tomato, *Capsicum* or potato seeds had comparatively little lectin activity, and that of *Nicandra* was barely detectable. However, relative lectin activity between different sources need not necessarily be an indication of the relative amounts of total lectin protein. Low lectin activity might result, for example, from the extraction of the lectin bound to saccharide inhibitors; alternatively the cell-type used to detect the activity might be inappropriate to the saccharide specificity of the lectin. Some evidence in favour of the latter interpretation is that the *Nicandra* lectin could be detected by double diffusion with anti (*Datura* lectin) serum at a concentration lower than the minimum required for agglutination of erythrocytes, whereas tomato fruit juice, potato tuber or potato fruit lectins only formed precipitation lines when used at a concentration thirty-fold or more greater than the minimum required to cause agglutination. Similarly, other seed lectins (apart from *Datura*) were able to form precipitation lines in double diffusion against anti (*Datura* lectin) serum at lectin activities significantly below the minimum required of non-seed lectin from potato or tomato under identical conditions. Furthermore, differences in saccharide specificity have been directly demonstrated by inhibition studies with *N*-acetylglusosamine oligomers. It is likely that if erythrocytes from a wide

variety of animals were compared, another source of erythrocytes would be found more suitable than human for detecting *Nicandra* lectin and possibly also the other seed lectins.

It is probable that all Solanaceous lectins originated from a common ancestor. The differences between lectins from different species could be accounted for by mutations occurring during the couse of evolution, but an alternative mechanism is required to explain the antigenic non-identity of lectins from tissues of the same plant. Two possibilities may be suggested. Firstly, that gene duplication occurred, and subsequently, after divergence, one gene came to be expressed in the seed, and the other gene came to be expressed in other tissues. Alternatively, tissue-specific lectins might arise from post-translational modification of the same gene product; for example, by differences in the degree or type of glycosylation.

It is of particular interest that all the Solanaceous seed lectins examined here, excluding *Datura*, are antigenically identical with respect to the anti-(*Datura* lectin) serum. This suggests that one part of the molecular structure of these lectins has been highly conserved throughout the evolution of those species, and implies that the same structure is necessary for the function of the molecule. Since we have demonstrated that the saccharide specificity of these lectins is not highly conserved, the inference is that precise saccharide specificity may not be essential to the function of these lectins. This inference is at variance with the generally held view that the function of a lectin must be related to saccharide specificity, upon which all (unproven) suggested functions for lectins have been based.

Acknowledgement

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