Purification and Some Properties of a Lectin from the Fruit Juice of the Tomato (*Lycopersicon esculentum*)

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In the tomato (*Lycopersicon esculentum*) plant, the fruit juice was found to be the richest source of agglutinating activity. The lectin responsible could be inhibited by oligomers of *N*-acetylglucosamine, and this property was exploited to purify the lectin by affinity adsorption on trypsin-treated erythrocytes. The lectin is a glycoprotein that cross-reacts immunologically with the lectin from *Datura stramonium* (thorn-apple).

Plant lectins are carbohydrate-binding protein or glycoprotein agglutinins that constitute up to 3% of the total protein of some seeds (Sharon & Lis, 1972). Lectins are usually detected in, and subsequently isolated from, dried seeds. This is because of the convenience of obtaining and working with seeds, and not necessarily because lectins are absent from other tissues. Indeed, lectins have been found in various other tissues, including potato (*Solanum tuberosum*) tubers (Marinkovitch, 1964), soya-bean (*Glycine max*) roots (Pueppke & Bauer, 1976) and the fruit skins of several species of the cucumber family (Sabnis & Hart, 1978).

Boyd & Reguera (1949) reported that tomato (*Lycopersicon esculentum*) seeds possessed no agglutinating activity towards human A-, B- or O-type erythrocytes. Yeoman *et al.* (1978), however, found agglutinating activity towards glutaraldehyde-fixed human erythrocytes in extracts from all parts of the tomato plant examined, including the seeds. This apparent inconsistency might simply reflect a difference between fresh and fixed cells used in the assay. Moreover, it is possible that Yeoman and co-workers were detecting a non-lectin agglutinin such as has been reported to occur in tomato extracts (Howard *et al.*, 1972).

I report here that tomato seed homogenate does indeed possess agglutinating activity towards fresh human erythrocytes, but the specific activity is very low. Other parts of the tomato plant were examined, and it was found that by far the highest specific agglutinating activity occurs in the juice of ripe fruits. The lectin responsible has been isolated and partially characterized.

Materials and Methods

Seeds and plants of the tomato (variety Ailsa Craig) were kindly given by Professor M. M. Yeoman, Department of Botany, University of Edinburgh, The King's Buildings, Edinburgh EH9 3JH, Scotland, U.K. Purified oligomers of *N*-acetylglucosamine (*NN'*-diacetylchitobiose, *NN'N''*-tri-acetylchitobiose and *NN'N''N'''*-tetra-acetylchitotetraose) were kindly given by Miss H. Marcan, Department of Botany, University of Hull, Hull, North Humberside, U.K. Monosaccharides were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. Cellobiose and sucrose were from BDH Chemicals, Poole, Dorset, U.K.

Antiserum to *Datura* lectin was obtained by injecting a rabbit with purified *Datura* lectin (Kilpatrick & Yeoman, 1978) as described by Kilpatrick *et al.* (1979). Rabbit antiserum to human serum was obtained from Travenol Laboratories, Costa Mesa, CA, U.S.A.

The lectin was purified as follows. The juice from ripe tomatoes (1 kg selected from a local green-grocer) was centrifuged (1000g; 10 min) in an MSE Superminor centrifuge at room temperature (22°C) to remove any seeds or contaminating debris. The clear juice (85 ml containing 170 mg of protein) obtained was mixed with an equal volume of 0.15 M-NaCl/0.1 M-sodium phosphate, pH 7.0. To this solution, solid (NH₄)₂SO₄ was slowly added to 50% saturation. After the solution had been left overnight at 4°C, the precipitate was collected by centrifugation (40000g; 1 h) at 4°C in an MSE SS50 centrifuge. The precipitate was then resuspended in

neutral saline (0.9% NaCl adjusted to pH 7.0 with Na_2HPO_4) and dialysed against 5 litres of neutral saline for 48 h at 4°C, with a change of saline after 24 h. Material that failed to redissolve was removed by centrifugation (40 000 g; 30 min) and discarded. The supernatant solution (50 ml) was added to trypsin-treated human (group-B) erythrocytes (20 ml packed volume), and the mixture was shaken gently at room temperature (22°C) for 15 min. The lectin-agglutinated erythrocytes were then harvested (1000 g; 5 min) at room temperature. The cells were then washed three times with 5 vol. of neutral saline. (It is necessary to use relatively vigorous shaking to divide the agglutinated cells into smaller clumps for efficient washing.) The lectin was recovered from the washed erythrocytes by resuspending the cells in 20 ml of a mixture of *N*-acetylglucosamine oligomers prepared as previously described (Kilpatrick & Yeoman, 1978). After being shaken for 5 min at room temperature, the cells were harvested (1000 g; 10 min) and the supernatant retained. A further 20 ml of the preparation of *N*-acetylglucosamine oligomers was added to the cell pellet and the procedure was repeated. The combined supernatants were then dialysed against 5 litres of neutral saline for 5 days at 4°C with changes of saline every 24 h. The dialysed preparation (approx. 40 ml) was first concentrated to about 2 ml by ultrafiltration in an Amicon cell fitted with a PM-30 membrane filter, then applied to a column (36 cm × 2.5 cm diam.) of Sephadex G-200 equilibrated with neutral saline. The column was eluted with neutral saline at a flow rate of 18 ml/h; 4.5 ml fractions were collected. The fractions containing greatest lectin activity were pooled to provide the purified lectin preparation.

The estimation of molecular weight by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed as described by Weber & Osborn (1969). The gels were stained either for protein by using Coomassie Brilliant Blue (Weber & Osborn, 1969), or for carbohydrate by the method of Glossman & Neville (1971), modified by using basic Fuchsin in acid ethanol (Borzynski *et al.*, 1972) instead of Schiff's reagent.

Periodate oxidation was carried out by incubating a suitable dilution of tomato lectin in neutral saline (200 µl) with an equal volume of 2% NaIO_4 in neutral saline at 4°C in the dark for 16 h. The NaIO_4 was then removed by dialysis against 2 litres of neutral saline at 4°C. A control consisting of neutral saline without NaIO_4 was treated in an identical manner.

Lectin (agglutinating) activity was assayed as previously described (Kilpatrick *et al.*, 1978) by using trypsin-treated human erythrocytes. Activity was taken to be the reciprocal of the titre (Kilpatrick & Yeoman, 1978). Human (group-B) erythrocytes were trypsin-treated by suspension (25%, v/v) in

neutral saline containing 0.25% trypsin ('1:250'; Difco Laboratories, West Molesey, Surrey, U.K.). After incubation at 37°C for 5 min, the cells were harvested (1500 g; 5 min) with an MSE Superminor centrifuge at room temperature. The cells were subsequently washed four times in neutral saline before use.

Protein concentration was measured by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma) as standard. Carbohydrate concentration was determined by the phenol/ H_2SO_4 method of Dubois *et al.* (1956), with glucose as standard.

Results

Various tissues from tomato (*Lycopersicon esculentum*) plants were homogenized in phosphate-buffered saline and tested for agglutinating (lectin) activity towards untreated or trypsin-treated human erythrocytes. Agglutinating activity was generally about four times greater with trypsin-treated cells, which were subsequently used as a routine. As Table 1 shows, agglutinating activity was found in all the extracts tested, but by far the highest specific activity was found in the juice of ripe fruits. The juice was therefore chosen as the source from which to investigate the active factor.

The agglutinin was precipitated from the fruit juice with $(\text{NH}_4)_2\text{SO}_4$ added to 50% saturation, and, after redissolving in, and dialysing against, neutral saline, the fraction was tested in the presence of a selection of saccharides (Table 2). None of the monosaccharides tested had any effect, but oligomers of *N*-acetylglucosamine were potent inhibitors. *N*-Acetylglucosamine monomer (66 mM) and cello-

Table 1. *Specific agglutinating activity of tomato tissue extracts*

All samples were homogenized in 0.15 M-NaCl/0.05 M-sodium phosphate, pH 7.2, by using a mortar and pestle, except the fruit juice, which was simply diluted into an equal volume of the same buffer. After overnight extraction at 4°C, the homogenates were strained through a layer of Miracloth before being assayed for agglutinating (lectin) activity and for protein. One unit of activity is the amount giving a titre of 1 under standard assay conditions. For further details, see the Materials and Methods section.

Tissue homogenate	Specific activity (units/mg of protein)
Dried seed	32
Seed from ripe fruits	67
Leaf	8
Stem	53
Fruit skin	914
Fruit juice	9846

Table 2. *Saccharide inhibition of tomato lectin activity*

Solutions of the above sugars were ultrafiltered through an Amicon PM-30 membrane to remove contaminating agglutinins (Kilpatrick *et al.*, 1978), then added to the standard agglutination assay to give the final concentration shown above. Results are expressed as a percentage of a control to which an equal volume of saline (0.9% NaCl) was used instead of sugar solution.

Sugar	Concn. (mM)	Percentage of control activity
Fucose	33	100
Galactose	33	100
Glucose	33	100
Mannose	33	100
<i>N</i> -Acetylglucosamine	66	100
<i>N</i> -Acetylgalactosamine	66	100
Cellobiose	33	100
Sucrose	33	100
<i>NN'</i> -Diacetylchitobiose	2	50
<i>NN'N''</i> -Triacetylchitotriose	1	50
<i>NN'N''N'''</i> -Tetra-acetylchitotetraose	1	6.25

biose (33mM) were without effect, but *NN'*-diacetylchitobiose was able to decrease the activity by 50% at a concentration of only 2mM. Moreover, amongst those oligomers tested, the inhibition increased with their molecular size. In view of this narrow saccharide specificity, it seemed reasonable to assume that the agglutinin responsible was a lectin.

The lectin was isolated from a fruit juice extract in three steps. The lectin was first precipitated with 50%-satd. $(\text{NH}_4)_2\text{SO}_4$. This resulted in a 4-fold increase in specific activity. Secondly, the lectin was adsorbed on to trypsin-treated erythrocytes and subsequently eluted with a mixture of *N*-acetylglucosamine oligomers. This step had the unfortunate side-effect of contamination by haemoglobin (and possibly other erythrocyte proteins) leaking from damaged cells. This circumstance necessitated the final step in which the lectin was separated from haemoglobin on Sephadex G-200. The lectin was eluted in a single peak, corresponding to a mol.wt. of 200 000 on the basis of precalibration of the column with molecular-weight markers (Andrews, 1965). Fractions containing lectin activity were also assayed for carbohydrate; the profile obtained was similar to that for lectin activity, indicating that the lectin might be a glycoprotein. Fractions with greatest lectin activity were pooled and found to contain 800 μg of protein. A 42-fold increase in specific activity over the fruit-judge extract was obtained with a 20% recovery. The lectin was estimated to contain equal amounts of protein and carbohydrate, and was able to agglutinate trypsin-treated erythrocytes at a concentration of 0.05 μg of protein/ml.

The purified lectin was incubated with sodium dodecyl sulphate and 2-mercaptoethanol and subjected to electrophoresis in polyacrylamide gels. A major protein band was obtained with a mobility corresponding to a mol.wt. of 74 000. A faint pro-

tein band (mol.wt. 63 000) was also present. A parallel gel stained for carbohydrate showed a single band corresponding to the major protein band.

As the purification method involved the risk of contamination by erythrocyte proteins, that possibility had to be investigated. An erythrocyte suspension was mixed with 5 vol. of water and shaken at room temperature for 30 min. After removal of particulate matter by centrifugation, the extract was subjected to Sephadex G-200 chromatography in the same way as the tomato lectin preparation. The erythrocyte fraction thus obtained was then subjected to electrophoresis at the same time as the tomato lectin preparation. A major protein band with a mobility corresponding to a mol.wt. of 65 000 was obtained. In addition, there were several faint bands of approx. mol.wt. 40 000, 27 000 and 24 000. When a parallel gel was stained for carbohydrate, no bands were detected. A mixture of lectin and erythrocyte fraction yielded two major protein bands (and one carbohydrate band, corresponding to the polypeptide of lower mobility), confirming their separate identities.

The purified tomato lectin was stable to incubation at 65°C for 30 min, but at 100°C its activity decayed with a half-life of approx. 1 min. When assayed in the presence of disodium EDTA (2mM) or trisodium citrate (17mM), the lectin showed no decrease in activity. The lectin was sensitive, however, to both Pronase and periodate. Overnight incubation with 1% NaIO_4 abolished the activity of a lectin sample; after incubation with Pronase (0.2%) for 2 h at 37°C followed by 2 min at 100°C, a lectin sample exhibited only 25% of the activity of a control to which Pronase had been added immediately before the boiling-water step.

The tomato lectin was subjected to double diffusion (Ouchterlony, 1958) with rabbit antiserum raised against the lectin from *Datura stramonium*

(thorn-apple). A single precipitin line was obtained, but it did not form a line of identity with *Datura* lectin placed in an adjacent well. There was no reaction between the tomato lectin and non-immune serum, nor did it react with a sample of commercial rabbit antiserum to human serum used as a further control.

Discussion

The tomato lectin was purified 42-fold by a procedure in which the key step was affinity chromatography using erythrocytes. The final preparation was apparently not appreciably contaminated by erythrocyte proteins, as the control erythrocyte preparation described gave a different polypeptide pattern after electrophoresis, and did not react with the carbohydrate stain. The purified lectin is a glycoprotein containing 50% carbohydrate. The subunit mol.wt. based on electrophoretic mobility is therefore likely to be considerably overestimated (Segrest & Jackson, 1972). The estimation of mol.wt. based on gel filtration might also be inaccurate (Andrews, 1965). Nevertheless, the much higher value obtained by gel filtration indicates that the lectin molecule in its native form consists of more than one of the reduced polypeptide subunits detected by electrophoresis. The minor (mol.wt. 63 000) polypeptide associated with the lectin might represent a trace impurity (possibly of erythrocyte origin), or it might be a minor subunit or fragment of the type associated with concanavalin A (Wang *et al.*, 1971).

The properties of the tomato lectin are very similar to those of the lectin from *Datura stramonium*. The latter is also a glycoprotein, with a very similar saccharide specificity (Kilpatrick & Yeoman, 1978). It is unaffected by EDTA and stable at 65°C, but not at 100°C (Kilpatrick *et al.*, 1978). It can agglutinate untreated human erythrocytes at a concentration of less than 1 µg/ml (Kilpatrick, 1979), and its activity towards trypsin-treated cells can be detected at around 0.1 µg/ml (D. C. Kilpatrick, unpublished work). The immunological cross-reactivity between the two lectins confirms their relatedness. The only other lectin to have been isolated from a plant of the family Solanaceae, the potato lectin (Marinkovitch, 1964; Allen *et al.*, 1978), is another glycoprotein with a saccharide specificity like that of the tomato and *Datura* lectins. The potato and *Datura* lectins also exhibit immunological cross-reactivity (D. A. Ashford & A. K. Allen, personal communication). It is likely that all three solanaceous lectins are closely related in structure, but if the tissue distribution of the lectins within their respective plants is different, it might be an indication that they have evolved different functions.

One can only speculate about the function of the tomato lectin. Complex carbohydrates containing *N*-acetylglucosamine occur widely in the plant kingdom (Pusztai, 1964; Sharon & Lis, 1979), and it is possible the lectin acts as a storage protein giving nourishment to the seeds. Another possible function is suggested by the presence of chitinase in other parts of the tomato plant (Pegg & Vessey, 1973). Chitin is known to be a cell-wall component of many fungi (Crook & Johnstone, 1962) including some which infect tomato plants. The lectin may be involved in protecting the seeds from fungal attack.

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