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# Isolation and properties of three lectins from mistletoe (*Viscum album* L.)

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Three lectins have been isolated from an extract of mistletoe (*Viscum album*) by affinity chromatography on partially hydrolysed Sepharose and human immunoglobulin–Sepharose. The lectins differ in molecular weight and sugar specificity (lectin I, mol.wt. 115000, D-galactose-specific; lectin II, mol.wt. 60000, both D-galactose- and *N*-acetyl-D-galactosamine-specific; lectin III, mol.wt. 50000, *N*-acetyl-D-galactosamine-specific). All three lectins react with human erythrocytes without specificity for the A, B and O blood groups. In contrast with abrin and ricin the mistletoe lectins cannot be divided into 'toxins' and 'haemagglutinins'.

Since ancient times the toxic seeds of jequirity (*Abrus precatorius*; Leguminosae) and castor bean (*Ricinus communis*; Euphorbiaceae) have been used in folk medicine for the treatment of different diseases. It is now known that both plants contain toxic lectins with D-galactose specificity. Likewise, preparations from mistletoe (*Viscum album*; Loranthaceae) have been used therapeutically for thousands of years, and this plant also has a toxic lectin with galactose specificity.

A comparison of the well-investigated castor-bean and jequirity lectins ricin and abrin with the lectin(s) of mistletoe seemed worthwhile. After having investigated the possibilities of purification of mistletoe lectin(s) by use of affinity chromatography (Franz *et al.*, 1977; Ziska *et al.*, 1978), we now report the isolation and identification of three different lectins from mistletoe and discuss possible relationships between the lectins of the three plants.

## Materials and methods

The lectins were isolated from ground plant material from mistletoe grown on the locust tree (*Robinia pseudoacacia*) by using affinity chromatography. Lectin I was isolated using acid-treated agarose as carrier (Ziska *et al.*, 1978). The non-adsorbed material was applied to a column (2.6 cm × 30 cm) of immunoglobulin–Sepharose and the column was washed with 0.15 M-NaCl. The affinity adsorbent had been prepared by coupling human immunoglobulin, mainly immunoglobulin G, to CNBr-activated Sepharose 4B (20 mg/ml of gel).

Lectin II was eluted with 0.2 M-D-galactose in 0.15 M-NaCl. The fractions containing the haemagglutinating protein were pooled, dialysed against water and freeze-dried. After the lectin II had been displaced from the column, lectin III was eluted with 0.2 M-glycine/HCl buffer, pH 2.6. The fractions containing active material were pooled and neutralized with $Na_2CO_3$. The neutral solution was put on a column (1.5 cm × 30 cm) of Sepharose–*N*-(6-aminohexanoyl)-β-D-galactosamine (Gordon *et al.*, 1972). The column was washed and the lectin III was eluted with glycine/HCl buffer, pH 2.6. The haemagglutinating proteins were pooled, dialysed against water and freeze-dried. Haemagglutinating activity was determined in a 1% suspension of washed human erythrocytes by using the microtitrator of Takatsy (1967). Haemagglutination-inhibition tests were performed as follows: to 0.05 ml of a 2-fold serial dilution of carbohydrate, 0.025 ml of lectin solution with a haemagglutinating activity of 4 units was added. After incubation at 37°C for 1 h, 0.025 ml of a 2% human erythrocyte suspension was added. After 2 h at 37°C the degree of agglutination was estimated. Stock solutions of 0.2 M-carbohydrate in 0.15 M-NaCl were prepared. T.l.c. for determination of the molecular weight was performed with Sephadex G-200 (Superfine grade). Proteins with known molecular weights were used as markers [cytochrome *c* (12400), chymotrypsinogen (25700), ovalbumin (45000) and bovine serum albumin (68000)].

For polyacrylamide-gel disc electrophoresis the method of Maurer (1968) was used; 7.5% polyacrylamide gels were used in a 35 mM-β-alanine/acetic acid buffer of pH 4.3. The molecular weights of the lectin chains were obtained in 10%-polyacrylamide gels in the presence of 1% sodium



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dodecyl sulphate. The lectins were treated with 1% sodium dodecyl sulphate and 1% β-mercaptoethanol (Weber & Osborn, 1969). Staining was performed with Coomassie Brilliant Blue R250 (for proteins) and periodic acid/Schiff reagent (for glycoproteins; Fairbanks *et al.*, 1971).

Anti-(lectin I) serum was prepared as follows. The purified lectin I was treated with 1% formaldehyde in 0.1 M-phosphate buffer, pH 7.5, for 3 days at 37°C, and excess formaldehyde was removed by dialysis against 0.85% NaCl solution. Immunization of a rabbit was initiated by subcutaneous injection of 0.5 mg of protein in complete Freund's adjuvant, followed by 0.5 mg booster doses with Freund's incomplete adjuvant intracutaneously every week. The antiserum used was obtained 6 weeks after the first immunization.

## Results

By using affinity chromatography with different carriers we have been able to isolate three lectins from crude extracts of mistletoe. The isolation, properties and chemical modification of D-galactosyl-specific lectin I was published previously (Ziska *et al.*, 1978, 1979). Only lectin I is bound by partially-hydrolysed Sepharose. After separation of the lectin I, the extract contains two lectins, which are adsorbed by the carbohydrate moieties of the immobilized immunoglobulins (immunoglobulins G, A or M). Lectin II can be eluted from this column by 0.2 M-D-galactose solution. Lectin III cannot be eluted with 0.5 M-D-galactose solution, but with 0.2 M-glycine/HCl buffer, pH 2.6. For further purification the solution of lectin III is applied to a column of Sepharose–*N*-(6-aminohexanoyl)-β-D-galactosamine and then eluted with glycine/HCl buffer (Scheme 1).

Lectins I and II each give a single band on disc electrophoresis, and lectin III shows a major band and three faint bands (Fig. 1). After reduction with 2-mercaptoethanol, each of the lectins shows two bands in polyacrylamide-gel electrophoresis in the presence of 0.1% sodium dodecyl sulphate. Disc electrophoresis of mixtures of lectins I and II, and I and III showed that they are not identical (Fig. 2).

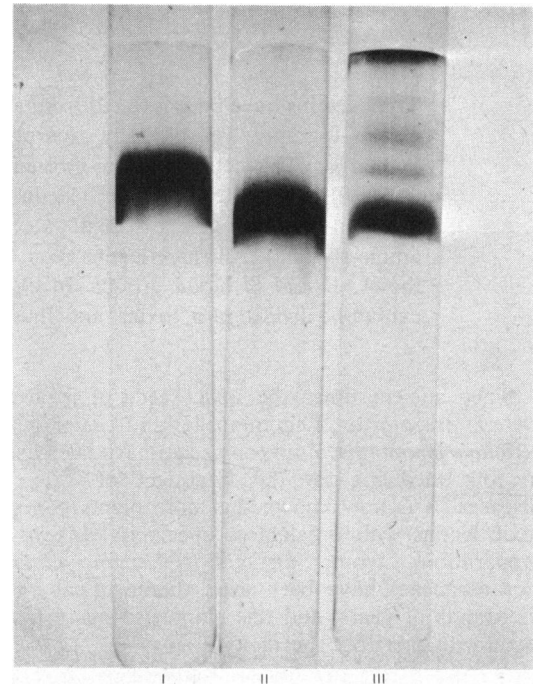


**Fig. 1.** ***7.5%-Polyacrylamide-gel electrophoresis (pH 4.3) of mistletoe lectins I, II and III***

Electrophoresis was performed at 4 mA per tube for 2.5 h. The gels were stained with Coomassie Brilliant Blue R250.

Mistletoe (100 g) mixed with 750 ml of 0.15 M-NaCl
↓ → Discard precipitate
Acid-treated Sepharose column
↓ → Elute with 0.2 M-D-galactose: **lectin I**
Effluent
↓
Immunoglobulin–Sepharose column
↓
Elute with 0.2 M-D-galactose: **lectin II**
↓
Elute with glycine/HCl, pH 2.6
↓
Neutralized
↓
Sepharose&*N*-(6-aminohexanoyl)-β-D-galactosamine column
↓
Elute with glycine/HCl, pH 2.6: **lectin III**

Scheme 1. *Flow sheet for the isolation of mistletoe lectins I, II and III*

Thus lectin I, in agreement with Luther *et al.* (1980), consists of two identical non-covalently bound subunits.

Lectins II and III and also the subunit of lectin I contain two chains linked by a disulphide bridge. Each of them show two bands on polyacrylamide-gel electrophoresis after reduction with 2-mercaptoethanol. The lectins differ in their molecular weights (Table 1). All three lectins agglutinated human erythrocytes with similar potency, but when tested against erythrocytes from other species, marked differences in potency were observed (Table 2). The sugar specificities of the three lectins were different (Table 3). Agglutination of human erythrocytes by lectin I was strongly inhibited by D-galactose and α- and β-methyl galactosides. Lectin II was inhibited with both *N*-acetyl-D-galactosamine and the galactosides, whereas lectin III was inhibited strongly only by *N*-acetyl-D-galactosamine.

All the six chains of the three lectins are different.

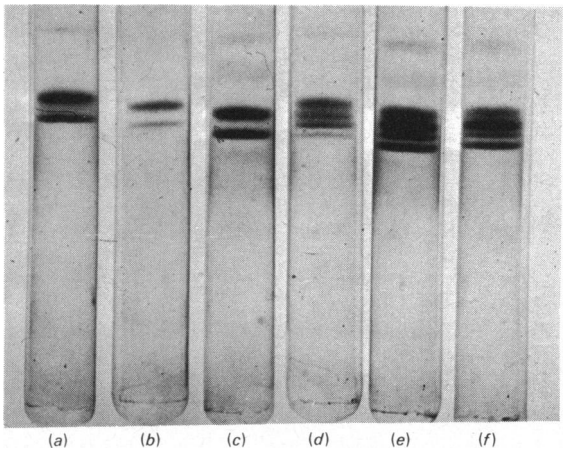


**Fig. 2.** *Chains of mistletoe lectins produced by β-mercaptoethanol treatment and analysed on sodium dodecyl sulphate/polyacrylamide gels*

The conditions are described in detail in the Materials and methods section. (*a*) lectin I; (*b*) lectin II; (*c*) lectin III; (*d*) mixture of lectins I and II; (*e*) mixture of lectins I and III; (*f*) mixture of lectins I, II and III.

However, lectins II and III cross-react with anti-(lectin I) antibody in radial immunodiffusion. Lectins I, II and III are stained by periodate/Schiff reagent.

Four of the chains of the three lectins can be separated, but there could be up to six different chains present. No mitogenic effect could be demonstrated on guinea-pig lymph-node cells, since the cells were killed within 6h by lectin I at a concentration of 25–80μg/ml ($10^6$ cells) (Scherbaum *et al.*, 1978). Lectin I inhibits protein synthesis in a lysate of rabbit reticulocytes with an $ID_{50}$ (concentration giving 50% inhibition) of 2.6μg/ml. This effect is enhanced ($ID_{50}$ 0.21μg/ml) if the lectin is first reduced with 2-mercaptoethanol. The lectin also inhibits protein synthesis by BL8L cells in culture. The $ID_{50}$ is 7ng/ml and the potency

Table 2. *Haemagglutination titres*

All lectin solutions used were diluted to a titre of 1:32 and made to react against human erythrocytes of group A.

| Source of erythrocytes | Mistletoe lectin ... | Titre I | II | III |
|---|---|---|---|---|
| Human | | 1:32 | 1:32 | 1:32 |
| Goat | | 1:2 | 0 | 1:32 |
| Sheep | | 0 | 0 | 1:2 |
| Rabbit | | 1:32 | 1:64 | 1:2 |
| Mouse | | 1:8 | 1:16 | 1:16 |
| Dog | | 1:32 | 1:16 | 1:8 |
| Cow | | 1:8 | 0 | 0 |
| Horse | | 0 | 1:4 | 1:4 |
| Pig | | 1:32 | 1:32 | 1:8 |
| Guinea pig | | 1:16 | 1:64 | 1:32 |

Table 3. *Inhibition by sugars*

The inhibition concentrations are expressed as μmol of carbohydrate/ml needed for complete inhibition of 4 haemagglutination units.

| Sugar | Lectin ... | Concentration I | II | III |
|---|---|---|---|---|
| D-Galactose | | 12 | 12 | 200 |
| *N*-Acetyl-D-galactosamine | | 200 | 6 | 3 |
| Methyl α-D-galactoside | | 12 | 6 | 200 |
| Methyl β-D-galactoside | | 6 | 3 | 50 |

Table 1. *Some properties of the mistletoe lectins*

| Lectin | Mol.wt. | Carbohydrate specificity | Blood-group specificity | No. of chains | Mol.wts. of chains |
|---|---|---|---|---|---|
| I | 115000 | D-Galactose | None | 4 | 34000 and 29000 |
| II | 60000 | D-Galactose/*N*-acetyl-D-galactosamine | None | 2 | 32000 and27000 |
| III | 50000 | *N*-Acetyl-D-galactosamine | None | 2 | 30000 and 25000 |

decreased after reduction of the lectin (Stirpe *et al.*, 1980).

## Discussion

Crude mistletoe extract contains three lectins with different molecular weights and differences in specificity. Disc electrophoresis showed that lectins II and III are not simple monomers of lectin I.

All three of the isolated lectins agglutinate human erythrocytes and react with immunoglobulins. A single mistletoe lectin described by Luther *et al.* (1980) may not be homogenous, because they used glutaraldehyde-fixed erythrocytes for the isolation of the lectin. Neither crude mistletoe extract nor solutions of purified lectins I, II and III show any specificity for human blood groups. Therefore all three lectins from mistletoe bind to human erythrocytes. These results are in contrast with the findings of Luther *et al.* (1973), who detected a B-blood-group specificity in mistletoe extracts. Immobilized immunoglobulins are useful for the affinity chromatography of mistletoe lectins (Franz *et al.*, 1977). When applied in conjunction with partially hydrolysed Sepharose, it is possible to fractionate the three lectins. Insolubilization of the immunoglobulins can be achieved in three ways: (1) cross-linking with glutaraldehyde; (2) heat aggregation; (3) fixation on CNBr-activated Sepharose. Pretreatment with neuraminidase is not necessary for the immunoglobulin to bind the lectins.

Lectin I from mistletoe was shown previously to be highly toxic (Stirpe *et al.*, 1980). Preliminary results indicate that lectins II and III are also toxic. The mistletoe lectins, like those from *Abrus precatorius* and *Ricinus communis*, comprise species with either two (lectins II and III) or four (lectin I) chains. It has yet to be determined whether the different chains of the three mistletoe lectins functionally correspond to the A- and B-chains of abrin and ricin (Olsnes & Pihl, 1976). It is clear, however, that the mistletoe lectins cannot be classified as either toxic or haemagglutinating species.

Viscotoxins, toxic peptides comprising 46 amino acids, extracted from mistletoe, have been investigated by Samuelsson (1973). It would be interesting to see whether the primary sequences described for the viscotoxins also appear in the mistletoe lectins.

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