Isolation and Comparison of Galactose-binding Lectins from *Abrus precatorius* and *Ricinus communis**

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SUMMARY

The agglutinins present in the seeds of *Abrus precatorius* and *Ricinus communis* have been separated from the toxins, abrin and ricin, and extensively purified. The procedure involves ion exchange chromatography on DEAE- and CM-cellulose columns, affinity chromatography on Sepharose 4B columns, and sucrose gradient centrifugation.

The purified agglutinins which possessed almost no toxic activity were found by analytical ultracentrifugation to have molecular weights of 134,000 (abrus agglutinin) and 120,000 (ricinus agglutinin). After treatment with sodium dodecyl sulfate and β-mercaptoethanol the agglutinins were split into four peptide chains, as revealed by polyacrylamide gel electrophoresis.

The binding of the agglutinins and the toxins to human erythrocytes was inhibited by galactose or galactose-containing carbohydrates, like lactose. Equilibrium dialysis experiments indicate that the agglutinins possess two binding sites for lactose, while the nonagglutinating toxins, abrin and ricin, have only one binding site.

The agglutinins as well as the toxins were found to consist of polypeptide chains of nearly the same size (30,000 to 35,000). Abrin and ricin consist of only two polypeptide chains, one of which is involved in the binding to cells. The agglutinins consist of four polypeptide chains, only two of which appear to be involved in the binding to cell surfaces. The data suggest that, within one kind of seed, the polypeptide chains involved in the binding of the agglutinins and the toxins to cell surfaces are identical or very similar.

Although numerous agglutinins or lectins[1] are known, only comparatively few have been isolated in pure form and their structure studied (for review see Refs. 1-3). It appears that most of the lectins exert their agglutinating activity by way of binding to carbohydrate residues on the cell surface (2). The recent finding that malignant cells are often more easily agglutinated by lectins than the corresponding normal cells (2-4) and the possibility that this selective agglutination might be

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[1] The term lectin is used also for proteins that bind to cells without the formation of cell aggregates (see "Discussion"). Thus, the term includes the toxins abrin and ricin.

used in cancer therapy has stimulated work on the structure and properties of lectins.

Lectins were first discovered in extracts from the seeds of *Abrus precatorius* L. and *Ricinus communis* L. (5-7), and such extracts were widely used in early immunological research (8-10). The high toxicity of the preparations used was originally attributed to their hemagglutinating activity (5, 6). More recent work has indicated that the toxic and the agglutinating properties of such extracts belong to separate proteins (11-16). Thus, the highly purified toxins abrin and ricin, which are present in the seeds of *A. precatorius* and *R. communis*, respectively, have very low agglutinating activity and, as we have earlier suggested (15, 16), the toxicity of purified agglutinin preparations from these seeds may be due to contamination by traces of residual toxins.

In previous papers we have isolated and studied the properties of the toxins abrin and ricin (15, 16). In the present paper we have purified two nontoxic agglutinating lectins from *A. precatorius* and *R. communis*. These lectins have been characterized with respect to molecular weight, subunit structure, and sugar-binding specificity, and their properties have been compared with those of abrin and ricin.

EXPERIMENTAL PROCEDURE

Materials—Semen jequiriti (the seeds of *A. precatorius*) was obtained from Norsk Medisinaldepot, Oslo. Castor beans (the seeds of *R. communis*) were kindly provided by Deutsche Rizinus-Oelfabrik Boley & Co., Krefeld-Uerdingen, West Germany. DE52 cellulose and CM52 cellulose (Whatman) were obtained from E. & R. Balstone Ltd., Springfield, Maidstone, Kent, England. Sepharose 4B was obtained from Pharmacia, Fine Biochemicals, Uppsala, Sweden. [1-^{14}C]Lactose (20 Ci per mole) was obtained from The Radiochemical Centre, Amersham, England.

Preparation of Crude Lectins—The procedures used for the extraction of lectins were the same as those previously used for the extraction of abrin and ricin (15, 16), except that the defatted castor beans were extracted with 0.14 M NaCl, rather than with distilled water. The solubilized material was dialyzed against distilled water and then, in the case of the abrus extract, against 10 mM Tris-HCl (pH 7.7) for 24 hours. In the case of the ricinus extract, the dialysis was carried out against 5 mM sodium phosphate (pH 6.5). After dialysis the extracts were centrifuged at 8,000 × *g* for 20 min, the pellets were discarded, and the supernatants were centrifuged once more in the same way. The final supernatants are referred to below as the crude lectins.

Isolation of Abrin and Ricin—Abrin and ricin were isolated from the crude lectin extracts as described earlier (15, 16).

Preparation of Antisera to Abrin and Ricin—Due to the high toxicity of the intact toxins, immunization of rabbits was initiated with formaldehyde-treated toxins. The immunization was carried out as described earlier (15, 16). Complement was inactivated by incubating the sera at 56° for 30 min.

Polyacrylamide Gel Electrophoresis—A solution of protein was treated with sodium dodecyl sulfate and submitted to polyacrylamide gel electrophoresis as described earlier (17, 18). In some cases the proteins were treated with 1% β-mercaptoethanol at room temperature for 1 hour before the electrophoresis.

Analytical Ultracentrifugation—The centrifugation was carried out in a Beckman-Spinco model E analytical ultracentrifuge at 20° with rotor speed of 10,000 rpm, as described by Olsen *et al.* (19). The samples were dissolved in 0.1 M NaCl and 10 mM Tris-HCl (pH 7.7). The partial specific volume was assumed to be 0.73 ml per g and the starting concentration was 0.5 to 1.5 mg per ml.

Hemagglutination Tests—Increasing amounts of protein were mixed with 1.6×10^7 thoroughly washed human erythrocytes, blood group B⁻, in 1 ml of 10 mM Tris-HCl (pH 7.7) in 0.15 M NaCl. In experiments with agglutinins the mixtures were incubated at room temperature for 9 hours and then the sedimented erythrocytes were resuspended. One drop of the solution was examined under the microscope. One unit of hemagglutinating activity was defined as the amount of lectin per ml needed to give clearly visible aggregation of human erythrocytes under these conditions.

In experiments with abrin and ricin, which in low concentrations do not spontaneously agglutinate cells, agglutination was studied by adding, after 1 hour, 5 μl of specific antisera against the toxins and examination after 8 more hours of incubation.

Toxicity Test—Various protein fractions were injected intraperitoneally into mice weighing 25 $\pm$ 3 g and LD₅₀ was measured as described earlier (15, 16).

Equilibrium Dialysis—The different lectins were concentrated by precipitation in the presence of 80% saturated ammonium sulfate, dissolved in a small volume, and dialyzed against 50 mM sodium phosphate (pH 7.1) and 0.02% sodium azide. The concentrated lectins contained 5 to 15 mg of protein per ml as measured by the absorbance at 280 nm. Equilibrium dialysis (20) was carried out with 200-μl samples which were dialyzed against 50 mM sodium phosphate (pH 7.1), containing varying amounts of unlabeled α-lactose and 17 nCi of [¹⁴C]lactose per ml. The dialysis was carried out at room temperature for 18 to 20 hours, then 25-μl samples were removed from the solution inside and outside of the dialysis bags and radioactivity as well as absorbance at 280 nm was measured in each sample. Control experiments showed that equilibrium was reached after 6 to 10 hours. Protein concentrations were determined using the following extinction coefficients ($E_{1\,cm}^{1\%}$) for the different protein fractions: abrin, 15.9; abrus agglutinin, 14.6; ricin, 11.8; ricinus agglutinin, 11.7; and Fraction Q, 11.2. The extinction coefficients were measured by weighing the lyophilized and desiccated proteins.

RESULTS

Isolation of Abrus Agglutinin—Crude abrus lectin, extracted as described under "Experimental Procedure," was purified first by chromatography on a DEAE-cellulose column. The protein was applied to the column in the presence of 30 mM NaCl in 10 mM Tris-HCl (pH 7.7) under which conditions only a minor part of the material was not adsorbed. The adsorbed proteins were
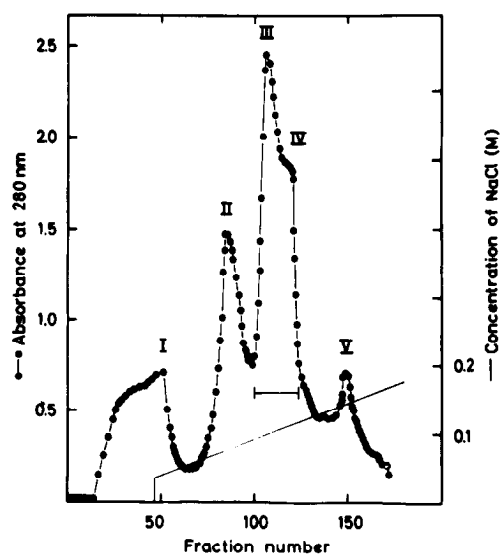

Fig. 1. DEAE-cellulose chromatography of proteins extracted from semen jequiriti. Crude lectin (600 ml) (see "Experimental Procedure"), was applied to a column, 2.6×40 cm, of DE52 cellulose equilibrated with 30 mM NaCl in 10 mM Tris-HCl (pH 7.7) and eluted with a 1600-ml linear gradient from 30 to 180 mM NaCl in the same buffer. Fractions (14 ml) were collected and the absorbance was measured.

TABLE I

Toxicity and hemagglutinating activity of different abrus fractions

Purification step	Fraction number	Hemagglutinating activity	Toxicity
		units/μg protein	*LD₅₀/μg of protein*
DEAE-cellulose chromatography (Fig. 1)	49 (I)	0	1.6
	86 (II)	0.1	6.6
	108 (III)	60	1.1
	119 (IV)	40	0.9
	149 (V)	3	0.3
Sepharose 4B chromatography (Fig. 2)	12 (VI)	0	<0.1
	25 (VII)	100	1.1
CM-cellulose chromatography (Fig. 3)	96 (VIII)	100	0.1
	110 (IX)	100	1.0
	120 (X)	10	10.0
Sucrose gradient centrifugation (Fig. 4)	12 (XI)	100	<0.005
	16 (XII)	100	0.005
	20 (XIII)	60	0.20

eluted with a linear NaCl gradient in the same buffer. As shown in Fig. 1 the proteins were eluted as four peaks, two of which were not separated. In agreement with previous findings (15) the second peak (*II*) was found to represent the toxic protein abrin (Table I), whereas most of the hemagglutinating activity was present in the double *Peaks III* and *IV*.

In the further purification advantage was taken of the fact that abrus agglutinin has a high affinity to Sepharose 4B (15, 21). The material in *Peaks III* and *IV* was pooled and applied to a Sepharose 4B column from which it was eluted with galactose as shown in Fig. 2. A number of contaminating proteins did not bind to the column and were removed by this procedure. However, since abrin also binds to a Sepharose 4B column and is
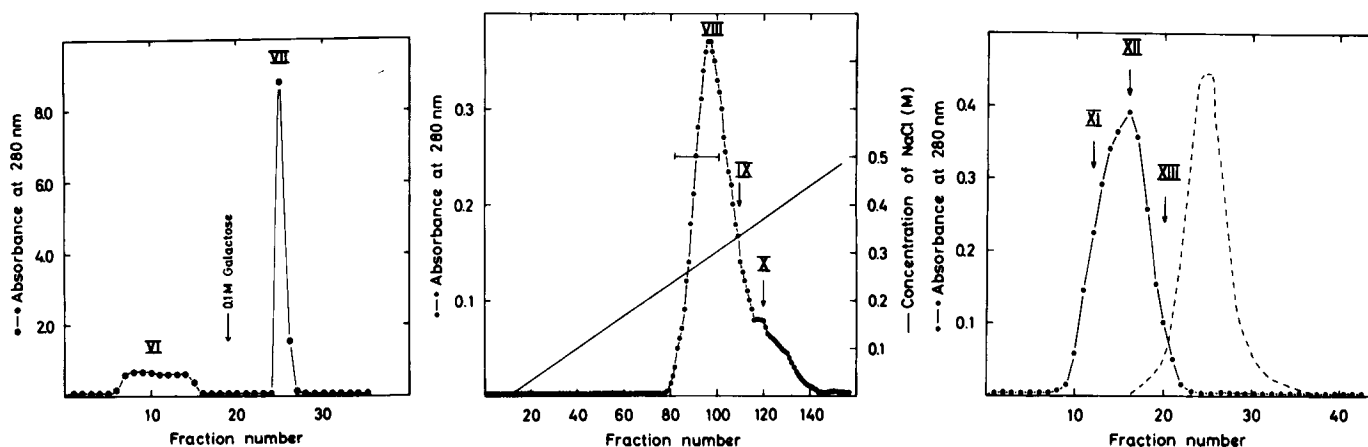
FIG. 2 (*left*). Affinity chromatography of abrus agglutinin on a Sepharose 4B column. The fractions (indicated with a *bar* in Fig. 1) were pooled and about half of the material was applied to a Sepharose 4B column, 2.6 × 20 cm, equilibrated with 10 mM Tris-HCl (pH 7.7). The column was washed with 100 ml of the same buffer and the lectin was eluted with 0.1 M D(+)-galactose in the same buffer at a speed of 40 ml per hour. Fractions (14 ml) were collected and the absorbance was measured.

FIG. 3 (*center*). CM-cellulose chromatography of abrus agglutinin. Agglutinin eluted from the Sepharose 4B column (Fig. 2, Fraction VII) was dialyzed against 25 mM sodium phosphate (pH 6.0) and then adsorbed on a CM52 column, 2.6 × 40 cm, equilibrated with the same buffer. The lectin was eluted from the column with a 1600-ml linear NaCl gradient (from 0 to 0.50 M

NaCl) in the same buffer at a speed of 20 ml per hour. Fractions (12 ml) were collected and the absorbance was measured.

FIG. 4 (*right*). Sucrose gradient centrifugation of abrus agglutinin. The fractions (indicated with a *bar* in Fig. 3) were pooled and concentrated by affinity chromatography on a Sepharose 4B column under similar conditions as in Fig. 2. One milliliter of the material eluted with 0.1 M D(+)-galactose was layered onto a 5 to 15% sucrose gradient made up in 0.15 M KCl, 1 mM MgCl$_2$, and 10 mM triethanolamine (pH 7.5). The gradient was centrifuged in rotor SW 40 at 198,000 × g_{av} for 24 hours. After centrifugation 0.3-ml fractions were collected by puncturing the bottom of the tube. Each fraction was diluted twice with H$_2$O and the absorbance at 280 nm was measured. The *dashed line* indicates the position of abrin sedimented under the same conditions in a parallel gradient.

eluted by galactose (15), this procedure fails to remove the traces of toxin which contaminated the agglutinin. To separate the two proteins, the material was chromatographed on a CM52 column as shown in Fig. 3. It is apparent from Table I that a fraction was obtained (VIII) which possessed a very high hemagglutinating activity but had a low toxicity in mice.

In attempts to remove the last traces of toxin, the material in Fig. 3 (indicated with a *bar*) was concentrated and submitted to sucrose gradient centrifugation. It is evident (Fig. 4) that the material sedimented as one symmetrical peak ahead of abrin. Studies on the toxicity of the different fractions showed (Table I) that as much as 200 μg of the more rapidly sedimenting material (Fraction XI) were not lethal to mice, while 5 μg of the slowly sedimenting material (Fraction XIII) were definitely toxic. The former fraction possessed the highest agglutinating activity (Table I). Thus, upon progressive purification of crude abrus agglutinin the toxicity concomitantly decreased and the highly purified abrus agglutinin was virtually nontoxic.

Isolation of Lectins from Ricinus communis—Crude ricinus lectin (see "Experimental Procedure") was further purified by chromatography on a CM52 column. As shown in Fig. 5 the adsorbed proteins were eluted as five peaks. The peak denoted D contained the highest concentration of the toxic protein ricin, whereas most of the hemagglutinating activity was present in Peaks E and F (Table II), in agreement with our previous findings (16).

The ricinus agglutinin, like the abrus agglutinin, was further purified by affinity chromatography on a Sepharose 4B column (Fig. 6). The proteins which did not bind to the column (Fraction G) possessed no hemagglutinating activity (Table II). Fraction H possessed strong hemagglutinating activity but still had considerable toxic activity. The material eluted from the Sepharose column with galactose was adsorbed to a DE52 cellulose column, and eluted with a NaCl gradient (Fig. 7). As

shown in Table II the material with the highest hemagglutinating activity (Fractions J and K) was only slightly toxic to mice.

We then tried to remove the last traces of toxin by submitting the material (indicated with a *bar* in Fig. 7) to sucrose gradient centrifugation (Fig. 8). Again the rapidly sedimenting fraction of the peak (the ascending limb) possessed a high agglutinating activity and very low toxicity, while the more slowly sedimenting material (Fraction N) possessed appreciable toxicity (Table II). Hence, as in the case of abrus agglutinin, the toxic effect disappeared upon purification of the agglutinin. The finding in Table II that removal of the toxic activity was not correlated with an increase in the specific agglutinating activity shows that the toxic contaminants removed were present only in trace amounts.

The data in Fig. 5 show a protein (*Peak C*) eluting in front of the main ricin peak (*D*). This fraction has a very low hemagglutinating activity but a high toxicity (Table II). When the material in *Peak C* (indicated with a *bar* in Fig. 5) was applied to a Sepharose 4B column, most of the protein was bound to the column and could be eluted with galactose in a similar way as the ricinus agglutinin in Fig. 6 (data not shown). The protein was further purified by chromatography on a DE52 column (Fig. 9). Two protein peaks were found, both of which were highly toxic in mice. When analyzed by polyacrylamide gel electrophoresis, Fraction R proved to contain ricin, identical with that present in Fraction D of Fig. 5, whereas the main peak (*Q*) contained ricinus toxin with slightly different properties (see below).

Structure of Lectins—The pure lectins were analyzed by analytical ultracentrifugation and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. It was found (Table III) that the native abrus agglutinin had a molecular weight of 134,000, whereas the agglutinin from *Ricinus communis* had a molecular weight of 120,000. After treatment with sodium
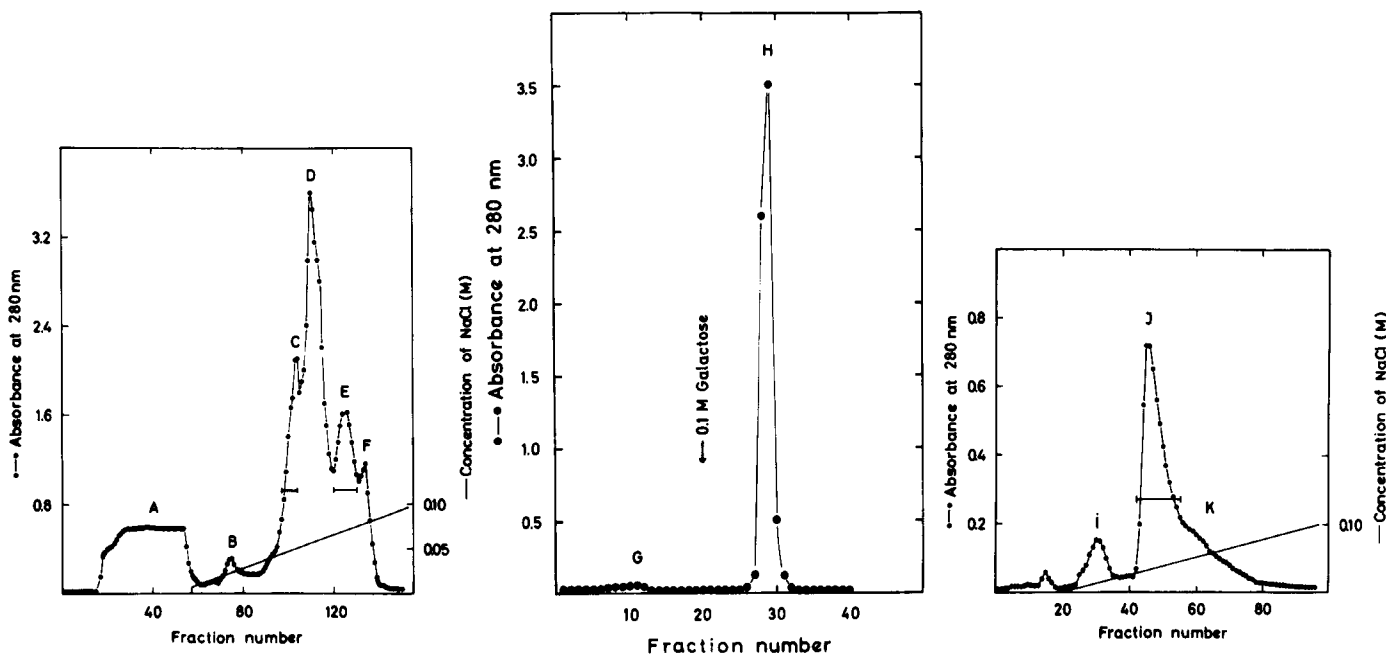
FIG. 5 (left). CM-cellulose chromatography of proteins extracted from castor beans. Crude lectin (600 ml), prepared as described under "Experimental Procedure," was applied to a column of CM52 (Whatman), 2.6 × 40 cm, equilibrated with 5 mM sodium phosphate (pH 6.5), and eluted at a speed of 80 ml per hour with a 1600-ml linear NaCl gradient (from 10 to 100 mM) in the same buffer. Fractions (14 ml) were collected and the absorbance was measured.

FIG. 6 (center). Affinity chromatography of ricinus agglutinin on a Sepharose 4B column. The fractions from Peak E (Fig. 5) (indicated with a bar) were pooled and applied to a column of Sepharose 4B, 2.6 × 20 cm, equilibrated with 10 mM Tris-HCl

(pH 7.7). The column was washed with 100 ml of the same buffer and the lectin was eluted with 0.1 M D(+)-galactose in 10 mM Tris-HCl (pH 7.7) at a speed of 40 ml per hour. Fractions (16 ml) were collected and the absorbance was measured.

FIG. 7 (right). DE52 cellulose chromatography of ricinus agglutinin. Fractions 28 and 29 (Fig. 6) were pooled, dialyzed against 10 mM Tris-HCl (pH 8.5), and about half of the material was applied to a DE52 column, 1.6 × 15 cm, equilibrated with the same buffer. The lectin was eluted from the column with a 500-ml linear NaCl gradient (from 0 to 0.1 M NaCl) in the same buffer at a speed of 20 ml per hour. Fractions (5 ml) were collected and the absorbance was measured.

TABLE II

Toxicity in mice and hemagglutinating activity of different ricinus fractions

| Purification step	Fraction number	Hemagglutinating activity	Toxicity
		units/μg of protein	$LD_{50}/\mu g$ of protein
CM52 cellulose chromatography (Fig. 5)	40 (A)	0.1	0.3
	75 (B)	0.5	1.2
	103 (C)	1	3.3
	109 (D)	3	5.0
	125 (E)	100	1.6
	133 (F)	100	0.3
Sepharose 4B chromatography (Fig. 6)	11 (G)	0	<0.1
	29 (H)	100	1.4
DE52 cellulose chromatography (Fig. 7)	29 (I)	10	3.3
	48 (J)	100	0.1
	62 (K)	100	0.1
Sucrose gradient centrifugation (Fig. 8)	13 (L)	100	<0.005
	16 (M)	100	0.007
	20 (N)	30	0.10
DE52 cellulose chromatography (Fig. 9)	48 (Q)	0.1	8.0
	56 (R)	1.0	6.6
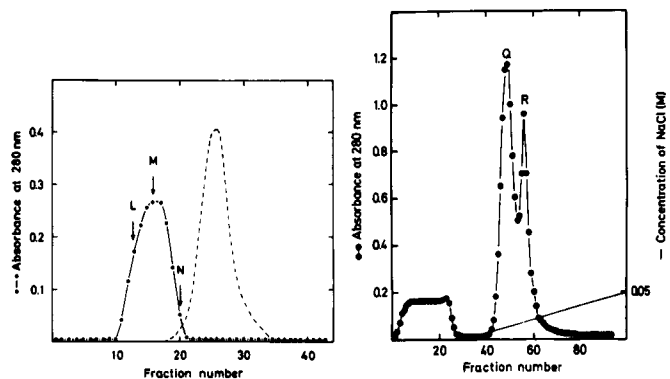
FIG. 8 (left). Sucrose gradient centrifugation of ricinus agglutinin. The material in the fractions indicated with a bar in Fig. 7 was concentrated and analyzed by sucrose gradient centrifugation as described for abrus lectin in Fig. 4. The dashed line indicates the position of ricin sedimented under the same conditions in a parallel gradient.

FIG. 9 (right). DE52 cellulose chromatography of ricin. The material in Peak C (indicated with a bar in Fig. 5) was pooled and applied to a Sepharose 4B column as described in Fig. 6 for ricinus agglutinin. The material eluted with 0.1 M galactose was dialyzed against 10 mM Tris-HCl (pH 8.5) and applied to a DE52 column, 1.6 × 15 cm, equilibrated with the same buffer. The lectin was eluted with a 500-ml linear NaCl gradient (from 0 to 50 mM NaCl) in the same buffer at a speed of 20 ml per hour. Fractions (8 ml) were collected and the absorbance was measured.

TABLE III

Molecular weights of abrus and ricinus lectins and their constituent peptide chains

Lectin	Native[a]	Denatured[b]	Denatured and dissociated[c]
Abrus agglutinin (Fraction VIII)	134,000	69,000 68,000	36,000 35,000 33,000
Abrin (Ref. 15)	63,000	65,000	35,000 30,000
Ricinus agglutinin (Fraction J)	120,000		34,000 31,000
Ricin (Ref. 16)	64,000	65,000	34,000 32,000
Fraction Q	67,000	66,000	34,000

[a] Measurement by analytical ultracentrifugation in 0.1 M NaCl, 10 mM Tris-HCl (pH 7.7).

[b] The lectins were treated with 5% sodium dodecyl sulfate and analyzed by polyacrylamide gel electrophoresis.

[c] The lectins were treated with 5% sodium dodecyl sulfate and 1% β-mercaptoethanol before electrophoresis.

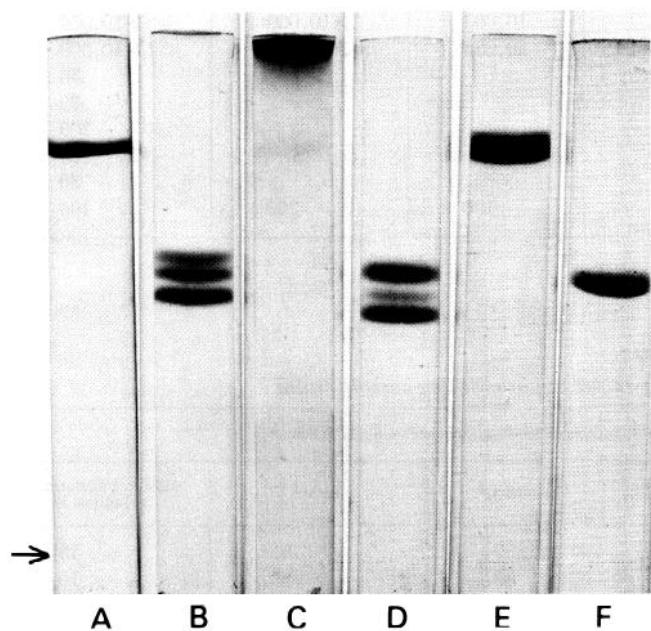
FIG. 10. Polyacrylamide gel electrophoresis of lectins before and after treatment with β-mercaptoethanol. The isolated lectins were treated with 5% sodium dodecyl sulfate in the presence and absence of 1% β-mercaptoethanol and subsequently analyzed by polyacrylamide gel electrophoresis in 13% gels containing 0.1% sodium dodecyl sulfate. A, abrus agglutinin; B, abrus agglutinin treated with β-mercaptoethanol; C, ricinus agglutinin; D, ricinus agglutinin treated with β-mercaptoethanol; E, Fraction Q; and F, Fraction Q treated with β-mercaptoethanol. The *arrow* indicates the position of the tracking dye (bromphenol blue) at the end of the electrophoresis.

dodecyl sulfate, the abrus agglutinin was split into two components with molecular weights of 68,000 and 69,000 (Fig. 10A). In the gel shown the two proteins were not separated, but, in other experiments where the gels were run for a longer period of time, two closely moving bands were found (data not shown).

On treatment with β-mercaptoethanol, the abrus agglutinin was converted into three bands moving at rates corresponding to molecular weights of 36,000, 35,000, and 33,000 (Fig. 10B). In the gel shown here, the fastest moving component appears to be

present in about twice as high amount as the other two. The relative amount of the two heavier chains differed from one preparation to another, and, when different fractions as shown in Fig. 3 were analyzed, it was found that the material which eluted first contained more of the chain with molecular weight of 36,000, whereas that eluting later contained more of the chain with molecular weight of 35,000. The amount of the lightest chain (mol wt 33,000) remained constant. It thus appears that two agglutinins differing in the heavy chains are present in the seeds of *A. precatorius* and that they are not separated under the conditions used.

In accordance with findings by other authors (22) the ricinus agglutinin did not move into gels under these conditions (Fig. 10C), but after treatment with β-mercaptoethanol it moved as two main bands corresponding to molecular weights of 31,000 and 34,000 (Fig. 10D). A faint band was always found between the two main bands. The reason why the ricinus agglutinin sticks to the top of the gel after treatment with sodium dodecyl sulfate alone is obscure. However, since it readily moves into the gel after additional treatment with β-mercaptoethanol, the reason could be that upon denaturation of the toxin with sodium dodecyl sulfate intermolecular S—S bonds are formed by oxidation of reactive —SH groups.

The native protein shown in Fig. 9, Fraction Q, was found to have a molecular weight of 67,000 (Table III), and almost the same value was found when the protein was analyzed by polyacrylamide gel electrophoresis (Fig. 10E). After treatment with β-mercaptoethanol, this protein moved as a single band corresponding to a molecular weight of 34,000. The material proved to have a toxicity almost identical with that of ricin. We have been able to separate the fractions into two different peptides having properties similar to those of the A and the B chain of ricin, and have shown that per intact molecule the protein contains one binding site for lactose (see below). It therefore appears that this protein is a variant of ricin, which differs only from the ricin discussed previously in having a slightly more heavy A chain.

The results together with our previous data on abrin and ricin demonstrate that different lectins present in the seeds of *A. precatorius* and *R. communis* all consist of peptide chains of nearly the same size.

Carbohydrate Specificity of Lectins—The effect of different mono- and disaccharides on the ability of the lectins to agglutinate erythrocytes was then studied. A lectin which can agglutinate cells spontaneously must be able to bind simultaneously to structures on the surface of at least two cells, thus forming a bridge between the cells. The abrus and ricinus agglutinins are able to agglutinate erythrocytes spontaneously, even in very low concentrations, indicating that they possess at least two binding sites. In similar concentrations, abrin and ricin are not able to agglutinate cells spontaneously, but they do agglutinate erythrocytes if specific antisera are added after the proteins have had time to bind to the erythrocytes (15, 16). This suggests that they are monovalent lectins. It is shown in Tables IV and V that in the absence of added carbohydrates as little as 10 to 100 ng per ml of the different lectins gave a visible agglutination of human as well as rabbit erythrocytes. In agreement with previous data (13, 23, 24) it was found that galactose inhibits agglutination by ricinus agglutinin and that carbohydrates containing a terminal nonreducing galactose residue are even more active. Thus, the agglutination of human erythrocytes by ricinus agglutinin (Table IV) was strongly inhibited by D(+)-galactose, α-D(+)-melibiose, α-methylgalactose, α- and β-lactose,

and also by α-D(+)-fucose. Similar results were obtained with the other lectins studied. The results suggest that these lectins agglutinate human erythrocytes by binding to galactose-containing carbohydrates on their cell surface. In similar experi-ments with rabbit erythrocytes (Table V) the agglutination with ricinus lectins was inhibited by those carbohydrates which inhibited the agglutination of human erythrocytes. On the other hand, the binding of abrus lectins to rabbit erythrocytes could

TABLE IV

Inhibition of lectin-induced agglutination of human erythrocytes by carbohydrates

Sugar added[a]	Concentration of lectin giving visible agglutination[b]				
	Abrin[c]	Abrus agglutinin (Fraction VIII)	Fraction Q[c]	Ricin[c]	Ricinus agglutinin (Fraction J)
None	30	10	30	100	10
D(+)-Glucose	100	10	100	100	10
D(+)-Galactose	10,000	3,000	10,000	10,000	>10,000
D(+)-Mannose	100	30	100	300	100
D(+)-Trehalose	30	100	30	100	300
L(+)-Arabinose	300	100	300	300	1,000
D(−)-Arabinose	30	30	100	100	100
D(+)-Xylose	30	30	100	300	100
α-D(+)-Fucose	300	300	3,000	10,000	10,000
D(−)-Fructose	100	30	100	300	100
D(−)-Ribose	100	30	300	300	300
Maltose	30	100	100	300	100
α-D(+)-Melibiose	1,000	1,000	10,000	>10,000	>10,000
α-Lactose	3,000	3,000	10,000	>10,000	>10,000
β-D(+)-Cellobiose		100			30
D(+)-Mannosamine		30			30
D(+)-Galactosamine		300			100
α-Methyl-D-galactose		300			100
3-O-Methylglucose		100			30
α-Methyl-D-mannoside	100	100	100	300	100

ᵃ Concentration: 10 mM.

ᵇ In nanograms of lectin per ml of erythrocyte suspension.

ᶜ Antiserum, 5 μl, was added 2 hours after the addition of lectin.

TABLE V

Inhibition of lectin-induced agglutination of rabbit erythrocytes by carbohydrates

Sugar added[a]	Concentration of lectin giving visible agglutination[b]				
	Abrin[c]	Abrus agglutinin (Fraction VIII)	Fraction Q[c]	Ricin[c]	Ricinus agglutinin (Fraction J)
None	30	10	30	30	10
D(+)-Glucose	30	10	30	30	10
D(+)-Galactose	300	100	10,000	>10,000	10,000
D(+)-Mannose	100	30	10	100	100
D(+)-Trehalose	30	30	30	100	10
L(+)-Arabinose	100	30	30	30	300
D(−)-Arabinose	100	30	100	100	100
D(+)-Xylose	100	30	100	100	100
α-D(+)-Fucose	300	100	1,000	1,000	1,000
D(−)-Fructose	100	100	30	100	10
D(−)-Ribose	100	10	100	100	100
Maltose	100	100	30	100	30
α-D(+)-Melibiose	300	100	10,000	>10,000	10,000
α-Lactose	300	100	10,000	>10,000	>10,000
β-D(+)-Cellobiose		10			10
D(+)-Mannosamine		30			10
D(+)-Galactosamine		100			30
α-Methyl-D-galactose		100			30
3-O-Methylglucose		10			30
α-Methyl-D-mannoside	100	100	30	30	30
N-Acetyl-D-galactosamine		100	100		100

ᵃ Concentration: 10 mM.

ᵇ In nanograms of lectin per ml of erythrocyte suspension.

ᶜ Antiserum, 5 μl, was added 2 hours after the addition of lectin.

not be inhibited by any of the carbohydrates tested. It is there-fore clear that abrus lectins differ somewhat from the ricinus lectins in their binding specificity.

Blood Group Specificity of Lectins—The ability of the different lectins to agglutinate human red blood cells from different blood groups was then tested. It is clear from Table VI that the abrus lectins agglutinate erythrocytes of blood groups B and O somewhat better than cells from blood group A, in accordance with earlier findings (14). With ricinus lectins no difference was found. On the other hand, when the incubation was carried out in the presence of a low concentration (1 mM) of α-lactose, the amount of both abrus and ricinus lectins necessary to agglutinate erythrocytes from blood groups O and B was lower than that required for the agglutination of erythrocytes from blood group A.

Equilibrium Dialysis of Lectins—Among the carbohydrates tested, lactose was the most efficient inhibitor of hemagglutination. To study the binding of lactose to the lectins, equilibrium dialysis with radioactively labeled lactose was carried out. From the data in Fig. 11 the binding constant for abrus lectins was calculated to be about 8×10^3 liters per mole and that of ricinus lectins about 1.5×10^4 liters per mole. From the data it was deduced that the agglutinins contain two binding sites for lactose per molecule, whereas abrin, ricin, and the material from Fraction Q in Fig. 9 contain only one binding site. After the completion of this work van Wauwe et al. (25) published data indicating that ricinus hemagglutinin has two binding sites for p-nitrophenyl-β-D-galactopyranoside. The binding constant was measured to be 1.65×10^4 liters per mole.

DISCUSSION

In this paper we have used the word lectin (from *legere*, to select (1)) not only for the seed proteins capable of agglutinating cells, but also for related proteins that bind in a similar fashion to cells but are unable to cause spontaneous agglutination. We feel that this use of the term is justified and rational since the agglutinating and nonagglutinating proteins prove to have many features in common.

Previously, we have shown (15, 16) that the toxic lectins abrin and ricin contain only one peptide chain (the B chain) capable of binding to galactose-containing carbohydrates. The present equilibrium dialysis experiments confirm that they only contain one binding site for lactose. The data here presented indicate that the nontoxic abrus and ricinus agglutinins contain two such chains and show that, as expected, they contain two binding sites for lactose. The evidence presented indicates that the different lectins present in one kind of seed may have identical or very similar B chains.

The data presented are consistent with the view that the purified abrus and ricinus agglutinins are nontoxic, and that they consist of two heavy and two light peptide chains. In the case of abrus agglutinins three different peptide chains were found. The data indicate, however, that in any one agglutinin molecule only two different peptide chains are present. This implies that the abrus agglutinin is heterogeneous and consists of two populations of molecules which differ only in their heavy peptide chains. Since the abrus agglutinins are split into two parts by treatment with sodium dodecyl sulfate it appears that the intact agglutinin consists of two complexes with molecular weight of 68,000 to 69,000, held together by weak interactions. Each of these complexes appears to consist of a heavy and a light chain held together by disulfide bonds.

In the case of ricinus agglutinin, a small amount of a third

TABLE VI

Binding of abrus and ricinus lectins to human erythrocytes of different blood groups

Blood group	Sugar added[a]	Concentration of lectin giving visible agglutination[b]				
		Abrin[c]	Abrus agglutinin (Fraction VIII)	Fraction Q[c]	Ricin[c]	Ricinus agglutinin (Fraction J)
A	None	300	100	30	30	10
B	None	30	10	30	30	10
O	None	30	10	30	30	10
A	α-Lactose	>10,000	3,000	>10,000	>10,000	3,000
B	α-Lactose	3,000	300	3,000	3,000	300
O	α-Lactose	3,000	300	3,000	3,000	300

[a] Concentration: 1 mM.

[b] In nanograms of lectin per ml of erythrocyte suspension.

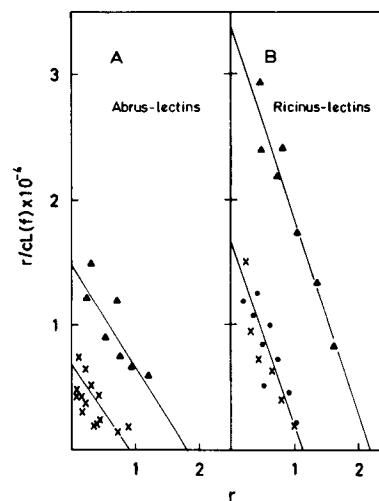[c] Antiserum, 5 µl, was added 2 hours after the addition of lectin.

FIG. 11. Binding of lactose to abrus and ricinus lectins as determined by equilibrium dialysis. Samples consisting of 200 µl of lectin solution were dialyzed against buffer containing different concentration of ^{14}C-labeled lactose, as described under "Experimental Procedure." In these experiments the molecular weights of both abrus agglutinin and ricinus agglutinin were assumed to be 130,000 and that of the other lectins 65,000. r, molar concentration of lactose bound/molar concentration of protein; $cL(f)$, molar concentration of free lactose; ×, abrin, ricin; ▲, abrus agglutinin, ricinus agglutinin; ●, Fraction Q.

peptide chain was always found in accordance with the reports of previous authors (22). It is therefore possible that this agglutinin, like the abrus agglutinin, consists of a mixture of two molecular species. In the case of ricinus agglutinin it can not be excluded that all four peptide chains are held together by disulfide linkages.

The pure toxins abrin and ricin possess a small, but definite, agglutinating activity (15, 16). Since, in spite of considerable efforts, we have not been able to remove this activity from the toxins, we believe that it is not due to contaminating agglutinins but rather to dimers or larger aggregates of the toxins proper. A similar situation may apply in the case of wheat germ lectin, which has only one binding site for N-acetylglucosamine (26), but nevertheless is capable of agglutinating cells.

Ricin has earlier been purified in different laboratories (11-13, 21, 22, 27). There is, however, a striking variability in the

hemagglutinating activity of the different ricin preparations. Thus, Gürtler and Horstmann (22) reported that ricin had a hemagglutinating activity of 1.7 units per mg, whereas the agglutinin had 2.4 units per mg. Nicolson and Blaustein (13) found that ricin had about 10% of the hemagglutinating activity of the ricinus agglutinin. On the other hand, Waldschmidt-Leitz and Keller (12), as well as Tomita et al. (21), found, in accordance with our own data, that ricin has only 1 to 3% of the hemagglutinating activity of ricinus agglutinin. One reason for the discrepancies could be that different conditions of isolation and storage may influence the extent of dimer formation of the toxin in solution. Contamination with agglutinin in some ricin preparations may be another explanation. Conversely, the high toxicity reported for some ricinus agglutinin preparations (13, 21, 27) is probably due to contamination with toxin.

Many agglutinins stimulate DNA synthesis and blastoid transformation in lymphocytes (2). In the case of abrus and ricinus agglutinins, such studies have been hampered by the fact that previous agglutinin preparations contained sufficient amounts of abrin and ricin to kill the lymphocytes. However, preliminary experiments with our highly purified agglutinins indicate that they indeed initiate DNA synthesis in lymphocytes.

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