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COMPARISON OF DEVELOPMENTALLY REGULATED LECTINS FROM THREE SPECIES OF CELLULAR SLIME MOLD

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Summary

Extracts of the cohesive forms of the cellular slime molds *Dictyostelium discoideum*, *Dictyostelium mucoroides* and *Dictyostelium purpureum* contain lectin activity, assayed as hemagglutination activity. The lectin activity from each species binds quantitatively to Sepharose 4B and can be eluted with D-galactose. The resultant purified lectins are abundant proteins representing, in the case of *D. purpureum*, up to 5% of the total soluble protein of cohesive cells. The preparations from each species are similar but distinct in amino acid composition and other properties. Each purified preparation gives rise to two protein bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the major band representing as little at 77% (*D. purpureum*) and as much as 96% (*D. mucoroides*) of the total protein in the two bands. The molecular weights of the pair of bands were different for each species, ranging between about 23 000 and 26 000. The two bands are believed to represent subunits of lectins made up of either one or a combination of these two proteins. The apparent molecular weights of the purified lectin activities determined by sucrose density gradient centrifugation were all in the range of 100 000. *N*-Acetyl-D-galactosamine was a potent inhibitor of the hemagglutination activity of each preparation; but there were some differences in the relative inhibitory potency of a number of other saccharides. Antiserum raised against each preparation, as well as univalent antibody fragments derived from these antisera, reacted best with the antigens to which they were raised; but showed some cross reaction measured both by precipitin reactions and by inhibition of hemagglutination activity of the purified lectins. The differences between the lectins from the different species could be trivial; but they also could be important for defining specific properties of these three species which reliably segregate into colonies of a single species when grown in mixed culture.

Abbreviations: ECT, 1 mM ethylenediamine-tetraacetic acid, 75 mM NaCl, 75 mM KCl, 15 mM Tris-HCl, pH 7.3; SDS, sodium dodecyl sulfate.

Introduction

Cellular slime molds contain polyvalent carbohydrate binding proteins, referred to as lectins, that can be extracted in soluble form and assayed as hemagglutinins [1—3]. Lectin activity is undetectable or barely detectable in extracts of vegetative non-cohesive cells; but is abundant when cells are induced to differentiate to a cohesive state by food deprivation [1—3]. Evidence has been presented that these developmentally regulated lectins [1—7] as well as substances that bind with these lectins [6,8] are present on the surface of cohesive slime mold cells. Available evidence is consistent with the hypothesis that the lectins play a role in cell cohesion [1—10].

When cells of two species of cellular slime molds are mixed in the absence of food they segregate to form distinct colonies each containing thousands of cells of one of the species [11—13]. Whereas some mixing is initially observed, segregation is eventually very marked if not complete. This segregation, frequently called 'sorting out', has been studied mostly with three species of Dictyosteliaceae, *Dictyostelium discoideum*, *Dictyostelium purpureum* and *Dictyostelium mucoroides*. It cannot be attributed to species-specific chemotactic factors since each of these species forms aggregates under the influence of the same chemotactic substance, 3′,5′-cyclic AMP [14]. We have previously raised the possibility that sorting out might be mediated to some extent by species-specific differences in cell surface carbohydrate-binding proteins. We have shown that extracts of cohesive forms of a number of species of slime molds contain measurable lectin activity; and that the relative potency of a series of saccharides in inhibiting hemagglutination activity of these lectins differs somewhat with the extracts of the different species [15]. In the present report we describe the purification of lectins from *D. discoideum*, *D. purpureum* and *D. mucoroides* and compare their properties. We show that they are closely related but distinct proteins by physicochemical and immunological criteria; and that they show some differences in the relative potency of saccharides as inhibitors of their hemagglutination activity. The differences observed could reflect mutations that have no functional significance; but they are consistent with a possible role of these proteins in species-specific sorting out.

Experimental procedures

Growth and differentiation of cells. D. discoideum strain NC-4, established in our laboratory for about 5 years was obtained from Dr. William F. Loomis. *D. mucoroides* (strain 11) and *D. purpureum* (strain 2) established in our laboratory for 4 years, were obtained from Dr. J.T. Bonner. Cells of all species were grown in association with *Klebsiella* on nutrient agar in large aluminium pans as described for *D. discoideum* [16]. The cells were harvested while still in the vegetative phase and differentiated in 16.7 mM sodium-potassium phosphate, pH 6.0, by gyration at room temperature as described [16] for *D. discoideum*. Differentiation was continued for 16 h at which time each species had substantial lectin activity.

Extraction and assay of lectin activity. The differentiated cells were centri-

fuged at 300 $\times g$ for 5 min and suspended at a concentration of $2 \cdot 10^8$ cells/ml in ECT containing 0.3 M D-galactose. The cells were lysed by freezing in liquid nitrogen and thawing under running cold water. The lysate was centrifuged at 90 000 $\times g$ for 75 min and the supernatant was assayed for lectin activity and used for purification.

All the lectins were assayed with formalinized rabbit erythrocytes prepared as described [16]. Assays were done in microtiter V-plates (Cooke Engineering) using serial 2-fold dilutions of the crude extracts of purified lectins. If necessary D-galactose in these materials was removed by dialysis; but in most cases lectin titers were so high that the galactose was diluted to trivial concentrations before the lectin endpoint was reached. Details of this assay and also of the method for determining the concentration of saccharides which inhibit hemagglutination activity by 50% have been presented [16]. Saccharides of the highest available purity were obtained from Pfanstiehl, Sigma or Calbiochem.

Affinity chromatography on Sepharose 4B. All lectins were purified by the same method used for discoidin [16]. To bind the lectins in crude extracts to Sepharose, it is necessary to remove the galactose. To this end, one volume of extract was mixed with 0.5 volumes of Sepharose 4B (Pharmacia Fine Chemicals) that had been equilibrated with ECT. This suspension was then placed in a dialysis bag and dialysed at 4°C overnight, against two baths, each containing 25 volumes of ECT. The contents of the dialysis bag were then layered on top of a 1 l Sepharose 4B column equilibrated with ECT at 4°C. The column was then washed with ECT at approx. 60 ml/h until all unbound material was eluted as indicated by monitoring the absorbance of the eluate at 280 nm. The elution buffer was then changed to ECT containing 0.3 M D-galactose and fractions were collected until all recoverable activity was eluted. Since we wished to make specific antibodies to each of the purified lectins, we reserved a separate Sepharose column for the extracts from each species.

Polyacrylamide gel electrophoresis. This was done in the presence of sodium dodecyl sulfate by the method of Laemmli [17] in a slab gel apparatus with 15% polyacrylamide sample gels. Samples were prepared for electrophoresis by boiling for 10 min in 0.063 M Tris-HCl buffer, pH 6.8, containing 5% β-mercaptoethanol and 1% sodium dodecyl sulfate. Gels were stained in 0.05% Coomassie Blue in 25% isopropanol, 10% acetic acid, 65% H_2O and destained in 10% acetic acid. To estimate relative protein concentration of different bands on the same gel, they were scanned in a densitometer devised and loaned by Dr. J. Garrels, Salk Institute. We scanned several samples containing a range of protein concentrations and determined the relative peak areas by cutting out multiple Xerox copies of each peak and weighing them on a microbalance.

Sucrose gradient centrifugation. Molecular weights of the lectins were estimated by the method of Martin and Ames [18] using sucrose gradients that had been equilibrated with 0.3 M D-galactose. All lectins were run concurrently in a six place swinging bucket rotor as were tubes containing the standard proteins, bovine serum albumin and human γ-globulin. The position of the lectins on the gradients was determined by assaying all fractions for hemagglutination activity. Comparability of identical fractions from different gradients was confirmed by estimating sucrose content of each fraction with a refractometer.

Isoelectric focusing. Isoelectric focusing was performed at room temperature

in an LKB isoelectric focusing apparatus using pH 3—10 or 4—6 ampholytes as described in the manual for this apparatus, with the exception that the ampholyte gradient contained 0.3 M D-galactose. All fractions were then assayed for hemagglutination activity and appropriate fractions were further evaluated by polyacrylamide gel electrophoresis in SDS.

Amino acid analysis. This was performed on acid hydrolysates in the laboratory of Drs. T. Vedvick and H. Itano, University of California at San Diego.

Immunological studies. Antibodies directed against each of the purified lectins were raised in rabbits by first subcutaneously injecting 0.50 mg of purified protein with 1.5 ml of Freund's complete adjuvant at multiple sites on the back followed 28 days later by injection of another 0.50 mg of antigen in Freund's incomplete adjuvant. Serum was obtained 5 and 7 days later. γ-Globulin was prepared by ammonium sulfate precipitation [19]. Univalent antibody (Fab) fragments were prepared by papain digestion, followed by ion-exchange chromatography [20]. Double gel diffusion was performed [19] in agar equilibrated with 0.3 M D-galactose to block any association of the lectins with the agar.

Results

Affinity chromatography of lectins

Extracts of cohesive cells of each of the species studied contained substantial lectin activity (Table I). Vegetative cells of all these species contained little or no detectable lectin activity, as shown before for *D. discoideum* [2]. Lectins from each of the three species of slime molds bound to a Sepharose 4B column as observed previously [21]. In each case no detectable lectin activity was found in the proteins which did not bind to the column. Upon elution of the column with D-galactose a sharp peak of protein containing lectin activity was recovered. An example of affinity chromatography of an extract from *D. purpureum* is shown in Fig. 1. With the various extracts, between 34 and 72% of the lectin activity applied to the column was recovered (Table I). Because of the inherent inaccuracies of the serial 2-fold dilution method of hemagglutination assay and the possibility that components of the crude extract could either inhibit or augment lectin activity somewhat, it is difficult to determine precisely the fraction of lectin activity that was recovered. Since none was lost with the proteins not bound to the column, losses would occur only because of failure to elute. It is possible that some lectin activity was so tightly bound to

TABLE I

PURIFICATION OF LECTINS FROM THREE SPECIES OF CELLULAR SLIME MOLDS

The specific acitivity is expressed as titer^{-1}. (mg protein/ml solution)$^{-1}$.

Species	Specific activity of starting material	Specific activity of purified lectin	Purification (-fold)	Recovery (%)
D. discoideum	48	6 400	133	72
D. purpureum	820	16 200	20	58
D. mucoroides	34	6 400	188	34

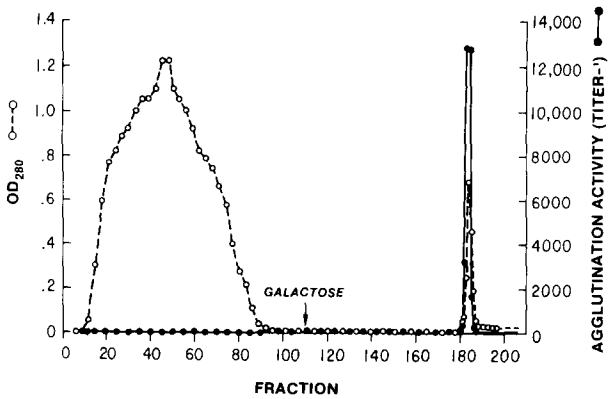

Fig. 1. Purification of agglutination activity from an extract of *D. purpureum* by affinity chromatography of Sepharose 4B. Extract was applied and the column was first eluted with ECT. At the point indicated by 'Galactose', elution was began with ECT containing 0.3 M D-galactose.

the column that it could not be eluted with galactose; but we have no direct evidence for this.

In all purification runs, recovery was relatively smallest with extracts from *D. mucoroides*. Because of the relatively poor recovery with these extracts we also attempted purification by absorption of lectin to formalinized human O erythrocytes and elution with D-galactose, a method used previously to purify the lectin from *Polysphondylium pallidum* [22]. With this method, recovery was in the same range of that found when we used Sepharose 4B as the affinity adsorbant.

Polyacrylamide gel electrophoresis in SDS

After Sepharose 4B affinity chromatography, the purified lectins from each species showed two distinct bands on polyacrylamide gel electrophoresis in SDS. The two bands in a preparation from *D. discoideum* are clearly seen in Fig. 2A as are the two very closely spaced bands seen in the preparation from *D. purpureum*. The minor band in the extracts from *D. mucoroides* is very difficult to see in the print since it represents only about 4% of the total stained material in the two bands and is more diffuse than the one from *D. discoideum*. In some preparations of the purified material from *D. mucoroides*, the minor band represented as much as 9% of the total stained material in the two bands as determined by densitometry. The minor band from *D. discoideum* accounted for 10% of the material determined this way. The minor more rapdily migrating band from *D. purpureum* represented 23% of the total in the two bands. Molecular weights of these bands are plotted in Fig. 2B. They range from about 23 000 to 26 000.

We have previously shown that the two bands found in the purified material from *D. discoideum* are subunits of distinct lectins that can be separated by DEAE-cellulose chromatography [23]. We have named the lectin mixture obtained upon elution from Sepharose 4B discoidin and the two distinct lectins discoidin I and discoidin II. By analogy we propose the names of purpurin and mucoroidin, respectively, for the purified lectins from *D. purpureum* and *D. mucoroides*. Whether or not the two bands in mucoroidin represent two

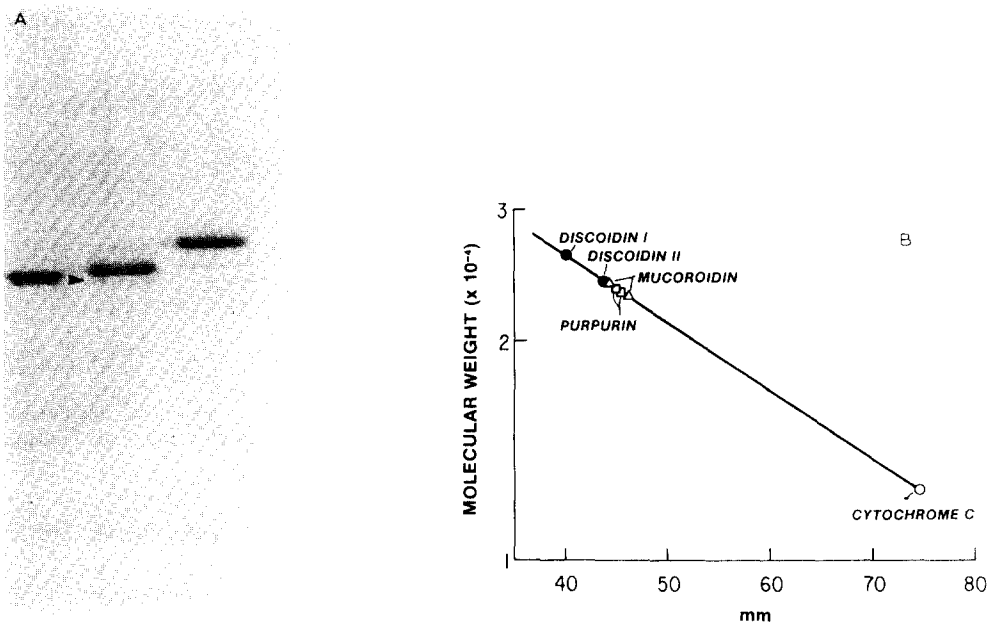

Fig. 2. (A) SDS polyacrylamide gel electrophoresis of lectins from *D. purpureum* (left), *D. mucoroides* (center) and *D. discoideum* (right) purified by affinity chromatography on Sepharose 4B. Samples were run concurrently in adjacent lanes on the same slab gel. The minor band in the preparation from *D. mucoroides* used in this experiment was especially faint and is indicated by the arrow. (B) Molecular weights of lectins from *D. discoideum* (discoidin I and discoidin II), *D. mucoroides* (mucoroidin) and *D. purpureum* (purpurin) estimated from SDS-polyacrylamide gel electrophoresis in 15% acrylamide sample gels. Standards used are discoidin I and cytochrome *C*. When bovine serum albumin and ovalbumin were used as standards the apparent molecular weights of all the proteins shown here were about 2000 daltons higher.

distinct lectins is not presently known. We have a small amount of evidence bearing on this in the case of purpurin as will be considered below.

Sucrose gradient centrifugation

In an attempt to estimate the state of aggregation of the subunits of the major lectin from each species, we subjected each purified lectin preparation to sucrose density gradient centrifugation and assayed hemagglutination activity of the gradient fractions. Discoidin, mucoroidin and purpurin each gave single sharp peaks upon sucrose density gradient centrifugation. The peaks in gradient tubes, all run concurrently, were in the same fraction and the density of sucrose in the peak fraction was identical, within experimental error, as determined by refractometry. This indicated that the state of aggregation of each of these lectins was the same. In previous studies with the sedimentation equilibrium technique [24] we determined that the molecular weight of discoidin was approx. 100 000, suggesting that it was composed of four subunits. The sucrose gradient centrifugation data suggest that the major lectins from *D. mucoroides* and *D. purpureum* also are tetramers. However, when molecular weights of each of these lectins was estimated by comparison with the sedimentation of the standards bovine serum albumin and human γ-globulin, different numbers were computed. Each of the lectins ran ahead of albumin and behind γ-globulin

but the calculated molecular weights were in all cases in the range of about 140 000. The reason for the discrepancy between the calculated molecular weight using discoidin as standard and using other proteins as standards is not known. Since discoidin is probably a more appropriate standard than these other proteins, we infer that the other slime mold lectins are also probably tetramers.

Isoelectric focusing

Because the minor subunit found with purpurin represented a much larger fraction of the total than was the case with discoidin or mucoroidin we attempted to determine if purpurin might be a single lectin made up of these two different subunits. If this were the case the two subunits should migrate together on isoelectric focusing in contrast with discoidin I and II which can be widely separated by isoelectric focusing [23]. Results with isoelectric focusing of purpurin (Fig. 3) did not completely resolve this question. Upon isoelectric focusing using ampholytes in the pH range of 3—10, only a single peak of lectin activity was found which contained all the applied lectin activity. When the fractions containing this activity were pooled and electrophoresed in SDS, both subunit bands were found. We then electrophoresed the two most widely separated major fractions (insets to Fig. 3). The most acidic major fraction contained, based on microdensitometry scanning, 64% major band, and 36% minor band. The least acidic major fractions contained 77% major band, and 23% minor band. Similar results were found in another experiment in which we used ampholytes in the pH range of 4—6. We also tested the relative potency of the most potent saccharide inhibitors of purpurin (see below) against the most widely separated fractions and found no significant differences. The results are consistent with the possibility that there are two distinct purpurins with very similar, indeed overlapping, isoelectric points. Alternatively purpurin may be composed of several combinations of two different subunits.

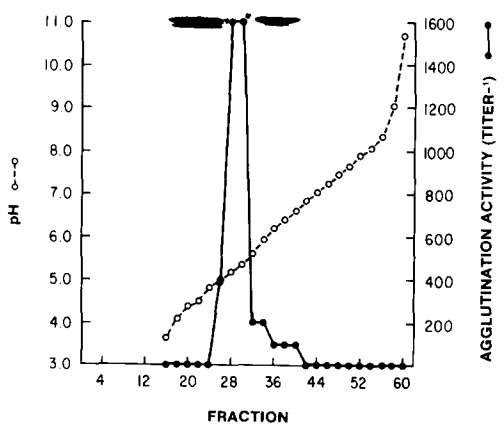

Fig. 3. Isoelectric focusing of purpurin. When isoelectric focusing was completed hemagglutination activity of alternate fractions was determined. Fractions 28 and 30 which had major lectin activity were analyzed by SDS-polyacrylamide gel electrophoresis and the ratio of the two protein bands in these fractions is shown in the insets of photographs of these bands, on the top of the figure.

Amino acid composition

The amino acid compositions of mucoroidin and purpurin are similar but distinct (Table II); and are also distinct from the amino acid compositions of discoidins I and II, published previously [23]. Neither mucoroidin nor purpurin has detectable methionine, whereas discoidins I and II contain this amino acid [23,24].

Immunological cross reactions

We raised antisera against discoidin, mucoroidin, and purpurin as well as against purified discoidin I and discoidin II. All antisera were potent as determined by precipitin tests. Some specificity and considerable cross reactivity was observed with precipitin reactions determined by double gel diffusion (Table III). Greater specificity was found with discoidin II which showed little or no reaction with antiserum raised against discoidin I, mucoroidin or purpurin. The antiserum raised against discoidin II also showed the greatest specificity in that it reacted very well with this antigen but not at all with discoidin I, mucoroidin or purpurin. Varying degrees of cross reactivity were observed with the other combinations (Table III). Both specificity and cross reactivity were observed when γ-globulin fractions of the antisera were assayed as inhibitors of hemagglutination activity of the various lectins (Table IV). The antiserum raised against a specific lectin always inhibited the hemagglutination activity of the lectin to which it was raised more than that of other lectins. In

TABLE II

AMINO ACID COMPOSITIONS OF MUCOROIDIN AND PURPURIN

Results are based on duplicate analysis of purpurin and a single analysis of mucoroidin.

Amino acids	Mucoroidin		Purpurin	
	Residue number	Integral number	Residue number	Integral number
Aspartic acid	23.3	23	25.3	25
Threonine	17.0	17	19.8	20
Serine	29.4	29	18.6	19
Glutamic acid	28.5	29	18.4	18
Proline	6.6	7	5.8	6
Cysteine	—**	0	6.1	6
Glycine	30.6	31	11.7	12
Alanine	19.8	20	17.5	18
Valine	13.3	13	14.0	14
Methionine	<0.2	0	<0.2	0
Isoleucine	11.4	11	7.7	8
Leucine	9.1	9	13.3	13
Tyrosine	4.0	4	4.0	4
Phenylalanine	9.6	10	9.2	9
Histidine	6.0	6	5.0	5
Lysine	7.0	7	5.9	6
Arginine	5.9	6	10.4	10
Total residues *		222		193
Calculated molecular weight *		26 745		24 165

* Does not include tryptophan.

** Uncertain due to technical problem.

TABLE III

PRECIPITIN REACTIONS OF ANTISERA WITH PURIFIED SLIME MOLD LECTINS

Precipitin reactions were determined by double gel diffusion in 1% agar containing 0.3 M D-galactose to inhibit lectin interactions with the agar. Center wells each contained 300 μg of a γ-globulin fraction from an antiserum raised in a single rabbit against each purified lectin. Peripheral wells contained 3 μg purified lectin. The intensity of the precipitin bands observed is graded from + to ++++. Similar relative activities were observed with a range of antigen and antibody concentrations.

Antiserum raised against	Antigen tested			
	Discoidin I	Discoidin II	Mucoroidin	Purpurin
Discoidin I *	++++	0	+/0	++++
Discoidin II *	0	++++	0	0
Mucoroidin	++	0	++	++
Purpurin	++	+/0	0	++++

* These antisera were raised against highly purified discoidin I and discoidin II prepared by David Lesikar.

some cases antiserum augmented hemagglutination activity of lectins other than those to which it was raised. One possible explanation for this is that these cross reactive sites are distinct from the carbohydrate binding sites. The antiserum might then bind lectin molecules together possibly improving their potency as hemagglutinins without interfering with the active sites. To evaluate this we made univalent antibody fragments which could not cross link separate lectin molecules. These Fab fragments inhibited hemagglutination activity of the lectins to which they were prepared (Table V) but also showed cross reactions. The Fab fragments prepared from antipurpurin did not share the augmenting effect of the parent substance in hemagglutination activity of discoidin or mucoroidin. Fab fragments made from antidiscoidin and antimucoroidin sera both had a slight augmenting effect on the hemagglutination activity of purpurin. The reason for this is not known.

TABLE IV

INHIBITION OF HEMAGGLUTINATION ACTIVITY OF PURIFIED LECTINS BY SPECIFIC ANTISERA

A range of concentrations of a γ-globulin fraction of antiserum were tested as inhibitors of hemagglutination activity of each purified lectin in the standard assay system. At high concentrations of antiserum hemagglutination activity was usually inhibited completely; and some preimmune sera had slight effects at high concentrations. To control the effects of preimmune sera, identical dilutions of these sera were used as the controls for the immune sera.

Lectin	Antiserum dilution that inhibits 50%			
	Anti-discoidin I	Anti-discoidin II	Anti-mucoroidin	Anti-purpurin
Discoidin	1 : 64	1 : 8	1 : 8	none *
Mucoroidin	1 : 16	none	1 : 64	none *
Purpurin	1 : 2	none	none *	1 : 160

* Addition of antiserum at dilutions up to about 1 : 20 doubled hemagglutination activity.

TABLE V

INHIBITION OF HEMAGGLUTINATION ACTIVITY OF PURIFIED LECTINS BY Fab FRAGMENTS PREPARED FROM SPECIFIC ANTISERA

A range of amounts of Fab fragments of each type were studied in standard hemagglutination assays with purified lectins. The amount of Fab (added to each assay well) that inhibited hemagglutination titer by 50% (the last step) is shown. In these experiments the last well that showed positive hemagglutination in the control case contained about 0.003 μg of the indicated lectin. Fab fragments prepared from pre-immune sera had no detectable effect on hemagglutination activity.

Lectin	Fab amount (μg) that inhibits 50%		
	Anti-discoidin	Anti-mucoroidin	Anti-purpurin
Discoidin	0.1	0.4	1.0
Mucoroidin	0.4	0.1	1.0
Purpurin	none *	none *	0.1

* No inhibition was observed at a range of concentrations; but 0.4—3 μg anti-discoidin Fab and 1—2 μg anti-mucoroidin Fab doubled the hemagglutination activity of purpurin.

Relative potency of saccharide inhibitors

We previously showed differences in the relative potency of a series of saccharides to inhibit the hemagglutination activity of crude extracts of *D. discoideum*, *D. mucoroides* and *D. purpureum* [15]. Some differences were also observed with the purified lectins (Table VI). However there were a great many similarities. For example *N*-acetyl-D-galactosamine is a potent inhibitor of all these lectins.

TABLE VI

RELATIVE POTENCY OF SACCHARIDES IN INHIBITING HEMAGGLUTINATION ACTIVITY OF PURIFIED LECTINS

Hemagglutination activity of lectins purified by affinity chromatography was determined with formalinized rabbit erythrocytes. Lectins from the three species were studied concurrently.

Saccharide	Concentration that inhibits 50% (mM)		
	Discoidin	Purpurin	Mucoroidin
N-Acetyl-D-galactosamine	3.13	6.25	3.13
D-Fucose	3.13	25	1.56
3-*O*-Methyl-D-glucoside	3.13	50	3.13
Methyl-α-D-glucoside	6.25	>100	1.56
D-Galactose	6.25	100	12.5
Lactose	12.5	3.13	25.0
Methyl-α-D-galactoside	12.5	100	3.13
Methyl-β-D-galactoside	12.5	12.5	12.5
L-Fucose	12.5	100	12.5
D-Mellibiose	50	100	12.5
L-Rhamnose	100	100	100
Methyl-β-D-glucoside	100	>100	>100
D-Glucose	>100	>100	>100
N-Acetyl-D-glucosamine	>100	>100	>100
D-Mannose	>100	>100	>100
Methyl-α-D-mannoside	>100	>100	>100
D-Trehalose	>100	>100	>100

Discussion

The present results confirm previous findings that there are developmentally regulated lectins in slime molds and that the lectins represent major proteins in the differentiated cells. This latter point is very strikingly demonstrated in the case of *D. purpureum* in that about 5% of the soluble protein of differentiated *D. purpureum* cells is lectin. We have solubilized and electrophoresed intact *D. purpureum* cells and found (data not shown) that two major protein bands on the SDS gels co-migrate with the two proteins found in pure purpurin.

In previous studies [23] we showed that discoidin is composed of two distinct proteins, discoidin I and discoidin II, each of which forms a distinct homotetramer. In the present experiments we find evidence for two distinct protein bands in preparations of mucoroidin and purpurin purified by affinity chromatography. In the case of purpurin, where the minor subunit is so abundant, we have attempted to determine by isoelectric focusing whether or not there are two distinct lectins. The available evidence is consistent with two distinct lectins with slightly different isoelectric points or with the possibility that the distinct molecular entities are not homopolymers of one subunit but rather combinations of the two subunits. We have not attempted to study this question with mucoroidin since recovery of this material was relatively low and the minor band was not prominent in several preparations.

The lectins that we have purified from the three species of slime molds are closely related proteins. They are similar in subunit molecular weights, amino acid compositions and aggregated molecular weights; but there are also distinct differences. Immunologically they are also distinct; but show cross reactivity as assayed both by precipitin reactions and by inhibition of hemagglutination activity. Similarities and differences are also observed in the relative potency of saccharides in inhibiting hemagglutination activity of the purified lectins.

It seems clear then that the presence of developmentally regulated lectins in slime molds is a general phenomenon; and that the lectins are a closely related group of proteins. Previous studies showed that slime mold lectins are detectable on the surface of cohesive slime mold cells [1—7]. Evidence suggesting a role in cell cohesion has also been presented [1—10]. Whether or not the differences in the lectins from the different species of Dictyosteliaceae play a role in their segregation in mixed cultures might be determined by using the lectins and antisera characterized here as reagents in experiments on cell aggregation. We have recently devised a method for direct quantitative analysis of species-specific adhesion of slime molds [25] which should facilitate this comparison.

Acknowledgements

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