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The effects of three lectins on the cellular agglutination and morphogenesis of Dictyostelium mucoroides.

William H. Roccaforte

THE EFFECTS OF THREE LECTINS ON THE CELLULAR AGGLUTINATION AND MORPHOGENESIS OF *DICTYOSTELIUM MUCOROIDES*

A Thesis

Presented to the

Department of Biology

and the

Faculty of the Graduate College

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In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

William H. Roccaforte

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

THESIS ACCEPTANCE

Accepted for the faculty of the Graduate College, University of Nebraska, in partial fulfillment of the requirements for the degree Master of Arts, University of Nebraska at Omaha.

Thesis Committee

Name	Department
E. J. Kennedy	Chemistry
Ann W Nederman	UNL
Dayan E Mathis	Biology

A T Roman Weber
Chairman

July 19, 1978
Date

ACKNOWLEGEMENTS

In the course of graduate events it becomes necessary to complete a thesis. It is during this educational experience that one discovers our forefathers were sages that spanned the ages when they penned those immortal words, "All men are berated equal."

I would like to say that this project has been all my own work and no one else deserves any credit for its conception and completion. Unfortunately, I cannot say this because it is not true. Dr. Weber's effervescent ever-presence made the work much more enjoyable than it should have been. Dr. Moshier contributed timely and pertinent suggestions throughout the course of this research. Also, I extend special thanks to both of them for endlessly (or so it seemed) reviewing the manuscript and making it a much better paper. Thanks go to Drs. Nickerson and Kemnitz for serving on my committee and to Dr. William O'Dell for willingly sharing equipment and his supplies with a lowly graduate student. And, of course, I thank my parents for getting me here in the first place and supporting me through the years.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Dictyostelium mucoroides was the first cellular slime mold reported (Brefeld, 1869) and it has since been found to have a worldwide distribution (Cavender, 1973). Increasingly, the cellular slime molds have become the object of intensive developmental studies, in part because their life cycles exhibit distinct periods of growth and differentiation. In *D. mucoroides*, whose life cycle is typical of these organisms, the growth phase begins upon germination of the spore which releases a single amoeba. The amoebae independently grow and divide until their food sources, bacteria and other microorganisms, become depleted. Then the individual amoebae aggregate into multicellular mounds, thereby initiating the differentiation phase of the life cycle. The amoebae in the aggregates form cone-shaped mounds that continue to rise until the influx of cells ceases, whereupon these masses usually lie flat on the substrate and migrate as pseudoplasmodia, producing a stalk as they proceed. Three distinct cell types can be recognized at this stage: prespore, presumptive stalk and mature stalk cells. At the end of migration stalk cells within the pseudoplasmodium accumulate on top of the basal-most stalk cells on the substrate lifting the developing spores off the surface. In this manner the spheroid sorus, the mature spore-bearing structure, comes to rest at the apex of the stalk, or sorophore. The whole,

differentiated body is referred to as the sorocarp (Bonner, 1967).

Some species of cellular slime molds are capable of entering an alternative morphogenetic cycle that leads to macrocyst formation (Blaskovics and Raper, 1957). In D. mucoroides the growth and division of amoebae is the same as that which precedes sorocarp development, but under conditions of high humidity, temperatures near 25°C and in the absence of light and phosphates the aggregated amoebae will differentiate into macrocysts (Nickerson and Raper, 1973a). This process begins with the formation of a primary wall around the aggregate. During wall deposition a large, centrally located cell ingests each amoeba in the aggregate and encloses each in a vacuole. A thick, secondary wall is secreted inside the primary wall. The digestion of the amoebae is completed and the vacuolar membranes disappear leaving a homogeneous plasm. An innermost tertiary wall develops around the cyst contents and contracts away from the secondary wall to complete the formation of the mature macrocyst (Nickerson and Raper, 1973b). After a period of dormancy the amoebal cell membranes reappear and the macrocyst walls rupture to release the individual amoebae (Erdos et al., 1973).

Lectins are protein or protein-containing compounds, usually extracted from plants, with the capacity to bind to specific carbohydrates (Lis and Sharon, 1973). It has

been known since the turn of the century that lectins can cause red blood cells to agglutinate (Sharon and Lis, 1972). Interest in this phenomenon increased when many types of cells, e.g. mouse thymocytes and fibroblasts, were found to be more susceptible to this agglutination action after they were transformed into cancerous cells (Aub et al., 1963). Agglutination patterns were also discovered to vary during differentiation in numerous systems (Nicolson, 1974). Several investigators utilized lectin-induced agglutination to examine the surface changes that occur during the life cycle of the cellular slime mold, Dictyostelium discoideum (Weeks, 1973; Weeks and Weeks, 1975; Kawai and Takeuchi, 1976). All such studies showed decreased cellular agglutination in the presence of concanavalin A (Con A) as development progressed toward sorocarp formation.

Since the lectins have been found to bind to sugar-bearing surface receptors it is possible that these same receptors are important in the differentiation process of the cellular slime molds. To test this possibility Gillette and Filosa (1973) and Weeks and Weeks (1975) exposed D. discoideum amoebae to Con A and then followed development. Both teams reported that a sustained treatment caused a delay in sorocarp formation.

The purpose of this research was to extend the study of cellular slime mold-lectin interaction. First, the involvement of various sugar-bearing receptors in lectin-in-

duced agglutination could be determined by using several lectins with different sugar-binding specificities. To this end, three lectins were used: Con A, wheat germ agglutinin (WGA) and Ricinus communis (castor bean) agglutinin (RCA_{120}) (see Table I for physical characteristics). Second, a cellular slime mold other than D. discoideum, i.e. D. mucoroides, was used, thus permitting a comparison of agglutination results from two closely related species. Third, this study followed the lectin-induced agglutination of D. mucoroides cells embarked on development toward macrocysts, as well as those developing under conditions favoring sorocarp formation. The final goal of this project was to determine if those lectins that were found to agglutinate D. mucoroides cells, and hence were considered to be bound to the cells, were attached to sites that were influential in the organism's morphogenesis.

TABLE I

Physical Characteristics of Con A, WGA and RCA_{120}*

Lectin	Source	Chemical Nature	Molecular Weight	Sugar Binding Specificity
Con A	*Canavalia ensiformis*	Protein	112,000	α-D-glucose α-D-mannose
WGA	*Triticum vulgaris*	Protein	35,000	N-acetyl-D-glucosamine
RCA_{120}	*Ricinus communis*	Glyco-protein	120,000	β-D-galactose

* From Lis and Sharon (1973) and Nicolson (1974)

MATERIALS AND METHODS

Organisms and Growth Conditions

Dictyostelium mucoroides strain DM7 amoebae were grown in 250 ml Erlenmeyer flasks containing 40 ml of liquid glucose-yeast-peptone medium (GYP) (2.0 g peptone, 1.0 g glucose, 0.5 g yeast extract, 0.84 g KH_2PO_4, 0.54 g Na_2HPO_4 in 1.0 l glass-distilled water) in association with *Escherichia coli* strain B/r. The flasks were inoculated with 0.5 ml of a *D. mucoroides* spore suspension containing 2.5×10^6 spore/ml and 0.1 ml of a bacterial suspension and were incubated in a gyratory water bath at 180 rpm and 21°C.

Preparation of Cells for Agglutination Assays

Amoebae were harvested at early exponential, late exponential and stationary growth phases (see Results for specific times). The contents of one flask were used for each agglutination assay. The cells were concentrated by centrifugation at 800 X g for 10 minutes and washed four successive times in 10 ml cold (4°C) Bonner's salt solution (BSS) (Bonner and Frascella, 1953). Amoebae were induced to develop to early and late aggregation by harvesting stationary phase cells from six flasks, washing as above and plating them on water agar (15 g agar and 1 l glass-distilled water). For sorocarp development 0.2 ml of the washed cell suspension at approximately 3.0×10^9

amoebae/ml was spread on water agar plates with a sterile, bent glass rod and was incubated at 21°C in constant light. Macrocyst formation was induced by inoculating water agar plates with 5 ml of an amoeba suspension containing 1.2×10^8 amoebae/ml and incubating them at 26°C in total darkness. Cultures were shielded from light by wrapping the plates in aluminum foil. Early and late aggregation cells were collected at 3-4 hours and 6-8 hours, respectively, after the start of incubation. Four sorocarp plates were flooded twice, and four macrocyst plates once, with 5 ml BSS while gently scraping with a wire loop. The cells were washed and resuspended as described for the vegetative amoebae. Microscopic inspection revealed that this treatment successfully disaggregated the cells.

Lectin-Induced Agglutination Assay

Washed cells were adjusted to a concentration of 1.5 to 2.5×10^6 particles/ml in BSS and 2 ml of the cell suspension was combined with 1 ml of the appropriate lectin solution, or solvent for the corresponding lectin, in a 10 ml flask. The final assay mixtures contained lectin at concentrations of 0 μg/ml, 4 μg/ml and 40 μg/ml. These are equivalent to 0.036μM and 0.36μM Con A, 0.114μM and 1.14μM WGA and 0.033μM and 0.33μM RCA_{120}. Duplicate flasks were prepared for each assay. Immediately after inoculation the flasks were shaken in a gyratory water bath at 200 rpm for Con A and RCA_{120} assays and at 350 rpm

for WGA assays. After 10 minutes of shaking at 21°C, particle concentration was determined by counts made in a haemocytometer under a phase contrast microscope at 100X. Three counts were made from each of the two duplicate flasks. Each single cell or distinct cell cluster was counted as one particle. The fraction of particles after lectin treatment relative to the number of particles in the initial assay suspension was employed as a measure of the degree of agglutination. The fractions presented are the compiled results of three experiments each for Con A and WGA and one experiment involving RCA_{120}.

Hapten Inhibition

A sugar hapten of each active lectin was used to determine whether any observed agglutination was induced by the specific binding of the lectin to the surface sugars. The proper hapten, bound to a specific lectin, makes the lectin unavailable to react with the cellular slime mold surface sugars. The sugars used were 1-O-methyl-α-D-glucopyranoside and α-methyl-D-mannoside for Con A and N-acetyl-D-glucosamine for WGA. RCA_{120} did not induce agglutination so no test was performed with its hapten. All sugars were tested at 1M and added to the added to the assay flasks immediately after the lectin. Assays for agglutination were completed as previously described.

Bound Lectin Effects on Development

To determine if bound lectins affected normal development of sorocarps and macrocysts two methods were used to treat washed, stationary phase cells. In the first, suspended cells at 2.5×10^7 cells/ml were incubated in the presence of 500 µg/ml Con A or WGA for 30 minutes prior to plating on water agar. Another aliquot of the same suspension was similarly exposed to 2.25M NaCl, the equivalent of the concentration present using saturated NaCl as the Con A solvent, to serve as a control. Since BSS served as both the cell suspension medium and the WGA solvent, an untreated suspension of cells was used as both the WGA and overall control. Treated and untreated cells were plated and incubated under conditions favorable for either sorocarp or macrocyst development, as described earlier. The second treatment involved prolonged exposure to Con A. Cells were incubated to form macrocysts or sorocarps on water agar containing 500 µg/ml Con A. The same procedure using water agar supplemented with 1.25% NaCl served as a control.

Chemicals

RCA_{120} was purchased from P-L Biochemicals Inc., WGA from United States Biochemical Corp. and Con A (2X crystallized) from Miles-Yeda Ltd. All sugar haptens were obtained from Sigma Chemical Co.

RESULTS

Growth of Vegetative Amoebae

Vegetative amoebae grown in GYP broth produced the curve shown in Figure 1. Amoebae for the early exponential, late exponential and stationary growth phase assays were harvested 39 hours, 48 hours and 60 hours after inoculation, respectively.

Agglutination Patterns

Con A induced the agglutination of amoebae at 4 µg/ml and 40 µg/ml with the effects of 40 µg/ml being greater at all stages of development (Figure 2 and Table II). At both concentrations agglutination was relatively high during exponential growth, decreased at stationary phase, increased during early aggregation for cells developing toward sorocarps and those that were forming macrocysts, and then decreased at late aggregation for both cell types. There was relatively little background agglutination in the control groups, however, aggregating cells developing toward macrocysts showed more spontaneous agglutinability than did aggregating cells destined to form sorocarps.

At 4 µg/ml and 40 µg/ml WGA induced agglutination but there was considerable background agglutination in the control group (Figure 3 and Table III). All treatments produced the same pattern of agglutination: the least amount during early growth, increasing during late growth,

FIGURE 1. The growth of Dictyostelium mucoroides DM7 amoebae from one experiment in liquid GYP medium at 21°C.

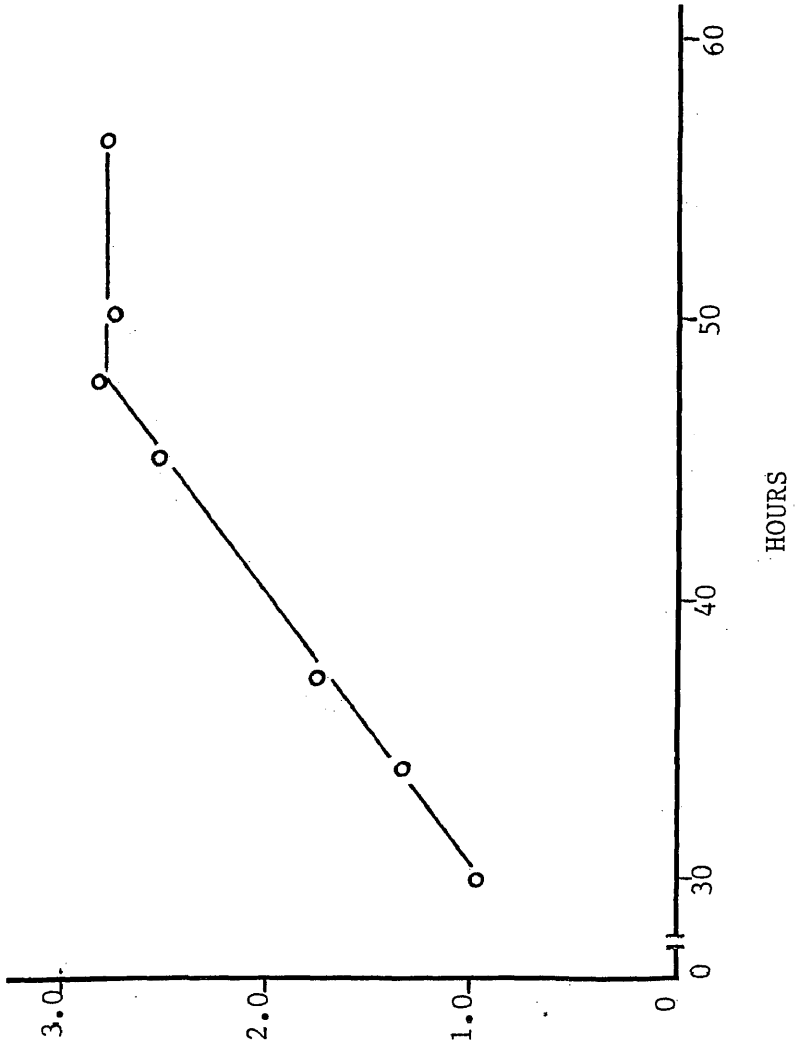
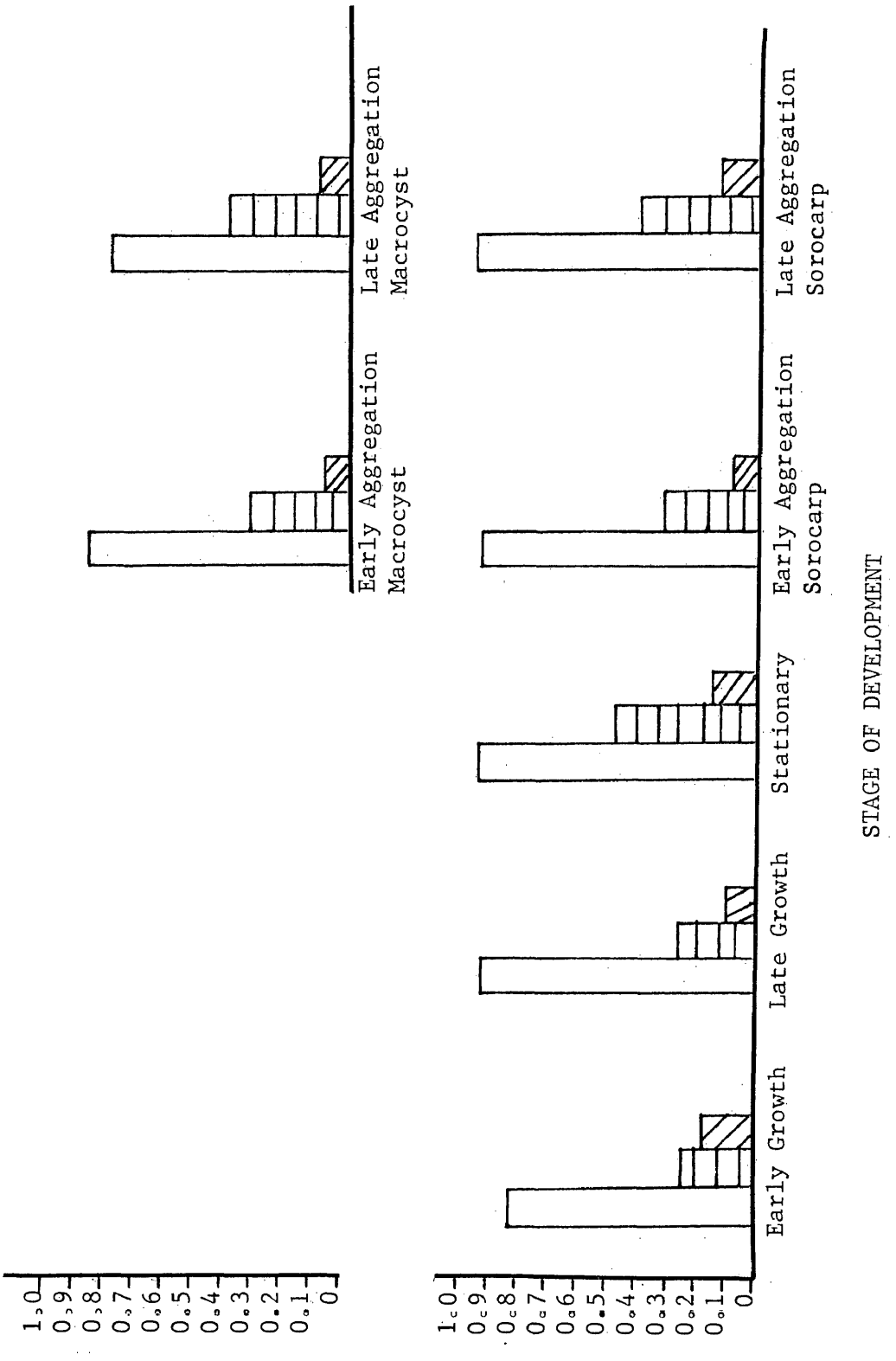

LOG NUMBER OF AMOEBAE/ML (X10⁻⁴)
3.0
2.0
1.0
0
0
30
40
50
60
HOURS

FIGURE 2. The average fraction of particles after Con A treatment relative to the pre-treatment particle concentration. Each bar represents the average of eighteen data points. In each of three separate experiments three counts were taken from both duplicate flasks.


1.0
0.9
0.8
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0.6
0.5
0.4
0.3
0.2
0.1
0
Early Growth
Late Growth
Stationary
Early Aggregation Sorocarp
Late Aggregation Sorocarp
Early Aggregation Macrocyst
Late Aggregation Macrocyst
FRACTION OF PRE-TREATMENT PARTICLES
STAGE OF DEVELOPMENT

TABLE II

Average fraction of particles after Con A treatment relative to the pre-treatment particle concentration

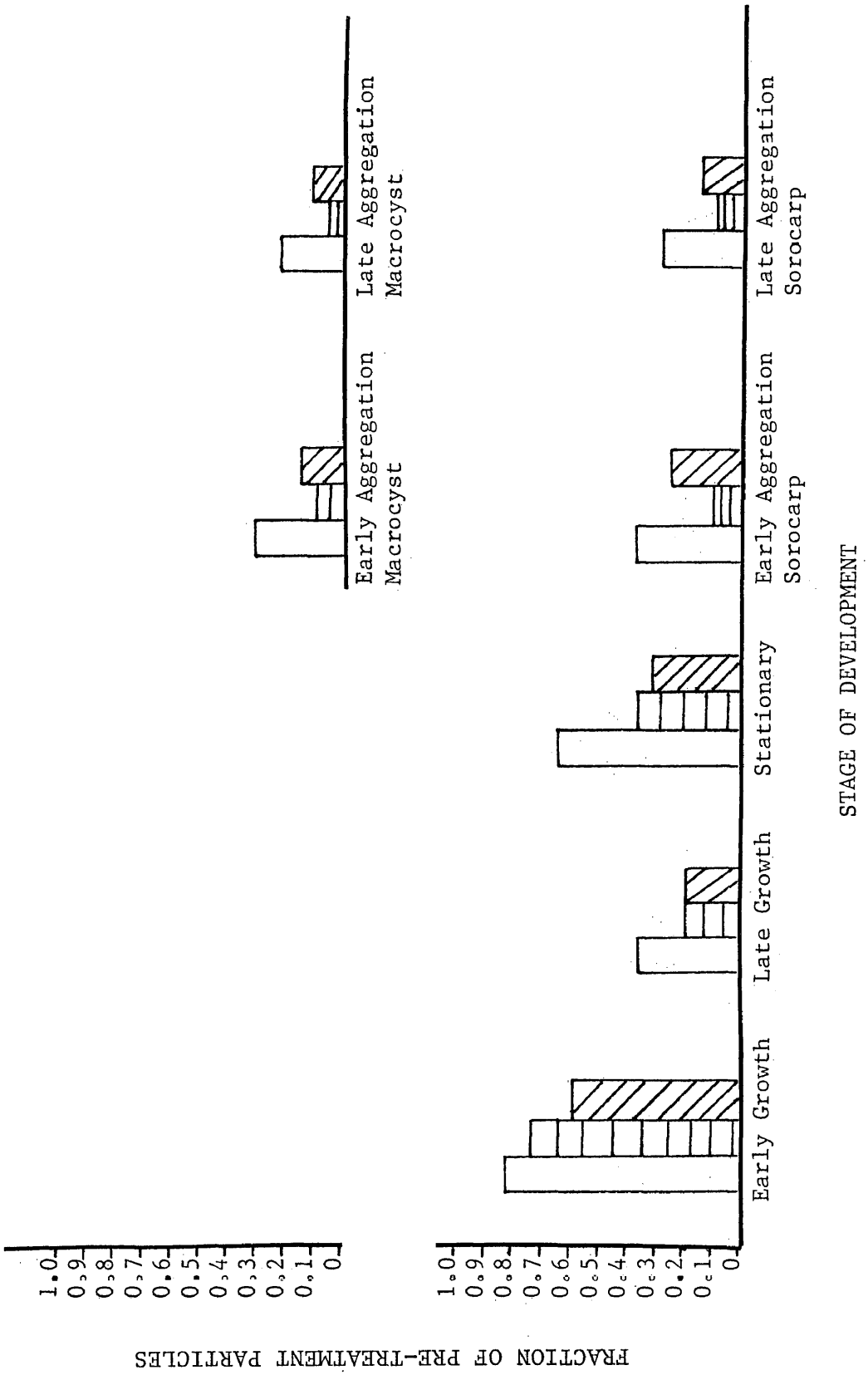
Stage of development	Concentration of Con A (μg/ml)		
	0	4	40
Early Growth	0.825	0.246	0.159
Late Growth	0.922	0.250	0.092
Stationary	0.918	0.466	0.143
Early Aggregation			
Sorocarp	0.930	0.300	0.072
Macrocyst	0.892	0.331	0.062
Late Aggregation			
Sorocarp	0.941	0.408	0.117
Macrocyst	0.819	0.396	0.089

TABLE III

Average fraction of particles after WGA treatment relative to the pre-treatment particle concentration

Stage of development	Concentration of WGA (μg/ml)		
	0	4	40
Early Growth	0.842	0.748	0.601
Late Growth	0.355	0.183	0.184
Stationary	0.638	0.355	0.318
Early Aggregation			
Sorocarp	0.369	0.105	0.244
Macrocyst	0.306	0.086	0.144
Late Aggregation			
Sorocarp	0.292	0.081	0.138
Macrocyst	0.213	0.055	0.109

FIGURE 3. The average fraction of particles after WGA treatment relative to the pre-treatment particle concentration. Each bar represents the average of eighteen data points. In each of three separate experiments three counts were taken from both duplicate flasks.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Early Growth
Late Growth
Stationary
Early Aggregation Sorocarp
Late Aggregation Sorocarp
Early Aggregation Macrocyst
Late Aggregation Macrocyst
FRACTION OF PRE-TREATMENT PARTICLES
STAGE OF DEVELOPMENT

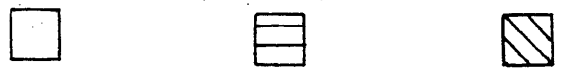
decreasing at stationary phase and progressively increasing during aggregation for cells embarked on development toward sorocarps and for those destined to produce macrocysts. The data from the three separate experiments using 40 µg/ml was erratic.

RCA_{120} did not cause agglutination at 4 µg/ml or 40 µg/ml but the control group did exhibit the same pattern of background agglutination as the WGA control group with the exception of the decrease in agglutination seen during aggregation (Figure 4 and Table IV).

One way analysis of variance results demonstrated that the agglutination pattern varied significantly when the stages of development were compared for each concentration of lectin (Table V). Only in the Con A control group was $p>0.05$, reflecting the low degree of background agglutination. Analysis of variance also showed that $p=0.816$ for the cumulative RCA_{120} agglutination data indicating that this lectin did not cause agglutination.

Student t tests comparing the agglutination of macrocyst-forming cells to those developing toward sorocarps were significant at $p<0.05$ during late aggregation in the Con A control group, early and late aggregation in the WGA control group and late aggregation in the group treated with 40 µg/ml RCA_{120} (Table VI). Therefore, there were no differences between the two cell types that were detectable by lectin agglutination.

FIGURE 4. The average fraction of particles after RCA_{120} treatment relative to the pre-treatment particle concentration. Each bar represents the average of six data points. Three counts were made from both duplicate flasks in one experiment.



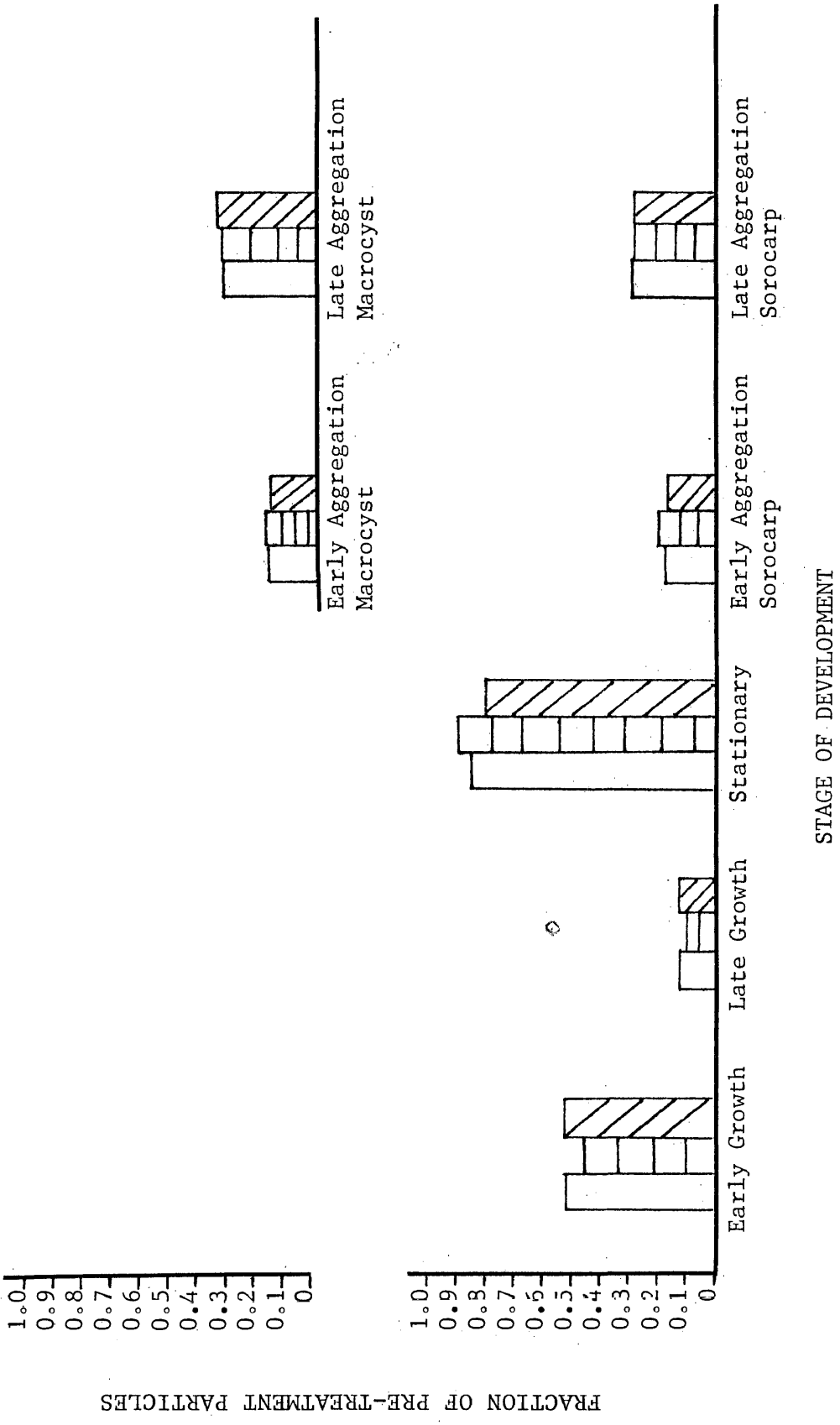

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Early Growth
Late Growth
Stationary
Early Aggregation Sorocarp
Late Aggregation Sorocarp
Early Aggregation Macrocyst
Late Aggregation Macrocyst
FRACTION OF PRE-TREATMENT PARTICLES
STAGE OF DEVELOPMENT

TABLE IV

Average fraction of particles after RCA_{120} treatment relative to the pre-treatment particle concentration

Stage of development	Concentration of RCA_{120} (µg/ml)		
	0	4	40
Early Growth	0.518	0.441	0.527
Late Growth	0.117	0.103	0.121
Stationary	0.846	0.898	0.820
Early Aggregation			
Sorocarp	0.173	0.191	0.177
Macrocyst	0.167	0.172	0.156
Late Aggregation			
Sorocarp	0.294	0.279	0.277
Macrocyst	0.312	0.311	0.323

TABLE V

Analysis of variance comparing agglutiation at the different stages of development

Lectin	(µg/ml)	F_c^*	p^{**}
Con A	0	2.129	0.055
	4	14.684	<0.001
	40	25.448	<0.001
WGA	0	182.369	<0.001
	4	134.980	<0.001
	40	74.371	<0.001
RCA_{120}	0	64.126	<0.001
	4	187.406	<0.001
	40	82.387	<0.001

* F_c is the calculated F value.

** p is the probability of obtaining the tested results by chance.

TABLE VI

Student t tests comparing agglutination of cells developing toward sorocarps and macrocysts during aggregation stages

		Concentration of Lectin (μg/ml)					
		0		4		40	
Lectin	Stage	t_c*	p**	t_c	p	t_c	p
Con A	Early Agg.	0.655	>0.25	1.445	0.1-0.05	1.165	0.25-0.1
	Late Agg.	2.774	<0.005	1.102	0.25-0.1	1.144	0.25-0.1
WGA	Early Agg.	3.716	<0.005	0.137	>0.25	1.610	0.1-0.05
	Late Agg.	3.236	<0.005	0.925	0.25-0.1	0.539	>0.25
RCA_{120}	Early Agg.	0.417	>0.025	0.793	0.25-0.1	1.196	0.25-0.1
	Late Agg.	1.507	0.25-0.1	0.807	0.25-0.1	3.774	<0.005

* t_c is the calculated t value.

** p is the probability of obtaining the tested results by chance.

Hapten Inhibition

The appropriate sugar haptens completely inhibited the Con A and WGA agglutination at 4 µg/ml and 40 µg/ml. The degree of agglutination in all instances was reduced to that of the respective control groups (Table VII).

Lectin Effects on Morphogenesis

Con A was found to inhibit both sorocarp and macrocyst formation. Thirty minute exposure of stationary phase cells to 500 µg/ml Con A delayed sorocarp development approximately 48 hours and macrocyst formation 10 hours. Con A added to the water agar on which development was to occur prevented the morphogenesis of stationary phase amoebae to sorocarps or macrocysts. No appreciable aggregation occurred in either group, although a very small number of flat aggregates were observed among the sorocarp-forming cells. Exposure of amoebae to 2.25M NaCl for thirty minutes prior to plating delayed the formation of sorocarps and macrocysts 3-5 hours, as did plating untreated cells on water agar containing 1.25% NaCl.

Incubation for thirty minutes in 500 µg/ml WGA prior to plating the cells for development delayed sorocarp formation by approximately 11 hours while macrocyst formation was not appreciably inhibited. The progress of morphogenesis was judged using a stereo microscope at 30X.

TABLE VII

The fraction of particles relative to the pre-treatment particle concentration after hapten inhibition tests and those of the respective control groups

Lectin	(μg/ml)	Hapten	Stage of Development	Fraction of Particles-Hapten	Fraction of Particles-Control
Con A	4	1-O-methyl-α-D-glucopyranoside	Late Growth	.901	.887
	4	α- methyl-D-mannoside	Late Growth	.905	.887
	40	1-O-methyl-α-D-glucopyranoside	Late Growth	.865	.887
	40	α-methyl-D-mannoside	Late Growth	.911	.887
WGA	4	N-acetyl-D-glucosamine	Stationary	.683	.700
	40	N-acetyl-D-glucosamine	Stationary	.673	.700

DISCUSSION

This study demonstrated that Con A and WGA induced agglutination of D. mucoroides cells during the growth and aggregation periods of development. Agglutination was observed during the sorocarp and macrocyst morphogenetic phases. These findings indicated that D. mucoroides cells had surface receptors that were able to bind to these two lectins and that these receptors were able to interact in such ways, as will be discussed later, that produced cellular agglutination (Lis and Sharon, 1973). RCA_{120}, however, did not cause these cells to agglutinate. According to Nicolson (1974) the usual factor responsible for the absence of agglutination was either the lack of specific surface receptors for a lectin or a binding constant that was too low.

Previous studies of the patterns of agglutination of D. discoideum cells induced by Con A showed that as the growth of amoebae ceased and the development proceeded toward sorocarp formation the cells became less susceptible to agglutination (Weeks, 1973; Weeks and Weeks, 1975; Kawai and Takeuchi, 1976). In this investigation D. mucoroides cells treated with Con A were found to follow the same pattern of agglutination except for an increase of agglutinability at early aggregation. Any of the conditions known to influence lectin-induced agglutination could have caused these differences in the agglutination patterns.

First, the degree of agglutination was influenced by the number of surface lectin receptors present (Nicolson, 1974). Perhaps more Con A receptors were present during early aggregation, hence causing the increased agglutination. Second, even if lectin receptors were present the conditions at the reaction site had to be favorable for the actual binding of the lectin to those receptors (Lis and Sharon, 1973). These reactions were influenced by the net surface charge, the polymeric state of the lectin, the pH of the microenvironment near the receptor and the binding constants (Nicolson, 1974). Third, the intracellular and intercellular aggregation of lectin-receptor complexes were important in the agglutination process (Lis and Sharon, 1973). Differences in the surface fluidity and topography, the nature of the receptor to which the lectin was bound and the presence or absence of other surface components could have influenced receptor mobility and so the agglutination of the cells (Nicolson, 1974).

WGA had been reported to induce the agglutination of D. discoideum cells (Reitherman et al., 1975) and in the present study it was found to cause D. mucoroides cells to agglutinate. The 40 µg/ml results were erratic so this discussion will refer to the 4 µg/ml data. Reitherman et al. (1975) demonstrated that in WGA's presence the vegetative amoebae of D. discoideum agglutinated more readily than cells that were starved for nine hours. This pattern

followed the usual trend of decreased cellular agglutinability with slower growth rates and increased differentiation (Lis and Sharon, 1973). D. mucoroides cells behaved differently in that there was little agglutination caused by WGA of cells that were in early exponential growth. However, the typical pattern, a high degree of agglutinability of late growth cells followed by less agglutination of stationary phase cells, was observed. The trend reversed again for cells in early and late aggregation since the latter cells agglutinated more than the former cells. As mentioned with respect to Con A, the possible reasons for these patterns were numerous.

While RCA_{120} did not induce the agglutination of D. mucoroides cells it has been found to cause starved D. discoideum cells to agglutinate more readily than vegetative cells (Reitherman et al., 1975). These results indicated that there were differences between the two species either in the presence of RCA_{120} receptors or in their interactions (Nicolson, 1974).

The differences in background agglutination patterns among the three lectin control groups observed in this investigation probably reflected variation of the cell adhesiveness of D. mucoroides in the presence of the lectins' solvents. These were saturated NaCl for Con A, BSS for WGA and 0.1M NaCl for RCA_{120}. There was little spontaneous agglutination in the Con A control group indicating

that the higher concentration of NaCl in these assay flasks, about 2.25M, effectively controlled this property. The cells in the RCA_{120} and WGA control groups showed an increase of cell stickiness during late exponential growth. Surface changes appear to have taken place in the individual amoebae before the cell-to-cell association occurred in aggregation. These surface alterations could reflect the transition of the amoebae from independently dividing cells to cells that interact during the formation of multicellular fruiting bodies. The RCA_{120} control group showed decreased background agglutination during aggregation while there was increased agglutination in the corresponding WGA group. The presence of different lectin solvents was probably responsible for this.

A comparison of the agglutination results of sorocarp-forming cells to those that were developing into macrocysts suggested that the latter were more adhesive than the former. Significant differences, i.e. $p<0.05$ using the Student t test, appeared during late aggregation in the Con A control group and during both early and late aggregation in the WGA control group. However, agglutination induced by these two lectins masked these variations since no significant differences were observed in the groups treated with Con A or WGA. These results indicated that differential surface alterations occurred during aggregation, although they were not detectable by Con A or WGA

agglutination. Such variations could have been the consequence of different morphogenetic programs and/or dissimilar environmental conditions.

In the present study the morphogenesis of D. mucoroides cells was found to be affected by Con A. Constant exposure of stationary phase cells to Con A inhibited aggregation when the cells were incubated in conditions favorable to sorocarp formation. In similar experiments by Weeks and Weeks (1975) aggregation of D. discoideum cells was also retarded but to a lesser extent. Brief exposure of D. mucoroides sorocarp-forming cells to Con A delayed development but complete differentiation did occur 43-48 hours later than in the control groups. The temporary retardation induced by brief exposure was probably due to the cells' ability to ingest or destroy Con A attached to their receptors (Darmon and Klein, 1976). Weeks and Weeks (1975) and Darmon and Klein (1976) found that brief exposure of D. discoideum cells to Con A had little or no effect on the rate of aggregation. Therefore, while the overall response of the two species appeared to be similar the D. mucoroides cells were more sensitive to its action.

Since the sorocarp-producing cells of both D. mucoroides and D. discoideum were affected by Con A it seemed reasonable to assume that this lectin influenced the same aggregation factors in both species. Cyclic adenosine 3':5'-monophosphate (cAMP) was implicated as an attracting agent

for cells undergoing aggregation (Konijn et al., 1967) and disruption of this chemotactic mechanism was suspected as a cause of the Con A effects. Cyclic AMP phosphodiesterase (PDE), which hydrolyzes cAMP, was shown to be instrumental to the cells' ability to respond to cAMP, and hence to aggregate (Malchow et al., 1972). Under normal conditions there was an increase of PDE activity just prior to the onset of aggregation, which usually occurred four to eight hours after starvation began (Bonner, 1967), followed by a decline of PDE activity upon the completion of aggregation (Klein and Darmon, 1975). Gillette and Filosa (1973) reported that constant exposure of D. discoideum cells to Con A led to a greater than normal PDE activity in the cells' membranes during the first hour after cell starvation had begun. The authors attributed the delay in aggregation to this increase of PDE activity. Weeks and Weeks (1975) and Darmon and Klein (1976) extended these studies by measuring the PDE activity of Con A treated cells past the first hour of interphase and they found that the initial rise in PDE activity was followed by a continuous decrease. Therefore, Con A retarded aggregation by causing a reduction in PDE activity at the time when aggregation would have taken place normally. Brief exposure of D. discoideum cells to Con A was shown to cause a slight stimulation of PDE activity even though there was little effect on their morphogenesis (Darmon and Klein, 1976).

The aggregation of D. mucoroides macrocyst-forming cells was prevented by constant exposure of Con A. Thus, it was possible that the chemotactic mechanism of aggregation for cells destined to produce either sorocarps or macrocysts was similar. Brief exposure of cells to Con A also delayed macrocyst formation but to a lesser extent than sorocarp development. This could have been due to the 25X dilution of the concentration of cells that were incubated to form macrocysts after the Con A treatment, since the direct relationship between the concentration of of Con A to which the cells were exposed and the length of the delay of sorocarp development was demonstrated by Gillette and Filosa (1973). It was also possible that there were subtle differences in the chemotactic response that gave the macrocyst-forming cells a different susceptibility to Con A's action.

Brief exposure of D. mucoroides cells to WGA caused a delay in sorocarp development but it was shorter than the one induced by Con A. Therefore, it appeared that lectin binding to at least two types of cell surface receptors was able to elicit the same type of response but to different degrees.

The failure of WGA to delay macrocyst formation could have involved the same factors that caused macrocyst-producing cells to be less susceptible to Con A than sorocarp-forming cells.

SUMMARY

Con A and WGA induced the agglutination of D. mucoroides cells. The degree of agglutination changed during the course of development but the pattern of agglutination caused by Con A differed from the one induced by WGA. RCA_{120} did not cause agglutination of these cells.

The background agglutination of sorocarp-forming cells was significantly different from that of the macrocyst-producing cells in the Con A and WGA control groups suggesting differential membrane alterations during aggregation. Lectin-induced agglutination masked these differences.

Constant exposure to Con A prevented the aggregation of D. mucoroides cells developing toward sorocarps and macrocysts while brief exposure delayed their formation. These results suggested that these two morphogenetic phases had similar chemotactic mechanisms.

Brief exposure of D. mucoroides cells to WGA delayed sorocarp development. Thus, the binding of lectins to at least two types of surface receptors, those that react with Con A and WGA, caused similar responses. Macrocyst formation was not appreciably retarded by the WGA treatment.

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