INHIBITION OF DICTYOSTELIUM DISCOIDEUM
DIFFERENTIATION IN MONOLAYERS IN VITRO BY
ENDOGENOUS AND EXOGENOUS LECTINS

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SUMMARY
Spore-cell differentiation in monolayers in vitro of two sporagenous mutants of Dictyostelium discoideum, HM18 and HM15, is markedly inhibited by relatively low concentrations of the exogenous lectins, Concanavalin A (ConA) and wheat germ agglutinin (WGA) and by somewhat higher concentrations of the endogenous lectin, discoidin. The selective inhibition of spore cell formation by ConA occurs toward the end of the differentiation process, as shown by studies involving the addition or removal of ConA at various times during the period of differentiation. In addition, ConA has no deleterious effect on the expression of the pre-spore cell specific enzyme, UDP-galactosyl transferase. It is likely, therefore, that the maturation of pre-spore cells into spore cells is the most sensitive locus of lectin inhibition.

At higher concentrations of WGA and ConA, both stalk- and spore-cell formation are inhibited, but increased concentrations of discoidin do not have this effect.

INTRODUCTION
The appearance of a number of cell surface glycoproteins in Dictyostelium discoideum is developmentally regulated (Geltosky, Siu & Lerner, 1976; Hoffman & McMahon, 1977; West & McMahon, 1977, 1979; Gilkes, Laroy & Weeks, 1979; Orłowski & Loomis, 1979; Parish & Schmidlin, 1979; Toda, Ono & Ochiai, 1980; Lam & Siu, 1981). Many of these glycoproteins bind either Concanavalin A (ConA) or Wheat Germ Agglutinin (WGA) (Geltosky et al. 1976; West & McMahon, 1977, 1979; West, McMahon & Molday, 1978; Burridge & Jordan, 1979), and previous studies have shown that the addition of ConA and WGA to differentiating cells can influence the process of differentiation (Gillette & Filosa, 1973; Weeks & Weeks, 1975; Yu & Gregg, 1975; Darmon & Klein, 1976; West & McMahon, 1981). In particular, Yu & Gregg (1975) found that ConA inhibits both pre-spore formation and the conversion of pre-spore cells into spore cells, and West & McMahon (1981) reported that WGA inhibits the further differentiation of disaggregated pseudoplasmodial cells. The latter workers had previously shown that pre-stalk and pre-spore cells display different WGA building proteins at the cell surface (West & McMahon, 1979), suggesting a possible role for these glycoproteins in pattern formation.

In addition, D. discoideum produces an endogenous, developmentally regulated lectin, discoidin (Rosen, Kafka, Simpson & Barondes, 1973). It has been proposed that this lectin is involved in the establishment of cell–cell contacts during aggregation, but definitive proof of this theory has not been forthcoming (Rosen & Barondes,
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1978). Recently, data have been presented that indicate that there is twice as much discoidin in pre-spore cells as in pre-stalk cells, suggesting that discoidin may be involved at a later stage in the differentiation process (Lam, Pickering, Geltosky & Siu, 1981).

Kay and co-workers have isolated a number of sporagenous mutants of V12-M2 that form stalk and spore cells in monolayers in the presence of millimolar levels of cyclic-AMP (Kay, Garrod & Tilly, 1978). Since both stalk- and spore-cell formation can be readily determined in these monolayers by phase-contrast microscopy, we have used this technique to look for possible effects of discoidin on the process of differentiation and to delineate further the effects of WGA and ConA.

MATERIALS AND METHODS

Organisms and growth conditions

HM18 and HM15, two sporagenous mutant strains of V12-M2 that form spores when plated on non-nutrient agar in the presence of cyclic AMP (Kay et al. 1978), were obtained from Dr R. R. Kay. The two strains were grown in association with Entebacter aerogenes as previously described (Sussman, 1966), until clearing of the bacterial lawn was readily discernable.

Materials

Wheat germ agglutinin (WGA) was obtained from Pharmacia. Concanavalin A (ConA), Ricinus communis agglutinin-60 (RCA-60) and Ulex europaeus agglutinin (UEA) were all obtained from Miles-Yeda. Limulin was a gift from Dr J. Gross. Discoidin was isolated from vegetative cells of D. discoideum strain Ax-2 by a previously published procedure (Rosen et al. 1973).

The radioactive substrates were from New England Nuclear.

Monolayer differentiation

Cells were harvested from growth plates into 20 mm-potassium phosphate (pH 6.1) containing 2 mm-MgSO$_4$ (K/Mg) and the cell suspensions were centrifuged at 700 g for 5 min. The cell pellets were resuspended in K/Mg and recentrifuged at 700 g for 2 min, a procedure that was repeated three times to remove the residual bacteria. The final cell pellet was resuspended in 20 mm-KCl, 20 mm-NaCl, 1 mm-CaCl$_2$. Small volumes of HM15 cell suspensions were added to Nunc tissue-culture dishes (5 cm) containing 2 ml of 20 mm-KCl, 20 mm-NaCl, 1 mm-CaCl$_2$, 1 mm-MgCl$_2$, 5 mm-cyclic-AMP, 15 µg/ml tetracycline, 200 µg/ml streptomycin sulphate, 10 mm-2-(N-morpholino)-ethane sulphonic acid (MES), pH 6.2 (MES suspension medium). For strain HM18, the MES (pH 6.2) was replaced by Tris-Cl (pH 7.5) (Tris-Cl suspension medium) since this substitution increases the level of spore formation (Gross et al. 1981).

The monolayers were incubated for 48 h and then scored for stalk and spore formation by phase-contrast microscopy (Kay et al. 1978). The suspension medium was removed and cells were viewed under a glass coverslip. Stalk- and spore-cell formation was determined as a percentage of the total cell population.

For the enzyme studies the in vitro monolayer conditions were identical to those described above, except that 8 ml of suspension medium was used in 9 cm Nunc tissue-culture dishes. At the indicated times of differentiation the monolayers were resuspended by gently scraping the bottom of the tissue-culture dish with a rubber-tipped glass rod. The cell suspensions were centrifuged at 700 g for 5 min and the pellets transferred to 2 ml Eppendorf tubes and stored at −70 °C prior to the enzyme assays.

Resuspended monolayer cells were assayed for cell viability by determining the number of colony-forming units (clear plaques on a confluent bacterial lawn), as described previously (Laroy & Weeks, 1982).
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**Assays**

The stored cell suspensions were broken by freeze-thaw treatment and assayed directly for protein (Lowry, Rosebrough, Farr & Randall, 1951), cyclic-AMP-dependent phosphodiesterase (Henderson, 1975), glycogen phosphorylase (Town & Gross, 1978), UDP-glactosyl polysaccharide transferase (Kay, 1979) and alkaline phosphatase (Das & Weeks, 1981) by the indicated procedures. Since the vegetative alkaline phosphatase is heat-activated (Das & Weeks, 1981), care was taken to maintain the broken cell suspension at 4°C or below, and this enzyme was routinely assayed first.

**RESULTS**

As shown in Table 1, low concentrations (50 μg/ml) of discoidin specifically inhibited spore-cell formation in monolayers of strain HM18. The inhibition was only partial and could not be increased by elevated levels of discoidin, but there was no effect on stalk-cell formation even at the highest levels of discoidin tested. ConA and WGA also specifically inhibited spore-cell formation at low concentrations (Table 1). Similar results were obtained when strain HM15 was used (Table 2). The inhibitory effects of discoidin, ConA and WGA on spore-cell formation were not due to cell death, since these lectins had no deleterious effects on cell viability for up to 30 h of monolayer incubation (data not shown). At higher concentrations of WGA and ConA, stalk-cell formation was also inhibited (Tables 1 and 2) but this non-specific inhibition was not investigated further. Limulin, RCA-60, SBA and UEA all had no effect on either stalk- or spore-cell differentiation when added at concentrations of 50 μg/ml (data not shown).

In an attempt to delineate the period during which spore cell formation was susceptible to the inhibitory lectins, ConA was added at progressively later times, at levels that almost totally eliminated spore-cell formation but had no effect on stalk cell formation. Fig. 1 indicates that the addition of ConA prior to 17 h totally suppressed

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**Table 1. Effects of discoidin, ConA and WGA on stalk- and spore-cell formation in strain HM18**

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Concentration (μg/ml)</th>
<th>% Cell population*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amoebae</td>
</tr>
<tr>
<td>None</td>
<td>—</td>
<td>25</td>
</tr>
<tr>
<td>Discoidin</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Discoidin</td>
<td>50</td>
<td>42</td>
</tr>
<tr>
<td>Discoidin</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>Discoidin</td>
<td>200</td>
<td>46</td>
</tr>
<tr>
<td>ConA</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>ConA</td>
<td>30</td>
<td>86</td>
</tr>
<tr>
<td>WGA</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td>WGA</td>
<td>30</td>
<td>98</td>
</tr>
</tbody>
</table>

*Cell density was 2.5 × 10⁴ cells/cm².*
Table 2. Effect of lectins on stalk and spore formation in monolayers of strain HM15

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Concentration (μg/ml)</th>
<th>% Total cell population*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>21 A 55 S 24</td>
</tr>
<tr>
<td>Discoidin</td>
<td>200</td>
<td>34 A 55 S 11</td>
</tr>
<tr>
<td>ConA</td>
<td>5</td>
<td>31 A 57 S 12</td>
</tr>
<tr>
<td>ConA</td>
<td>30</td>
<td>60 A 40 S 0</td>
</tr>
<tr>
<td>WGA</td>
<td>5</td>
<td>23 A 59 S 18</td>
</tr>
<tr>
<td>WGA</td>
<td>30</td>
<td>68 A 28 S 4</td>
</tr>
</tbody>
</table>

* Cell density was 2·5 × 10⁴ cells/cm².

spore formation. However, when ConA was added at later times, progressively more spore cells were formed. The converse experiment was also performed, whereby ConA was added at the initiation of differentiation and then removed after the indicated times (Fig. 1). Spore-cell formation was inhibited after relatively short periods of incubation in the presence of ConA. However, if cells were briefly rinsed

Fig. 1. The effect of adding or removing ConA at various times during differentiation of strain HM18 in monolayers. Washed cells (5 × 10⁶) of strain HM18 were added in 2 ml of Tris Cl standard suspension medium to 5 cm Nunc tissue-culture dishes to give a cell density of 2·5 × 10⁴ cells/cm² at the bottom of the dish. Three experimental variations (O, Δ, ⊖) were performed. (1) ConA was added (6μg/ml) at the indicated times (Δ); (2) ConA was added (6μg/ml) at 0 h differentiation, and at the indicated times the 2 ml of suspension medium was removed and replaced by 2 ml of Tris-Cl standard suspension medium containing no ConA (⊖); and (3) ConA was added (6μg/ml) at 0 h differentiation, and at the indicated times the suspension medium was removed and replaced by 2·0 ml of Tris-Cl standard suspension medium containing 0·3 mM α-methyl mannoside (O). After 10 min incubation this suspension medium was replaced by 2 ml of Tris-Cl standard suspension medium.

After 48 h incubation at 22°C, the tissue-culture dishes were viewed by phase-contrast microscopy and the percentage of the population that had formed spores was scored.
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with α-methyl mannoside then the ConA inhibitory effect could be reversed as late as 17 h of differentiation (Fig. 2). When removed at times later than 17 h, however, there was a reduction in the number of spore cells. The fact that ConA inhibition can be prevented by brief treatment with α-methyl mannoside suggests that ConA remains bound to the surface of the cells under these conditions for the first 17 h of differentiation. Similar results were obtained with low concentrations of WGA (data not shown).

The timing of the ConA inhibitory effect on spore-cell differentiation was also examined by determining the effects of ConA on the activities of four developmentally regulated enzymes during in vitro differentiation in monolayers. The four enzymes chosen for this study increase in activity at different developmental stages. Cyclic-AMP-dependent phosphodiesterase accumulates during the pre-aggregation phase of

Fig. 2. The effect of ConA on the expression of four enzymes during the in vitro monolayer differentiation of strain HM18. Cells were incubated at a density of 10⁵ cells/cm² under Tris-Cl standard suspension medium containing either 8 μg/ml ConA (Δ) or no ConA (○). At the indicated times the cells were harvested as described in Materials and Methods and assayed for the activities of the four enzymes: A, cyclic-AMP-dependent phosphodiesterase; B, glycogen phosphorylase; C, UDP-galactosyl polysaccharide transferase; and D, alkaline phosphatase.
development and the activity then decreases during subsequent differentiation (Brookman, Town, Jermyn & Kay, 1982). Glycogen phosphorylase accumulates during the post-aggregative period (Firtel & Bonner, 1972; Loomis, 1975) and appears in both pre-stalk and pre-spore cells, although the specific activity in the former is higher (Rutherford & Harris, 1976). UDP-galactosyl polysaccharide transferase is also produced during the post-aggregative period, but is localized in pre-spore cells (Newell, Ellingson & Sussman, 1969). Alkaline phosphatase increases in activity during culmination (Loomis, 1969) and is localized in the maturing stalk cells (Quiviger, Benichou & Ryter, 1980; Armant, Stetler & Rutherford, 1980).

ConA was without effect on the cyclic-AMP-dependent phosphodiesterase and in fact slightly enhanced the expression of UDP-galactosyl polysaccharide transferase and alkaline phosphatase in strain HM18 (Fig. 2). There was a slight inhibitory effect on the onset of the accumulation of glycogen phosphorylase, but eventually the lectin-treated cells produced more enzyme than the control.

It should be noted, however, that in HM18 cells the expression of these four developmentally regulated enzymes (under the in vitro differentiation conditions described here) was somewhat unusual. In wild-type V12-M2 cells, differentiating either in vivo (Brookman et al. 1982) or in vitro in submerged monolayers (Weeks & Neave, unpublished data), the cyclic-AMP phosphodiesterase activity dropped drastically after the pre-aggregation period, and both the glycogen phosphorylase and the UDP-galactosyl polysaccharide transferase exhibited far more pronounced accumulations during the post-aggregative period. In addition, the accumulation of alkaline phosphatase activity occurred at later times during either in vivo or in vitro differentiation in V12-M2 cells (Sobolewski, Weeks & Neave, unpublished data). This divergence from the normal pattern of enzyme expression is clearly related to the precise conditions used for in vitro differentiation, since there was a striking similarity in the accumulation of glycogen phosphorylase and UDP-galactosyl polysaccharide transferase during the differentiation of HM18 cells in monolayers on non-nutrient agar (Peacey & Gross, 1981) and during in vivo differentiation of V12-M2 cells (Brookman et al. 1982). However, since the inhibitory effects of the lectins were far less pronounced and more variable during in vitro differentiation on non-nutrient agar, no attempt was made to study enzyme expression under these conditions.

The fact that the accumulation of the four developmentally regulated enzymes is relatively unaffected by ConA in strain HM18 is further corroboration of the idea that ConA inhibition of spore-cell formation occurs late during the differentiation process. However, since the expression of some of the developmentally regulated enzymes in in vitro monolayers of HM18 cells was somewhat unusual, the experiment was repeated in strain HM15 (see Fig. 3).

The developmentally regulated expression of the four enzymes in strain HM15 were more characteristic of wild-type in vivo development. The production of the cyclic-AMP-dependent phosphodiesterase was slightly delayed in the presence of ConA, and the normal decrease in activity did not occur. However, UDP-galactosyl polysaccharide transferase and alkaline phosphatase were unaffected. The onset of glycogen phosphorylase accumulation was also slightly delayed in this strain, but the
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Fig. 3. The effect of ConA on the expression of four enzymes during the differentiation of strain HM15 in monolayers. Experimental protocol was identical to that described for Fig. 2 except that MES standard suspension medium was used and ConA was used at a concentration of 20μg/ml.

levels of activity eventually attained in the presence of ConA were similar to those in controls. Again, these results suggest that ConA has but a minimal effect on the expression of the four developmentally regulated enzymes, and is consistent with the idea that ConA blocks spore-cell differentiation at a late stage. Similar results on the effect of WGA on enzyme accumulation were observed in strain HM15 (data not shown).

DISCUSSION

The results presented here indicate that relatively low concentrations of the plant lectins, WGA and ConA, specifically inhibit spore-cell formation in monolayers in vitro of the sporogenous mutants of D. discoideum, HM15 and HM18. The endogenous lectin, discoidin, also specifically inhibits spore-cell formation, but higher concentrations are required and the inhibition is never more than partial. Under the
conditions of the present study, spore-cell formation can first be detected 18 h after
the onset of differentiation and is essentially complete by 28 h (unpublished observa-
tions). The inhibition of spore-cell formation by ConA or WGA occurs at a relatively
late stage in the differentiation process, since the expression of the enzyme UDP-
galactosyl polysaccharide transferase is unaffected by levels of ConA or WGA that
totally inhibit spore-cell formation. This enzyme is specific for the pre-spore pathway
of development and accumulates during pseudoplasmodium formation (Newell et al.
1969). In addition, total removal of ConA or WGA from in vitro monolayers at times
prior to 17 h of differentiation reverses the lectin-mediated inhibition of spore-cell
formation (Fig. 1), whereas if the lectins are removed after 20 h of differentiation,
spore-cell formation is decreased. Conversely, the lectins inhibit spore-cell formation
if added before 17 h differentiation, but the inhibition is reduced when lectin is added
at later times (Fig. 1). This data are consistent with the idea that the most sensitive
locus for the lectin-mediated inhibition of spore-cell formation is a terminal step in the
process. It should be noted that Yu & Gregg (1975) found that ConA inhibited both
pre-spore and spore-cell formation during in vivo differentiation, but these workers
used considerably higher concentrations than those used in the present study.

Peacey & Gross (1981) have recently shown that spore-cell formation in monolayers
in vitro of strain HM18 is selectively inhibited by the addition of low concentrations
of protease, and this inhibition can be pinpointed to a late stage during differentia-
tion. Both protease and lectin treatments might interfere with the cell surface process-
ing that must accompany the formation of the spore cell wall. Alternatively, if some
form of cell–cell interaction is required for spore-cell formation, then it might be
interrupted by these agents. More work will be required to distinguish these
possibilities. It should be pointed out, however, that Kay and co-workers have obser-
vied spore formation in certain sporagenous mutants, at cell densities sufficiently low
to preclude the possible involvement of cell–cell interaction (Kay & Trevan, 1981;
Kay, 1982). However, while spore-cell formation is independent of cell density in
strain HM15, spore-cell formation in strain HM18 remains density-dependent
(Weeks, unpublished observations).

Spore-cell formation is inhibited by ConA, WGA and discoidin, but it is conceiv-
able that all three lectins react with a single component at the cell surface, since some
D. discoideum membrane glycoproteins react with both ConA and WGA (West &
McMahon, 1979; West et al. 1978). The differences in the sensitivity of stalk- and
spore-cell formation to WGA might be related to the finding of West & McMahon
(1979) that there are different WGA receptors on the surfaces of pre-stalk and pre-
spore cells. However, these workers detected no differences in ConA binding sites on
the surfaces of the two cell types, and in the present study, the inhibitory effects of
ConA and WGA were very similar. The inhibition of spore-cell formation by the
endogenous lectin, discoidin, may indicate a possible role for discoidin in spore-cell
differentiation. In this respect, it is interesting that pre-spore cells appear to have
higher levels of discoidin than pre-stalk cells (Lam et al. 1981).

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REFERENCES


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