

EFFECT OF CONCAVALIN A ON CELLULAR SLIME MOLD DEVELOPMENT:
PREMATURE APPEARANCE OF MEMBRANE-BOUND CYCLIC AMP
PHOSPHODIESTERASE

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Summary: Concanavalin A delays aggregation of slime mold amoebae, apparently by interfering with the cells' response to the chemotactic agent, cyclic AMP. Concanavalin A also induces the premature appearance in non-aggregating cells of a membrane-bound cyclic AMP phosphodiesterase normally found only at the time of aggregation. The appearance of this enzyme is not due to activation of an inactive form of the enzyme.

After a period of vegetative growth as solitary amoebae, cells of Dictyostelium discoideum move together into multicellular aggregates each of which differentiates into a fruiting body. The attraction of the dispersed cells into aggregation centres is in response to cyclic AMP secreted by the amoebae (1). Malchow *et al.* (2) have demonstrated in D. discoideum the presence of a membrane-bound cyclic AMP phosphodiesterase (mPDE) which dramatically increases in activity just preceding aggregation and then returns to very low levels after aggregation. These investigators suggest that this phosphodiesterase participates both in the detection of cyclic AMP and the chemotactic response during cell aggregation. We present here a study showing that Concanavalin A (Con A), a plant lectin known to bind to carbohydrate determinants of cell surfaces (3-8), affects the process of aggregation in slime mold development.

MATERIALS AND METHODS

For all developmental studies late log phase amoebae were washed free of Escherichia coli B, plated on 1% purified agar (Difco), and incubated at 22°C in the light. To test for cyclic AMP responsiveness, washed cells were sensitized by aging at 4°C and assayed according to Konijn (9). Cyclic AMP phosphodiesterase activity of whole cells was examined which includes both membrane-bound enzyme exposed at the cell surface and soluble enzyme released from the cells. Total whole cell phosphodiesterase

Table 1. Effect of Con A in delaying aggregation of Dictyostelium discoideum amoebae.

		Time of completion of the first aggregate (hr)			cells/mm ²
		2.5 x 10 ³	7.5 x 10 ³	1.25 x 10 ⁴	
Con A	0 µg/ml	23	23	22	
	50	30	27	24	
	100	30	28	27	
	200	42	30	30	
	300	38	32	31	
	400	53	34	33	
α-MG	5 x 10 ⁻² M	21	20	20.5	
Con A	200 µg/ml				
+ α-MG	+ 2.5 x 10 ⁻² M	21	21	22	

Stock cultures of slime molds were maintained in association with Escherichia coli B on buffered nutrient agar (11). Hydrophobic 1% purified agar (12) in slime mold saline was prepared; Con A (Sigma, Grade IV) or α-MG (Sigma) was added where necessary and the mixture poured into sterile 35mm plastic Petri dishes. Cells were grown from spores on E. coli at 22°C in the dark. The amoebae were washed free of bacteria and 2µl droplets of cell suspension were applied to the hydrophobic agar with a 10µl Hamilton microsyringe. Each cell density was prepared in duplicate or triplicate; the experiment was repeated four times.

activity was corrected for soluble activity (2) in order to determine the specific activity of enzyme localized at the cell surface. In addition, a particulate fraction consisting of the 27000 x g pellet of twice freeze-thawed cells was prepared (2). This crude particulate preparation includes surface as well as internal membranes. All enzyme assays were according to Malchow et al. (2) with modifications described in the legends. Protein was determined by the method of Lowry (10).

RESULTS AND DISCUSSION

Effect of Con A on slime mold development

When late log phase amoebae are plated on Con A agar and allowed to develop, a striking delay in aggregation is observed (Table 1), although morphology of the resulting fruiting bodies is normal. The extent of the delay is related to Con A concentration and the density at which the cells are plated.

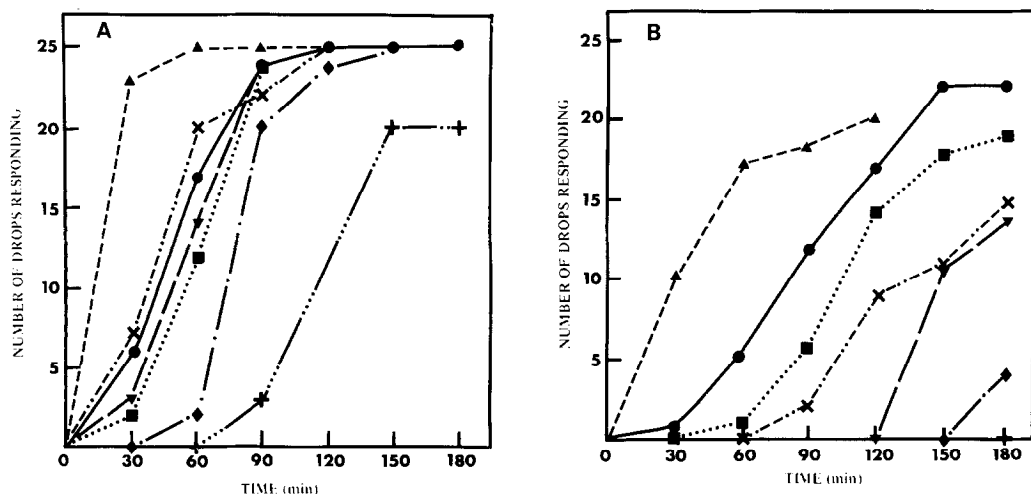


Figure 1A: Response of *D. discoideum* to 10^{-3} M cyclic AMP. Vegetative cells were deposited on hydrophobic agar (12) in 0.1 μ l droplets at a density of 3.7×10^3 cells/mm². Cyclic AMP (Sigma) in saline was applied with a microsyringe in a 0.1 μ l drop 0.1-0.2 mm from the edge of each droplet of sensitized amoebae. Movement of amoebae outside the perimeter of the drop was considered a response to cyclic AMP (9). The rate of response was scored for cells incubated on various concentrations of Con A:

● ———, 0 μ g/ml; ■ ·····, 50 μ g/ml; X — · — ·, 100 μ g/ml;
 ▼ ———, 200 μ g/ml; ◆ · — · — ·, 300 μ g/ml; + ··· —, 400 μ g/ml;
 ▲ - - - -, 200 μ g/ml + 2.5×10^{-2} M α -MG.

Figure 1B: Response of *D. discoideum* to 10^{-6} M cyclic AMP. Procedure and symbols as in Fig. 1A.

Since aggregation has been shown to be mediated by the chemotactic action of cyclic AMP, the possibility that Con A might be interfering with the response of amoebae to cyclic AMP was tested. The Konijn test (9) was performed in which aggregation-competent cells in the presence of Con A were tested for their ability to respond to exogenously supplied cyclic AMP. When cells on agar containing 50-200 μ g/ml of Con A are exposed to high levels of cyclic AMP (10^{-3} M), they respond at a rate very similar to controls (Fig. 1A). Above 200 μ g Con A/ml there is inhibition of the response, the degree of inhibition increasing with Con A concentration. This suggests that cyclic AMP reverses the Con A mediated inhibition. If so, lowering the cyclic AMP concentration should result in reduction

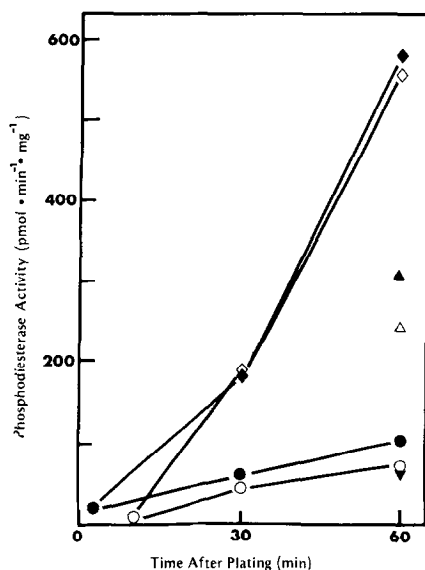


Figure 2: Effect of Con A on phosphodiesterase activity of crude particle fraction; two separate experiments. Abscissa: minutes after plating cells. Ordinate: pmol of cyclic AMP broken down per min per mg of protein. Assays for mPDE were performed at 19°C using about 5µg particle protein in 110µl of a reaction mixture that included 200 pmol [³H]-cyclic AMP (20.7 Ci/mmol Amersham Searle) in .05M Tris, pH 7.4 containing .02M MgSO₄(2). Under these conditions the protein concentration was rate-limiting and 5'AMP production was proportional to the protein concentration. The reaction was stopped with TCA, and the mixture chromatographed on paper to separate cyclic AMP and 5'AMP which were counted in a Beckman LS250 scintillation counter (2). For these experiments cells were plated at a density of 1 x 10⁴ cells/mm² on agar containing:

○, ●, agar only; ◇, ◆, 300µg Con A/ml;

▼, 3.75 x 10⁻²M α-MG; △, ▲, 300µg/ml Con A + 3.75 x 10⁻²M α-MG.

of the cyclic AMP response at the lower concentrations of Con A. This predicted shift in response was found. Using 10⁻⁶M cyclic AMP the response of D. discoideum is depressed by all Con A concentrations in a dose dependent manner (Fig. 1B) while at 300 and 400µg Con A/ml inhibition of the cyclic AMP response is essentially complete.

The effects of Con A on both aggregation and the response to cyclic AMP are prevented by addition of α-methyl-D-glucoside (α-MG), a competitive inhibitor of Con A binding (3) (Fig. 1A, 1B).

Effect of Con A on membrane-bound cyclic AMP phosphodiesterase.

Since it has been well-established for other organisms that Con A interacts with cell surfaces, it is possible that this lectin exerts its effect on slime molds in the same manner. Indeed, both vegetative and differentiating amoebae exhibit surface staining with fluorescein-labeled Con A and agglutination with unlabeled Con A, indicating the presence of Con A determinants on the cell surface (Gillette, unpublished results). Both of these reactions are inhibited by α -MG.

Because the membrane-bound cyclic AMP phosphodiesterase (mPDE) has been implicated in the chemotactic response of aggregating amoebae (2), it is possible that Con A may affect aggregation by changing the activity of this enzyme through its interaction with the cell surface. Accordingly, phosphodiesterase activity of whole cells and crude particulate preparations was examined.

Within one-half hour after plating on 300 μ g/ml Con A, the specific activity of mPDE in the particulate fraction is four times greater than that of controls (Fig. 2). When mPDE activity is measured for the surface only of intact cells, a marked difference in specific activity between Con A-treated and control cells does not appear until one hour after treatment begins (Table 2). This suggests that the enzyme is first incorporated into internal membranes and then transported to the cell surface. The effect of Con A on activity of both whole cells and particles is reduced by the haptenic sugar α -MG, indicating that the specificity of Con A binding to the cell is critical for this response.

The specific activity of particulate mPDE in amoebae which have been treated with Con A for one hour is near the level normally observed at the time of aggregation, which occurs at 12 hours under the conditions used in these experiments. This suggests that Con A may prematurely induce this enzyme. A requirement for RNA synthesis during Con A-mediated stimulation is demonstrated by treatment of cells simultaneously with Con A (300 μ g/ml) and actinomycin D (125 μ g/ml) in the dark. Sussman *et al.* (15) have shown that [3 H]-uridine incorporation is depressed by about 75% at this concentration of actinomycin D. Particles prepared from cells which were incubated on Con A and actinomycin D for 3 hours showed only 15% of the mPDE activity of cells kept on Con A alone for the same period. This result supports the notion that the increase in activity in the presence of Con A is due to synthesis of the enzyme rather than activation of an

Table 2. Effect of Con A on Phosphodiesterase activity of whole cells

Time after plating (minutes)	Phosphodiesterase activity (pmol.mg ⁻¹ .min ⁻¹)	
	<u>cells plated on 1% agar</u>	<u>cells plated on 300µg Con A/ml agar</u>
2.5	4.7	
30	16.8	27.0
60	31.7	289.0
60	29.7 (plus α-MG 3.75 x 10 ⁻² M)	70.0 (plus α-MG 3.75 x 10 ⁻² M)

Late log phase amoebae were collected from shaking cultures (13, 14), washed free of bacteria with saline, and plated on 1% purified agar at 1×10^4 cells/mm². Cells were collected from plates at the times indicated and washed with Sørensen's phosphate, pH 6.0. For enzyme assays either 8.3×10^5 or 1.7×10^6 cells in 160µl of phosphate buffer containing 150pmol of [³H]-cyclic AMP were incubated at 19°C. Enzyme activity is expressed as number of pmol of substrate broken down per min per mg of protein.

inactive form of the enzyme. Furthermore, in experiments in which untreated whole cells and particulate preparations were assayed for phosphodiesterase activity in the presence of Con A, there was no increase in specific activity above control values.

Inhibitors of the cyclic AMP phosphodiesterase (2) which normally appears during aggregation are also effective against the mPDE of cells treated with Con A. Five millimolar cyclic-3',5'-inosine monophosphate, a competitive inhibitor, completely inhibits activity of the particulate mPDE of such cells. Also, 10mM glutathione reduces the activity to 40% of control values. These results suggest that the enzyme induced by Con A is identical to that normally produced at the time of aggregation.

The data above indicate that Con A interferes with the cells' response to cyclic AMP, and increases mPDE activity prior to its normal increase at aggregation. This premature stimulation of mPDE might explain the delay in aggregation if the enzyme, by hydrolyzing secreted cyclic AMP, reduces extracellular levels of the nucleotide below the threshold required to trigger aggregation. Then, if no other part of the

aggregation machinery is affected by Con A, a longer period of time would be required for cyclic AMP to build up to an effective level. The increase in mPDE activity in the presence of the lectin appears to be due to a true stimulation of enzyme synthesis rather than activation of previously existing enzyme molecules. Since α -MG significantly reduces the increase in mPDE activity produced by Con A, it can be concluded that the effect of the lectin on this enzyme is very likely brought about by binding of the lectin to carbohydrate moieties on the cell surface.

These results raise the question of whether Con A is mimicking a factor produced during normal development which enhances mPDE activity at the time of aggregation. This would imply that such a factor produces its effect by binding to a receptor on the cell surface which in turn is linked to the internal metabolic machinery involved with synthesis of the enzyme and its incorporation into membrane.

From the standpoint of slime mold development our results are of interest in that attachment of a foreign protein to the surface of amoebae changes the temporal program for membrane-bound PDE. Furthermore, we believe that these results have significance as well for studies on lymphocyte activation by mitogens. Activation is thought to result from binding of a variety of lectins to the cell surface (16). Smith *et al.* (17) have shown that after 6 hours of incubation in the lectin Phaseolus vulgaris phytohemagglutinin (PHA), lymphocytes have a lower level of cyclic AMP than controls. They have also found that compounds which elevate cyclic AMP levels inhibit lymphocyte transformation. In view of these findings it would be of value to examine PHA and Con A stimulated lymphocytes for increased phosphodiesterase activity as a possible cause of the lowered cyclic AMP levels apparently required for activation.

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