EFFECT OF NH_3 ON c-AMP ASSOCIATED ACTIVITIES AND EXTRACELLULAR c-AMP PRODUCTION IN DICTYOSTELIUM DISCOIDEUM*

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Received October 19, 1977

SUMMARY: A model of morphogenetic regulation in Dictyostelium discoideum (1) is based on the assumption that NH_3 inhibits the synthesis and/or release of extracellular 3',5'-cyclic AMP and that by topographical restriction of c-AMP production to specified zones within the cell aggregate, NH_3 is presumed to set up the conditions for apical dominance and directed morphogenetic movements. This study indicates that: exposure of preaggregative cells to exogenous NH_3 + NH_4^+ inhibits the acquisition of c-AMP-induced properties associated with aggregation competence (accumulation of specific contact sites required to form EDTA resistant aggregates and the synthesis of extracellular and membrane-bound c-AMP phosphodiesterase); exposure of aggregation competent cells which are actively producing extracellular c-AMP to exogenous NH_3 + NH_4^+ is followed by the immediate cessation of extracellular c-AMP release. The pH dependence of these effects suggests that the active species is NH_3.

INTRODUCTION

Two compounds, 3',5'-c-AMP and ammonia, have been shown to act as specific morphogens regulating the course of morphogenesis and gene expression in D. discoideum. Prior to cell aggregation, c-AMP appears to induce alterations in the plasma membrane associated with the acquisition of specific cell cohesivity (2) and to induce the synthesis of membrane-bound and extracellular phosphodiesterase (3). During aggregation it serves as a chemotactic agent facilitating the movements of the responding cells toward the aggregative centers (4). During slug migration and fruiting body construction it plays a major role in the maintenance of polarity and apical dominance, and possibly in directing morphogenetic movements (5). Finally, it may be involved in the choice of pathways of cytodifferentiation elected by the component cells (6,7).

Ammonia plays a decisive role in determining which pathway of morphogene-

*This work was supported by a grant from the National Science Foundation, PCM 76-03993. **Andrew Mellon Predoctoral Fellow.

ISSN 0006-291X

sis the newly formed cell aggregate will elect to enter. The latter can either construct a fruiting body directly at the site of aggregation or transform into a migrating slug and move away. After a migratory period lasting from hours to days, it can stop and re-enter the fruiting mode (8). All of these morphogenetic transitions are accompanied by profound changes in the program of gene expression as reflected by the patterns of accumulation and disappearance of specific proteins (9,10). Recent experiments (11) have shown that the transformation of the newly formed aggregate into a migrating slug depends on the local accumulation of NH_3 as influenced by: the rate of $NH_3 + NH_4^+$ production by catabolic degradation; the ambient pH (which determines the NH_3:NH_4^+ ratio); and the rate of evaporation of NH_3 and its diffusion into the substratum. Similarly, the abandonment of slug migration and re-entrance into the fruiting mode depends on the dissipation of NH_3 either by changes in the parameters listed above or the experimentally contrived removal of NH_3 by applying to the surface of the slug 0.5 μl of an enzyme reaction mixture containing glutamate dehydrogenase, α-ketoglutarate, and NADH.

The results described in the present communication indicate the existence of a direct interaction between ammonia and c-AMP during D. discoideum development. Specifically, they demonstrate that: (a) exogenous NH_3 (supplied as ammonium carbonate) inhibits c-AMP-induced phenotypic changes in stationary phase cells which normally are associated with the acquisition of aggregative competence and; (b) exogenous NH_3 inhibits the production and/or release of c-AMP by cells which have already achieved aggregation competence.

RESULTS

Ability of Ammonia to Inhibit the Acquisition of Contact Sites by Pre-aggregative Cells

Previous studies (12,13) have demonstrated that the acquisition of aggregation competence by stationary phase cells is accompanied by the appearance of immunochemically specific contact sites at the cell surface. The accumulation of these sites can be followed quantitatively by assaying the ability of the

cells in gently shaken suspensions to form aggregates in the presence of EDTA (14). Addition of c-AMP to preaggregative wild type cells has been shown to induce the premature acquisition of these sites and to induce certain aggregateless mutant strains to acquire the sites and to actually aggregate (15). The results of the experiment summarized in Fig. 1 show that addition of exogenous NH_3 + NH_4^+ to preaggregative wild type cells prevents the accumulation of these contact sites, but does not interfere with the actual formation of EDTA-resistant aggregates by cells that have already attained aggregation competence. The pH dependence of this inhibition, shown in Fig. 2, indicates that NH_3 is the active species. (The pK of the reaction $NH_3 + H^+ \rightleftharpoons NH_4^+$ is 9.2. Hence at pH 7.2 the ratio NH_3:NH_4^+ would be 1:200 and at pH 6.2, 1:2000.)

Ability of NH_3 to Inhibit Phosphodiesterase Synthesis by Pre-aggregative Cells

Confirming previous work (16,17), the results shown in Fig. 3 indicate that the acquisition of aggregation competence by cells in gently shaken suspension is accompanied by the accumulation of both extracellular and membrane-bound phosphodiesterase. Addition of exogenous c-AMP to stationary phase cells has been found to induce the premature accumulation of these enzyme activities (3). Moreover, as in the case of contact site acquisition, phosphodiesterase activity could be induced by exogenous c-AMP in aggregateless mutants incapable of doing so in its absence (18). The results summarized in Fig. 3 show that addition of exogenous NH_3 + NH_4^+ prevented the accumulation of both extracellular and membrane-bound activities. The pH dependence of this response (not shown) indicated that the active species is NH_3. The presence of NH_3 + NH_4^+ in the assay mixture did not interfere with the activity of the enzyme. In contrast to the above, the pattern of N-acetyl-glucosaminidase accumulation remained unaffected by the presence of NH_3 + NH_4^+. Previous work (19) has shown that the accumulation of this enzyme appears to be associated with entrance of the cells into the stationary growth phase and is not connected with the acquisition of either aggregation competence nor presumably with changes in c-AMP metabolism.

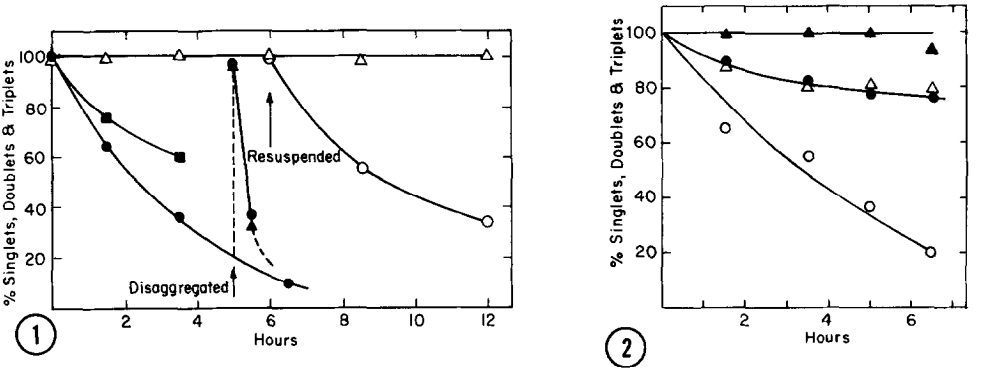


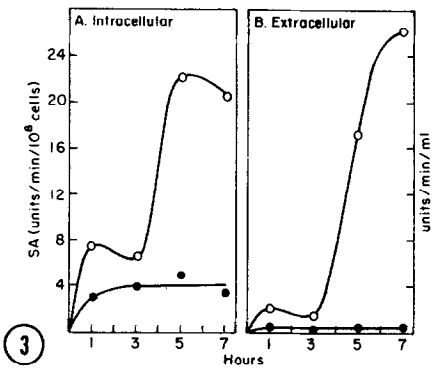
Figure 1: Effect of ammonia on the acquisition of contact sites in Dictyostelium discoideum. D. discoideum, strain NC4 (haploid), was grown in association with Aerobacter aerogenes as previously described (23). Aliquots of washed stationary phase cells were delivered to 125 ml Erlenmeyer flasks containing 10 ml volumes of 17 mM phosphate buffer: at pH 6.25, (●); pH 7.25, (■); pH 7.25 + 14 mM NH_3 + NH_4^+, (△). All flasks contained 200 μg/ml of streptomycin sulfate and were incubated at 22°C on a gyratory shaker rotating at 100 rpm. The final cell concentration was 10^7 cells/ml. At various times cells, from an entire flask, were harvested by centrifugation and the pellet resuspended in 1 ml of 17 mM phosphate buffer, pH 6.25, containing 10 mM EDTA. The proportion of single cells, doublets, and triplets were scored by counting replicate samples in a hemocytometer.

To determine if NH_3 + NH_4^+ interfered with aggregation (as opposed to the acquisition of aggregative capacity) cells that had been incubated for 5 hrs at pH 6.25, were harvested by centrifugation and mechanically disaggregated (24) to yield single cell suspensions. Aliquots were then resuspended in 17 mM phosphate buffer, pH 6.25, (●), and pH 7.25 + 14 mM NH_3 + NH_4^+ (▲). At intervals, replicates were assayed for EDTA resistant aggregation as described above. To determine if the effect of NH_3 + NH_4^+ was reversible cells that had been incubated at pH 7.25 + 14 mM NH_3 + NH_4^+ for six hr were harvested by centrifugation, resuspended in pH 6.25 buffer and incubated further, (○).

Figure 2: pH dependence of ammonia effect on the acquisition of contact sites. Cells were prepared for experiment as described in the legend to Fig. 1 and aliquots were delivered into 10 ml volumes of 17 mM phosphate buffer plus: 1.4 mM NH_3 + NH_4^+, pH 6.25, (○); 1.4 mM NH_3 + NH_4^+, pH 7.25 (●); 14 mM NH_3 + NH_4^+, pH 6.25 (△); 14 mM NH_3 + NH_4^+, pH 7.25 (▲). Final cell concentrations were 10^7 cells/ml. Assays for EDTA resistant contact sites were conducted as described in the legend to Fig. 1.

Ability of NH_3 to Inhibit the Accumulation of Extracellular c-AMP by Aggregation Competent Cells

Previous studies (20,21) have shown that the acquisition of aggregation competence is accompanied by the pulsatile synthesis and release of c-AMP which results in a dramatic increase in its extracellular concentration. The



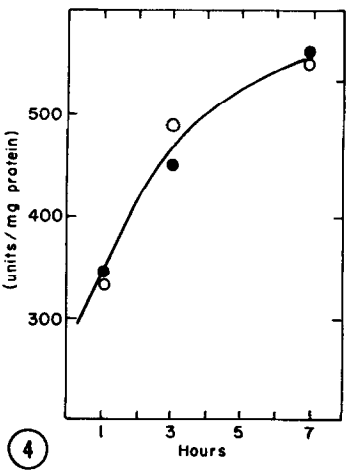


Figure 3: Determination of intracellular and extracellular phosphodiesterase activity. Cells were prepared for experiment as described in the legend to Fig. 1 and aliquots were suspended in phosphate buffer, pH 6.25, (O) or pH 7.25 containing 14 mM NH_3 + NH_4^+, (●). At intervals flask contents were centrifuged. Supernatants and frozen-thawed pellets were treated as described elsewhere (3) and assayed for phosphodiesterase activity (25).

Figure 4: Determination of N-acetylglucosaminidase activity. Preparation of cells, suspensions and symbols as in legend to Fig. 3. Flask contents were harvested at intervals and centrifuged. Frozen-thawed pellets were assayed for N-acetylglucosaminidase activity as described elsewhere (26).

results summarized in Fig. 5 demonstrate that addition of exogenous NH_3 + NH_4^+ is followed immediately by the cessation of extracellular c-AMP accumulation. Here again the pH dependence of the inhibition would suggest that NH_3 is the active species.

DISCUSSION

We have recently presented a model of morphogenetic regulation in *Dictyostelium discoideum* which is based on actions of NH_3 and c-AMP and interactions between them. The model accounts in principle for all the primary aspects of morphogenesis and cytodifferentiation in this organism, including the choice of morphogenetic pathways (slug migration or fruit construction) by the newly formed aggregate, the maintenance of apical polarity within the migrating slug, the directed movements of the cells during actual fruiting body construction,

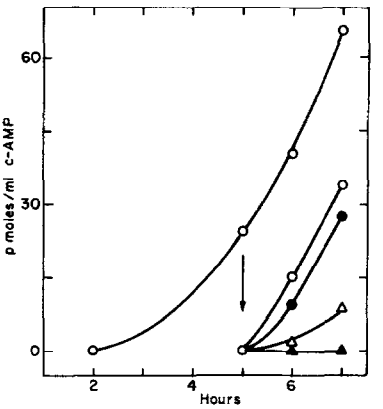


Figure 5: Determination of extracellular c-AMP in cell suspensions of D. discoideum. Cells were treated as described in the legend to Fig. 1 and aliquots were suspended in phosphate buffer pH 6.25, (○). After 5 hr incubation with shaking, flask contents were centrifuged and aliquots resuspended in phosphate buffer: pH 6.25, (○); pH 7.25 (●); pH 6.25 containing 14 mM NH_3 + NH_4^+, (△); pH 7.25 containing 14 mM NH_3 + NH_4^+, (▲). At intervals 0.5 ml samples were spun in a microfuge 2 min at 5,000 x g. Supernatants were heated at 80°C for 10 min to inactivate phosphodiesterase. c-AMP was determined by the Gilman saturation method (27) as modified by Weller et al. (28).

and the choice of pathways of cytodifferentiation. One crucial assumption upon which the model is based is that NH_3 inhibits the synthesis and/or release of c-AMP by cells within the developing aggregates. The results described in this communication indicate that the assumption is valid.

Previous results have suggested the possibility of an interaction between NH_3 and c-AMP. Thus, exogenous NH_3 + NH_4^+ present from zero time have been shown to inhibit the aggregation of D. discoideum cells incubated on filter paper circles over absorbent pads (8). In D. mucoroides, c-AMP and NH_3 appear to have counteractive effects on the size and the numbers of cell aggregates, i.e. exogenous c-AMP appears to decrease the size and increase the number of aggregates and NH_3 appears to do the opposite (22). Furthermore, incubation of preaggregative cells of D. mucoroides in an atmosphere containing NH_3 inhibited their aggregation and prevented the appearance of extracellular c-AMP. The effect was reversible since, when shifted to an NH_3-free atmosphere, the cells

subsequently aggregated and did accumulate extracellular c-AMP.

The concentrations of NH_3 + NH_4^+ used in our experiments are within the physiological range. That is, they are comparable to the concentrations that D. discoideum cells normally accumulate prior to and during aggregation (11).

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