PROMOTION OF CONIDIA AGGREGATION IN ASPERGILLUS NIGER BY CYCLIC AMP AND 5'-GMP

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Summary

Conidia of A. niger aggregated into clumps, and then germinated to form masses of mycelia (pellets) when grown in agitated, submerged, liquid cultures. Cyclic AMP and 5'-GMP promoted the aggregation of conidia, while adenosine, AMP, ADP, dibutyryl cyclic AMP, cyclic GMP, guanosine, GDP, GTP and theophylline were ineffective. ATP and 2', 3'-cyclic AMP elicited a weak response. Conidia in the center of clumps did not germinate. The increase in "adhesiveness" of conidia by cyclic AMP and GMP may have implications in the regulation of germination, as well as the eventual growth and development of hyphae.

Filamentous fungi often grow in the form of pellets in agitated, submerged, liquid cultures (1-4). These mycelial aggregates arise from the initial clumping of conidia and germlings (1,4). Although neither the causes nor the effects of hyphal aggregation in liquid cultures are well understood, studies of fungi grown on solid media (5) and studies of higher organisms (6,7) both indicate that contiguity is of prime importance in the growth and differentiation of cells.

Adenosine 3', 5'-cyclic monophosphate (cyclic AMP) is known to mediate a wide variety of physiological responses (8), including some associated with cell surfaces. It increases the adhesion of cultured cells to the substratum (9,10), decreases the aglutinability of transformed cells by plant lectins (11,12), regulates contact inhibition (11-14), increases the "adhesiveness" of the amoebae of the slime mold Dictyostelium discoidium during the aggregation phase (15), and inhibits platelet aggregation during blood clotting (16).

In this report we demonstrate that cyclic AMP and 5'-GMP promote

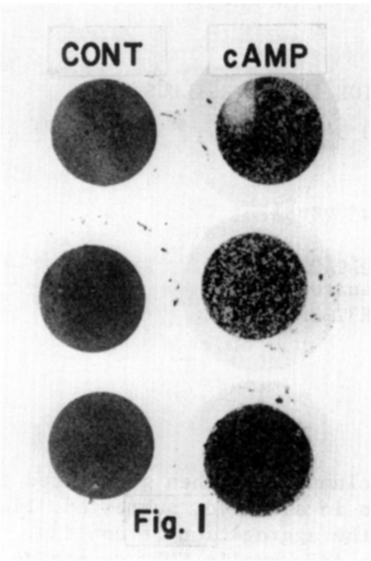


Fig. 1

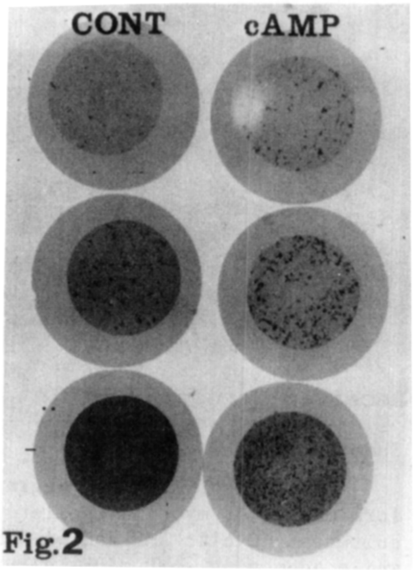


Fig.2

Figure 1 Cyclic AMP (2.5 mM) stimulation of conidia aggregation as compared to controls (nothing added). The three replicate samples illustrate the degree of variability commonly obtained in a set of five flasks. The controls (left column) appear as a dense film of relatively unaggregated conidia, while the cyclic AMP treated conidia (right column) have clumped so that the white Millipore filter can be seen in the background. 8.2×10^6 conidia per flask were used in this experiment.

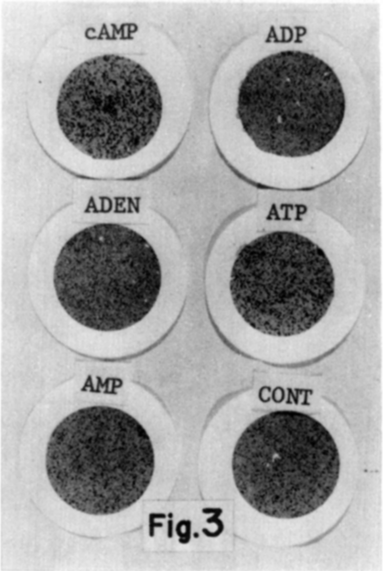
Figure 2 Cyclic AMP (1.0 mM) stimulation of conidia aggregation at different conidia concentrations. Top, 2.4×10^5 conidia; middle, 9.5×10^5 conidia; bottom, 2.4×10^6 conidia.

the clumping of conidia in Aspergillus niger. Subsequently we will show that cyclic AMP appears to be involved in a complex way with the "adhesiveness" of vegetative hyphae, actually causing de-aggregation under certain conditions (manuscript in preparation).

Materials and Methods

Conidia of A. niger NRC-A-1-233 were maintained and subcultured on agar slants of a previously described liquid medium containing Fe^{+++}, Mn^{++}, and Ca^{++} ions (17). For the clumping experiments conidia were prepared on agar slants of a metal deficient synthetic medium (18), as these conidia seemed more adhesive and more sensitive to cyclic AMP.

Growth conditions were as before (18), with each flask receiving



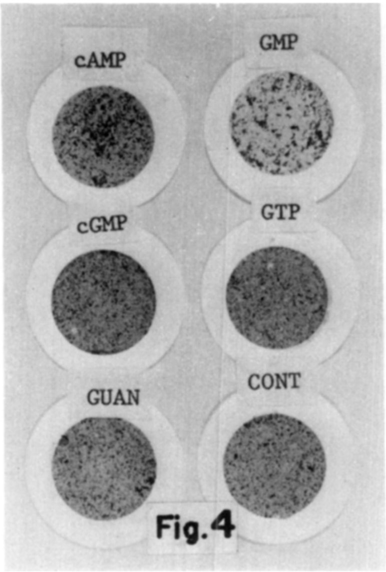


Figure 3 Influence of adenine nucleotides and cyclic AMP (all 1.5 mM) on conidia aggregation. (5.0 x 10^6 conidia).

Figure 4 Influence of guanine nucleotides and cyclic AMP (all 1.5 mM) on conidia aggregation. GDP (not shown) was the same as the control. (4.0 x 10^6 conidia).

0.5 ml of an homogeneous Tween-80 conidia suspension. Cyclic AMP and other effectors were added at inoculation, and the cells were normally harvested soon after the conidia had sunk beneath the surface of the medium. Aggregation was scored by filtering the contents of the flasks through Millipore filters (0.45 μ) and visually observing the clumping patterns on the filter. At least 5 flasks were used for each test, and the photographs represent typical results.

Micrographs were obtained by first placing the conidia-bearing Millipore filters on a microscope slide, dissolving the filter in dioxane, and photographing the undisturbed conidia through a Zeiss photomicroscope 11 with a Zeiss Icarex 35 overhead camera.

Results and Discussion

The biology of pellet formation in our system will be described

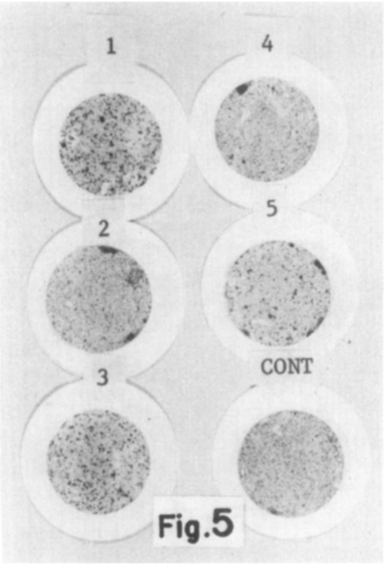


Fig.5

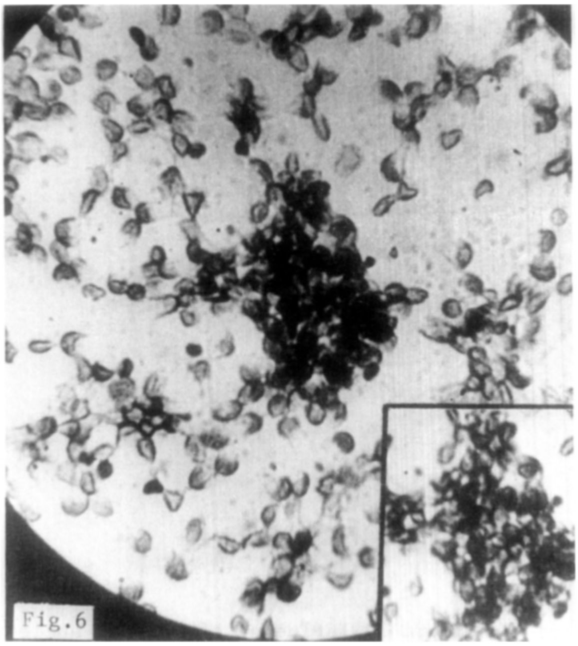


Fig.6

Figure 5 Influence of cyclic AMP analogues on conidia aggregation. (1) 1.5 mM cyclic AMP (2) 3 mM theophylline (3) 1.5 mM cyclic AMP plus 3 mM theophylline (4) 1.5 mM N^6-2'-O-dibutyryl adenosine monophosphate (5) 1.5 mM 2', 3'-cyclic AMP (4.5 x 10^6 conidia).

Figure 6 Micrograph illustrating that conidia aggregation inhibits germination. INSERT: the conidia clump at a slightly different focus. The conidia in the center of the clump are small, unswollen, and devoid of germination tubes. The conidia outside the clump are much larger, some show germination tubes, and many of the others have translucent sections of the conidia wall. (x 1000).

elsewhere. Our observations were essentially as described by Galbraith and Smith (4) except that the kinetics of clumping appeared to be more rapid and vigorous under our conditions. Briefly, single conidia gradually aggregated both while floating on the surface of the medium, and after sinking beneath the surface (5-7 hours). Aggregation continued, accompanied by swelling and germination, until, after 15 hours, large, irregular clumps had formed. These then seemed to "break up" to form the eventual pellet structure (4,19). The process depended upon a nutrient medium, and was poisoned by cyanide (4, confirmed by us).

Cyclic AMP caused a large increase in aggregation (Fig. 1) which was observed at all conidia concentrations tested (Fig. 2), and either before or after germination had occurred. The response was dose dependent, with a lower limit of about 0.5 mM. The following nucleotides were ineffective at 1.5 mM: adenosine, AMP, ADP, (Fig. 3); cyclic GMP, guanosine, GDP, GTP, (Fig. 4); and dibutyryl cyclic AMP (Fig. 5, expt. 4).

ATP (Fig. 3) and 2',3'-cyclic AMP (Fig. 5, expt. 5) both evoked a weak response. The ATP effect may be the result of its conversion to cyclic AMP via adenyl cyclase; ATP similarly had mimicked cyclic AMP stimulation of citric acid accumulation by this organism (18). The response to 2',3'-cyclic AMP may be due to the structural similarity.

Theophylline (2-5 mM) had no effect (Fig. 5), either alone (expt. 2) or in combination with cyclic AMP (expt. 3). This compound often increases cyclic AMP effects in biological systems, presumably by inhibiting the phosphodiesterase which breaks down cyclic AMP (20). Although theophylline had increased cyclic AMP stimulation of citric acid accumulation (18), in this case it may be that the phosphodiesterase activity in the conidia was too low for cyclic AMP hydrolysis to be an important factor.

5'-GMP had a profound influence on clumping, larger than cyclic AMP (Fig. 4). Interesting in this respect is the recent report of the isolation of N,N-dimethylguanosine from *D. discoidium*, a structural analogue of GMP, and a putative germination inhibitor (21). Autoinhibition of germination of spores and conidia, especially under crowded conditions, has been reported previously in fungi and Actinomycetes (22). Fig. 6 illustrates that the conidia in the centers of clumps do not germinate, a result that has been suggested before (2). Those in the center of the clump (INSERT) are small, unswollen, and devoid of germination tubes, while the individual conidia surrounding the clump are large, swollen, and have emitted or are about to emit germination tubes.

These observations suggest a possible physiological role for conidia aggregation: that cyclic AMP and 5'-GMP may be acting alone or synergistically to effect a population control mechanism; the number of conidia which germinate is regulated by clumping. Since the vegetative pellets arise from the conidia aggregates, it follows that any agent which promotes clumping would reduce the number of pellets formed. This would ensure that under crowded conditions, such as within a fructification, there would be an adequate nutrient supply to maintain a viable population with full reproductive capacity. Evidence for this view is that the conidia/pellet ratio increases sharply once a certain conidia density is reached (manuscript in preparation), and that the conidia concentration is one of the most important parameters in pellet formation (1-4). In addition, unfavorable nutrient conditions are believed to favor the pellet type of growth, rather than the more luxurious filamentous type (hyphae individually dispersed throughout the milieu) (2).

This report demonstrates that cyclic AMP and 5'-GMP increase the "adhesiveness" of conidia and germlings of *A. niger*. Since cell-cell contacts in the pellet are probably important in the developmental control of this organism, these results are in accord with the view that cyclic AMP operates as a regulator of growth and differentiation in biological systems.

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