Vol. 80, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1978 Pages 858-865

THE EFFECTS OF CYCLIC AMP ON DISAGGREGATION-INDUCED CHANGES IN ACTIVITIES OF DEVELOPMENTALLY REGULATED ENZYMES IN DICTYOSTELIUM DISCOIDEUM

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Received January 3, 1978

SUMMARY

The addition of cyclic AMP to the shaking medium of cells disaggregated from pseudoplasmodia of Dictyostelium discoideum suppressed the accumulation of cell-bound phosphodiesterase which normally occurs(1) after disaggregation. The suppression was not secondarily brought about by its possible inhibitory effect of cyclic AMP on protein synthesis or by its stimulating effect on the release of the enzyme into the medium. The effect was reversible and specific to cyclic AMP. On the other hand, the inhibitory effect of cyclic AMP on the disaggregation-induced inactivation of UDP-galactose transferase was not apparent in the initial period, but thereafter it slowed down the decrease in the enzyme activity. These results indicate that exogenous cyclic AMP mimics at least in part the regulatory effects of cell-to-cell contact on certain enzymes.

Aggregation of Dictyostelium discoideum amoebae into a multicellular complex (pseudoplasmodium) of a slug shape is followed by differentiation of the presumptive spore cells, which is characterized by the synthesis of the specific antigen known to be acid mucopolysaccharide(2). The importance of cell contact for such differentiation has been suggested by many investigators.

When cells are disaggregated from slugs and incubated without being allowed to reaggregate, they not only lose the antigen(3), but also are induced to accumulate cell-bound PDE and to inactivate UDP-galactose transferase(1). A similar induction of cell-bound PDE was also reported by Darmon(4) with cells disaggregated from 8-hr aggregates. Likewise, it has been previously shown that cells disaggregated from slugs cannot continue their program of accumula-

Abbreviations: cAMP: 3', 5'-cyclic AMP; PDE: phosphodiesterase;

0006-291X/78/0804-0858$01.00/0

tion of UDP-glucose pyrophosphorylase(5) and of glycogen phosphorylase(6) when prohibited from reaggregation. Gross et al.(7) recently found that rapid shaking of the suspension of starved amoebae inhibited formation of large agglomerates as well as the arrest of cell-bound PDE accumulation and the induction of UDP-glucose pyrophosphorylase, which normally occurs with slow shaking. These results suggest that formation and retention of cell contact are required for the programmed changes in activity of certain developmentally regulated enzymes.

On the other hand, it has been demonstrated that the addition of cAMP to the incubation medium inhibited disaggregated slug cells from losing the specific antigen(3). It was also recently shown by Rickenberg et al.(8) that amoebae under conditions where agglomeration is inhibited are still able to accumulate alkaline phosphatase when pulsed with cAMP.

To examine whether exogenous cAMP mimics cell contact in regulating the synthesis or the breakdown of certain enzymes, we investigated the effects of cAMP on both the accumulation of PDE and the inactivation of transferase induced by disaggregation of slugs. As a result, cAMP was shown to inhibit both events, either completely or partially.

MATERIALS AND METHODS

Dictyostelium discoideum NC-4 was used in all experiments. Exponentially growing cells were harvested by centrifugation, washed three times, and streaked on 2 % non-nutrient agar plate containing 40 mM each of NaCl and KCl(9). Pseudoplasmodia thus formed were mechanically disaggregated by means of repeated forced pipetting in 40 mM phosphate buffer (pH 6.4) containing 20 mM KCl. Disaggregated cells were washed and resuspended in 20 mM phosphate buffer (pH 6.0) containing 2 mM EDTA at a concentration of 0.8-2 x 10^7 cells/ml, and shaken at 25°C. at 150 strokes per min. After various times of shaking, an aliquot of the culture was taken and centrifuged at 2500 rpm for 3 min. Cell lysates were prepared by adding 1 % Emulgen 109P (Kao-Atlas Co.) in 20 mM phosphate buffer (pH 7.1) to pelleted cells and used for assays of cell-bound PDE or UDP-galactose transferase. The supernatant was used for assays of extracellular PDE and PDE inhibitor.

The activity of transferase was measured essentially by the method as described(1). For assaying cell-bound PDE, 10 µl of extensively dialyzed cell lysates were incubated with 10 µl of [^{3}H]cAMP (250 nCi=8.3 pmole) at 25°C for 10 min. Radioactivities of 5'-[^{3}H]AMP produced were then counted after separation by paper chromatography(10). PDE inhibitor and extracellular PDE were assayed as described by Gerisch et al.(11) and by Klein and Darmon(12), respectively. Prior to determinations of PDE activities, samples were extensively dialyzed against 20 mM phosphate buffer (pH 7.1) at 4°C to remove exogenous cAMP.

RESULTS

The effect of cAMP on the disaggregation-induced accumulation of cell-bound PDE

Cells disaggregated from slugs were incubated in the presence or the absence of 1 mM cAMP and lysed at various times, as described in Materials and Mathods. After extensive dialysis of cell lysates, cell-bound PDE was assayed. No effects of the dialysis was observed on the enzyme activity (data not shown). As shown in Fig. 1, exogenous cAMP completely suppressed the accumulation of PDE which normally occurs after disaggregation.

The suppression was not due to the production of an inhibitor, since the mixture of the lysates of cells incubated for 210 min in the presence and the absence of cAMP had an activity equal to the sum of the individual activities. Furthermore, it was also unlikely that the high concentration of cAMP inactivated the enzyme, because the addition of 1 mM cAMP to the lysate of cells incubated in the absence of cAMP had no effect on the enzyme activity after dialysis.

Analyses of the effect of cAMP

It was previously shown that cycloheximide (250 μg/ml) completely suppresses the increase in activity of cell-bound PDE after disaggregation(1). To test whether or not the effect of cAMP on the accumulation of PDE is secondarily brought about by its inhibitory effect on protein synthesis, incorporation of [^{3}H]leucine into protein of disaggregated cells was examined in the presence or the absence of cAMP. Chloramphenicol (250 μg/ml) was added to the incubation medium to exclude incorporation into contaminating bacteria, if any. An aliquot of cell suspension was collected at various times, and cellular radioactivities were determined. Fig. 2 shows that the incorporation was as active in the presence of cAMP as in its absence.

It is possible that exogenous cAMP does not actually suppress the synthesis of the enzyme but rather stimulates the excretion of the intracellular enzyme

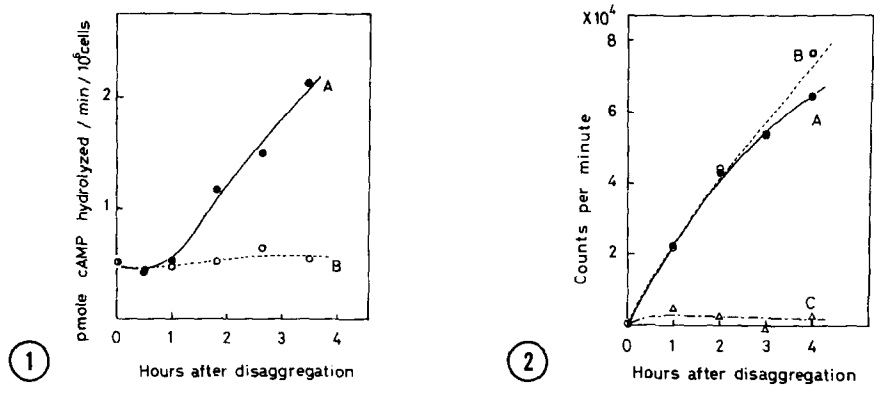


Fig. 1. The effect of cAMP on the increase in activity of cell-bound PDE induced by disaggregation of pseudoplasmodia (slugs). Disaggregated slug cells were incubated in the presence (B) or the absence (A) of 1 mM cAMP. At intervals, a portion of the culture was taken, and a cell lysate was prepared as described in text. For each assay, a lysate from 3.6×10^4 cells was used.

Fig. 2. Incorporation of [^{3}H]leucine into disaggregated cells. Cells disaggregated from slugs were incubated with [^{3}H]leucine (10 μCi=0.27 nmole) and chloramphenicol (250 μg/ml), in the presence (B) or the absence (A) of 1 mM cAMP or in the presence of cycloheximide (250 μg/ml) (C). An aliquot of cells (1.5×10^6 cells) was taken into 0.2 N NaOH, and trichloroacetic acid-insoluble radioactivity was counted.

into the medium. To examine this possibility, the effect of cAMP both on the activities of intra- and extracellular PDE was studied. After 1.5 hrs of disaggregation, cells were collected by centrifugation and suspended in fresh medium with or without 1 mM cAMP. As shown in Fig. 3, the addition of cAMP completely inhibited the subsequent accumulation of the intracellular PDE, with accompanying no more increase in the activity of the extracellular enzyme than in the control. When cAMP was removed after 1 hr of treatment, cells soon began to accumulate PDE at the same rate as those incubated without cAMP from the beginning (data not shown), indicating the reversibility of the effect of cAMP.

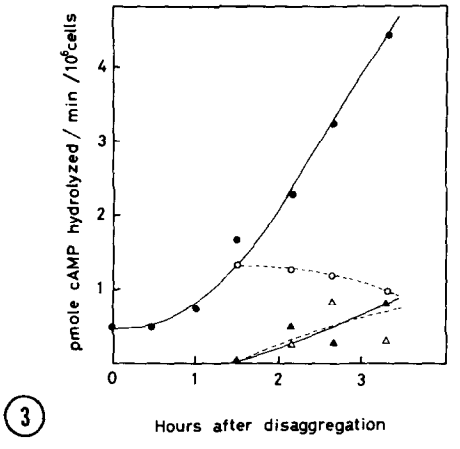
To examine the specificity of the cAMP effect, a variety of nucleotides were tested for their ability to inhibit the accumulation of PDE after disaggregation. As shown in Table 1, ATP, ADP, 5'-AMP and cGMP had no effect, and dibutyryl cAMP was a less potent suppressor than cAMP.

Table 1. The effect of a variety of nucleotides on the accumulation of PDE in disaggregated slug cells

Nucleotides	Relative increase in PDE activity (%)
control	100
cAMP	2.0
5'AMP	74.0
ADP	90.8
ATP	95.1
cGMP	117.8
dbcAMP*	40.8

* N^6-2'-O-dibutyryl cAMP

Nucleotides (1 mM) as indicated were added at 0 time to the culture and the activity of cell-bound PDE was assayed after 4.7 hrs. Increases in activity were expressed as percentages of that in control (2.1 pmole cAMP hydrolyzed/ min/10^6 cells).



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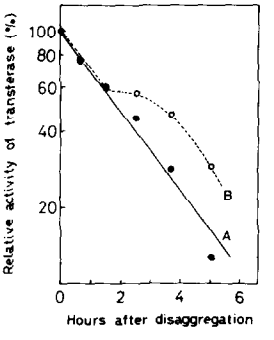

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Fig. 3. The effect of cAMP on the accumulation of intra- and extracellular PDE in disaggregated slug cells. After incubation of 1.5 hrs, cells were collected and resuspended in fresh medium and incubated with (broken line) or without (solid line) 1 mM cAMP. Both intra-(○ , ●) and extracellular (△ , ▲) PDE's were assayed at intervals.

Fig. 4. The effect of cAMP on the inactivation of UDP-galactose transferase after disaggregation. Disaggregated slug cells were incubated in the presence (B) or the absence (A) of 1 mM cAMP. At intervals, a portion of the culture was taken, and transferase in cell lysates was assayed. For each assay, a lysate from 8.1 x 10^4 cells was used, and the activity at 0 time was 1.3 pmole [^{14}C] galactose transferred/min/10^8 cells.

The effect of cAMP on disaggregation-induced inactivation of transferase

It has been shown that disaggregation of slugs brings about an immediate decrease in activity of UDP-galactose transferase(1). The effect of exogenous cAMP on this change was examined, and the results are shown in Fig. 4. In the presence of 1 mM cAMP, transferase activity decreased with the same half-life of 60 to 120 min as in the absence of cAMP, but the decrease was slowed down after about 100 min of incubation. Accordingly, the inhibitory effect of cAMP on the disaggregation-induced changes in transferase became apparent only after a certain period in contrast to the case of cell-bound PDE.

DISCUSSION

When cells disaggregated from slugs were incubated with cAMP under conditions where no reaggregation was allowed, the accumulation of cell-bound PDE was completely suppressed. Several experiments in this report indicate that the addition of cAMP brought about the inhibition of de novo synthesis of the enzyme induced by the loss of cell-to-cell contact. Microscopic observations showed that cells formed no larger aggregates in the presence of cAMP than in its absence.

On the other hand, although exogenous cAMP had little effect on the decrease in transferase activity immediately after disaggregation, it showed an inhibitory effect after about 100 min. The fact that the decrease in the enzyme activity is not inhibited by cycloheximide up to 120 min after disaggregation(1) indicates that the decrease is caused by an inactivating machinery (probably a proteolytic enzyme) which has already formed in slug cells. It is possible that cAMP is not effective for the preformed machinery to act on the enzyme, but causes the de novo synthesis of the enzyme after a lag of a certain period. There is also another possibility that exogenous cAMP inhibits de novo formation of the inactivating machinery after disaggregation.

The inhibitory effect of exogenous cAMP on disaggregation-induced changes was also observed with glycogen phosphorylase (unpublished). The enzyme was

shown to begin accumulating at the aggregation stage and to increase in activity through development until the culmination stage(6). When aggregating cell masses were disaggregated after 9 hrs of starvation, the activity of the enzyme was decreased. In the presence of exogenous cAMP, however, the enzyme activity continued to rise in the absence of cell-to-cell contact. It is concluded from all of these results, that exogenous cAMP mimics cell-to-cell contact in regulating the synthesis of certain enzymes.

An analogous effect of exogenous cAMP was also revealed by Gross et al.(7), using a different experimental system. They found that turing-off of cell-bound PDE and turning-on of UDP-glucose pyrophosphorylase coincide with cells becoming associated in streams and that inhibition of extensive agglomeration by rapid shaking of starved amoebae results in sustained accumulation of PDE and suppression of the accumulation of UDP-glucose pyrophosphorylase. Addition of cAMP under these conditions, however, brought about the normal enzymatic changes(13). Using a similar shaking culture, Rickenberg et al.(8) found no effect of exogenous cAMP on the formation of UDP-glucose pyrophosphorylase. We have also examined the effect of cAMP on this enzyme after disaggregation of slugs, but were so far unable to obtain the consistent result that exogenous cAMP sustains formation of this enzyme in disaggregated cells (unpublished). The reasons for such discrepancy must await for further studies.

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