CHANGES IN ACTIVITIES OF TWO DEVELOPMENTALLY REGULATED ENZYMES INDUCED BY DISAGGREGATION OF THE PSEUDOPLASMODIA OF DICTYOSTELIUM DISCOIDEUM

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SUMMARY

Changes in activities of two developmentally regulated enzymes were examined after disaggregation of the multicellular complex (pseudoplasmodium) of Dictyostelium discoideum. When disaggregated cells were incubated with shaking in a buffer containing EDTA, the activity of UDP-galactose transferase began to decrease almost immediately after disaggregation, with a half-life of 60-80 min (at 25°C). This decrease was induced even in the presence of cycloheximide and proceeded normally up to about 2 hrs of incubation. On the other hand, disaggregation caused an increase in the activity of cell-bound phosphodiesterase for cAMP. This process was sensitive to cycloheximide. These results indicate that at least two categories of changes are induced by loss of cell contact in the cellular slime mold.

When vegetative cells of Dictyostelium discoideum are starved, they form an aggregate and differentiate into at least two types of cells -- prespore and prestalk cells. In contrast, cells which are not allowed to aggregate are unable to differentiate under normal conditions. When cells are isolated from a multicellular complex (pseudoplasmodium) and incubated without being allowed to reaggregate, they gradually undergo "dedifferentiation", as evidenced by disappearance of a prespore specific antigen (1) which is presumably AMPS (2). Furthermore, cells disaggregated from pseudoplasmodia cannot continue their program of accumulation of UDP-glucose pyrophosphorylase when prohibited from reaggregating (3). These facts suggest that cell interaction plays an impor-

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Abbreviations: AMPS: acid mucopolysaccharide; PDE: phosphodiesterase; cAMP: 3', 5'-cyclic AMP; BAL: 2,3-dimercaptopropanol.

tant role in cell differentiation during the development of this organism.

To understand the role of such cell interaction from a biochemical standpoint, changes in the activities of two developmentally regulated enzymes were examined after disaggregation of multicellular pseudoplasmodia. The enzymes chosen for this purpose were UDP-galactose:polysaccharide galactosyl transferase and cell-bound PDE for cAMP. The former has been shown to be synthesized only in the prespore cells (4) and is most likely to participate in the synthesis of AMPS (5). The latter is known to reach a peak of activity when cells enter the aggregation stage (6). It has been suggested that the activity of this enzyme closely correlates to the ability of cell aggregation (7).

MATERIALS AND METHODS

D. discoideum NC-4 was used throughout. Pseudoplasmodia were disaggregated by the use of pronase and BAL (8). After being washed with 20 mM phosphate buffer (pH 6.0) containing 2 mM EDTA, disaggregated cells were resuspended in the same buffer at a concentration of ca. 2×10^7 cells/ml and were shaken at 25°C. Prespore cells containing AMPS in the prespore vacuoles were identified by staining with fluorescein-conjugated anti-*D. mucoroides* spore serum (9).

UDP-galactose transferase was assayed essentially by the method of Sussman and Osborn (5), except that incubation was carried out at 0°C for 2 hrs and that ethanol-insoluble materials were collected by filtration on a Whatman glass filter (GF/C). Acceptor polysaccharide was prepared from fruiting bodies of a sporeless mutant of *D. mucoroides*. Each incubation mixture (40 µl) contained: 20 µl of cell lysate, 10 µl of acceptor, and 12.5 nc = 54 pmole of UDP-[^{14}C]galactose. The activity of cell-bound PDE was measured by incubating 10 µl of washed cells with [^{3}H]cAMP (final concentration: 12.5 µc = 5 nmole/ml) at 35°C for 30 min in the presence of 10 mM glutathione and by counting 5'-[H^{3}]AMP produced after separation by paper chromatography (7).

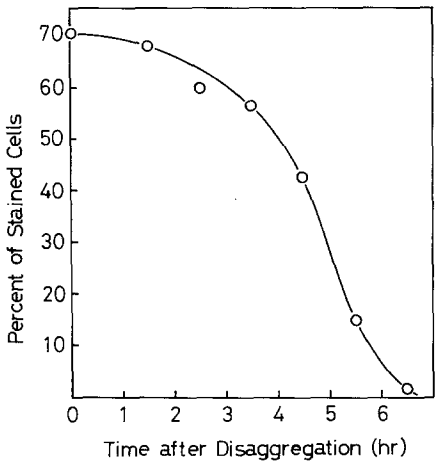

Fig. 1. Disappearance of AMPS from cells incubated in a shaking culture after disaggregation of pseudoplasmodia. At indicated times, a portion of the culture was taken and added to an equal volume of a NaCl (0.4 M)-EDTA (0.04 M) solution. Small cell agglutinates in the solution were dispersed by repeated pipetting. The cell suspension was then diluted 10 times with Bonner's salt solution (10), and placed on cover glasses, and cells were stained as described (1). The numbers of stained and unstained cells were counted. The cells which contained less than a few stained granules were counted as "unstained".

RESULTS

Process of dedifferentiation in a shaking culture

When cells disaggregated from pseudoplasmodia were shaken at 25°C in phosphate buffer containing EDTA, they formed only small agglutinates. Under such conditions, dedifferentiation proceeded normally: AMPS which had been accumulated in prespore cells began to disappear after disaggregation and became undetectable in almost all cells after about 6 hrs of shaking (Fig. 1). The extent of cell lysis during 5 hrs of shaking was insignificant, as judged by measurements of UV-absorbing materials released into the medium during the incubation. The time course of disappearance of AMPS is comparable to that observed in the previous experiments (1) where disaggregated cells in Bonner's salt solution (10) were placed on glass under a sparsely populated condition. The use of the shaking culture, in contrast to the previous plating method, permitted us to deal with a large quantity of dedifferentiating cells needed for biochemical analysis.

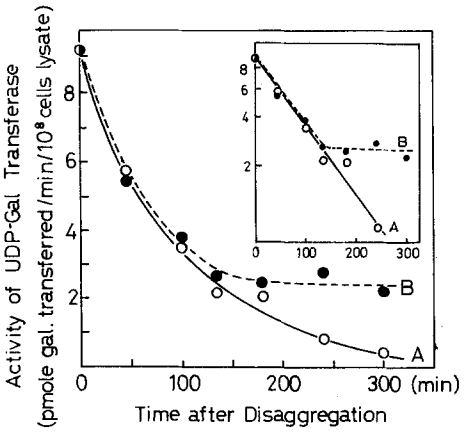

Fig. 2. Decrease in activity of UDP-galactose transferase after disaggregation. At intervals, a portion of the shaking culture was taken. Cells were pelleted by centrifugation, subjected to freezing-thawing, and lysed with 20 mM phosphate buffer (pH 7.1) containing 1 % Triton-X100. For each assay, a lysate from 2 x 10^5 cells was used. A: no drug added; B: cycloheximide added at 0 time. Insert: Enzyme activities plotted in a log scale.

Changes in activity of UDP-galactose transferase after disaggregation

When cells disaggregated from pseudoplasmodia were shaken at 25°C, the activity of UDP-galactose transferase rapidly declined with a half-life of 60 to 80 min, as shown in Fig. 2. There was almost no lag before the onset of the decrease in the enzyme activity. Even when cycloheximide (250 μg/ml) was added prior to disaggregation, the decrease occurred with essentially the same half-life, suggesting that synthesis of no new protein is involved in this change induced by disaggregation of pseudoplasmodia. However, in the presence of the antibiotic, the decrease in the enzyme activity was halted after about 2 hrs of incubation, and the level of the enzyme did not change thereafter. Measurements of the enzyme activity in the mixture of the lysates of cells collected after 0 and 6 hrs of incubation excluded the possibility that an enzyme inhibitor appears after disaggregation (data not shown).

Changes in activity of cell-bound PDE after disaggregation

Contrary to UDP-galactose transferase, the activity of cell-bound PDE was

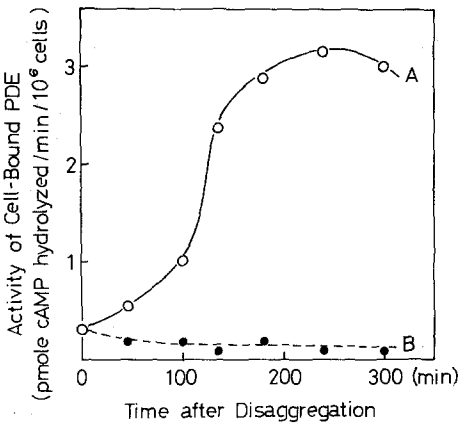

Fig. 3. Increase in activity of cell-bound PDE after disaggregation. At indicated times, a portion of the shaking culture was taken. Cells were pelleted by centrifugation and resuspended in 20 mM phosphate buffer (pH 7.1) at a concentration of 2.6 x 10^6 cells/ml. For each assay conditions, see text. A: no drug added; B: cycloheximide added at 0 time.

found to increase after disaggregation of pseudoplasmodia, with a lag of about an hour, reaching a peak after about 4 hrs of incubation (Fig. 3). The extent of increase varied considerably from experiment to experiment, ranging from 2- to 10-fold. The increase of the enzyme activity was completely blocked if cycloheximide (250 μg/ml) was added at the time of disaggregation, indicating that protein synthesis is required for this change to occur.

Changes in enzyme activities of mechanically disaggregated cells

Since disaggregation in the above experiments was conducted with pronase and BAL and disaggregated cells were incubated in a solution containing EDTA, it could be argued that the observed changes in the enzyme activities might be caused by these agents rather than by loss of cell contact. To test this possibility, pseudoplasmodia were mechanically disaggregated by means of repeated forced pipetting, and disaggregated cells (mostly in the form of small aggregates composed of several cells) were spread over agar surface at a density of 1 x 10^4 cells/cm^2 so as not to reaggregate. At intervals, cells were collected and assayed for the enzyme activities. As Fig. 4 shows, the

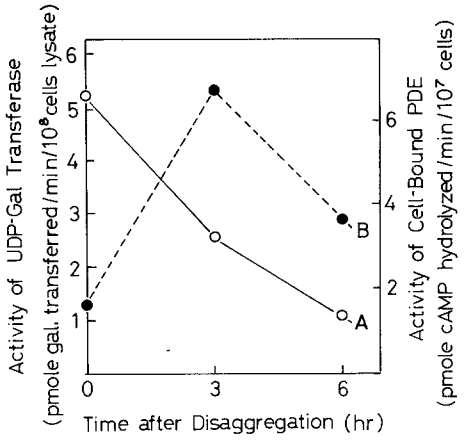

Fig. 4. Changes in enzyme activities of mechanically disaggregated cells. At indicated times, cells were collected from agar plates, and a portion was lysed with 1 % Triton-X100 for UDP-galactose transferase assay. The remainder was used for assay of cell-bound PDE. A: UDP-galactose transferase; B: cell-bound PDE.

patterns of the changes in activities of the two enzymes were essentially the same as those obtained in the above experiments. Therefore, it is concluded that the changes in the enzyme activities as described above were indeed the direct effect of disaggregation.

DISCUSSION

It was shown in the present work that disaggregation of the multicellular complex induces at least two categories of changes in enzyme activities in this organism. One of the enzymes examined, UDP-galactose transferase exhibited an almost immediate decrease in its activity after disaggregation. This decrease is obviously not due to either cell death or degeneracy, since (a) essentially all cells were actively moving at the end of experiments when observed under a phase contrast microscope, (b) incorporation of [^{3}H]leucine into an acid-insoluble fraction continued at a constant rate over 4 hrs after disaggregation (data not shown), and (c), as discussed below, disaggregated cells could synthe size PDE. Furthermore, the previous study (1) showed that every cell was able to grow when fed on bacteria.

Although no significant turn-over of UDP-galactose transferase occurs in intact pseudoplasmodia, as indicated by Sussman's experiments (11), once they were lysed with detergent the enzyme activity began to decrease with time (unpublished observation). In addition, the initial decrease in the enzyme activity in disaggregated cells proceeded normally even in the presence of cycloheximide. These indicate that disaggregation (or loss of cell contact) brings about a change in the cellular state in such a way that the transferase now becomes exposed to an inactivating (or unstabilizing) machinary, which has been present in the cell but somehow blocked in intact pseudoplasmodia from acting on the enzyme. The verification of this model, however, awaits further experimentation.

Disaggregation of the multicellular complex also induces another kind of changes. The activity of cell-bound PDE was increased by disaggregation in a cycloheximide-sensitive manner. Our preliminary experiments (unpublished) indicate that this increase is probably due to de novo synthesis of the enzyme rather than to translocation of the enzyme already present in the cells, and that the synthesis is regulated at the transcriptional level rather than the translational.

It is conceivable that cell interaction affects not only the two enzymes studied, but many other enzymes in the cell. In view of the fact that differentiation and dedifferentiation of slime mold cells depend on the presence and absence of cell-to-cell contact, elucidation of the mechanisms by which the informations based on cell contact is transmitted and expressed in the cell would be one of the most intriguing problems.

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