Cyclic AMP Phosphodiesterase in Some Mutants of
Dictyostelium purpureum

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Received April 4, 1974

Changes in levels of PDase at different stages of development in the wild type and some
mutants of D. purpureum were investigated. The enzyme levels were measured in sub-cellular
fractions, i.e., extra-cellular, wash, intra-cellular and pellet fraction. Some differences were
observed in patterns of enzyme activity among the wild type and the mutants. Impairment in
gross morphological differentiation seems to be reflected readily in changes of levels of PDase
during the course of development in this organism. The significance of PDase in the aggre-
gation processes of Dictyostelaceae is also discussed.

In many instances, hormones activate the
production of adenosine-3',5'-cyclic mono-
phosphate (cAMP) which acts as a second
messenger. The cellular slime mold is the
only known system in which cAMP itself is
secreted out of the cells to transmit informa-
tion. The cells of this organism are gathered
with the aid of this information transmitter,
cAMP. After the discovery of cAMP as
a chemotactic agent of this organism, at least
three factors have been reported in relation
to this aggregation system, that is, adenyl
cyclase (EC 3.1.4.1), cAMP phosphodi-
esterase (PDase) and a proteinaceous inhibi-
tor of PDase. According to Rossomando and Sussman, specific activity of
adenyl cyclase remains constant throughout
all stages of vegetative growth and fruiting
body construction in Dictyostelium discoideum. It would be of great interest, therefore, to
study the levels of PDase activity during the
course of development of the cellular slime
mold. The PDase activity of this organism
has been studied by several investigators under
different conditions. However, no one
has measured the distribution of PDase ac-
tivity in sub-cellular fractions systematically
and has checked the effect of washing the cells
on the enzyme levels. Here, we report the
change and the distribution in the levels of
PDase in several sub-cellular fractions at dif-
ferent stages of development in the wild type and some mutants of Dictyostelium purpureum.
The existence of two types of extra-cellular
PDase and in vitro interconversion of these
enzyme types have been reported. One has
a high Km value (2 mM) and the other a low
Km value (15 μM) for cAMP. Since it was
suggested that during the aggregation process,
physiologically significant form is the one
which has low Km value, we measured the
activity of low Km PDase in the present ex-
periments.

MATERIALS AND METHODS

D. purpureum, SA3 (wild type) and the mutants, 1002
aggregateless) and Dps (sporeless) were grown on
nutrient agar in association with Aerobacter aerogenes. These mutants were isolated from the wild type SA3 by
treating with N-methyl-N'-nitro-N-nitrosoguanidine. At
several stages, cells were harvested and suspended in
30 ml of cold salt solution at a density of 1–2 x 10⁷
cells/ml. The growth of cells were synchronized by
the method of Sussman. The growth was of cells
were shaken at 22°C for 30 min. The cells were removed by centri-
figation (1500 rpm, 5 min). The supernatant was
designated as the extra-cellular fraction.

Ten milliliters of the cell (10⁷ cells/ml) were centri-
fuged at 1500 rpm for 5 min. The supernatant was called the wash fraction. The pellet was washed once with 0.01 M Tris (hydroxyethyl) aminomethane-hydrochloride (Tris-HCl) buffer (pH 7.5). The activity of PDase in the supernatant was negligible. The cells were resuspended in 2 ml of the same buffer and homogenized in a Teflon homogenizer until most of the cells were broken examining under a microscope (×400), since homogenization varied considerably depending on the developmental stages of the cells used. The homogenate was then centrifuged twice at 15,000 rpm for 30 min. The pellet fractions were combined and resuspended in 0.01 M Tris-HCl buffer (pH 7.5) and stored at −20°C; this was called the pellet fraction. The supernatant was dialyzed overnight against the same buffer in order to lower the extinction of this fraction at 265 nm. This preparation was designated as the intracellular fraction. The enzyme activity was not influenced by dialysis. The entire process was carried out at 0−4°C. The procedure of fractionation is summarized in Fig. 1.

For the determination of PDase activity, the 5′-AMP derived from cAMP in the diesterase reaction was converted to inosine with the aid of alkaline phosphatase and adenosine deaminase, and the decrease of extinction at 265 nm (2.5 × 10^4 nmoles CAMP hydrolyzed/10 optical density unit) was measured. The reaction mixture contained the following components (μmoles): Tris-HCl buffer (pH 7.5), 20; MgCl₂, 14; cAMP, 0.1; alkaline phosphatase (EC 3.1.3.1, from E. coli, Boelinger, Germany), 50 μg; adenosine deaminase (EC 3.5.4.4 from intestinal mucosa, Boelinger, Germany), 0.04 μg and the enzyme preparation in a total volume of 2.0 ml. The reaction was started by adding cAMP and incubated at 35°C. The reaction time varied from 5 to 30 min depending on the rate of the reaction. Under these conditions reaction rate was constant over at least 30 min.

Enzyme activity of the intra-cellular and the pellet fractions was expressed as units per mg of protein; one unit is that quantity of the enzyme which converts 1 n mole CAMP to inosine per min at 35°C in 0.01 M Tris-HCl (pH 7.5) containing 7 × 10^-3 M MgCl₂. Enzyme activity of the extra-cellular and the wash fraction was expressed as units per 10⁷ cells. The protein content of these fractions was relatively small. The amount of protein was assayed according to the method of Lowry et al. All assays were duplicated, and the averaged values are presented.

RESULTS

The stages of development at which the activity of the enzyme was determined were: growing, preaggregation, early aggregation, and culmination. At the growing stage, the cells were still feeding on bacteria. About 3 hr after the exhaustion of bacteria, cells produce mucus. At this stage, which we called preaggregation, the cells did not show any sign of aggregation when examined under a dissecting microscope. The early aggregation stage started about 6 hr after the exhaustion of bacteria. At this stage, cells began to form multi-cellular aggregates, which after about 3 hr became slugs at the culmination stage.

The level of PDase activity in each fraction is illustrated in Figs. 2 and 3. Activity of bacterial PDase was found to be negligible under these conditions. In the wild type, the enzyme activities of all the fractions were highest at the preaggregation stage and decreased when the wavy aggregation pattern had appeared.
The mutant Dps forms stalks and very tiny sori without spores. In this mutant, the pattern of the transition in enzyme activity during the course of development was not so much different from that of the wild type, although the levels of the enzyme activities of all the fractions were about 40 per cent higher in Dps compared to those of the wild type at the preaggregation stage.

The mutant 1002 is an aggregateless; its development proceeds normally to the pre-aggregation stage as far as microscopic observation can determine. Then the surface of the culture shows slight signs of a wavy pattern,
and development stops at this stage. Samples for PDase assays were taken at times corresponding to those at which wild type cells would have changed from one stage to another. In this mutant, the profile of PDase activity during the developmental process was very different from that of the wild type. At the pre-aggregation stage, the activity of the extra-cellular fraction was 3.5 fold higher than that of the wild type (Fig. 3-c). The activity of PDase of the intra-cellular and the pellet fraction was similar to those of the wild type (Fig. 2-c). Although the enzyme activity of the intra-cellular and the pellet fraction of the wild type dropped at the early aggregation and culmination stages (Fig. 2-a), the PDase activity of this mutant did not decrease at these stages. On the other hand, those of the extra-cellular and the wash fraction dropped considerably at the early aggregation stage, although they did not reach the same level as those of the wild type. These results indicate that the enzyme activity in cells not always correlated with the extra-cellular one especially in mutant strains.

Distribution of PDase activity in each fraction is summarized in Table I. This result shows that the distribution pattern of PDase activity is diverse depending on the developmental stages and strains. About 65~75% of the total activity appeared in wash fraction and a negligible enzyme activity was recovered at the second centrifugation (Fig 1). The enzyme activity recovered in the pellet fraction, which has been dealt with by many investigators, is only one-fourth to one-fifth of the total enzyme activity of all fractions.

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* Relative activity of PDase in percentage to the total activity.
DISCUSSION

The results obtained here indicate that the enzyme activity of each fraction changes independently from those of other fractions in some mutants. Therefore, it is important to examine the different patterns of the enzyme activity among the fractions during the course of development, especially in the case of mutant strains.

In 1002, the ability to produce PDase seems not to be impaired. But this mutant strain could complete a normal developmental process when external cAMP (4 x 10^{-3} M) was added to the medium constantly to the end of aggregation stage, after food deprivation. Moreover, this mutant completed its normal developmental process synergistically with a mutant strain of other developmentally distinguishable classes (See Table I of reference 17). These results may suggest the possibility that the disorder is due to high PDase activity of the extra-cellular fraction, which is thought to have a function to make a cAMP gradient. Malchow et al. proposed and Konijn and Jastorff supported the hypothesis that the membrane-bound PDase acts itself as a cAMP receptor site. Recently supporting results were reported by Malkinson and Ashworth and Malchow et al.

Malchow et al. also found that, in non-aggregating mutants, the activity of particle-bound PDase was absent or smaller compared to the activities found in the wild type during aggregation-competence. They measured the enzyme activity in a liquid culture in which gross morphological differentiation is usually not observed. In contrast, we assayed the enzyme levels in cells cultured on agar plates, examining morphological differentiation under a microscope.

At present, we have examined only two mutants out of nearly one hundred mutant stocks. Studies on other mutants are left for future work.

Regardless of the complexity of the aggregation process as a developmental event in Dictyostelium, dissection of PDase will offer fruitful results for the understanding of regulatory mechanisms of development in these organisms.

Acknowledgement. We thank Miss Miyako Morikawa for her excellent technical assistance.

REFERENCES