REGULATION OF EXTRACELLULAR CYCLIC-AMP-PHOSPHODIESTERASE ACTIVITY DURING DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM

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SUMMARY

The cAMP-PDE* released into the medium of D.discoideum cultures during growth is inhibited by a factor which is produced early in the phase of transition from growth to cell aggregation. In a non-aggregating mutant, active PDE is overproduced and not depressed in this developmental stage, although the enzyme is sensitive to wild-type inhibitor. In two other mutants, only low PDE activities were measured throughout the growth and transition phases. The cAMP-PDE inhibition may trigger development by rising the external cAMP level.

During development of the cellular slime mold Dictyostelium discoideum, a shift occurs from the growth stage of single cells to aggregation-competence, at which stage cells interact with each other by chemotaxis, contact formation and other communication systems to form multicellular associations. Cyclic AMP participates in these cell interactions as a chemotactic agent (1,2). Moreover, other developmental functions have been reported or suggested to be controlled by cAMP (3,4,5). A cAMP-destroying phosphodiesterase (EC 3.1.4.1) is released into the medium during growth of Dictyostelium cells (6,7). This enzyme appears to participate in the control of chemotaxis by regulating the extracellular cAMP level (3). If the PDE would not only participate in the chemotactic reaction system but also in the trans-

* Abbreviations: cAMP cyclic 3',5'-adenosine phosphate
PDE phosphodiesterase

formation of growth phase cells to aggregation competent ones, changes of PDE activity would be expected to occur during a critical phase at exhaustion of nutrient supply, when this transformation begins. This paper reports marked inhibition of cAMP-PDE activity by a factor produced in this developmental stage.

METHODS

Dictyostelium discoideum wild-type and mutant cells were grown in suspension with 1 x 10^{10}/ml washed E.coli B/r cells as a nutrient source (8). For timing development, the date of complete consumption of bacteria (t_o) was used as a reference point. At this point, the concentration of amoebae was approximately 1 x 10^7 cells/ml.

For determination of cAMP-PDE activity, the culture fluid was freed from amoebae and bacteria and dialyzed against 0.01 M triethanolamine buffer pH 7.4. The 5'-AMP produced in the diesterase reaction was converted to inosine with alkaline phosphatase (EC 3.1.3.1) and adenosine-deaminase (EC 3.5.4.4) (9), and the change of extinction at 265 nm recorded with a Beckman DB spectrophotometer against a blank containing the complete reaction mixture except cAMP. The mixture contained 7 x 10^{-3} M Mg^{++}, 0.1 M triethanolamine pH 7.4 and the enzymes. The reaction was started by adding 150 nmoles cAMP to a total volume of 3 ml, and measured at 35°C. cAMP and bovine heart muscle cAMP-PDE were purchased from Boehringer Mannheim. For enzyme inhibition, the PDE containing samples were pre-incubated with culture supernatants in 0.01 M triethanolamine buffer at 23°C.

RESULTS AND DISCUSSION

Evidence for phosphodiesterase inhibition. In suspension cul-

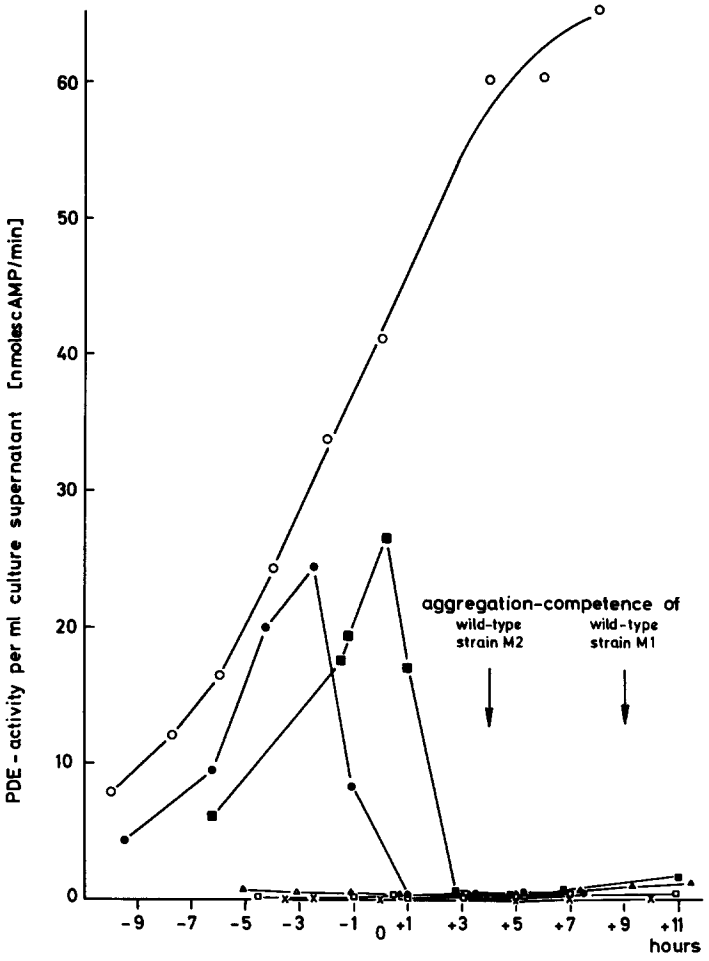

Fig. 1. PDE-activities in culture supernatants of D. discoideum cells grown in E.coli B/r. Wild-type strains M 1, ■———■ and M 2, ●——● ; aggregateless mutant aggr 50 (M2), O———O ; mutants forming giant aggregation territories ga 86 (M1), □——□ and ga 88 (M1), Δ———Δ ; control suspension of bacteria without D. discoideum cells, ×———×. The 0-point on the abscissa indicates the time of complete bacteria consumption.

tures, cAMP-PDE activity increased in the culture medium during growth of D.discoideum wild-type cells, and declined within 2 hours after consumption of food bacteria, reaching a level of 2 percent of the original activity (fig. 1). When culture medium harvested after depression of PDE activity ("late" supernatant), was added to medium harvested before depression ("early" super-

natant), the total activity in the mixture decreased with increasing amounts of "late" supernatant. The inhibiting factor was not removed by dialyzing "late" supernatant for 3 days. The time dependence of inhibition (fig. 2) suggests an enzyme mediated transfer reaction from a donor (or to an acceptor) as a possible mechanism of cAMP-PDE inhibition in D.discoideum. The factor did not inhibit cAMP-PDE from bovine heart muscle.

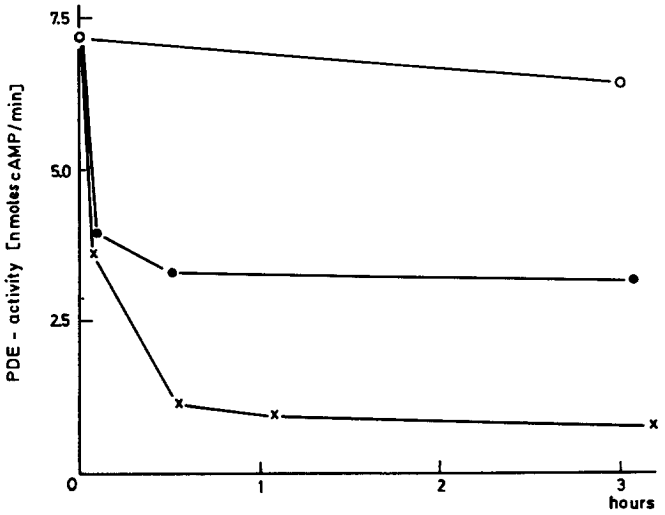

Fig. 2. Inhibition of PDE in an "early" culture supernatant of D. discoideum wild-type M1, by culture supernatant of mutant ga 86 after various incubation times (abscissa) at 23°C. The wild-type supernatant was harvested shortly before t_o. 250 µl "early" supernatant incubated without ga 86 supernatant, o——o; with 75 µl, ●——●, and with 100 µl ga 86 supernatant, x——x. The total volume of mixtures was 350 µl.

Overproduction of PDE in a non-aggregating mutant. The mutant aggr 50 produced active PDE up to a higher level than the wild-type, and it retained this activity after having passed the period during which PDE activity in the wild-type is depressed. To establish if the mutant produces an increased enzyme to inhibitor ratio, or if the mutant enzyme is insensitive to inhibition, aggr 50 medium was tested with wild-type "late" super-

natant. In this experiment, no difference in sensitivity of aggr 50 and wild-type PDE was found, suggesting that inhibitor production is deficient in this mutant.

Low-PDE-mutants. cAMP influences the size of aggregation territories (3). Accordingly, searching for mutants that form giant aggregation territories should be a method to select for strains which are aberrant in PDE-regulation. In our collection were two UV-induced mutants, ga 86 and 88, which showed this morphogenetic aberration when cultivated on agar plates. Both these mutants concurred in exhibiting reduced PDE activities. In suspension cultures, this reduction of enzyme activities was most prominent in the growth phase, and - contrary to expectation - leveled out in the aggregation-competent stage (fig.1). Both these mutants produced inhibitor already in a stage in which PDE activity still increased in the wild-type (6 hours before exhaustion of bacteria).

cAMP-PDE inhibition - a trigger of cell differentiation? Provided that the decline of cAMP-PDE in the wild-type is not compensated by a parallel change in adenyl-cyclase activity, the results would mean that extracellular cAMP concentration is adjusted to a higher level several hours predatory to aggregation-competence. One may speculate that turning off of the PDE is rendered necessary in this stage for initiating or maintaining the shift from growth to aggregation by increasing the external cAMP concentration.

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