THE RELATIONSHIP OF PHOSPHODIESTERASE TO THE DEVELOPMENTAL CYCLE OF *DICTYOSTELIUM DISCOIDEUM*

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Received September 4,1975

SUMMARY

Determination of the rate of total phosphodiesterase production by *Dictyostelium discoideum* shows that a dramatic rise in enzyme production occurs after 3 hours of cell starvation. Use of imposed cAMP pulses indicate that this increase is related to the developmental program of the amoebae and is probably due to a stimulation of adenyl cyclase.

Dictyostelium discoideum amoebae undertake their developmental cycle when they have exhausted their food supply or are transferred into buffer. After an initial period of starvation,amoebae display aggregation competence (1,2) as defined by the capacity to attract each other and establish specific intercellular contacts. During the aggregation phase, cell movement is directed by an acrasin (3,4), which is probably cAMP (5,6). Differentiating cells excrete pulses of cAMP (7) and show enhanced chemotactic responses to this cyclic nucleotide with increasing periods of starvation (8,9). Phosphodiesterase, which specifically hydrolyzes cAMP, is also produced by these amoebae (10). However, the existence of both extracellular (11) and cellular (12) phosphodiesterases, as well as an extracellular inhibitor of the enzyme (13), makes it difficult to determine the actual rate of enzyme production by starved cells. Here we describe an analysis which permits such a determination. The results show that an increase in enzyme production occurs during the early hours of cell starvation.

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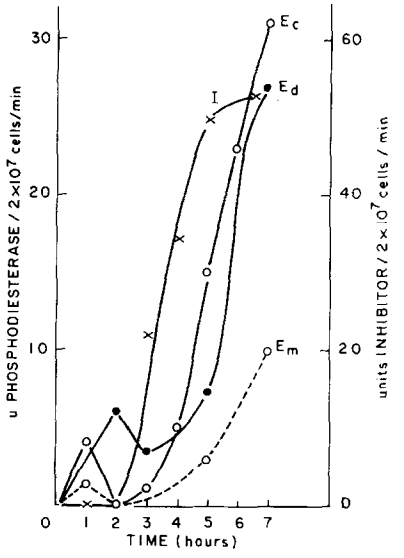

Figure 1. Changes in phosphodiesterase and inhibitor activities during differentiation of amoebae to aggregation-competence. Axenically grown Ax2 amoebae (14) were harvested from exponentially growing cultures by centrifugation. Cells were washed once with KPB (17 mM phosphate buffer, pH 6.2) and starved in this buffer as described by Beug *et al.* (15). At the indicated times, aliquots were taken for inhibitor and enzyme determinations. Phosphodiesterase was measured as described previously (16). Cellular activity is defined as the total phosphodiesterase activity of a cell lysate. Membrane-bound activity is that associated with the pelleted fraction of a cell lysate centrifuged at 30,000 x g for 20 min (13). A unit of phosphodiesterase is defined as the enzyme activity which hydrolyzes 1 nmole of cAMP in one minute at 30°. Inhibitor activity was measured according to Gerisch *et al.* (13). A unit of inhibitor is defined as the activity which neutralyzes one unit of phosphodiesterase. Membrane-bound phosphodiesterase (E_m) O--O; Cellular phosphodiesterase (E_c) O-O; Extracellular phosphodiesterase (E_d) ●-●; Phosphodiesterase inhibitor (I) x-x.

Moreover, experiments indicate that this increase is related to the developmental program of these amoebae.

RESULTS

Kinetics of Enzyme Appearance. Figure 1 shows the changes in phosphodiesterase activities which occur during the first 7 h of starvation of strain Ax2, an axenic derivative of NC_4 (14). A small, early rise in cellular activity precedes the major increase observed after 4-5 h of starvation. In both cases, 40% of the

peak activity is membrane-bound (Em). Changes in cellular enzyme activity precede the changes in extracellular phosphodiesterase seen at 2 and 6-7 h respectively. As reported by Gerisch *et al.* (13) and shown here, inhibitor activity increases after cells have been starved for 2 h. The kinetics of appearance of cellular and extracellular phosphodiesterase suggest that these activities represent one enzyme which is synthesized in the cytoplasm and subsequently excreted. As in the case of most "exported" proteins, the membrane-bound activity probably represents an intermediate form necessary for enzyme excretion. Other lines of evidence support this hypothesis (17,18).

The changes in phosphodiesterase activity observed when cells are starved in buffer may not reflect the actual enzyme production, since a substantial amount may be excreted but masked by the phosphodiesterase inhibitor. However, since the inhibitor is a tight-binding inhibitor (19,17), it is possible to calculate the amount of total extracellular enzyme E_e present at any particular time using the equation $E_e = E_d + (I_t - I_f)$, where E_d is the extracellular activity detected, I_t is the total amount of inhibitor, and I_f is the amount of free inhibitor. The quantity $I_t - I_f$ represents the amount of enzyme complexed with inhibitor, and E_c (cellular enzyme) + E_e equals the amount of total enzyme (E_t). The figures obtained in a typical experiment are shown in Table 1. Enzymic activity detected in the media (E_d) increases during the first 2 h of starvation, declines upon appearance of the enzyme inhibitor, and remains low until cells have become fully aggregation competent (6-7 h). However, the quantity of phosphodiesterase complexed with inhibitor ($I_t - I_f$) gradually increases during this period indicating that synthesis and excretion of the enzyme continues. After 7 h, when all the inhibitor is

Table 1: Effect of starvation on extracellular phosphodiesterase excretion and on total enzyme production

	Inhibitor units/ $2x10^7$ cells		Phosphodiesterase (units/$2x10^7$ cells)				
Time (h)	I_t	I_f	E_c	E_d	I_t-I_f	E_e	E_t
0	-	-	1.5	-	-		1.5
1	-	-	4.2	2.4	-	2.4	6.6.
2	4.5	-	0.5	2.0	4.5	6.5	7.0
3	33.5	32	3.0	2.0	1.5	3.5	6.5
4	45	40	5.3	2.9	5	7.9	13.2
5	45	40	11.3	4.0	5	9.0	20.3
6	45	28	30	6.0	17	23	53
7	45	7	42	15	38	53	95

The results are representative of three experiments. Cells were incubated as described in the legend of fig.1. The amount of inhibitor not complexed with enzyme, I_f, was measured as described (13) except that samples were not heated. Heating inactivates phosphodiesterase and permits a quantitative recovery of the inhibitor (I_t) which was present in both free and enzyme-complex forms (13). I_t, total inhibitor; I_f inhibitor not complexed with enzyme; I_t-I_f, phosphodiesterase bound to inhibitor; E_c, cellular enzyme activity; E_d, extracellular enzyme activity; E_e, total calculated extracellular phosphodiesterase; E_t, sum of cellular and total calculated extracellular enzyme.

saturated with enzyme, the increase in extracellular activity is observed. A plot of these data is shown in fig.2. The curve E_t represents the rate of total enzyme production, as defined by its rate of synthesis and degradation. The small changes in enzyme activities observed during the early hours of starvation probably reflect changes in the rate of enzyme excretion, since total phosphodiesterase production remains constant. However, a striking rise in enzyme production occurs after 4 to 5 h of starvation, and is proportional to the increase in cellular

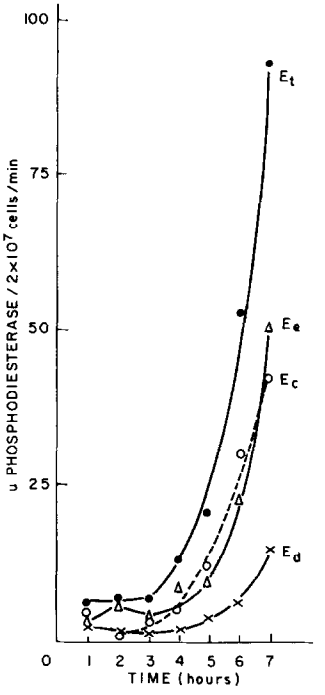

Figure 2. Changes in phosphodiesterase production during starvation. The data is taken from Table 1. Time zero is not represented since the extracellular enzyme present at that time was eliminated in the transferral of cells from growth to starvation media. Extracellular phosphodiesterase (E_d) x-x; cellular phosphodiesterase (E_c) O--O; Total extracellular phosphodiesterase (E_e) Δ-Δ; Total enzyme production (E_t) ●-●.

enzymic activity also seen at that time.

Relationship to Cell Differentiation. The changes in phosphodiesterase activity seen during the first 3 h of starvation also occur whenever cells are centrifuged and resuspended in fresh buffer (unpublished observations) suggesting that these variations are artefacts of the method used to harvest amoebae. To investigate whether the increase in enzyme production which occurs after 3-4 h of incubation in buffer is actually related to the differentiation of cells to aggregation competence or is an independant event of cell starvation, we measured the changes in phosphodiesterase activity in amoebae subjected to pulses of

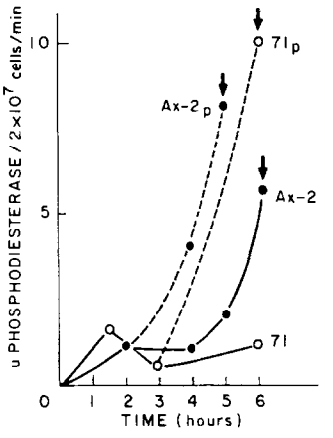

Figure 3. Relationship between phosphodiesterase production and cell differentiation. Cells were prepared as described in the legend of fig.1. Starved amoebae were subjected to cAMP pulses giving a final concentration of 10^{-7}M cAMP in the media, as previously reported (20). Unpulsed cultures were used as controls. At the times indicated, aliquots were removed and the cellular phosphodiesterase activity determined. Criteria used in determining aggregation competence have been described (20). Arrows indicate the time that cells displayed full aggregation-competence. Pulsed Ax-2 cells ●--●; pulsed mutant 71 O--O; unpulsed Ax2 ●-●; unpulsed mutant 71 O-O.

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cAMP. We have previously shown that this treatment advances aggregation competence in Ax2 cells and also induces this state in a class of aggregateless mutants normally incapable of differentiating (20). A mutant of this class, mutant 71, has been used for this experiment since it normally displays low levels of phosphodiesterase activity during starvation. Fig 3 shows that in both Ax2 and mutant 71 an increase in phosphodiesterase production is correlated in time with the progression of amoebae to aggregation competence. In these experiments cellular phosphodiesterase activity was determined since it accurately reflects the kinetics of total enzyme production (see fig.2). Aggregation competence of Ax2 amoebae starved in the presence of cAMP pulses is expressed 1 1/2 h earlier than unpulsed control cells and is associated with an increase in phosphodies-

terase. Similar treatment induces both phosphodiesterase production and aggregation competence in the mutant 71.

DISCUSSION

Shaffer has proposed that extracellular phosphodiesterase may regulate the cAMP signal to noise ratio (7). We have shown here that continuous changes in the ratio of extracellular enzyme to its tight-binding inhibitor (which affect the efficiency of cAMP hydrolysis) occur during starvation. The resulting changes in the background of unhydrolyzed cyclic nucleotide may change the intensity of the cAMP signal perceived and may therefore influence the pattern of cell aggregation.

Other experiments reported here show that an increase in phosphodiesterase production is brought about by applied pulses of cAMP and is related to the developmental program of the cells. The fact that high concentrations of cAMP when added to the media of starved cells induce phosphodiesterase production while low concentrations, if not administered as pulses, do not effect this rise (18) indicates that an amplification of the chemotactic signal and, therefore, a stimulation of cAMP synthesis is required for the induction of enzyme production.

ACKNOWLEDGEMENTS

This work was supported by grants from the Centre National de la Recherche Scientifique (France). C.Klein is recipient of an award from the Philippe Foundation. The authors wish to thank Dr.Luiz Pereira da Silva for his critical reading of this manuscript.

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