ADENYL CYCLASE IN DICTYOSTELIUM DISCOIDEUM: A POSSIBLE CONTROL ELEMENT OF THE CHEMOTACTIC SYSTEM

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SUMMARY In this report we describe the identification and preliminary characterization of an adenyl cyclase in cell free extracts of D. discoideum. The specific activity of this enzyme was found to remain constant throughout growth and fruiting body construction, and the enzyme does not appear to turn over at an appreciable rate at any time. Its peculiar reaction kinetics as observed in crude extracts offer one possible explanation of the pulsating feature of the chemotactic attraction (1). In addition, a c-AMP stimulated disappearance of ATP has been noted. At least part of the latter can be ascribed to the action of a phosphokinase.

INTRODUCTION

The cells of Dictyostelium discoideum grow exponentially either in association with bacteria or in axenic medium (2). After cessation of growth, they collect into organized, multicellular aggregates and construct fruiting bodies. The actual formation of aggregates is preceded by the chemotactic attraction of the outlying cells toward central collecting points. Recently it was observed that the deposition of exogenous adenosine 3'5' cyclic monophosphate (c-AMP) in the midst of aggregating cells provides an attractant signal (3). Furthermore, D. discoideum ameobae have been shown to secrete c-AMP and both the secretion of c-AMP by the cells and their sensitivity to it as a chemotactic agent increase by about 100-fold just prior to aggregation (4). In addition, the cells secrete appreciable amounts of a phosphodiesterase specific for c-AMP (5, 6). These findings suggest that c-AMP and the phosphodiesterase are parts of the chemotactic apparatus. A deeper understanding of this system obviously requires the characterization of the remaining elements, one of them being adenyl cyclase. Hence, the present study.

EXPERIMENTAL

Preparation of Cell Extracts. Aliquots of 6 X 10^8 cells were lysed by suspension in 1 ml volumes containing: 20 mM Tris-HCl pH 7.4; 1 mM dithiothreitol; 100 mM NaF; 0.4% triton X-100. After vortexing and centrifuging at 5000 x g for 10 min, the supernatant fluid (S-5) was collected and stored at -80°C. Proteins were determined by the Lowry procedure (7) using bovine serum albumin as standard.

Adenyl Cyclase Assay Conditions. Volumes of 100 ul contained samples of S-5 plus final concentrations of the following components: 20mM Tris-HCl, pH 7.4; 1 mM dithiothreitol; 100 mM NaF; 0.4% triton X-100; 170 nmoles of (^{3}H) ATP (1.7 X 10^3 cpm/nMol); varying amounts of unlabelled c-AMP. The mixtures were incubated 30 min at 37°C and the reactions stopped by addition of trichloroacetic acid (TCA) to a final concentration of 10%. The entire reaction mixture was then deposited in 10 spots on an Eastman Chromatogram Sheet and developed for 70 min. in n-butanol, acetone, glacial acetic acid, conc NH_4OH, water (14:10:6:1:8) at room temperature and air dried (8). Marker spots of c-AMP, adenosine, 5' AMP, and ATP, were located under UV light and the corresponding spots from the chromatographed reaction mixture were cut out and assayed for radioactivity.

RESULTS

Adenyl cyclase catalyzes the reaction, ATP $\rightleftharpoons$ c-AMP + PP_i. The demonstration of this enzyme in extracts of D. discoideum wild type is complicated by the presence of appreciable phosphodiesterase activity. Therefore initial experiments were conducted with extracts of mutant strain R-46, isolated from the wild type after Nitrosoguanidine treatment and recognized by an altered aggregative pattern. This mutant has about 1% of the wild type level of phosphodiesterase. In addition, unlabelled c-AMP was added to the reaction mixture to dilute the labelled product. The data in Fig. 1 show that about 24 nmoles unlabelled c-AMP conferred complete protection in the mutant extract whereas 120-240 nmoles are required in

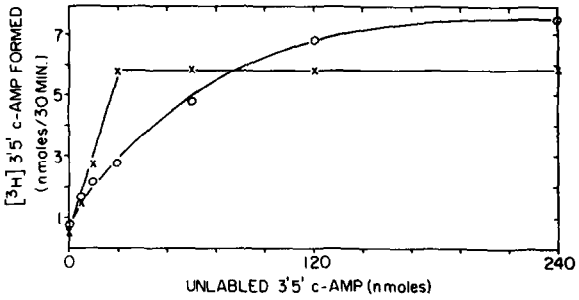

Fig. 1 - Effect of addition of varying amounts of unlabelled c-AMP on the amount of radioactive c-AMP recovered. (-X——X-) Extracts of mutant strain R46; (-o——o-) Extracts of wild type D. discoideum

corresponding experiments carried out with wild type extracts. These results plus the fact that the labelled product co-chromatographed with an authentic sample of c-AMP in two different solvent systems provide proof that the reaction product is c-AMP.

Under the assay conditions described in Experimental, the accumulation of (^{3}H) c-AMP is linear with time for the first 12 minutes and the rate of accumulation proportional to the concentration of extract. One unit of cyclase activity is defined as that amount of enzyme required to produce 1 nmole c-AMP/min. during the first 10 min. incubation at 37°C. The enzyme activity of the S-5 fraction is quantitatively recovered in the supernatant after centrifugation at 100,000 x g for 180 min. (S-100) with a 3-fold increase in specific activity. The activity was appreciably higher at pH 7.4 than either 4.7 or 9.5. It rose 2-fold between 23° and 30° with an additional 15% between 30° and 37°.

Extracts were prepared from D. discoideum cells grown in liquid axenic medium (9) and harvested at mid-exponential phase (2 X 10^6 cells/ml) or at the beginning of the stationary phase (1 X 10^7 cells/ml). Other stationary phase cells were washed and deposited on solid substratum (2) and allowed to form multicellular aggregates (about 8 hours) and construct fruiting bodies (about 24 hours). At intervals cells were harvested and extracts prepared for enzyme assay. The results (Table 1) indicate that the level of enzyme

Table 1

	Stage of Development	Adenyl Cyclase Specific Activity (units/mg cell protein)
	exponential growth	3.4
	beginning stationary phase	3.4 ± 0.5
0 hrs	(cells deposited on solid substratum)	
1		3.5 ± 0.4
2		2.6 ± 0.3
3	formation of multicellular	4.4 ± 0.1
4	aggregates	2.8 ± 0.5
7		3.0 ± 0.5
12		3.1 ± 0.6
14	construction of fruiting	3.5 ± 0.6
18	bodies	3.9 ± 0.1
		Mean: 3.4 ± 0.4

activity does not vary significantly at any time during fruit construction nor is it significantly different from the specific activities of growing or stationary phase cells.

In order to determine if molecules of the cyclase are turned over and replaced during fruit construction, cells were harvested, washed, and deposited on solid substratum in the presence of cycloheximide at a concentration (500 ug/ml) known to inhibit morphogenesis and the incorporation of amino acids into protein (10). The level of cyclase activity remained constant over many hours. Hence the enzyme molecules do not appear to be turned over and replaced.

Fig. 2 shows the kinetics of (^{3}H) ATP disappearance and the accumulation of (^{3}H) 5'-AMP in the presence and absence of unlabelled carrier c-AMP. In the absence of carrier c-AMP, less than 10 nmoles of ATP disappeared, but in the presence of carrier c-AMP about 50 nmoles disappeared. About 10

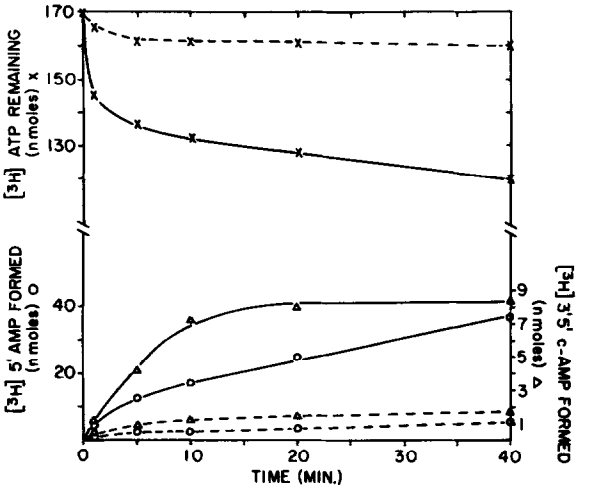

Fig. 2 - The time course of (^{3}H) ATP disappearance and (^{3}H) c-AMP and (^{3}H) 5'-AMP accumulation. Solid lines: The reaction in the presence of 240 nmoles unlabelled c-AMP. Dotted lines: The reaction carried out in the absence of unlabelled c-AMP.

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nmoles had been converted to c-AMP (corresponding to the 10 nmoles that disappeared in the absence of carrier and presumably were converted from c-AMP to 5'-AMP by the phosphodiesterase) and an additional 40 nmoles of (^{3}H) ATP disappeared, all of it recovered as (^{3}H)5'-AMP. Protein phosphokinase activity which is stimulated by c-AMP has been detected in these extracts (unpublished observations) and could conceivably account for at least part of the anomalous ATP disappearance. The addition of guanosine 3', 5' cyclic monophosphate in equimolar quantities failed to stimulate ATP disappearance.

The data in Fig. 2 also indicate that the rate of c-AMP production though initially linear rapidly falls to zero. The following results indicate that the inactivation of the enzyme is not easily explained and may have significance for the chemotactic process: (a) Samples of extract pre-incubated for 20 min at 37°C showed the same initial activity and produced the same total amount of c-AMP as control samples; (b) If an inhibitor of the cyclase does appear in the course of the incubation, it does not accumulate in excess

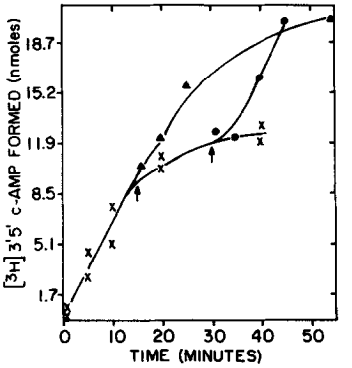

Fig. 3 - The adenyl cyclase activities of sequentially added aliquots of extract. Additional aliquots of wild type S-100 extract were added to the first at either arrow.

since the addition of fresh extract was followed immediately by further c-AMP accumulation at a rate equal to the initial rate (Fig. 3).

DISCUSSION

The foregoing data demonstrate the presence of adenyl cyclase in extracts of D. discoideum cells taken from all stages of vegetative growth and fruiting body construction and indicate that the specific activity remains constant throughout. Thus if the cyclase plays a significant role in regulating the chemotactic process, it must do so via the modulation of its activity rather than by differential enzyme synthesis and/or destruction.

The chemotactic migration in D. discoideum has been shown by time-lapse cinematography to involve concerted, pulsed, radial movements of the attracted cells toward aggregative centers at constant frequency and amplitude[6]. This could conceivably result from the pulsed production and extracellular release of c-AMP. The peculiar kinetics of the cyclase reaction in vitro might be the source of this pulsation in vivo. One necessary condition is that the inhibition of adenyl cyclase activity observed in Fig. 2 and 3 be transient, i.e., that the inhibited enzyme be reactivated by some physiological meaningful condition. After further purification of the cyclase we should be in a position to investigate these possibilities.

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