Adenylate Cyclase and Guanylate Cyclase in *Myxococcus xanthus*

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Myxococcus xanthus M300 vegetative cells contained significant amounts of adenylate and guanylate cyclase activity. The latter was distributed between the 100000 *g* supernatant and pellet fractions, required divalent cations for activity and exhibited an apparent K_m of 1 mM. Adenylate cyclase activity was detected both in the 100000 *g* supernatant and pellet. The supernatant enzyme had an apparent K_m of 220 μ M with a Hill coefficient of 1.9, whereas the pellet enzyme had a K_m of 72 μ M and a Hill coefficient of 1.0. The isoenzymes differed in their pH optima and divalent cation requirements for optimal activity. During development of *Myxococcus xanthus*, the nucleotide cyclase activities exhibited changes that were substantially consistent with the roles postulated for each in a previously proposed model.

INTRODUCTION

Addition of cyclic nucleotide phosphodiesterase (PD) to developing *Myxococcus xanthus* increased territory size and accelerated aggregation; guanosine 3':5'-monophosphate (cGMP) was antagonistic to the PD effect (McCurdy *et al.*, 1978). Exogenous adenosine 3':5'-monophosphate (cAMP), however, increased the number of fruiting bodies but did not influence the PD effect. These findings and results from other laboratories (Parish *et al.*, 1976; Zusman, 1978; Yajko & Zusman, 1978) led to the proposal that cAMP functions as a derepressor of development-specific genes and that cGMP is involved in aggregation chemotaxis (McCurdy *et al.*, 1978).

Consistent with this hypothesis was the report that cAMP concentration peaked immediately after induction, that cGMP peaked intracellularly between 18 and 28 h and exhibited an extra-cellular peak corresponding to aggregation and that the PDs for both nucleotides were maximal at the times of aggregation (Ho & McCurdy, 1980). Furthermore, under fruiting conditions cGMP was a chemoattractant for *M. xanthus* (Ho & McCurdy, 1979). In this paper we report results of an examination of adenylate and guanylate cyclase and their behaviour during development.

METHODS

Organism and growth conditions. Methods of vegetative cultivation, fruiting body induction and cell harvest at various stages of development of *Myxococcus xanthus* M300 were as described previously (Ho & McCurdy, 1980).

Enzyme preparation. Toluene treatment of cells was done according to Harwood & Peterkofsky (1975): 10 μ l toluene was added to 1 ml of cell suspension containing approximately 0.5 mg protein ml⁻¹, and incubated at 30 °C for 10 min.

For preparation of crude homogenate and preliminary fractionation, cells were suspended in homogenizing buffer consisting of 50 mM-Tris/HCl, pH 7.5; 10 mM-MnSO₄; 3 mM-mercaptoethanol and 20% (v/v) glycerol. The cells were broken by two bursts of sonication of 20 s duration with a Bronwill Biosonik (Bronwill Scientific, Rochester, NY, USA), set at full power. The cell extract obtained after low speed centrifugation (7000 *g*) was centrifuged at 100000 *g* for 90 min at 4 °C. The clear supernatant was removed and the pellet was resuspended in homogenizing buffer to a final volume equivalent to the original volume.

Enzyme assays. Adenylate cyclase was measured essentially by the method of Terasaki *et al.* (1978). The 100 μ l

Abbreviation: PD, phosphodiesterase.

reaction mixture contained 50 mM-Tris/HCl, pH 7.5; 2 mM-MnSO₄; 2 mM-creatine kinase; 2 mM-phosphocreatine, 3 mM-sodium azide; 0.05 mM-ethanol and 2 mM-1-methyl-3-isobutylxanthine (a cyclic nucleotide phosphodiesterase inhibitor in *M. xanthus*, Ho & McCurdy, 1980); 2 mM-mercaptoethanol and 0.1 mM-ATP. The reaction was incubated at 30 °C for 10 min and then stopped by adding 85 µl 0.2 M-acetic acid at 4 °C, followed by boiling for 3 min. After cooling, 15 µl 0.8 M-NaOH was added to neutralize the reaction mixture and cAMP determinations were made by using the radioimmune assay of Steiner *et al.* (1972). Data analysis was by the computer program of Brooker *et al.* (1979).

Guanylate cyclase was measured essentially by the method of Garbers & Murad (1979), except that 1 mM-GTP was used as the substrate. Product was measured by the radioimmune assay of Steiner *et al.* (1972) and the data analysis program of Brooker *et al.* (1979). Tris/HCl, Tris/maleate, glycine/NaOH and HEPES buffers were used for pH optimum determinations for both guanylate and adenylate cyclases.

The method of Lowry was used for protein determinations, with bovine serum albumin as the standard.

Biochemicals. The following compounds were obtained from Sigma: GTP (lithium salt), 1-methyl-3-isobutylxanthine, 2-mercaptoethanol, sodium azide, creatine kinase, phosphocreatine, Tris, HEPES, maleic acid and glycine. The radioimmunoassay (RIA) kits were obtained from New England Nuclear.

For liquid scintillation counting, the filmware plastic bags (10 ml) were obtained from Nalge, Rochester, NY, USA. Triton X-100 was obtained from Rohm and Haas, West Hill, Ontario, Canada. 2,5-oxazole (PPO) and 1,4-di-2-(5-phenyloxazolyl)benzene (POPOP) and toluene were obtained from Fisher Chemical Co.

RESULTS

Nucleotide cyclase activities during development

When *M. xanthus* M300 was induced to fruit by inoculation onto FM (fruiting medium, McCurdy *et al.*, 1978), maximum aggregation occurred at approximately 40 h. Fruiting bodies were clearly delimited at about 60 h and myxospore differentiation was completed between 75 and 85 h. Both adenylate and guanylate cyclases were detectable at levels of activity which varied with the time of development.

The level of adenylate cyclase activity in toluenized vegetative cells was 2–4 pmol (mg protein)⁻¹ min⁻¹, but during development it exhibited much higher peaks between 10 and 20 h and between 40 and 50 h (Fig. 1). There was also a third, smaller but reproducible peak at about 75 h.

Guanylate cyclase activity in toluenized cells was initially 3–4 pmol (mg protein)⁻¹ min⁻¹ but then rose sharply to a peak between 8 and 20 h, decreased until 40 h and then rose again to a less dramatic maximum at about 75 h (Fig. 1).

Guanylate cyclase

Guanylate cyclase activity was detectable in both the 100 000 g supernatant and pellet (Table 1). Both activities exhibited concave substrate-velocity curves. However, the insolubility of the substrate prevented our obtaining data from higher concentrations which, assuming the usual

Fig. 1. Activities of cellular adenylate cyclase (●) and guanylate cyclase (■) in toluenized *Myxococcus xanthus* during development plotted as the mean results of five or six separate experiments, with bars showing highest and lowest values detected.

Fig. 2. Substrate-velocity plot of guanylate cyclase activity. The enzyme was assayed in 100000 *g* soluble (■) and particulate (●) fractions of *M. xanthus*. Inset: Hill plot of guanylate cyclase activity obtained using 100000 *g* soluble (■) and particulate (●) fractions at various concentrations of GTP from 10^{-2} M to 10^{-6} M.

Table 1. Cyclase activities in the 100000 *g* supernatant and pellet fractions

	Specific activity [pmol (mg protein) ⁻¹ min ⁻¹]
Adenylate cyclase	
Supernatant fraction	1.81
Pellet fraction	12.1
Guanylate cyclase	
Supernatant fraction	3.3
Pellet fraction	4.3

Table 2. Effect of pyruvate, GTP, NaF and cAMP on guanylate cyclase activity

Addition	Concn (mM)	Guanylate cyclase activity (%)
Control	—	100
Pyruvate	4.5	223
cAMP	5.0	200
NaF	5.0	99
ATP	1.0	100

saturation kinetics, would have yielded a sigmoidal curve. Both the supernatant and pellet enzymes had a K_m of 1 mM and a Hill coefficient of 2.6 (Fig. 2). Accordingly, it appeared that the same enzyme was detected in both fractions and that activity was consistent with positive cooperativity. Guanylate cyclase was dependent on divalent cations: Mn^{2+} was more effective than Mg^{2+} or Ca^{2+} , producing optimal activity at 0.15 mM, whereas the concentration of Mg^{2+} for optimal activity was 1.5 mM. The enzyme had a sharp pH optimum at pH 7.0. It was stimulated by cAMP and pyruvate, but neither ATP (Macchia *et al.*, 1981) nor F^- (Sun *et al.*, 1974; Macchia *et al.*, 1975) had any effect (Table 2).

Fig. 3. Substrate-velocity plot of adenylate cyclase activity. The enzyme was assayed in the 100000 *g* soluble (■) and particulate (●) fractions from the vegetative cells of *M. xanthus*. Inset: Hill plot of adenylate cyclase activity obtained using 100000 *g* soluble (■) and particulate (●) fractions.

Fig. 4. Adenylate cyclase activity in 100000 *g* soluble fraction (■) and in 100000 *g* particulate fraction (●) of *Myxococcus xanthus* during development. Points represent the mean of two separate determinations.

Table 3. Effect of pyruvate, ATP and NaF on soluble and particulate adenylate cyclase activity

Addition	Concn (mM)	Adenylate cyclase activity (%)	
		Supernatant	Pellet
Control		100	100
Mn ²⁺	1.5	736	163
Mg ²⁺	1.5	271	105
Pyruvate	4.5	270	103
NaF	5.0	562	98
GTP	0.05	1200	101

Adenylate cyclase

Adenylate cyclase activity was located in both the 100000 *g* supernatant (soluble enzyme) and pellet (particulate enzyme) fractions (Table 1). The K_m for the latter was 72 μ M and the Hill coefficient was 1. The supernatant enzyme had an apparent K_m of 220 μ M and a Hill coefficient of 1.9, which may indicate positive cooperativity towards the substrate (Fig. 3).

The supernatant enzyme exhibited a sharp pH optimum at pH 8.5, whereas the pellet enzyme had a broader range of optimal activity between pH 7.5 and 9.0. Both enzymes required Mn^{2+} or Mg^{2+} , with Mn^{2+} being more effective (Table 3). Ca^{2+} had no effect on either the soluble or the particulate enzyme.

Soluble enzyme activity was stimulated by pyruvate, NaF and GTP, while the particulate enzyme was not (Table 3).

To determine if the activity peaks seen during development reflected one or both enzymes, changes in soluble and particulate enzyme activities were examined separately during development. Soluble activity exhibited two peaks, one at around 8 h and another at approximately 75 h (Fig. 4). Pellet enzyme activity was initially high in vegetative cells, declined and then rose to a peak at around 40 h (Fig. 4). Hence, while the specific activities determined using toluenized cells were not the resultant from combined specific activities of pellet and supernatant enzymes (the two enzymes perhaps being differentially extracted), the results do seem consistent with the assumption that the first and third peaks observed were attributable to supernatant enzyme, while the second peak was attributable to the pellet enzyme.

DISCUSSION

The guanylate cyclase of *M. xanthus* resembled other prokaryotic enzymes in its response to F^- , Mn^{2+} and ATP, as well as its K_m (Clark & Bernlohr, 1972; Macchia *et al.*, 1975; Sun *et al.*, 1974). On the other hand, it resembled the eukaryotic enzymes in its cooperative response to substrate (Garbers *et al.*, 1975; Chrisman *et al.*, 1975; Craven & DeRubertis, 1975).

The observation of two forms of adenylate cyclase differing in their solubility and responses to pyruvate accords with findings in other prokaryotes (Ide, 1971; Khandelwal & Hamilton, 1971). However, the soluble form resembled the eukaryotic enzyme in being stimulated by GTP and NaF (Bhat *et al.*, 1980).

Adenylate cyclase activity exhibited peaks at critical periods in development. The first peak of activity occurring around 8 h could be responsible for the peak of cAMP concentration previously reported to occur between 0 and 20 h (Ho & McCurdy 1980). The 40 h peak, while coincident with aggregation, did not correlate with any observed increase in cAMP concentration (Yajko & Zusman, 1978; Ho & McCurdy, 1980). This may have been due to high PD activity observed at that stage (Ho & McCurdy, 1980). The third peak occurred around 75 h when microcyst formation within fruiting bodies was initiated. Previous studies (McCurdy *et al.*, 1978; Yajko & Zusman, 1978) had demonstrated a sharp peak in cAMP content during early development of glycerol induced myxospores.

McCurdy *et al.* (1978) suggested that cAMP is involved in derepressing differentiation specific operons at two critical times: first, shortly after induction of fruiting body formation; second, on initiation of myxospore induction. Consistent with these expectations, it was the soluble, apparently allosteric form of adenylate cyclase which exhibits maxima at these critical times.

Guanylate cyclase activity exhibited a peak of activity between 0 and 20 h, a time which slightly precedes intracellular and subsequent extracellular accumulation of cGMP associated with aggregation (Ho & McCurdy, 1980). Similarly, the second peak in the activity corresponds to the second rise in cGMP concentration late in development, as reported earlier (Ho & McCurdy, 1980).

If one takes into account the PD activities, it appears that cyclic nucleotide accumulation is largely a reflection of cyclase activities which exhibit a pattern consistent with a role in differentiation in *M. xanthus*.

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