ADENYL CYCLASE OF Escherichia coli

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Received May 20, 1969

SUMMARY

Adenyl cyclase activity was found in the cell-free extract of Crooke's strain of Escherichia coli. The activity was observed exclusively in the particulate fraction of the extract. Adenyl cyclase of E. coli required Mg^{++} for its activity, but no other cofactor was necessary. The enzyme activity was inhibited by pyridoxal phosphate and oxalacetate. A weak stimulatory effect was observed with GMP and phosphoenolpyruvate.

Two species of bacteria have so far been reported which make adenosine 3',5'-cyclic phosphate (cyclic 3',5'-AMP). Brevibacterium liquefaciens, a bacterium isolated in our laboratory from sewage, excretes in its culture fluid a large amount of cyclic 3',5'-AMP (Okabayashi et al., 1963). Its cell-free extract contains adenyl cyclase which catalyzes the synthesis of cyclic 3',5'-AMP from ATP (Hirata and Hayaishi, 1967; Ide et al., 1967). Adenyl cyclase of this bacteria requires pyruvate as a cofactor. The adenylcyclase of B. liquefaciens has been isolated in a soluble form.

The other bacterium which makes cyclic 3',5'-AMP is Escherichia coli. Makman and Sutherland (1965) reported the conditions under which this nucleotide accumulates in the cells and culture media of Crooke's strain of E. coli, but attempts to demonstrate adenyl cyclase in the cell-free system have been unsuccessful.

Recently, Perlman and Pastan (1968) reported that cyclic 3',5'-AMP participates in the induction of catabolite repressible enzymes of E. coli, and it is expected that the investigation of the in vitro synthesis of cyclic 3',5'-AMP by an E. coli cell-free system will provide valuable information on the role of this nucleotide in bacteria.

This communication reports the synthesis of cyclic 3',5'-AMP using adenyl cyclase

of E. coli and describes some of the properties of this enzyme.

MATERIALS AND METHODS

Preparation of E. coli Adenyl Cyclase: Crooke's strain of E. coli (ATCC 8739) was cultured at 37°C to late logarithmic phase in 5 liters of the medium composed of glucose, 1%; $(NH_4)_2SO_4$, 0.1%; K_2HPO_4, 0.7%; KH_2PO_4, 0.3% and $MgSO_4 \cdot 7H_2O$, 0.01% under forced aeration. The cells (20 g) were harvested by centrifugation, suspended in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM $MgCl_2$ and 5 mM β-mercaptoethanol, and were disrupted in a French pressure cell. The extract was centrifuged and the precipitate that was obtained between 3,000 x g and 30,000 x g was suspended in 20 ml of 10 mM Tris-HCl buffer (pH 8.0) containing 5 mM β-mercaptoethanol and was used as the enzyme preparation. This preparation contained only little, if any, cyclic 3',5'-AMP phosphodiesterase.

Assay of Adenyl Cyclase: The standard assay medium contained, unless otherwise specified, 50 mM glycine buffer (pH 9.75), 5 mM β-mercaptoethanol, 30 mM $MgCl_2$, 2.5 mM ATP-8-C^{14} (4.0 μCi/μmole, Schwarz BioResearch Inc.), enzyme preparation 0.025 ml and the final volume was adjusted to 0.1 ml. The reaction was carried out at 30°C for 30 min and was stopped by cooling the mixture in an ice bath and by adding 1.5 μmoles of ATP. Aliquots of the reaction mixture were cochromatographed on paper with cold cyclic 3',5'-AMP using ethanol-1M ammonium acetate buffer (pH 7.5) (75 : 30 v/v) as the solvent. The cyclic 3',5'-AMP was eluted from the paper and rechromatographed using isopropanol-HCl-H_2O (65 : 16.7 : 18.3 v/v) as the solvent. The spots corresponding to cyclic 3',5'-AMP were cut out and were measured for radioactivity with a Beckman DPM-100 liquid scintillation counter. One unit of enzyme was defined as the amount producing 1 μmole of cyclic 3',5'-AMP per min under standard assay conditions. The protein content was determined according to Lowry et al. (1951).

The validity of this assay system was confirmed by 1) quantitative recovery of

radioactivity after rechromatography of the cyclic 3',5'-AMP spots with six different solvent systems and 2) formation of 5'-AMP after hydrolysis with phosphodiesterase from rabbit brain (Drummond and Perrot-Yee, 1961).

RESULTS AND DISCUSSION

Table 1 shows the adenyl cyclase activity of the fractions obtained during fractionation. The activity was observed exclusively in the particulate fraction. Treatment with a sonic disintegrator and with several detergents failed to make the enzyme soluble. The rather weak activity of the crude extract may be due to the presence of some inhibitory compounds as described below. Maximum activity of the enzyme was observed between pH 9.5 and 10.0, and the Km for ATP was 1.0 mM. The presence of Mg^{++} was necessary for the enzyme to be active and maximum activity was observed at 30 mM of Mg^{++}. The reaction proceeded without any other cofactor and compounds such as pyruvate, a cofactor of adenyl cyclase of B. liquefaciens (Hirata and Hayaishi, 1967; Ide et al., 1967), and catecholamines, cofactors of mammalian enzymes, did not show any distinct effect on the activity of adenyl cyclase of E. coli.

About one hundred compounds were tested for their effect on the E. coli enzyme. Among them pyridoxal phosphate, oxalacetate and malate were found inhibitory, whereas

Table 1

Distribution of adenyl cyclase

Fraction	Adenyl cyclase	Protein
	units x 10^3	g
Crude extract	4.0	2.1
3,000 x g supernatant	3.4	1.9
30,000 x g supernatant	0.2	0.8
30,000 x g precipitate	7.1	1.0

GMP and phosphoenolpyruvate showed a weak activating effect (Table 2). The inhibition by pyridoxal phosphate was the most pronounced. Pyridoxamine phosphate, pyridoxamine and pyridoxine showed no effect, however, pyridoxal was somewhat inhibitory. The inhibitory effect by pyridoxal phosphate has also been found on several enzymes (Anderson et al., 1966; Kaldor and Weinbach, 1966; Rippa et al., 1967; Shapiro et al., 1968), but its physiological significance is yet undetermined. Among the organic acids tested, oxalacetate demonstrated the most clear cut inhibition. Malate may have an inhibitory effect in itself, but it is also possible that it is converted into oxalacetate during the incubation. Phosphoenolpyruvate and GMP showed only weak stimulatory effects.

Whether the effects of these compounds have any physiological meaning within E. coli cells is open to further investigation. However, the fact that the compounds

Table 2

Effect of various compounds on E. coli adenyl cyclase*

Compound added	Enzyme activity
	%
None	(100)
Pyridoxal phosphate, 1 mM	20.0
Pyridoxal phosphate, 10 mM	0.5
Oxalacetate, 1 mM	78.7
Oxalacetate, 10 mM	10.4
Malate, 10 mM	38.5
GMP, 10 mM	155
Phosphoenolpyruvate, 10 mM	148
GMP, 10 mM + Phosphoenolpyruvate, 10 mM	186

* Concentration of ATP-8-C^{14} was 0.625 mM in this experiment.

closely related to pyruvate metabolism affect the enzyme may suggest that the metabolic flow around pyruvate has some relationship to the regulation of E. coli adenyl cyclase. The importance of pyruvate metabolism in the on and off mechanism of catabolite repression in E. coli has been suggested (Okinaka and Dobrogosz, 1967). Furthermore, a recent report by Perlman and Pastan (1968) suggests that cyclic 3',5'-AMP reverses the catabolite repression by glucose in E. coli. Further investigation is necessary to clarify these points.

Adenyl cyclase of E. coli was found in the particulate fraction of the E. coli culture extract, and in this respect resembles mammalian enzymes rather than the adenyl cyclase of B. liquefaciens which has been isolated in a soluble form (Hirata and Hayaishi, 1967). Whether this implies some intrinsic difference in the physiological function of this enzyme and of cyclic 3',5'-AMP between E. coli and B. liquefaciens remains to be determined.

ACKNOWLEDGEMENTS

I would like to thank Dr. Tadashi Okabayashi for his advice and stimulating discussions. The technical assistance of Mrs. Miyuki Mitsugi is gratefully acknowledged.

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