STIMULATION OF COLICIN E_1 SYNTHESIS BY CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE IN MITOMYCIN C-INDUCED ESCHERICHIA COLI

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SUMMARY

Optimal colicin E_1 production in a mitomycin C-induced colicinogenic strain of Escherichia coli requires incubation without aeration in the presence of glucose. Under these conditions, cyclic 3',5'-adenosine monophosphate stimulated the rate of the colicin synthesis. Colicin E_1 was synthesized at a lower rate in the colicinogenic adenyl cyclase-defective mutant than its revertant. Upon addition of cyclic AMP to the mutant, the synthesis was markedly stimulated. The effect was specific for cyclic AMP and the approximate half maximal concentration was 0.06 mM after the Tris-EDTA treatment.

Colicin E_1 is an antibiotic protein, whose action on sensitive bacteria involves a cessation of all macromolecular syntheses and a decrease of intracellular ATP levels as well as a block of active transport across the membrance (1). The ability to synthesize colicin E_1 depends on the presence of colicinogenic factor E_1 (ColE_1), a bacterial plasmid (2).

ColE_1 is normally dormant with regard to active colicin production, but it is readily induced to synthesize the colicin by agents such as mitomycin C and ultraviolet. Although the induced production of colicin E_1 has been shown to be accompanied by an increase in the amount of ColE_1 deoxyribonucleic acid (3), little is known on the control mechanism of the induction. In this paper we present evidence indicating that cyclic 3',5'-adenosine monophosphate plays a role in the synthesis of colicin E_1.

MATERIALS AND METHODS

Bacteria: E. coli CA 7902, a mutant deficient in adenyl cyclase originally

isolated by Dr. J. Beckwith (4) was obtained from Dr. T. Yokota. Strain CA 7902 fails to ferment lactose, arabinose, maltose, mannitol, glycerol, xylose, and rhamnose unless cyclic AMP is supplied in the medium (T. Yokota and J. S. Gots, personal communication). E. coli Y 20 (ColE$_1$) that requires thiamine, threonine and leucine for growth was the gift of Dr. S. E. Luria. E. coli NT01, a mutant deficient in adenyl cyclase and colicinogenic for colicins E$_1$ and Ib was made by Dr. Kiyoshi Mizobuchi using CA 7902, Y 20 (ColE$_1$) and Salmonella typhimurium LT-2 his 712 (ColIb), according to the method of Smith, Ozeki and Stocker (5). Strain NT09 is a revertant from NT01, that can ferment lactose in the absence of cyclic AMP and still produces colicin E$_1$ and colicin Ib. E. coli W 3110 colicin Ib^r and W 1655 met$^-$ λ^r colicin E$_1{}^r$ (R 15) were donated by Dr. K. Mizobuchi. The former strain was used as an indicator for colicin E$_1$, and the latter for colicin Ib.

Media: A basal medium contained 1 g NH_4Cl, 6 g Na_2HPO_4, 3 g KH_2PO_4, 5 g NaCl, 0.12 g $MgSO_4$, 11 mg $CaCl_2$, 0.16 mg $FeCl_3$, 1 mg thiamine-HCl, and 1.5 g vitamine-free casamino acids per liter. The basal medium was supplemented with 0.4% glucose in most of the experiments. Indicator stains were grown in the nutrient broth medium. Growth of the bacteria was carried out at 37° with constant bubbling.

Induction of colicin: When absorbancy at 660 nm of the culture reached 0.400, mitomycin C was added to the cell culture (2 μg/ml), followed by the addition of either cyclic AMP (1 mM) or distilled water. Routinely, one ml of the mixture was incubated without aeration at 37°. When aeration is required, 3 ml of the mixture were bubbled constantly at 37°.

Colicin assay: The incubation mixture was read for absorbancy at 660 nm and treated with sonic oscillation for 90 sec to obtain the colicin solution. Colicin activity was determined by serially diluting the colicin solution with 0.06 M phosphate buffer, pH 7.0 and spotting a drop of each dilution on a nutrient agar plate spread with 2.7 ml of nutrient soft agar containing 0.2 ml of a chilled overnight culture of an indicator strain. The plates were incubated for 5 hours at 37°. The number of colicin units per ml was defined

Table I. Effect of various carbon sources on the synthesis of colicin E_1 in Y 20 (ColE_1)

Strain Y 20 (ColE_1) was aerobically grown to log phase in the basal medium supplemented with various carbon sources at 20 mM. Induction of colicin E_1 was performed for one and 6 hours in the medium for growth as described in Materials and Methods. The colicin activity is expressed as units per absorbancy at 660 nm of the incubation mixture.

Carbon source	Aeration	Colicin E_1 activity at		
		0 time	1 hour	6 hours
Glucose	-	2.7	220	330
	+	-	19	60
Glycerol	-	8.5	15	4.0
	+	-	19	34
Lactate	-	3.9	12	13
	+	-	19	5.0

as the highest dilution giving a clear zone of inhibition of growth.

RESULTS AND DISCUSSION

In an effort to search for optimal conditions for producing colicin E_1 in Y 20 (ColE_1), the cells were incubated with mitomycin C in the presence of various carbon sources, with or without aeration (Table I). Under the conditions without aeration, a large amount of colicin E_1 was synthesized in the medium containing glucose. Aeration of the medium increased the level of the colicin in the presence of glycerol, but the levels attained were less than those in the presence of glucose.

Since a number of protein synthesizing systems are regulated by cyclic AMP (6), it would be of interest to examine the effect of the nucleotide on the colicin synthesis. When the cells were induced for 45 min in the presence of cyclic AMP at 1 mM, approximately 4-fold increase in colicin E_1 level was obtained in the presence of glucose without aeration. There was, however, essentially no stimulation of the nucleotide in the presence of glycerol or lactate both with and without aeration. A slight stimulatory effect was observed in the presence of glucose on aeration. Therefore, the presence of

Table II. Time course of colicin E_1 induction and the effect of cyclic AMP

Growth of the cells and the incubation for the induction were carried out in the basal medium supplemented with 0.4% glucose as described in Materials and Methods. The specific colicin activity was expressed as units per absorbancy at 660 nm of the incubation mixture. The increase in absorbancy at 660 nm during the incubation was within 20%. There was no significant difference in absorbancy at 660 nm in the presence and absence of cyclic AMP after incubation. For the assay of colicin E_1, colicin Ib-resistant cells were used as an indicator.

Strain	Cyclic AMP (1 mM)	Colicin E_1 activity at 30 min	60 min	120 min	180 min
Y 20 (ColE_1)	-	2.3	340	580	760
	+	14	400	730	870
NT01 (ColE_1, ColIb)	-	< 1	2.6	35	54
	+	2.6	61	300	460
NT09 (ColE_1, ColIb)	-	1.3	190	460	850
	+	10	420	1300	1300

glucose and incubation without aeration are the conditions most effective for the colicin E_1 synthesis as well as its stimulation by cyclic AMP.

The effect of cyclic AMP on the colicin E_1 synthesis was further analyzed with respect to the time course of the induction in Y 20 (ColE_1). As shown in Table II, a significant stimulation of the colicin synthesis by the added nucleotide was observed before 60 min of incubation, and there was a little effect after 60 min.

These findings prompted an examination of the effect of lower intracellula cyclic AMP concentration on colicin E_1 synthesis. In a colicinogenic adenyl cyclase-defective mutant (NT01), colicin E_1 was synthesized at a very low rate and 10- to 20-fold stimulation was observed upon addition of cyclic AMP* (Table II). The stimulation was also more remarkable in the early phase of incubation. The colicin synthesis in NT01 in the absence of the added cyclic AMP may be due to the intracellular cyclic AMP accumulated during the incubation,

* The synthesis of colicin Ib was also dependent on cyclic AMP in NT01, whereas no dependency was observed in NT09. Although the levels of colicin Ib activity were about one-fiftieth those of colicin E_1, the degree of stimulation by the nucleotide was almost the same as in colicin E_1.

since the strain CA 7902 still contains a slight activity of the enzyme although it is below one-tenth that of a wild type (T. Yokota and J. S. Gots, personal communication). In a revertant (NT09), however, colicin E_1 was rapidly synthesized and there was a slight stimulation of the colicin production by the added cyclic AMP as in Y 20 (ColE_1) (Table II).

There was no difference in the induced colicin levels between additions of cyclic AMP at 0 time and 30 min prior to mitomycin C treatment. Moreover, when cyclic AMP was included 30 min after the addition of mitomycin C, the nucleotide was also able to stimulate the synthesis. Cyclic AMP alone, without mitomycin C, did not induce the colicin production. Therefore, the effect of cyclic AMP is dependent on mitomycin C, but not vice versa.

The effect of varying concentration of cyclic AMP on colicin E_1 synthesis

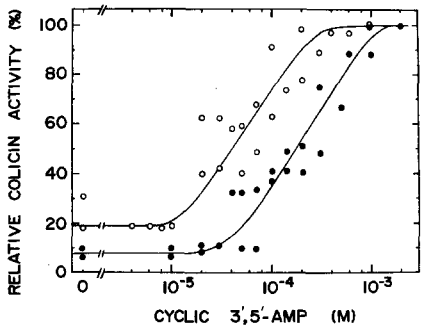

Figure 1. Effect of cyclic AMP concentration on colicin E_1 induction. Growing, log phase cells of NT01 (ColE_1, ColIb) were treated with Tris and EDTA according to the method described by Pastan and Perlman (10). Treated (○) and non-treated (●) cells were incubated with mitomycin C (2 μg/ml) and varying concentrations of cyclic AMP in the basal medium containing 0.4% glucose for 30 min at 37°. Colicin E_1 activity was expressed as units per absorbancy at 660 nm of the incubation mixture.

in NT01 is shown in Fig. 1. The maximal effect was observed at 1 mM and a half maximal effect at 0.2 mM. Treatment with Tris-EDTA shifted the saturation curve to the left and a half maximal concentration of 0.06 mM was obtained. The stimulatory effect on the colicin production was specific for cyclic AMP; neither adenine, adenosin, 5'-AMP, 2'(3')-AMP, ADP nor ATP at 1 mM was effective in the Tris-EDTA treated cells.

Colicin E_1 producing system seems different in several respects from the other systems that are regulated by cyclic AMP. In most of the induction of

the catabolic enzymes, glucose as a catabolite repressor counteracts cyclic AMP with respect to the protein synthesis (6). In contrast, glucose and cyclic AMP are cooperative in the colicin production. As shown in Table I, the presence of glucose in the medium is essential for the colicin synthesis. Moreover, the effect of glucose, in turn, is dependent on cyclic AMP, as seen from the results with a colicinogenic cyclic AMP-deficient mutant. Glucose, therefore, appears not a repressor, but it may positively play a role in the induction mechanism in the present system.

With regard to the protein whose production is determined by the plasmid, R factor-coded chloramphenicol acetyl transferase and streptomycin adenyl transferase are the systems that are reported to be subject to cyclic AMP regulation (7). These systems are, however, catabolite repressible similarly to β-galactosidase system.

Furthermore, on the mechanism whereby cyclic AMP controls the protein synthesis, the regulatory target of cyclic AMP for β-galactosidase system is the site of the specific messanger RNA synthesis with the participation of the cyclic AMP receptor protein (8, 9). In R factor-coded protein synthesis several lines of evidence suggest that the regulatory site affecting R factor replication was very unlikely (7). In the colicin E_1 producing system, however, it has been already shown that DNA molecule of colicinogenic factor E_1 is multiplied after mitomycin C induction and the magnitude of the increase in the DNA is approximately proportional to the level of induced colicin production (3). In view of above considerations, the regulatory site that cyclic AMP affects may not be restricted to transcription and translation in this case. It might be the level of replication of the colicinogenic factor. Studies along this line are in progress.

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