PLEIOTROPIC DEFICIENCY OF CARBOHYDRATE UTILIZATION IN AN ADENYL CYCLASE DEFICIENT MUTANT OF ESCHERICHIA COLI

Robert L. Perlman and Ira Pastan

Diabetes Section and Endocrine Biochemistry Section
CLINICAL ENDOCRINOLOGY BRANCH
NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

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A strain of E. coli deficient in adenyl cyclase activity has been isolated. This strain neither ferments nor grows on lactose, maltose, arabinose, mannitol or glycerol, and ferments weakly and grows slowly on glucose, fructose and galactose. Cyclic AMP restores the normal utilization of all of these carbon sources. The mutant makes very little β-galactosidase in the absence of cyclic AMP. It is concluded that cyclic AMP is necessary for the normal utilization of a number of carbon sources by E. coli, because it is required for the synthesis of enzymes involved in their metabolism.

Adenyl cyclase, the enzyme that catalyzes the synthesis of cyclic adenosine 3',5'-monophosphate (cyclic AMP), has recently been detected in E. coli (1, 2). Cyclic AMP stimulates the synthesis of a number of inducible enzymes in E. coli, including β-galactosidase, galactokinase, glycerokinase, and L-arabinose permease (3,4). If cyclic AMP is involved in the normal regulation of the synthesis of these enzymes, mutant strains deficient in adenyl cyclase activity should be defective in the synthesis of these cyclic AMP-responsive enzymes, and in the utilization of those carbon sources whose metabolism depends upon these enzymes. This paper describes the isolation and some properties of a mutant deficient in adenyl cyclase activity, and impaired in the utilization of a large number of carbon sources.

MATERIALS AND METHODS. E. coli K12 strains 1100, 1101 and 1103 (5) were a gift from Dr. C. F. Fox, and strain 903-C (6) was provided by Dr. M. L. Morse. Strain 1100 was treated with N-methyl-

N'-nitro-N-nitrosoguanidine (NTG) according to the method of Adelberg et al (7).

Medium A contains 14.0 gm K_2HPO_4, 6.0 gm KH_2PO_4, 2.0 gm $(NH_4)_2SO_4$, 0.2 mg $MgSO_4$, and 5 mg thiamine per liter, and was supplemented with carbon sources as indicated. MacConkey agar contains the standard MacConkey ingredients, without lactose. Tetrazolium agar contains 25.5 gm Antibiotic Medium #2 (Difco) and 50 mg 2,3,5-triphenyl tetrazolium chloride per liter. The tetrazolium was autoclaved before use, so positive colonies appear white, and negative colonies are red. Both kinds of agar were supplemented with carbon sources as indicated.

β-galactosidase was induced by incubation with 10^{-3}M isopropyl-β-D-thiogalactoside, and assayed as described previously (3). Adenyl cyclase was assayed by the method of Ide (1), except that the assay was performed at 34°, α-^{32}P-ATP was the substrate, and, following incubation, ^{32}P-cyclic AMP was isolated as described by Taunton et al (8). Protein was measured by the method of Lowry et al (9).

RESULTS AND DISCUSSION. Because cyclic AMP stimulates the synthesis of both β-galactosidase and galactokinase (4), we reasoned that a strain deficient in adenyl cyclase activity would ferment lactose and galactose poorly. Accordingly, NTG-treated cells were plated on tetrazolium agar containing 0.5% lactose and 0.5% galactose, and negative (red) colonies were isolated. Several mutants were found which made little β-galactosidase unless cyclic AMP was added. These were screened for adenyl cyclase activity. One mutant, strain 5336, was markedly deficient in adenyl cyclase activity (Table 1). The assays reported in Table 1 are with the 100,000 x g particulate fraction, which normally contains almost all of the adenyl cyclase activity (1). No additional activity was detected when whole extracts or the soluble fraction of strain 5336 was assayed. Mixing experiments between

TABLE 1

Adenyl Cyclase Activity of E. coli Strains

Strain	Adenyl Cyclase (picomoles ^{32}P-cyclic AMP formed/30 min/ml)	Protein (mg/ml)	Specific Activity (picomoles/30 min/mg)
1100	5720	18.4	311
5336	200	10.4	19
1101	4720	21.2	223
1103	4430	20.4	217
903-C	9520	10.2	933

Table 1: Cells were grown to late-log phase in nutrient broth, centrifuged, suspended in 0.05M Tris, pH 9.5, containing 0.01M $MgCl_2$ and 0.001M dithiothreitol (DTT), and put through a French press. The French press extract was centrifuged for one hour at 100,000 X g, and the pellet resuspended in the Tris-$MgCl_2$-DTT buffer. Aliquots of this preparation were incubated for 30 minutes at 34° in the same buffer, but containing 0.02M Mg^{++} and 6.4 mM ^{32}P-ATP. The reaction was stopped by the addition of 10 volumes of a solution containing 8 mM ATP and 1 mM ^{3}H-cyclic AMP, 0.15 mC/mmole, followed by heating for 2 minutes at 100°. Cyclic AMP was isolated as described by Taunton et al. (8).

strains 5336 and 1100 did not reveal an inhibitor in the mutant. The inability of strain 5336 to accumulate cyclic AMP was not due to rapid hydrolysis of the nucleotide by a cyclic AMP phosphodiesterase, since phosphodiesterase activity in strain 5336 and in strain 1100 was undetectable at pH 9.5.

Table 2 shows the fermentation characteristics of the mutant on MacConkey and tetrazolium agar supplemented with various carbon sources. The parent strain, 1100, ferments all of the carbon sources. In contrast, the mutant is unable to ferment lactose, maltose, arabinose, glycerol or mannitol unless cyclic AMP is present. 5'-AMP has no effect on the fermentation of sugars by strain 5336. Cyclic AMP itself is not

TABLE 2

Utilization of Carbon Sources by Strain 5336

Carbon Source	Fermentation				Doubling Time	
	Mac Conkey		Tetrazolium		Cyclic AMP	
	Without Cyclic AMP	With Cyclic AMP	Without Cyclic AMP	With Cyclic AMP	-	0.002M
Lactose	-	+	-	+	>720	85
Maltose	-	+	-	+	>720	90
Arabinose	-	+	-	+	>720	70
Glycerol	-	+	-	-	>720	90
Mannitol	-	+	-	+	>720	65
Glucose	+	+	-	+	110	65
Fructose	+	+	-	+	110	70
Galactose	+	+	-	+	110	65

Table 2: Fermentation was estimated on indicator agars supplemented with the indicated carbon sources at 1%. One drop of a 0.1M solution of cyclic AMP in medium A was added to a sterile paper disc on the surface of the agar. Strain 1100 ferments all of the carbon sources on Mac Conkey agar, and is positive to all but glycerol on tetrazolium agar. To measure doubling times, cells were grown at 37° overnight in medium A containing 50 µg/ml each of glutamate and histidine, and 0.4% glucose, and were then diluted into fresh medium A, containing glutamate, histidine, and the carbon sources indicated at 0.4%, with and without 0.002M cyclic AMP. Doubling times are given in minutes.

fermented. Glucose, fructose, and galactose are fermented by the mutant. However, on tetrazolium agar, a less sensitive indicator of sugar fermentation, the mutant is negative to these sugars as well. Cyclic AMP restores the normal fermentation of these three sugars.

Table 2 also gives the doubling times of the mutant in various carbon sources, with and without cyclic AMP (2×10^{-3}M). In these experiments, glutamate and histidine (50 µg/ml of each) were also added, since they were required for optimal growth of the mutant. Strain 1100 grows rapidly (doubling time 50-80 minutes) on all of these carbon sources, with and without cyclic AMP. Strain 5336 does not grow

(doubling time greater than 12 hours) on lactose, maltose, arabinose, mannitol and glycerol, and grows slowly (doubling time about 110 minutes) on glucose, fructose and galactose. With cyclic AMP, the mutant grows well on all of these carbon sources. The mutant does not grow on cyclic AMP alone. The mutant grows very slowly (doubling time greater than 5 hours) on succinate, puruvate and lactate; cyclic AMP also stimulates growth on these carbon sources. The effect of cyclic AMP is not restricted to the utilization of carbohydrates, since in addition, it promotes growth of the mutant on thymidine. Cyclic AMP is known to stimulate synthesis of thymidine phosphorylase (4).

We compared the rates of β-galactosidase synthesis in strains 1100 and 5336, in an attempt to examine the enzymatic basis for the inability of the mutant to utilize lactose. Without cyclic AMP, strain 5336 makes less than 10% as much β-galactosidase as does strain 1100 (Table 3). Cyclic AMP at 2×10^{-3} M increases the differential rate of β-galactosidase synthesis in glucose-grown cultures of strain 1100 about two-fold. In contrast, the nucleotide stimulates enzyme synthesis in the mutant over 20-fold, restoring it to near wild-type levels. The inability of the mutant to synthesize β-galactosidase without cyclic AMP is one explanation for its impaired utilization of lactose. An inability to synthesize other cyclic AMP-responsive enzymes (4) presumably underlies its failure to utilize other carbon sources in the absence of the nucleotide.

We were interested in the relationship between strain 5336 and other E. coli strains with pleiotropic defects in carbohydrate fermentation. Therefore, we measured adenyl cyclase activity in three such mutants: strains 1103 and 1101, deficient in enzyme I and HPr of the PEP-phosphotransferase system, respectively (5), and strain 903-C, which is also defective in carbohydrate transport (6). As shown in Table 1, all of these strains contain significant amounts of adenyl cyclase activity. It should be noted that the pattern of carbohydrate utilization seen

TABLE 3

β-Galactosidase Synthesis in Strains 1100 and 5336

Strain	Cyclic AMP (M)	β-Galactosidase Synthesis (Units/ml/minute)	Protein Synthesis (μg/ml/min)	Differential Rate (Units/μg)
	---	1.59	0.87	1.83
	$2x10^{-3}$	3.14	0.86	3.65
	---	.04	0.38	0.11
	$2x10^{-3}$	1.47	0.82	1.79

Table 3: Strain 1100 was grown in medium A containing 0.5% glucose, and strain 5336 was grown in the same medium supplemented with 50 μg/ml of histidine and sodium glutamate. Cultures of both strains were diluted to the same O.D. at the start of the experiment. Rates of β-galactosidase synthesis were determined from samples taken at 10 and 20 minutes after the addition of inducer, and rates of protein synthesis during this period were estimated from samples taken at 3, 35, and 65 minutes.

in strain 5336 is somewhat different than that described for the other strains (5,6).

Previously, we have shown that cyclic AMP stimulates the synthesis of a number of inducible enzymes in E. coli, and overcomes the repression of enzyme synthesis produced by glucose (3, 4, 10). Since glucose lowers the concentration of cyclic AMP in E. coli (11), we proposed that the glucose repression of enzyme synthesis was mediated by decreased cyclic AMP levels. The properties of the adenyl cyclase deficient mutant described in this paper suggest that cyclic AMP is required for the utilization of a large number of carbon sources. Coincident with its loss of adenyl cyclase activity, the mutant is unable to utilize normally a number of carbon sources. The fact that cyclic AMP restores the utilization of all these compounds strongly suggests that the metabolic defects are all secondary to the adenyl cyclase deficiency.

Several puzzling properties of this mutant await clarification.

The enzymatic basis for the defect in glucose utilization is not apparent, since cyclic AMP is not known to stimulate the synthesis of any enzyme required for glucose metabolism. The nucleotide may be required for the normal synthesis or activity of the PEP-phosphotransferase system (12), or of one of the enzymes of intermediary metabolism. In addition, it is surprising that the mutant grows on galactose and fructose in the absence of cyclic AMP, since galactokinase and fructose enzyme II (PEP-phosphotransferase system) are both subject to cyclic AMP control (4). The isolation and study of other adenyl cyclase deficient mutants will aid in answering these questions.

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