ON THE REGULATION OF CYCLIC 3',5' ADENOSINE MONOPHOSPHATE LEVELS IN BACTERIA. STIMULATION OF ADENYL CYCLASE ACTIVITY IN VITRO BY GLUCOSE IN A MUTANT OF E. COLI B/r.

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Received February 15,1973

SUMMARY

A mutant of E. coli B/r insensitive to glucose catabolite repression was found to be stimulated by glucose in its synthesis of inducible β-galactosidase and tryptophanase and also in its ability to synthesize cyclic 3',5'-adenosine monophosphate (cAMP) (1). Determination of the effect of glucose on the activity of the adenyl cyclase enzyme in the membrane fractions of the mutant (ABDR01), showed a 2.4 fold stimulation of the adenyl cyclase activity. Solubilization of the adenyl cyclase by ammonium sulfate precipitation resulted in a preparation insensitive to glucose stimulation. It is proposed that glucose may interact directly or indirectly with a membrane-bound adenyl cyclase system thereby regulating the level of cAMP in bacteria, in a manner analogous to hormonal activation of adenyl cyclase in mammalian systems.

INTRODUCTION

Glucose repression of inducible enzyme synthesis has been shown to be due, in part, to the lowering of the level of cAMP in bacteria (see reviews by Magasanick, (7) and Jost and Rickenberg, (5). Addition of exogenous cAMP relieves the glucose effect as originally shown with the lactose system (8) and subsequently with many inducible enzyme systems. A mutant of E. coli B/r isolated in this laboratory (2) was found to be insensitive to glucose repression as indicated by its ability to synthesize inducible β-galactosidase and tryptophanase in glucose minimal medium and also to synthesize cAMP under the same conditions (1). Glucose was, in fact, stimulatory in its effect on these enzymes and cAMP synthesis (1). In an attempt to analyze this glucose stimulation, the effect of glucose on the adenyl cyclase activity was determined in vitro. In this communication the results of this analysis are reported.

1 R. Nardi is a predoctoral fellow supported by USPHS GM-T-505.

MATERIALS AND METHODS

Organisms: A wild type Escherichia coli B/r (EE1001) and a d-ribose positive mutant (ABDR01) induced by N'-methyl-N'-nitrosoguanidine as previously described (2) were used in this investigation.

Culture Growth and Extract Preparation: A 10% inoculum of an overnight culture grown in 0.4% glycerol minimal medium (3) was transferred to fresh media and incubated on a gyrotory incubator shaker (New Brunswick Scientific) at 37° C until the culture turbidity reached 80 Klett units (blue filter). Cultures were immediately transferred to centrifuge bottles and centrifuged at 6000 rpm in a refrigerated Sorvall centrifuge (RC2-B) for 20 minutes. The media was discarded and the cells resuspended in a tris-hydroxy-methylaminomethane-HCl (Tris-HCl) buffer (0.04 M, pH 8) and sonicated at 4° C in a Heath Systems Sonifier (whole extract). Sonicates were centrifuged for 20 minutes at 10,000 rpm as above. The supernatant was removed and the pellet resuspended in a tris-HCl buffer (0.04 M, pH 8) containing 1 mM dithiothreitol (DTT) (pellet fraction). Preparations were stored under liquid nitrogen.

Adenyl Cyclase Assay: Adenyl cyclase activity was determined as described by Krishna, Weiss and Brodie (6) with a modified reaction mixture. Reaction mixture in our assays contained 40 µl tris-HCl buffer (0.25 M, pH 8.5), 20 µl $MgSO_4$ (0.2 M), 25 µl theophylline (0.033 M), 5 µl DTT (0.01 M), 15 nanomoles ATP-H^3 (10^6 cpm) and 25 µl of the enzyme extract in a total volume of 200 µl. Reaction was performed at 34° C for 30 minutes and stopped by a 2-minute incubation in a boiling water bath. Chromatographic separation of cAMP in the assay mixture was performed using Dowex-50-H+ as previously described (6).

RESULTS AND DISCUSSION

Effect of Glucose and Glycerol on Adenyl Cyclase Activity of ABDR01 and EE1001 Strains: Determination of the adenyl cyclase activity, as described above, in the whole extract and in the pellet fraction, showed that

TABLE 1

Effect of Glucose on the Adenyl Cyclase Activity

Experiment #	Bacterial Strain	Enzyme Source	Additions	Adenyl Cyclase Activity Nanomoles cAMP/30 minutes/mg Protein	Total Activity per ml Pellet*	Adenyl Cyclase Activity as Percent of Control
(a)	ABDR01	10,000 rpm pellet	none	0.158	1.153	100
			glucose	0.210	1.533	133
(b)	EE1001	16,000 rpm pellet	none	0.184	1.82	100
			glycerol	0.202	2.0	109.8
			glucose	0.149	1.48	81
	ABDR01	"	none	0.254	2.36	100
			glycerol	0.313	2.91	123
			glucose	0.452	4.20	178
(c)	ABDR01	40,000 rpm pellet	none	3.161	32.9	100
			glucose	8.607	89.5	272.3

* In the preparation of the pellet fractions, a standard procedure was used in which, cells collected from 1500 ml culture at 80 Kletts were resuspended in 9 ml buffer, sonicated and pellet resuspended in 2 ml buffer using the appropriate centrifugation procedure as described in text.

the adenyl cyclase activity was mainly associated with the particulate fraction of the extract as previously shown by Ide (4). Determination of the adenyl cyclase activity in the 10,000 rpm pellet showed a very low activity (Table 1, experiment a). In an effort to collect more of the enzyme from the whole extract in the pellet fraction, two centrifugation procedures were used: in one the whole extract was centrifuged for 20 minutes at 16,000 rpm while in the second procedure, the whole extract was centrifuged at 2,500 rpm for 10 minutes; pellet discarded, then the supernatant was centrifuged at 40,000 rpm for another 60 minutes, at 4° C. The pellet fractions collected from the 16,000 and 40,000 rpm centrifugations were resuspended in tris-HCl-DTT buffer as described above (Materials and Methods). Determination of the effect of glucose and glycerol at a concentration of 0.2 M on the adenyl cyclase activity showed a 19% repression by glucose and a 9.8% stimulation by glycerol in the wild type (EE1001) (Table 1, experiment b). Response of the adenyl cyclase of the mutant strain (ABDR01), however, was much more striking showing a 78% increase in the enzyme activity in the presence of glucose. The addition of glycerol resulted in a 23% increase in enzyme activity. Determination of the adenyl cyclase activity and the effect of glucose on the 40,000 pellet fraction of ABDR01 (Table 1, experiment c) showed a large increase in the specific activity and the total activity of adenyl cyclase, and a high stimulation response to glucose (272% stimulation).

Effect of Glucose Concentration: Variation of the glucose concentration in the adenyl cyclase reaction mixture showed an optimum glucose concentration of 0.2 M for maximum stimulation of the enzyme activity (Table 2).

Effect of Ammonium Sulfate Precipitation: Nonspecific precipitation of whole extract proteins by $(NH_4)_2SO_4$ at a 70% saturation, followed by the solubilization of the ammonium sulfate precipitate in tris-HCl buffer and the removal of nonsoluble precipitate by centrifugation, resulted in the loss of the stimulation effect of glucose on the adenyl cyclase activity (Table 3).

TABLE 2

Effect of Glucose Concentration on the Stimulation of Adenyl Cyclase Activity of ABDRO1

Glucose Concentrate (M)	Adenyl Cyclase Activity * Nanomoles cAMP/30 minutes/mg Protein
0	3.26
0.1	9.92
0.2	12.86
0.4	4.73

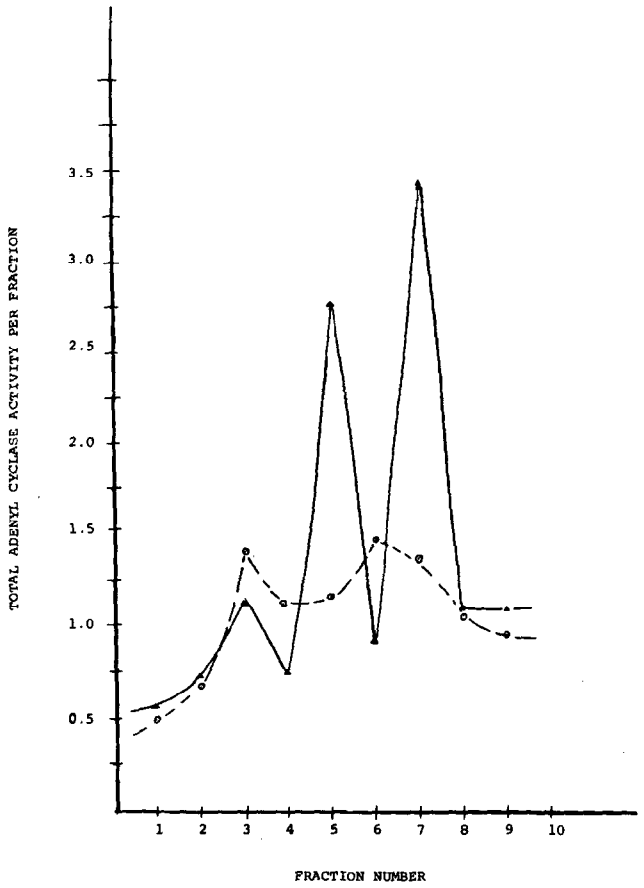
*Assays performed with 40,000 rpm pellet preparation.

TABLE 3

Effect of Ammonium Sulfate Precipitation

Additions	Adenyl Cyclase Activity Nanomoles cAMP/30 minutes/mg Protein
None	0.906
Glucose (0.2 M)	0.881

Separation of "Whole Extract" Components on a Sucrose Density Gradient: Fractionation of the components of the whole extract, particulate and soluble, was performed on a 14-ml density gradient column 10-60% w/w in sucrose and 0.04 M tris-HCl, pH 8 buffer, in a SB283 rotor at 35,000 rpm for 18 hours. One-half-ml fractions were collected, diluted to 3 ml with buffer and centrifuged at 50,000 rpm for 1 hour. Supernatants were discarded and pellets resuspended in 0.2 ml buffer for assay of adenyl cyclase. Determinations of the adenyl cyclase activity in each fraction, with and without glucose, showed two stimulation peaks with 230 and 250% activities, while other fractions showed up to 40% repression of the enzyme activity (Fig. 1). The existence of multiple peaks of stimulation has been confirmed in similar experiments.



LEGEND TO FIGURE 1

Fractionation of the membrane-bound adenyl cyclase system responsive to glucose regulation by sucrose density gradient centrifugation (10-60% w/w). Adenyl cyclase activity was determined in each fraction with and without glucose. Total enzyme activities per fraction were calculated as nanomoles cAMP produced per 30 minutes per 0.2 ml pellet (Fraction volume).

---o---o--- = total activities without glucose.

—Δ—Δ— = total activities with glucose.

From these results, we feel that the stimulatory effect of glucose in ABDROl is due to an interaction between glucose and components of a membrane bound adenyl cyclase enzyme system. In addition, this interaction might have a negative as well as positive effect on the activity of the system. This suggests to us a general mechanism of regulation for the variation in the bacterial cAMP level in response to different growth conditions.

ACKNOWLEDGEMENT

We are deeply indebted to Dr. Carl Price for his valuable help in the sucrose density gradient analysis and in the preparation of this manuscript. This work was supported by Rutgers Research Council Grant #07-2134 and Biomedical Science Support Grant PHS RR 7058-07.

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