JOURNAL OF BACTERIOLOGY, May 1983, p. 992-994
0021-9193/83/050992-03$02.00/0

Vol. 154, No. 2

Effects of Aerobic and Anaerobic Shock on Catabolite Repression in Cyclic AMP Suppressor Mutants of *Escherichia coli*†

JONG HO LEE‡ AND WALTER J. DOBROGOSZ*

Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27650

Received 28 December 1982/Accepted 10 February 1983

Cultures of *Escherichia coli* K-12 grown on glucose or gluconate under aerobic conditions exhibited catabolite repression of β-galactosidase synthesis. Derepression occurred when these cultures were subjected to anaerobic shock. These states of repression and derepression were found to be associated with low and high differential rates of cyclic AMP synthesis, respectively. This observation is consistent with the view that cyclic AMP plays a central role in the catabolite repression phenomenon. We report here, however, that identical stages of repression and derepression occur in mutant strains possessing *cya crp*(Csm) genotypes and therefore unable to synthesize cyclic AMP. These results suggest that cyclic AMP is not the sole regulator involved in catabolite repression.

It is known that cyclic AMP (cAMP) and the cAMP receptor protein play important roles in the catabolite repression (CR) phenomenon (3, 17). Cyclic AMP binds to the cAMP receptor protein to form a genetic regulatory complex which activates the transcription of catabolite repressible operons. The formation of this complex is thought to be dependent on the availability of cAMP, which in turn is dependent on the state of adenylate cyclase activity. Growth on repressor substrates is believed to inhibit this activity, and thus it is generally assumed that a correlation exists between the synthesis of cAMP and CR such that repression occurs when adenylate cyclase is inactivited, thereby decreasing cAMP availability. Conversely, derepression occurs when adenylate cyclase is activated (or de-activated), and cAMP levels are thereby elevated. Such correlations have been observed by some workers (5, 10) but not by others (11, 14, 19), and this issue has yet to be resolved.

New information in this regard is reported here. We determined the differential rates of cAMP and β-galactosidase synthesis in *Escherichia coli* cultures grown under conditions known to cause rapid and dramatic shifts in the state of CR of the organism. Cohn and Horibata (7) and subsequently our laboratory (9, 16) reported that CR by glucose or gluconate was reversed by anaerobic shock. Evidence was obtained that CR was closely associated with the reduction of terminal electron acceptors such as O_2, NO_3^-, or H^+, resulting in the formation of water, NO_2^-, and H_2, respectively. Interference with these processes resulted in catabolite derepression (CdeR). We were able to manipulate cultures along these lines to produce phases of CR and CdeR at will during growth on repressor substrates (16).

In the experiments reported below, alternate conditions of aerobic and anaerobic shock were used to promote repression and derepression of β-galactosidase synthesis in *E. coli* K-12 (*cya*$^+$ *crp*$^+$) and in two mutant strains which lack adenylate cyclase activity, *E. coli* NCR30 [*cya-57 crp-51*(Csm)] and *E. coli* NCR91 [*cya-57 crp-28*(Csm)]. These latter strains were isolated and characterized as described previously (15). They have a second-site mutation in the *crp* locus which suppresses the cAMP requirement conferred by the primary *cya* mutation. These strains are able to synthesize catabolite-repressible enzymes in the absence of cAMP or other cyclic nucleotides.

The total amount of cAMP produced during growth of these cultures was determined essentially as described by Gilman (12), using binding protein isolated from beef kidney by the method of Cheung (6) as described by Botsford (2). The total amount of cAMP (intra- and extracellular) present in a 0.5-ml culture sample was calculated as picomoles per milliliter and plotted on a differential basis against cell mass (micrograms of cells [dry weight] per milliliter). All analyses were carried out during exponential growth.

† Paper no. 8457 of the Journal Series of the North Carolina Agricultural Research Service of Raleigh, Raleigh, NC 27650.

‡ Present address: Department of Microbiology and Cell Sciences, University of Florida, Gainesville, FL 32611.

Induction and measurement of β-galactosidase activity were conducted as described earlier (13).

Differential rates of cAMP and β-galactosidase synthesis in wild-type *E. coli* K-12 grown on both glucose and gluconate under aerobic and anaerobic conditions are shown in Fig. 1. It is evident that CR of β-galactosidase synthesis during aerobic growth was overcome for a period of time after anaerobic shock. The differential rates of cAMP synthesis closely paralleled the rates of β-galactosidase synthesis. These findings tend to corroborate the view described above, namely, that CR is a consequence of decreased cAMP synthesis and that CdeR, on the other hand, is the consequence of increased cAMP synthesis. These same correlations were observed when the wild-type strain was subjected to an alternating series of aerobic and anaerobic shocks (data not shown) rather than just the one sustained anaerobic shock shown in Fig. 1. In either case, however, CR observed under aerobic conditions was completely overcome by the addition of 1 to 5 mM concentrations of cAMP to the culture medium (13). These observations are consistent with the hypothesis that cAMP plays a central bioregulatory role in CR.

However, if CdeR and CR are determined primarily by the respective activity or inactivity of adenylate cyclase, it would follow that these regulatory functions would not occur in strains possessing *cya* mutations. The data presented in Fig. 2 prove that this supposition is incorrect. The data showed that sustained anaerobic shock or alternating aerobic and anaerobic shock treatments resulted in the normal CR and CdeR responses that had been observed in wild-type cells; yet this strain (NCR91) was unable to synthesize cAMP. Essentially identical results were obtained (data not shown) when strain NCR30 was tested in this manner. Both of these strains are able to synthesize β-galactosidase (as well as other catabolite-repressible enzymes) in the absence of cAMP because of a suppressor mutation within their *crp* locus (15). Also, CR in these strains is completely overcome by the addition of 1 to 5 mM concentrations of cAMP (data not shown), just as with wild-type cells. Susceptibility of such suppressor strains to CR and its reversal by cAMP have been reported previously by others as well (1, 4, 8, 15, 18).

The data presented in this report demonstrate with exceptional clarity a dilemma that has thus emerged concerning our understanding of the mechanism underlying the CR phenomenon. On the one hand, our analyses of the wild-type K-12 strain tend to confirm the view that CR is inversely related to the ability of the organism to synthesize cAMP. On the other hand, our analyses of the *cya* suppressor strains show that

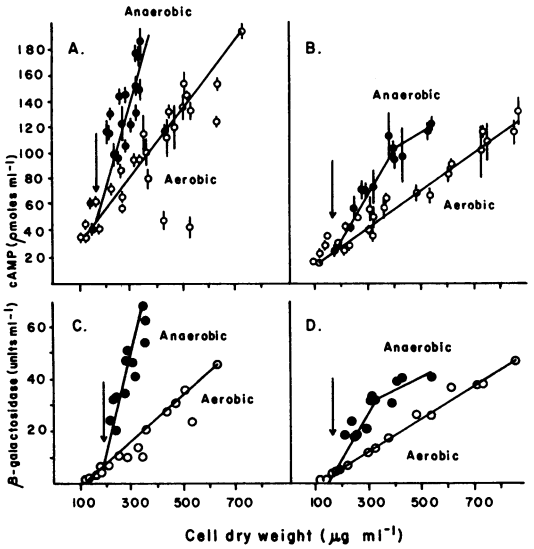

FIG. 1. Effect of anaerobic shock on differential rates of cAMP and β-galactosidase synthesis in *E. coli* K-12. Cultures grown on 20 mM gluconate (A and C) or 20 mM glucose (B and D) were analyzed for cAMP (A and B) and β-galactosidase (C and D) under aerobic (○) or anaerobic (●) conditions. Growth of the cultures was measured using a Spectronic 70 spectrophotometer and expressed as micrograms of cells (dry weight) per milliliter of culture. The cAMP concentrations (expressed as picomoles per milliliter of culture) and β-galactosidase levels (expressed as units of enzyme per milliliter of culture) were plotted on a differential basis against the culture mass as indicated. The arrows indicate the time at which one set of cultures in each case was made anaerobic by sparging the culture medium for 5 min with a mixture of 95% N_2 and 5% CO_2 as described elsewhere (16). The mineral salts basal medium contained 0.25% vitamin-free acid-hydrolyzed casein plus the substrates added as described previously (16). A unit of β-galactosidase activity was defined as that amount of enzyme that hydrolyzes 1 μmol of *o*-nitrophenyl-β-D-galactopyranoside h^{-1} at 30°C in the presence of 2 mM *o*-nitrophenyl-β-D-galactopyranoside, 1.24×10^{-4} M glutathione (red), and 0.05 M sodium phosphate buffer (pH 7.5). Aerobic cultures of strain K-12 grew on glucose and gluconate with generation times of 45 and 50 min, respectively. The anaerobic generation times on these substrates were 70 and 97 min, respectively. The vertical bars for the cAMP assays indicate the variability range noted in these assays. The circles indicate the average value determined on the basis of five assays. The upper and lower ends of the bars indicate the high and low values obtained in each set of these assays.

patterns of CR and CdeR identical to those observed in K-12 cultures are observed in cultures devoid of the adenylate cyclase-cAMP system. On the basis of these observations, it seems reasonable to conclude that (i) whereas the *cya* gene products, adenylate cyclase and

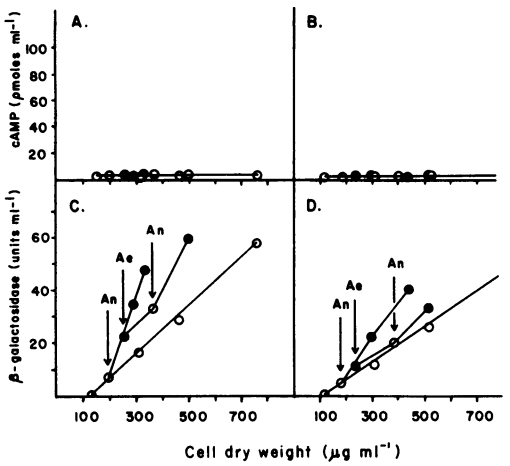

FIG. 2. Differential rates of cAMP and β-galactosidase synthesis in *E. coli* NCR91. These data were obtained and plotted as described in the legend to Fig. 1, except that strain NCR91 was used. Cultures grown on 20 mM gluconate (A and C) or 20 mM glucose (B and D) were analyzed for cAMP (A and B) and β-galactosidase (C and D) under aerobic (○) or anaerobic (●) growth conditions. The cultures were grown under three different conditions. One set was maintained under aerobic conditions (○) throughout the experiment. Another set was maintained under anaerobic conditions (●) throughout the experiment. A third set was alternated between aerobic (Ae) and anaerobic (An) conditions as indicated by the arrows. Aerobic cultures of this strain grew on glucose and gluconate with generation times of 90 and 95 min, respectively. Anaerobic cultures grew on these substrates with generation times of 240 and 220 min, respectively.

cAMP, are participants in the CR phenomenon, their role in this regard is neither central nor necessary; (ii) inasmuch as CR observed in *cya* suppressor strains is reversible by cAMP, the cAMP receptor protein is the central, key element in this regulatory system; and (iii) regulatory effectors other than cAMP are involved in the CR phenomenon.

LITERATURE CITED

1. **Alexander, J. K.** 1980. Suppression of defects in cyclic adenosine 3',5'-monophosphate metabolism in *Escherichia coli*. J. Bacteriol. **144**:205–209.
2. **Botsford, J. L.** 1975. Metabolism of cyclic adenosine 3',5'-monophosphate and induction of tryptophanase in *Escherichia coli*. J. Bacteriol. **124**:380–390.
3. **Botsford, J. L.** 1981. Cyclic necleotides in procaryotes. Microbiol. Rev. **45**:620–642.
4. **Botsford, J. L., and M. Drexler.** 1978. The cyclic adenosine 3':5'-monophosphate receptor protein and regulation of cyclic adenosine 3':5'-monophosphate synthesis in *Escherichia coli*. Mol. Gen. Genet. **165**:47–56.
5. **Buettner, M. J., E. Spitz, and H. V. Rickenberg.** 1973. Cyclic adenosine 3',5'-monophosphate in *Escherichia coli*. J. Bacteriol. **114**:1068–1073.
6. **Cheung, W. Y.** 1972. Cyclic 3',5'-nucleotide phosphodiesterase. Effect of binding protein on the hydrolysis of cyclic AMP. Biochem. Biophys. Res. Commun. **46**:99–105.
7. **Cohn, M., and K. Horibata.** 1959. Physiology of the inhibition by glucose of the induced synthesis of the β-galactoside-enzyme system of *Escherichia coli*. J. Bacteriol. **78**:624–635.
8. **Dessein, A., M. Schwartz, and A. Ullman.** 1978. Catabolite repression in *Escherichia coli* mutants lacking cyclic AMP. Mol. Gen. Genet. **162**:83–87.
9. **Dobrogosz, W. J.** 1965. The influence of nitrate and nitrite reduction on catabolite repression in *Escherichia coli*. Biochim. Biophys. Acta **100**:553–556.
10. **Epstein, W., L. B. Rothman-Denes, and J. Hesse.** 1975. Adenosine 3',5'-cyclic monophosphate as a mediator of catabolite repression in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:2300–2304.
11. **Frazer, A. D. E., and H. Yamazaki.** 1978. Construction of an *Escherichia coli* strain which excretes abnormally large amounts of adenosine 3',5'-cyclic monophosphate. Can. J. Microbiol. **24**:1423–1425.
12. **Gilman, A. G.** 1970. A protein binding assay for adenosine 3',5'-cyclic monophosphate. Proc. Natl. Acad. Sci. U.S.A. **67**:305–312.
13. **Goldenbaum, P. E., R. L. Broman, and W. J. Dobrogosz.** 1970. Cyclic 3',5'-adenosine monophosphate and *N*-acetylglucosamine-6-phosphate as regulatory signals in catabolite repression of the *lac* operon in *Escherichia coli*. J. Bacteriol. **103**:663–670.
14. **Haggerty, D. M., and R. F. Schleif.** 1975. Kinetics of the onset of catabolite repression in *Escherichia coli* as determined by *lac* messenger ribonucleic acid initiations and intracellular cyclic adenosine 3',5' monophosphate levels. J. Bacteriol. **123**:946–953.
15. **Melton, T., L. L. Snow, C. S. Freitag, and W. J. Dobrogosz.** 1981. Isolation and characterization of cAMP suppressor mutants of *Escherichia coli* K12. Mol. Gen. Genet. **182**:480–489.
16. **Okinaka, R. T., and W. J. Dobrogosz.** 1967. Catabolite repression and pyruvate metabolism in *Escherichia coli*. J. Bacteriol. **93**:1644–1650.
17. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 5'-monophosphate in *Escherichia coli*. Bacterial. Rev. **40**:527–551.
18. **Takebe, Y., M. Shibuya, and Y. Kaziro.** 1978. A new extragenic suppressor of *cya* mutation. J. Biochem. **83**:1615–1623.
19. **Wayne, P. K., and O. M. Rose.** 1974. Cyclic 3',5'-adenosine monophosphate in *Escherichia coli* during transient and catabolite repression. Proc. Natl. Acad. Sci. U.S.A. **71**:1436–1440.