JOURNAL OF BACTERIOLOGY, Feb. 1989, p. 1196–1198
0021-9193/89/021196-03$02.00/0

Vol. 171, No. 2

Induction of the Alkylation-Inducible *aidB* Gene of *Escherichia coli* by Anaerobiosis

MICHAEL R. VOLKERT,[1]* LAUREL I. HAJEC,[1] AND DINH C. NGUYEN[2]

Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655,[1] and Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709[2]

Received 22 July 1988/Accepted 8 November 1988

Induction of the adaptive response to alkylation damage results in the expression of four genes arranged in three transcriptional units: the *ada-alkB* operon and the *alkA* and *aidB* genes. Adaptive-response induction requires the *ada* gene product and occurs when cells are treated with methylating agents. In previous studies we noted that *aidB*, but not *alkA* or *ada-alkB*, was induced in the absence of alkylation damage as cells were grown to stationary phase. In this note we present evidence that *aidB* is induced by anaerobiosis. Thus, *aidB* is subject to dual regulation by *ada*-dependent alkylation induction and *ada*-independent anaerobic induction.

The adaptive response to alkylation damage is induced when cells are treated with methylating agents (7, 10, 16). This induction requires a functional *ada* gene (4, 11, 12). Ada protein functions as a methyltransferase that removes methyl groups from specific sites in DNA and transfers them to two of its own cysteine residues: methyl groups removed from O^6-methylguanine and O^4-methylthymine are transferred to cysteine residue 321, while methyl groups removed from methylphosphotriesters are transferred to cysteine residue 69. When the Cys-69 site is methylated, Ada protein becomes a transcriptional activator that binds to a site adjacent to the *ada* and *alkA* promoters (8, 14) and, presumably, the *aidB* promoter.

The *aidB* gene was originally identified as one of several methylation-inducible genes during the screening of random insertions in the *Escherichia coli* chromosome of the fusion vector Mu d1(*bla lac*) for increased β-galactosidase activity upon treatment with methyl methanesulfonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The function of the *aidB* gene is unknown. Two phenotypic variants have been identified among the seven independent isolates of *aidB* fusion mutants. One class of *aidB* mutants is more resistant to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine than is the wild type; the other class is identical to the wild type and has no known phenotype.

In addition to its induction by methylating agents, induction of *aidB* gene expression has been noted when cells are grown to stationary phase (17). This induction is unique to *aidB*; *ada-alkB* and *alkA* do not show increased expression under similar conditions. In this study we examined the induction of *aidB* in undamaged cells. Induction of *aidB* was monitored by assaying β-galactosidase activity in extracts obtained from strains containing fusions of Mu d1(*bla lac*) to *aidB*. Strain MV1563 carries the *aidB2*::Mu d1(*bla lac*) allele, and strain MV1701 is an *ada-10*::Tn*10* derivative of MV1563. Cells were grown at 30°C in minimal medium containing E salts (15), glucose (0.4%), Casamino Acid hydrolysate (0.2%; Difco Laboratories), and thiamine (0.2 μg/ml). All experiments were repeated at least three times, and representative data are shown in Fig. 1 and 2.

Since cells were not aerated during treatment in our previous studies, we first examined the effects of aeration on *aidB* gene expression. Cells were grown overnight, diluted in fresh medium, and grown with aeration to a density of 5×10^7 cells per ml. Cultures were then divided into two aliquots; one was incubated with aeration and the other was incubated without aeration. Aerated cultures were grown in flasks in a water bath shaker and agitated at a rate of approximately 300 rpm. Unaerated cultures were grown in tubes incubated in a water bath without agitation.

β-Galactosidase activity remained low in the aerated culture, indicating that aeration inhibited induction of *aidB*::Mu d1(*bla lac*). However, *aidB*::Mu d1(*bla lac*) was induced to high levels in the unaerated culture (Fig. 1). This induction of *aidB* was independent of *ada*, since it occurred in both *ada*$^+$ and *ada-10*::Tn*10* derivatives. The *ada-10*::Tn*10* mutant consistently showed a slightly lower level of *aidB*::Mu d1(*bla lac*) induction than did the *ada*$^+$ strain. We presume that this was due to the lower growth rate of the *ada-10*::Tn*10* mutant strain (Fig. 1). Since the unaerated culture conditions were sufficiently anaerobic to cause induction of a *lac* fusion to *frdA* (data not shown), a gene that is induced by anaerobiosis (5), we suspected that *aidB* may be induced by anaerobiosis.

To test this directly, *aidB*::Mu d1(*bla lac*) cells were grown overnight, diluted in fresh medium, and grown aerobically to a density of 5×10^7 cells per ml. Cultures were then divided into two 15-ml aliquots; one was grown aerobically by forcing air through the culture, and the other was grown anaerobically by forcing 95% N_2–5% CO_2 through the culture. Both gas mixtures were passed through a filter to maintain sterility and bubbled through the culture at a rate of 1 to 2 ml/sec. Induction of *aidB*::Mu d1(*bla lac*) occurred only in the cultures that were grown anaerobically (Fig. 2). Induction of *aidB*::Mu d1(*bla lac*) was also seen when cultures were grown in small volumes incubated in an anaerobic tank containing an atmosphere of H_2 and CO_2 (data not shown).

The *aidB* gene maps to a locus near the anaerobically inducible fumarate reductase (*frd*) operon (95 min) (1, 18). To define its locus more precisely and to determine whether it is an *frd* allele, *aidB* was mapped relative to *frdA* and *mutL* by P1 transduction in a three-point cross. P1 grown on MV2018 (*aidB*$^+$ *mutL218*::Tn*10 frdA11*) was used to transduce MV1563 [*aidB*::Mu d1(*bla lac*) *mutL*$^+$ *frdA*$^+$]. Tetracycline-resistant (Tet^r) recombinants (*mutL218*::Tn*10*) were

* Corresponding author.

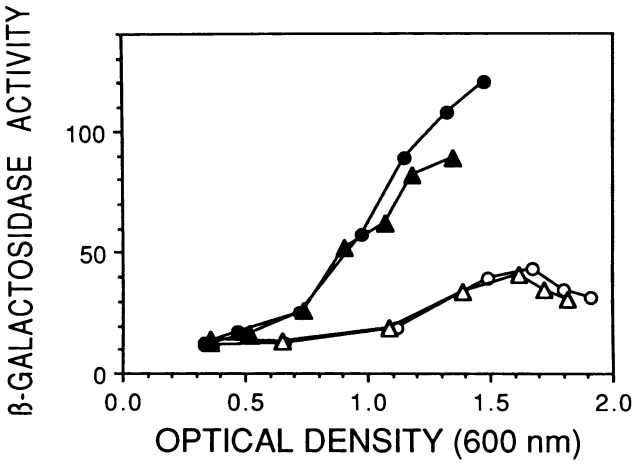

FIG. 1. Induction of *aidB*::Mu d1(*bla lac*) in aerated cultures (open symbols) and in unaerated cultures (closed symbols). Symbols: ○ and ●, MV1563 *aidB2*::Mu d1(*bla lac*); △ and ▲, MV1701 *aidB2*::Mu d1(*bla lac*) *ada-10*::Tn*10*. Hourly time points are shown. β-Galactosidase activity is expressed as units per optical density unit (600 nm).

selected and screened for loss of the *aidB*::Mu d1(*bla lac*) allele by testing for ampicillin sensitivity. Incorporation of the *frdA11* mutation of the donor was determined by testing for inability to grow anaerobically on glycerol fumarate plates (13).

Of *frdA*$^+$ transductants, 33 and 31% exhibited unselected phenotypes Ap^r and Ap^s, respectively. Of *frdA* mutant transductants, 21 and 14% exhibited Ap^r and Ap^s, respectively. A total of 159 Tet^r transductants were tested. These results indicate that *frdA* and *aidB* exhibit cotransduction frequencies with *mutL218*::Tn*10* of 35 and 45%, respectively, and lie on opposite sides of *mutL*. When the Tn*10* insert present in the donor strain is taken into account, *frdA* and *aidB* are placed approximately 0.45 and 0.6 min from *mutL*. The genetic map location, together with the finding that anaerobic induction of *aidB* is unaffected by the *nirR1* mutation (also called *fnr-1*) (data not shown), a mutation that blocks induction of *frdA* and several other anaerobically inducible genes (2, 6, 9; data not shown), allows us to rule out the possibility that *aidB*::Mu d1(*bla lac*) is an allele of the *frd* operon and shows that anaerobic induction of *aidB* is *nirR* independent.

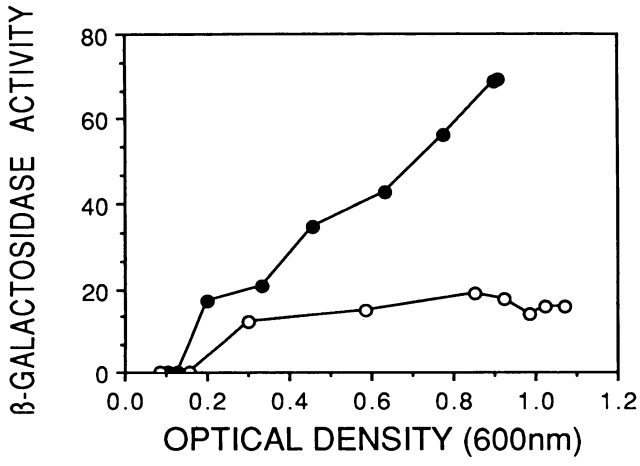

FIG. 2. Induction of *aidB*::Mu d1(*bla lac*) during anaerobic growth. MV1563 *aidB2*::Mu d1(*bla lac*) was grown in the presence of air (○) or 95% N_2–5% CO_2 (●). Hourly time points are shown. β-Galactosidase activity is expressed as units per optical density unit (600 nm).

Our results lead to the conclusion that the *aidB* gene of *E. coli* is induced by anaerobiosis in an *ada*-independent fashion as well as by alkylation damage to DNA in an *ada*-dependent fashion. Thus, there are at least two links between alkylation damage to DNA and anaerobiosis: the dual regulation of *aidB* described here and the requirement for anaerobiosis for alkylation induction of *aidC*. *aidC* is an *ada*-independent gene that is induced by treatment with a variety of alkylating agents only when cells are grown anaerobically (3, 18; H. H. Volkert, F. H. Gately, and L. I. Hajec, Mutat. Res., in press). Further work will be required to define the effects of anaerobiosis on alkylation damage and its repair and to determine the regulatory elements involved in anaerobic induction of the *aidB* gene.

We thank J. Weiner, S. T. Cole, S. Iuchi, and E. C. C. Lin for strains and advice and S. Deschenes, A. Poteete, M. Marinus, and Z. Matijasevic for critical comments on the manuscript.

This work was supported by Public Health Service grant GM37052 from the National Institutes of Health.

LITERATURE CITED

1. **Bachmann, B. J.** 1987. Linkage map of *Escherichia coli* K-12, edition 7, p. 807–876. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
2. **Chippaux, M., V. Bonnefoy-Orth, J. Ratouchniak, and M. Pascal.** 1981. Operon fusions in the nitrate reductase operon and study of the control gene *nirR* in *Escherichia coli*. Mol. Gen. Genet. **182:**477–479.
3. **Fram, R. J., M. G. Marinus, and M. R. Volkert.** 1988. Gene expression in *E. coli* after treatment with streptozotocin. Mutat. Res. **198:**45–51.
4. **Jeggo, P.** 1979. Isolation and characterization of *Escherichia coli* K-12 mutants unable to induce the adaptive response to simple alkylating agents. J. Bacteriol. **139:**783–791.
5. **Jones, H. M., and R. P. Gunsalus.** 1985. Transcription of the *Escherichia coli* fumarate reductase genes (*frdABCD*) and their coordinate regulation by oxygen, nitrate and fumarate. J. Bacteriol. **164:**1100–1109.
6. **Lambden, P. R., and J. R. Guest.** 1976. Mutants of *Escherichia coli* K12 unable to use fumarate as an anaerobic electron acceptor. J. Gen. Microbiol. **97:**145–160.
7. **Lindahl, T., B. Sedgwick, M. Sekiguchi, and Y. Nakabeppu.** 1988. Regulation and expression of the adaptive response to alkylating agents. Annu. Rev. Biochem. **57:**133–157.
8. **Nakabeppu, Y., and M. Sekiguchi.** 1986. Regulatory mechanisms for induction of synthesis of repair enzymes in response to alkylating agents: Ada protein acts as a transcriptional regulator. Proc. Natl. Acad. Sci. USA **83:**6297–6301.
9. **Newman, B. M., and J. A. Cole.** 1978. The chromosomal location and pleiotropic effects of mutations of the *nirA*$^+$ gene of *Escherichia coli* K12: the essential role of *nirA*$^+$ in nitrite reduction and in other anaerobic redox reactions. J. Gen. Microbiol. **106:**1–12.
10. **Samson, L., and J. Cairns.** 1977. A new pathway for DNA repair in *Escherichia coli*. Nature (London) **267:**281–283.
11. **Sedgwick, B.** 1982. Genetic mapping of *ada* and *adc* mutations affecting the adaptive response of *Escherichia coli* to alkylating agents. J. Bacteriol. **150:**984–988.
12. **Sedgwick, B.** 1983. Molecular cloning of a gene which regulates the adaptive response to alkylating agents in *Escherichia coli*. Mol. Gen. Genet. **191:**466–472.
13. **Spencer, M. E., and J. R. Guest.** 1973. Isolation and properties

of fumarate reductase mutants of *Escherichia coli*. J. Bacteriol. **114**:563–570.
14. **Teo, I., B. Sedgwick, M. W. Kilpatrick, T. V. McCarthy, and T. Lindahl.** 1986. The intracellular signal for induction of resistance to alkylating agents in *E. coli*. Cell **45**:315–324.
15. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification of some properties. J. Biol. Chem. **218**:97–106.
16. **Volkert, M. R.** 1988. Adaptive response of *Escherichia coli* to alkylation damage. Environ. Mol. Mutagen. **11**:241–255.
17. **Volkert, M. R., and D. C. Nguyen.** 1984. Induction of specific *Escherichia coli* genes by sublethal treatments with alkylating agents. Proc. Natl. Acad. Sci. USA **81**:4110–4114.
18. **Volkert, M. R., D. C. Nguyen, and K. C. Beard.** 1986. *Escherichia coli* gene induction by alkylation treatment. Genetics **112**:11–26.