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Structure and Transcriptional Regulation of the *Escherichia coli* Adaptive Response Gene *aidB*

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Expression of the *Escherichia coli aidB* gene is induced in vivo by alkylation damage in an *ada*-dependent pathway and by anaerobiosis or by acetate at pH 6.5 in an *ada*-independent fashion. In this report, we present data on *aidB* gene structure, function, and regulation. The *aidB* gene encodes a protein of ca. 60 kDa that is homologous to several mammalian acyl coenzyme A dehydrogenases. Accordingly, crude extracts from an *aidB*-overexpressing strain showed isovaleryl coenzyme A dehydrogenase activity. *aidB* overexpression also reduced *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced mutagenesis. Both *ada*- and acetate/pH-dependent induction of *aidB* are regulated at the transcriptional level, and the same transcriptional start point is used for both kinds of induction. Ada protein plays a direct role in *aidB* regulation: methylated Ada is able to bind to the *aidB* promoter region and to activate transcription from *aidB* in an in vitro transcription-translation system using crude *E. coli* extracts.

In *Escherichia coli*, exposure to sublethal doses of DNA-methylating agents like methyl methanesulfonate (MMS), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and *N*-methyl-*N*-nitrosourea (MNU) activates the expression of several genes via positive regulation mediated by Ada protein in a process termed adaptive response (6, 24). The molecular mechanism of this gene activation has been described in detail (21, 28). Ada protein transfers methyl groups from damaged DNA to two of its own cysteine residues; in doing so, it becomes a transcriptional activator (17, 28). The methylated protein is able to bind specific sequences of DNA in the promoter region of its own operon, *ada/alkB*, and at least one other gene, *alkA* (22). The *alkA* gene encodes a glycosylase, involved in the removal of methylated bases from DNA (16). The *ada* promoter is also responsible for the transcription of another gene, *alkB*, whose function is unknown (7, 11). In both of these promoters, Ada-binding sequences have been identified (18).

The *aidB* gene is *ada* regulated in vivo (32) but is also subject to an *ada*-independent mechanism of regulation (31). *aidB* is induced by anaerobiosis or by addition of sodium acetate to growth medium, at a slightly acidic pH (ranging 6.0 to 6.8 [26]). This second pathway of expression is mediated by *rpoS* (30), a gene which encodes an alternative sigma factor of RNA polymerase, mainly active in late-logarithmic and stationary phases of *E. coli* growth (9, 27).

Another important difference with the other adaptive response genes is the phenotype induced by mutations: *ada*, *alkA*, and *alkB* mutants are usually more sensitive to methylating agents (4, 6, 7). The *aidB*::*lacZ* insertion mutants showed two different phenotypes: one class of mutants had the wild-type phenotype, the second was more resistant to MNNG and not significantly affected in sensitivity to other alkylating agents (32).

In this study, we provide information about the structure of the *aidB* gene and about the role of the AidB protein produced. Moreover, initial characterization of the promoter structure and of the mechanism of *aidB* activation by Ada is described.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or description	Reference or source
E. coli strains		
MV1161[a]	Wild type	32
MV1561	*aidB1*::Mu d1 (*lac bla*)	32
MV2176[b]	*aidB1*::Mu d1 (*lac bla*)^{TR}	12
MV2805	MV1161/pMV132	This study
MV3039	MV1161/pMV156	This study
MV3357	MV1161/pMV175	This study
MV3590	RB791/pMV435	This study
MV3594	RB791/pTrc99A	This study
MV3626	MV1161/pTrc99A	This study
MV3628	MV1161/pMV435	This study
RB791	*lacI*^{q}	25
Plasmids		
pMV120	Vector: pUC18 *aidB*: *Bgl*II-*Eco*RI[c]	This study
pMV132	Vector: pRS1274 *aidB*: *Bgl*II-*Eco*RI	This study
pMV156	Vector: pRS1274 *aidB*: *Rsr*II-*Sty*I	This study
pMV175	Vector: pRS1274 *aidB*: *Bam*HI-*Eco*RI	This study
pMV180	Vector: pSL1180 *aidB*: *Bgl*II-*Pst*I	This study
pMV201	Vector: pBW2 *aidB*: *Pst*I-*Pst*I	This study
pMV240	Vector: pSL1180 *aidB*: *Xho*I-*Pst*I	This study
pMV 426	Vector: pUC18 *aidB*: *Bam*HI-*Kpn*I	This study

[a] *rfa-550* derivative of AB1157 that carries the following additional markers: *argE3 hisG4 leu-6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 rpsL31 supE44 tsx-6 rfa-550.*
[b] Spontaneous 42°C-resistant derivative of MV1561.
[c] Positions of restriction sites in *aidB* are shown in Fig. 1.

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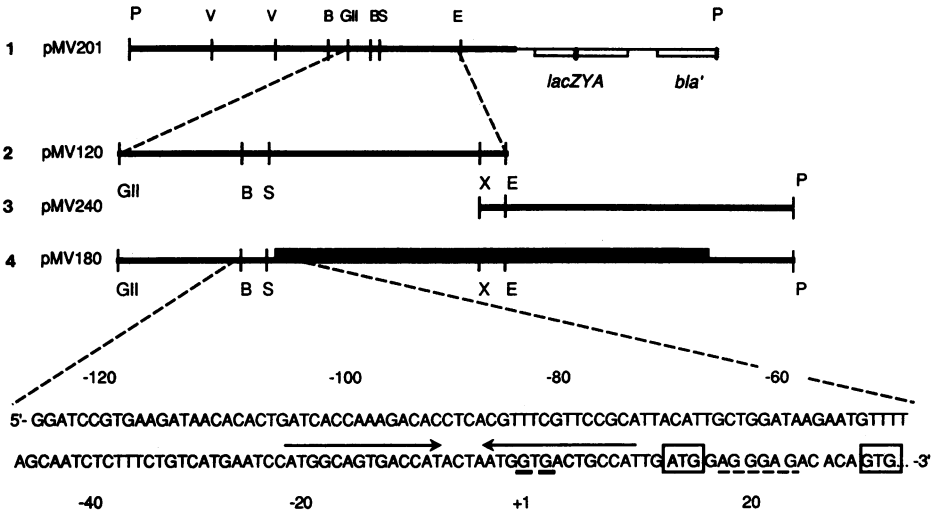


FIG. 1. Cloning and structure of the *E. coli aidB* gene. Line 1 shows the 9.2-kb *aidB*::Mud1 (*bla lac*) fusion mutation which was cloned as a *Pst*I fragment, using pBW2 as a vector (33), to produce pMV201. The dark line indicates chromosomal DNA (6.2 kb); the thin line indicates DNA of the Mu d1 (*bla lac*) phage. The 1.5-kb *Bgl*II-*Eco*RI fragment (line 2) was subcloned from the initial pBW2-*aidB-lac* clone to produce pMV120. The same fragment was transferred to M13 mp18 and mp19 and sequenced. The remaining 1.4 kb of the wild-type *aidB* gene (line 3) was subcloned from λ654 (λ1G10) of the Kohara collection (8) as an *Xho*I-*Pst*I fragment producing pMV240 and rejoined at the *Xho*I site to reconstruct the wild-type gene (pMV180). The gray box indicates the *aidB* open reading frame deduced from its DNA sequence. The DNA sequence of the upstream region required for regulation and expression is shown on the last two lines. The solid underlining indicates the two transcriptional start sites, the dotted underline indicates the presumptive Shine-Dalgarno sequence, the arrows indicate the inverted repeat which overlaps the transcriptional start sites. The two potential translational start sites are indicated by boxes. Restriction site abbreviations: B, *Bam*HI; V, *Eco*RV; GII, *Bgl*II; R, *Rsr*II; S, *Sty*I; X, *Xho*I; E, *Eco*RI; K, *Kpn*I; P, *Pst*I.

MATERIALS AND METHODS

Chemicals. Restriction endonucleases were from Boehringer Mannheim Corp. (Indianapolis, Ind.). The bandshift kit and plasmids pUC18, pSL1180, and pTrc99A were from Pharmacia Inc. (Piscataway, N.J.). RNA polymerase and the *E. coli* cell-free transcription-translation kit were from Promega Corp. (Madison, Wis.). [^{32}P]CTP, [^{35}S]methionine, and [^{32}P] dATP were from Amersham (Arlington Heights, Ill.). The DNA Sequenase kit was from U. S. Biochemical (Cleveland, Ohio). Other chemicals were from Sigma (St. Louis, Mo.).

Bacterial strains and media. The bacterial strains used are described in Table 1. Strains were grown in minimal medium containing E salts (31), glucose (0.4%), Casamino Acids (0.2%), and thiamine 0.2 μg/ml (EgluCAAB$_1$). When required, ampicillin was added either at 25 (MV2176) or 50 (plasmid-bearing strains) μg/ml. For induction of *aidB* in β-galactosidase (15) and primer extension experiments, log-phase cells were collected at Klett 10 and resuspended either in the same medium supplemented with 0.05% MMS (*ada*-dependent induction) or in medium adjusted to pH 6.5 and supplemented with 50 mM sodium acetate (acetate/pH induction) or with no additions (control cultures).

Dehydrogenase assays. Dehydrogenase assays were performed as described previously (3) except that addition of phenazine methosulfate was omitted. Crude extracts (120 μg of protein) from strains MV3590 (carrying *aidB* under control of the pTrc promoter) and MV3594 (pTrc99A plasmid in the same strain background) were used.

MNNG mutagenesis. MNNG mutagenesis was performed essentially as described previously (29) except that colonies were counted after 3 days of incubation at 37°C. Overnight cultures grown in EgluCAAB$_1$ were diluted 1:100 in fresh medium and grown for 4 h. Isopropylthiogalactopyranoside (IPTG) was added when necessary, and growth was prolonged for an additional 1.5 h. Optical densities (600 nm) of the different cultures were adjusted so that approximately 10^7 cells were plated.

In vitro techniques. Primer extension experiments were performed as described previously (23), using 10 μg of total cell RNA extracted with the RNAid plus kit (Bio 101, La Jolla, Calif.). The primer used was 5'-GGTATAGGTTGATTAAA AACGGTGAGTTTG-3' (Operon Technologies Inc., Alameda, Calif.). The reverse transcription products were resolved on a denaturing 6% acrylamide gel.

RNA polymerase assays and in vitro protein synthesis in presence of Ada protein were performed as described by Teo et al. (28). Purified Ada protein was a kind gift of M. Sekiguchi (Kyushu University) or G. Walker (Massachusetts Institute of Technology). Methylated Ada was produced by incubating the protein with MNU-methylated calf thymus DNA as described by Nakabeppu and Sekiguchi (17).

Nucleotide sequence accession number. Nucleotide and deduced amino acid sequences of the *Bgl*II-*Pst*I fragment (Fig. 1) are available from GenBank accession number L20915.

RESULTS

Cloning and structural analysis of *aidB*. The *E. coli aidB* gene was initially cloned as a *Pst*I fragment from the *aidB*::Mu d1 (*bla lac*) fusion strain MV1561, using the pBW2 vector designed for this purpose (33). Restriction analysis and subsequent deletion analysis and insertion mutagenesis (data not shown) indicated that the gene began at coordinate 4446 on the Kohara physical map (8). Thus, subcloning of the *Bgl*II-to-*Eco*RI fragment (Fig. 1, line 2) was expected to produce a fragment bearing all regulatory sequences and upstream portions of the *aidB* gene. Evidence that this is the case is presented below.

The remainder of the *aidB* gene was subcloned from λ654

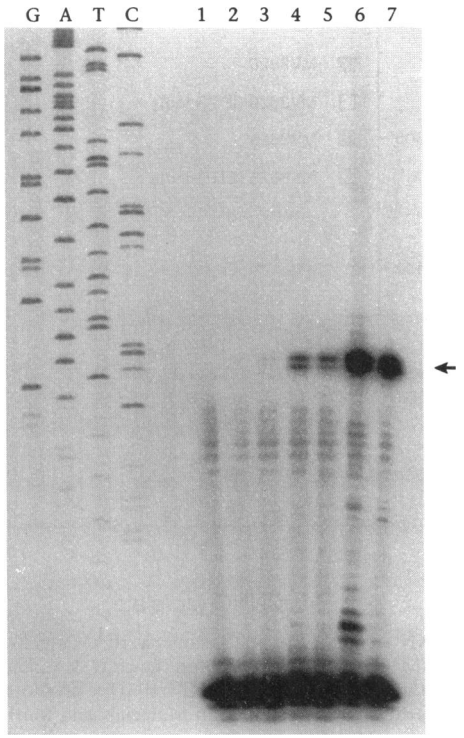
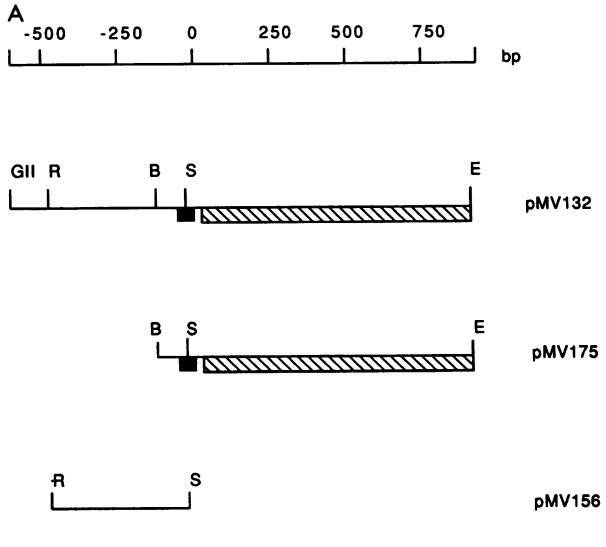


FIG. 2. Primer extension analysis of *aidB* transcription. Lane 1, primer, no RNA hybridized; lanes 2 to 4, MV2176 (chromosomal *aidB*::*lacZ* fusion); lanes 5 to 7, MV2805 (plasmid-borne *aidB*::*lacZ* fusion pMV132). Total RNA was isolated from uninduced cultures (lanes 2 and 5), acetate/pH-induced cultures (lanes 3 and 6), and MMS-induced cultures (lanes 4 and 7). The sequencing ladder (GATC) generated from the same oligonucleotide used for the primer extension is shown as a size standard. The arrow indicates the position of the primer extension products.



B

strains time (h)	MV2805 (pMV132)	MV3357 (pMV175)	MV3039 (pMV156)
0	523	432	67
5 uninduced	1098	1031	145
MMS-ind.	19163	16278	634
pH-ind.	5165	5420	121

FIG. 3. Expression of *lacZ* fusions with different fragments of the *aidB* region. (A) Positions of the different *aidB* fragments used. All fragments are fused to the *lacZ* gene carried by plasmid pRS1274. pMV132 carries the same *Bgl*II-*Eco*RI fragment as pMV120 (see Fig. 1); pMV156 and pMV175 carry the specific fragments shown. Restriction sites are abbreviated as in Fig. 1. (B) *lacZ* expression measured as β-galactosidase activity (Miller units). Cells were induced as described in Materials and Methods.

(λ1G10) of the Kohara collection (8). DNA sequencing of the overlapping *Xho*I-*Eco*RI region confirmed that this fragment is contiguous with the upstream *aidB* DNA fragment (Fig. 1, line 3) and that the gene was reassembled by joining the two fragments at the *Xho*I site present in both.

Nucleotide sequence. The DNA sequence of the *aidB* gene revealed an open reading frame of 1,642 nucleotides; the putative protein produced has an expected molecular mass of 60.5 kDa. Putative promoter and upstream DNA sequences are shown in Fig. 1. Numbering starts with the first transcriptional start identified by primer extension (see below). An ATG codon is present at the +14 position, with no apparent Shine-Dalgarno sequence; a putative ribosomal binding site is instead present before a possible +29 GTG start codon. We suggest that this could be the actual translational start. No consensus −10 or −35 sequence was found. It is noteworthy that the transcriptional start is internal to an inverted repeat, ranging from nucleotides −25 to +15.

Primer extension. To obtain information about the start site of *aidB* mRNA and, consequently, the location of its promoter, primer extension experiments were performed. Strains MV2176 (chromosomal *aidB*::*lacZ* fusion [12]) and MV2805 (*Bgl*II-*Eco*RI fragment of *aidB* fused to *lacZ* on plasmid pMV132) were grown in noninducing conditions or under *ada* or acetate/pH induction. As shown in Fig. 2, two different guanine residues (numbered +1 and +3) could be used as the start point of transcription.

Fusions of *aidB* deletions to *lacZ*. Several different fragments of *aidB* were subcloned in pRS1274 to give transcriptional fusions with *lacZ*. Strains transformed with these plasmids were tested for β-galactosidase activity under inducing conditions. Figure 3A shows the positions of the different restriction sites used.

Results of β-galactosidase assays are shown in Fig. 3B. Fusions to *lacZ* of *Bgl*II-*Eco*RI and *Bam*HI-*Eco*RI regions of *aidB* were fully active in inducing transcription of *lacZ*, while the *Rsr*II-*Sty*I fragment was unable to direct *lacZ* transcription, showed only a poor induction by MMS and no induction by 50 mM acetate at pH 6.5.

Interaction of Ada with the *aidB* promoter in vitro. To determine if the *ada*-mediated induction of *aidB* was due to direct interaction between Ada and the *aidB* promoter, in vitro RNA polymerase assays were performed. In reaction conditions in which methylated Ada protein clearly stimulated expression of the *ada* promoter, no Ada-induced transcription from the *Bgl*II-*Eco*RI fragment of *aidB* was detected (data not shown). However, a direct binding of Ada protein to the *Bam*HI-*Sty*I fragment of *aidB* was observed in a gel retardation assay (Fig. 4). Ada binding was competed for by a 200-fold excess of the unlabeled fragment but not by the presence of 1 μg of pUC18 DNA. No binding to *aidB*

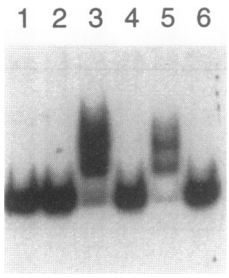


FIG. 4. Gel retardation analysis with an end-labeled *Bam*HI-*Sty*I fragment from pMV120 (*aidB*). Lane 1, no Ada protein added; lane 2, 5 pmol of unmethylated Ada protein; lane 3, 5 pmol of methylated Ada; lanes 4 to 6, as lane 3 with the following additions: 50 μg of proteinase K (lane 4), 1 μg of pUC18 DNA (lane 5), and 0.25 μg of unlabeled *Bam*HI-*Sty*I fragment from *aidB* (lane 6).

was observed if proteinase K was added to the reaction mixture.

Plasmid pMV426, a pUC18 derivative that harbors a *Bam*HI-*Kpn*I fragment (Fig. 1) including the whole *aidB* gene, was used in a cell-free coupled transcription-translation assay with crude *E. coli* extract. In this assay, methylated Ada was required for the expression of a protein of the apparent molecular mass of 56 kDa (Fig. 5). This molecular mass is in good agreement with the predicted size of the AidB protein. MNU-treated DNA used for methylation of Ada protein (see Materials and Methods) had no effect when added alone to the reaction mixture (data not shown).

MNNG mutagenesis. The *Sty*I-*Pst*I fragment of *aidB*, which includes the whole coding region for the AidB protein but lacks its regulatory sequence, was subcloned into pTrc99A, under the control of the IPTG-inducible promoter pTrc, in order to obtain inducible overexpression of the protein. The effect of AidB overexpression on MNNG mutagenesis was tested. Figure 6 shows that when strain MV3628 (*aidB* under pTrc control) is exposed to different concentrations of MNNG, mutagenesis is reduced only when *aidB* expression is induced by IPTG. In strain MV3626 (carrying the vector plasmid pTrc99A), mutation frequency was not affected by IPTG induction and remained at the level of uninduced MV3628. MNNG doses used had little or no effect on viability, and no difference in viability between strains was detected (data not shown).

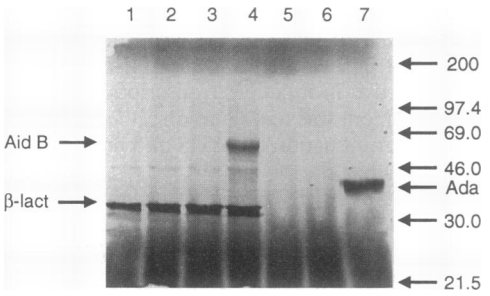


FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel analysis of in vitro protein synthesis reactions. Lane 1, pUC18; lanes 2 to 4, pMV426 (*aidB*); lanes 5 to 7, pGW2067 (*ada*) (11). In each reaction, 1.25 μg of plasmid was used. Reactions were performed without Ada protein (lanes 1, 2, and 5) or with addition of 10 pmol of Ada (unmethylated; lanes 3 and 6) or of 10 pmol of methylated Ada (lanes 4 and 7). Positions of Ada (39 kDa), AidB and β-lactamase (31.5 kDa), and molecular weight markers (in kilodaltons) are indicated.

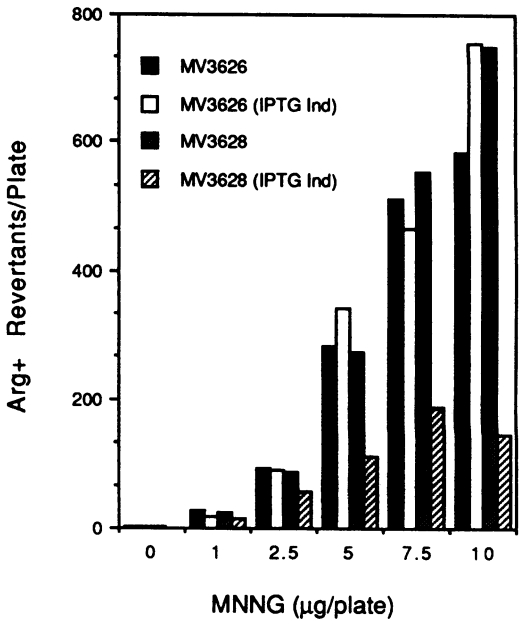


FIG. 6. MNNG mutagenesis. Strains were MV3626 (carrying pTrc99A) and MV3628 (carrying *aidB* on the pTrc99A plasmid under the control of the IPTG-inducible [IPTG Ind] pTrc promoter). Experiments were performed as described in Materials and Methods.

Dehydrogenase activity of AidB. The deduced amino acid sequence of the putative AidB protein was used in homology searches in Swiss Protein and GenBank-EMBL databases. No homology was found with Ada, AlkA, or AlkB, the other proteins involved in the adaptive response, nor does any other DNA repair protein show any significant homology with AidB. On the contrary, significant homology was detected with precursors of several mammalian acyl coenzyme A (acyl-CoA) and isovaleryl-CoA dehydrogenases. The highest homology was detected with human acyl-CoA dehydrogenase precursor (28.7% identity), rat short-branch acyl-CoA (27.6%), and human isovaleryl-CoA dehydrogenase (IVD) precursor (24.6%). Alignment of AidB with these protein precursors is shown in Fig. 7.

Dehydrogenase activity was tested in crude extracts from the AidB-overexpressing strain MV3590 as well as in control strain MV3594. Crude extracts from IPTG-induced MV3590 showed IVD activity (Fig. 8); this activity is undetectable in uninduced MV3590 as well as in IPTG-induced and uninduced MV3594. Activities of other dehydrogenases, such as butyryl-CoA and acetoacetyl-CoA dehydrogenases, were tested in the same extracts; no significant differences could be detected between the two strains, with or without IPTG induction.

DISCUSSION

The *aidB* gene is part of the adaptive response. It has already been demonstrated that activation by alkylating agents of *aidB* in vivo requires a functional *ada* gene (31). In this report, we have shown that the mechanism of *ada*-dependent induction of *aidB* expression is similar to the activation of the other adaptive response genes. Methylated Ada protein interacts directly with the *aidB* regulatory region in vitro (Fig. 4). While methylated Ada protein is able to induce transcription of the

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                                      10
       AidB                    VHWQTHTVFNQPIPLNNSNLYLSDGALCE
       ACD                     -----------------------------
       IVD                     -------------------MATATRLLGW
       Consensus               -----------------------------
                 50
AVTREGAGWDSDFLASIGQQLGTAESLELGRLANVNPPELLRYDAQGRRLDDVRFHPAWHL
MAAALLARASGPARRALCPRAWRQLHTIVQSVELPETHQMLLQTCRDFAEKELFPIAAQVD
RVASWRLRPPLAGFVSQRAHSLLPVDDAINGLSEEQRQLRQTMAKFLQEHLAPKAQEIDRS
-------------------------------------------------------------
 .:            :  .:   .       :.  :..     .       . :  :
       100                                              150
LMQALCTNRVHNLAWEEDARSGAFVARARFMLHAQVEAGSLCPITMTFAATPLLLQMLPAP
KEHLFPAAQVKKMGGLGLLAMDVPEELGGAGLDYLAYAIAMEEISRGCASTGVIMSVNNSL
NEFKNLREFWKQLGNLGVLGITAPVQYGGSGLGYLEHVLVMEEISRASGAVGLSYGAHSNL
---------------------------G-G-------------------------------
       .   :::  .    ..  . :  *     .  :   *:.. ::: :     .
                                             200
FQDWTTPLLSDRYDSHLLPGGQKRGLLIGMGMTEKQGGSDVMSNTTRAERLEDGSYRLVGH
YLGPILKFGSKEQKQAWVTPFTSGDKIGCFALSEPGNGSDAGAASTTA-RAEGDSWVLNGT
CINQLVRNGNEAQKEKYLPKLISGEYIGALAMSEPNAGSDVVSMKLKAEK-KGNHYILNGN
-----------------------------------EP--GSD-------A----G-----NG-
  :      :.  :   :.   . . :  .:::*  .***. : .  *.: .:. . * *
                                     250
K-WFFSVPQSDAHLVLAQT--AGGLSCFFVPRFLPDGQRNAIRLERLKDKLGNRSNASCEV
KAWITNAWEASAAVVFAST--DRALQNKGISAFLVPMPTPGLTLGKKEDKLGIRGSSTANL
KFWITNGPDADVLIVYAKTDLAAVPASRGITAFIVEKGMPGFSTSKKLDKLGMRGSNTCEL
K--ITN-------------------------F-V-----G--------K-G-R---T---
* *: :  ::.. :* * *  .       :. *:      :: *.:  **** *::.: ::
                              300
EFQD---AIGWLLGLEGEGIRLILKMGGMTRFDCALGSHAMMRRAFSLAIYHAHQRHVFGN
IFEDCRIPKDSILGEPGMGFKIAMQTLDMGRIGIASQALGIAQTALDCAVNYAENRMAFGA
IFEDCKIPAANILGHENKGVYVLMSGLDLERLVLAGGPLGLMQAVLDHTIPYLHVREAFGQ
-FED---P----L-----G----M------R---A----------------Y---R--FG-
 *:*   : : :**  . *: : :.  ::.*:  *  : :: : .:. :: . : * .**.
                  350
PLIQQPLMRHVLSRMALQLEGQTALLFRLARAWD-RRADAKEALWARLFTPAAKFVICKRG
PLTKLQVIQFKLADMALALESARLLTWRAAMLKDNKKPFIKEAAMAKLAASEAATAISHQA
KIGHFQLMQGKMADMYTRLMACRQYVYNVAKACDEGHCTAKDCAGVILY-----SAECATQ
--------Q---A------------------------------------------------
 : :  :::  :: *  .* :     .. *.  *  :   *:   . *      . .
       400                                            450
MPFVAEAMEVLGGIGYCEESELPRLYREMPVNSIWEGSGNIMCLDVLRVLNKQAGVYDLLS
-------IQILGGMGYVTEMPAERHYRDARITEITEGTSEIQRLVIAGHLLRSYRS-----
VA--LDGIQCFGGNGYINDFPMGRFLRDAKLYEIGAGTSEVRRLVIGRAFNADFH------
--------Q--GG-GY----P--R---DA----I--GT-----L-----------------
       ::  ** ** .:    *  *:  : .* .*::::  * :
                                              500
EAFVEVKGQDRYFDRAVRRLQQQLRKPAEELGREITHQLFLLGCGAQMLKY
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FIG. 7. Alignment of the predicted *E. coli* AidB protein with human acyl-CoA dehydrogenase (ACD) and IVD precursors and with the consensus sequence for acyl-CoA dehydrogenases proposed by Matsubara et al. (13). Asterisks indicate identical amino acids; colons indicate highly conservative substitutions; dots indicate moderately conserved substitutions.

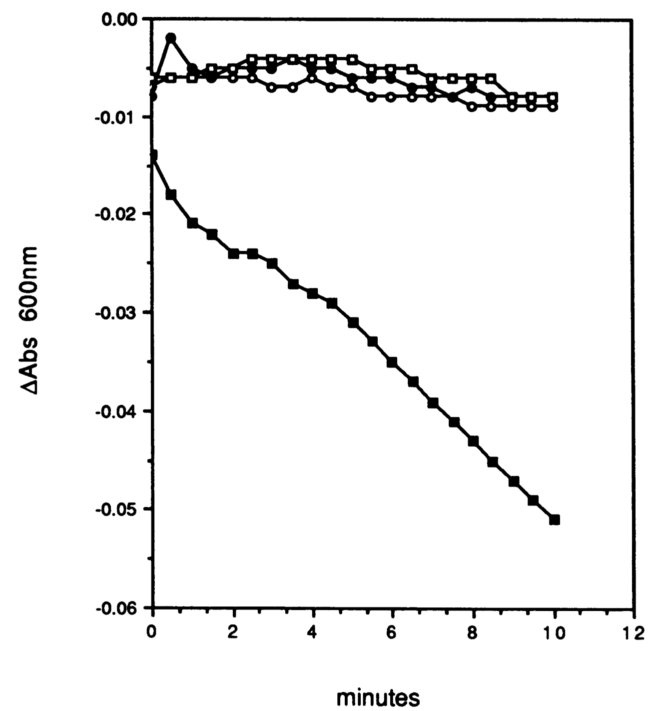

FIG. 8. IVD activity in crude extracts from strains MV3594 (carrying the pTrc plasmid; ○, ●) and MV3590 (carrying *aidB* on the pTrc99A plasmid; □, ■) with (●, ■) or without (○, □) IPTG induction. IVD activity was monitored as the reduction in A_{600} of 2,6-dichlorophenolindophenol in the presence of 10 mM isovaleryl-CoA (3). Data are from a single experiment. Three similar experiments gave very similar results.

ada and *alkA* promoter in a simple in vitro assay with RNA polymerase holoenzyme (17, 28), *aidB* was not transcribed in vitro in similar conditions. However, in a cell-free transcription-translation assay performed with *E. coli* crude extracts, a protein with the expected molecular weight was produced from the *aidB* gene only when methylated Ada was added. These experiments suggest that activation of transcription from the *aidB* promoter is triggered by direct interaction with methylated Ada protein, but another factor, possibly a protein present in the extract, might be required.

In vitro experiments such as cell-free transcription-translation (Fig. 5) and in vivo expression of *aidB* (32) showed a clear difference in Ada-dependent expression levels between the *aidB* and *ada* promoters. Since no sequence immediately comparable to Ada boxes of *ada* and *alkA* promoter upstream regions was found in *aidB*, a lower level of *ada*-dependent expression might reflect a lower affinity of the Ada protein for the regulatory region of *aidB*. The presence of two retarded bands in the bandshift experiment (Fig. 4) might suggest that more than one molecule of Ada is able to bind to *aidB*. Characterization of the Ada binding site of *aidB* is currently under investigation.

The structure of the *aidB* gene displays some unusual characteristics: there is no usual consensus −35 and −10 sequence for RNA polymerase. The position of the transcriptional start is internal to a 40-bp inverted repeat, and this was a possible problem for the primer extension experiments: premature stop of reverse transcriptase enzyme in cDNA synthesis can in fact occur when secondary structures are present in the template RNA. A confirmation that transcription of *aidB* is not driven by a promoter upstream of the inverted repeat comes from the *aidB*::*lacZ* fusion studies: when *lacZ* is fused to a fragment upstream of the inverted repeat (*Rsr*II-*Sty*I fragment; Fig. 3), very little expression of β-galactosidase is detected, and induction is lost or strongly affected. Results of *lacZ* fusion experiments suggest that all of the regulatory elements lie downstream of a *Bam*HI site (numbered −126 in Fig. 1), with an unidentified −10 box internal to the inverted repeat.

AidB homology with mammalian precursors of acyl-CoA dehydrogenases was interestingly high, with most of the conserved amino acids matching the consensus sequence of mammalian acyl-CoA dehydrogenase proposed by Matsubara et al. (13) (Fig. 7). The homology data are confirmed by the IVD activity detected in a crude extract from an *aidB*-overexpressing strain. The IVD activity seemed to be specific, although AidB showed a broader homology with several acyl-CoA dehydrogenases: no significant increase of other dehydrogenase activities was detected. A possible role of AidB as an acyl-CoA dehydrogenase could explain its expression when cells are shifted into anaerobiosis. This class of enzymes is involved in energy production during fermentation and anaer-

obic metabolism (2). Acetate at a reduced pH could act as an inducer of some anaerobic pathways: it is known that both anaerobiosis and supplementing acetate to the growth medium at a pH range of 6.0 to 6.8 results in a decrease of the internal pH of *E. coli* of around 0.7 (1, 19); this decrease could be a signal for *aidB* activation as well as for activation of other anaerobic genes.

aidB::Mu d1 (*bla lac*) fusions fall into two phenotypic categories with respect to MNNG sensitivity: those that are more resistant to its lethal and mutagenic effect and those that are identical to wild type. Neither type of mutant affects sensitivity to other alkylating agents such as MMS or MNU (32). We tested the effect of *aidB* overexpression on mutagenesis by MNNG. Surprisingly, overexpression of *aidB* reduced the mutagenic effect of MNNG (Fig. 6). The finding that the same phenotype can be produced both by insertional mutation and by overexpression of the *aidB* gene is apparently paradoxical. It has been observed that all of the *aidB*::Mu d1 (*bla lac*) fusions, regardless of phenotype, lie in the terminal third of the gene (unpublished observations). It is possible that a truncated AidB protein of at least two-thirds of the full-size wild-type protein is produced in these strains. It is therefore possible that the insertion mutants produce a functional fragment of AidB protein. According to this hypothesis, the *aidB*::Mu d1 (*bla lac*) fusion mutants that are more resistant to MNNG produce an AidB fragment which is either more stable or more active than the wild-type protein, whereas mutants that are phenotypically similar to the wild type produce an AidB fragment that is functionally similar to wild-type AidB protein.

Our finding that the AidB protein may have an isovaleryl-CoA activity does not provide any precise information on the possible role of the protein in counteracting MNNG-directed mutagenesis. However, similar examples have already been reported in the literature: Greenberg and Demple (5) found that in the *soxRS* system, the *zwf* gene encoding glucose-6-phosphate dehydrogenase is part of that oxidative damage response. In human cells, it has been found (14) that uracyl-*N*-glycosylase is actually a subunit of glyceraldehyde-3-phosphate dehydrogenase.

Since sensitivity to only MNNG is affected in the insertional mutants, *aidB* could be involved either in repair of MNNG-specific DNA lesions or in an MNNG detoxification pathway. MNNG, in fact, must be metabolically activated to exert its mutagenic and lethal activity (10). In *E. coli*, such a pathway involves glutathione, a nonprotein thiol: glutathione-deficient mutants are generally more resistant to MNNG (25). *aidB* might be involved in this or in an alternative detoxification pathway, either by acting directly on MNNG or by synthesis or modification of some MNNG-reactive compound. It is noteworthy that when *E. coli* is exposed to sodium acetate in a medium with a final pH of 6.5 (inducing conditions for *aidB*), it becomes more resistant to the mutagenic effect of MNNG (20).

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