Identification of the *Geobacter metallireducens* BamVW Two-Component System, Involved in Transcriptional Regulation of Aromatic Degradation[▽]

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Regulation of aromatic degradation in obligate anaerobes was studied in the Fe(III)-respiring model organism *Geobacter metallireducens* GS-15. A two-component system and a σ^{54} -dependent promoter were identified that are both involved in the regulation of the gene coding for benzoate-coenzyme A ligase, catalyzing the initial step of benzoate degradation.

Geobacter metallireducens GS-15 is an obligately anaerobic Fe(III)-respiring deltaproteobacterium with the potential to remediate environments contaminated with aromatic compounds (8, 9). A chromosomal 300-kb catabolic island has been shown to encode pathways for degradation of different aromatic compounds, such as benzoate, *p*-hydroxybenzoate, *p*-cresol, or phenol, which are all converted to the central intermediate benzoyl coenzyme A (CoA) (3, 14, 15, 16). The benzoate-induced gene cluster II contains the *bamY* gene encoding the benzoate-CoA ligase that generates benzoyl-CoA as the initial activating step in the anaerobic catabolism of benzoate. In the vicinity of the *bamY* gene, the *bamV* and *bamW* genes were suggested to control the expression of other *bam* genes (Fig. 1A) (15). The expression of the *bamW* gene was specifically increased by aromatic compounds, such as *p*-cresol, *p*-hydroxybenzoate, and benzoate (13, 15), suggesting that it plays a role in the catabolism of aromatics in *G. metallireducens* GS-15. In this work, we study the regulatory system that controls the expression of a catabolic gene involved in the degradation of aromatics in a strict anaerobic bacterium.

BamVW are the specific transcriptional activators of *bamY*. To study the expression of the *bamY* gene, its 5' region, the *Py* promoter, was cloned as a translational fusion with the *lacZ* reporter gene. The *Py* promoter was PCR amplified from the *G. metallireducens* GS-15 genome by using oligonucleotides 5'BCoAgeo (5'-CGGTACCGGTTTGTTCATCTCTCCCCG-3') and 3'BCoAgeo (5'-GCTCTAGACCCATTGTGGACCTCCGGCAGC-3') and was cloned into the pSJ3 promoter probe vector (1), generating plasmid pSJ3Py. The *Py::lacZ* fusion was also subcloned into the pUTminiTn5Km2 vector (1), which was then used to deliver by transposition the corre-

sponding translational fusion into the chromosome of the host strain *Escherichia coli* AFMC as described previously (1). We then monitored β -galactosidase activity in *E. coli* AFMCPy (*Py::lacZ*) cells harboring plasmid pCK01BamVW that contains the *bamVW* genes cloned under the control of the *Plac* promoter in the pCK01 vector (1). To control the expression of the *bamVW* genes, we used plasmid pIZ1016, which produces the LacI repressor (12). The induction of the *bamVW* genes into *E. coli* AFMCPy(pCK01BamVW, pIZ1016) cells was achieved by the addition of 0.5 mM IPTG (isopropyl- β -D-thiogalactopyranoside) into the culture medium. As shown in Fig. 2A, a minor induction of the *Py::lacZ* expression is observed in permeabilized cells grown anaerobically in IPTG-containing minimal medium. However, only in the presence of the aromatic substrate benzoate, *p*-cresol, or *p*-hydroxybenzoate (which are all funneled to the common benzoyl-CoA intermediate in *G. metallireducens* GS-15 [13, 15]) was the expression of *Py::lacZ* significantly induced (Fig. 2A).

To check whether both *bamV* and *bamW* were necessary for the activation of *Py*, these genes were cloned and expressed independently in two compatible plasmids—i.e., pCK01BamV, a pCK01 derivative harboring the *bamV* gene, and pBBR5BamW, a pBBR1MCS5 derivative harboring the *bamW* gene. Neither *E. coli* AFMCPy(pCK01BamV, pIZ1016) nor *E. coli* AFMCPy(pBBR5BamW, pIZ1016) cells anaerobically grown in the presence of IPTG and *p*-hydroxybenzoate gave a significant β -galactosidase activity (Fig. 2A), suggesting that both BamW and BamV have to act together to activate the *Py* promoter.

The N-terminal region (residues 1 to 340) of BamV shows two putative transmembrane helices flanking a periplasmic sensory domain (residues 29 to 318) similar to that of the C₄-dicarboxylate DctB membrane sensor (4). The C-terminal region (residues 377 to 603) shows significant similarity to the cytoplasmic kinase core of NtrB histidine kinases (5, 10). BamW shows a clear similarity to response regulators of the NtrC/DctD subfamily, including the three functional domains: (i) the N-terminal receiver domain (residues 1 to 114) containing the putative Asp-52 phosphoacceptor residue; (ii) the central AAA+ ATPase domain (residues 143 to

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FIG. 1. Transcriptional control of *bamY* in *G. metallireducens* GS-15. (A) Proposed model for the regulation of *bamY*. The *bamY* gene (Gmet_2143) is shown in black; *bamV* (Gmet_2146) and *bamW* (Gmet_2145) genes are represented with gray arrows; other *bam* genes are represented with white arrows. The aromatic inducers interact with BamV, triggering its autophosphorylation. Phosphate (P) is transferred to the BamW regulator that is then able to activate the transcription (+) at the *Py* promoter in collaboration with σ^{54} RNA polymerase holoenzyme (triangle). (B) View of the *Py* promoter from positions -146 to +45 is shown. The major transcription start site (+1) and the inferred -12 and -24 boxes are indicated. The ribosome-binding site (RBS) is boxed, and the ATG start codon of the *bamY* gene is indicated in italics. (C) Identification of the putative transcription start site(s) in the *Py* promoter. Total mRNA was isolated from *G. metallireducens* GS-15 cells grown anaerobically in 3 mM *p*-hydroxybenzoate (lane 1) or 20 mM acetate (lane 2) by using 50 mM Fe(III) citrate as the terminal electron acceptor (15). The sizes of the larger extension product (thick arrow) and those of the shorter extension products (thin arrows) were determined by comparison with the DNA sequencing ladder (lanes A, T, C, and G) of the *Py* promoter. Primer extension and sequencing reactions were performed with oligonucleotide *GeobamY* 3'.2 (GCTTGTCGCCTCTCCCCTCGC) that hybridizes in the coding strand, as previously described (1).

312) for transcriptional activation of σ^{54} -dependent promoters that includes the consensus GAFTGA motif (6), and (iii) the C-terminal domain (residues 403 to 446) with a DNA-binding motif typical of the Fis family of transcriptional regulators (5). Therefore, BamVW could function similarly to the DctBD two-component regulatory system, with BamV acting as a periplasm-sensing histidine kinase that recognizes the aromatic inducer, and BamW representing the transcriptional regulator that receives the signal from BamV and activates *Py* through the σ^{54} subunit of the RNA polymerase (Fig. 1A).

***Py* is a σ^{54} -dependent promoter.** Primer extension analysis revealed that the transcription initiation site of the larger extension product, which was specifically induced in *G. metallireducens* GS-15 grown in the presence of *p*-hydroxybenzoate, was mapped 42 nucleotides upstream of the ATG translation initiation codon of the *bamY* gene (Fig. 1C). Interestingly, the *Py* promoter showed the -12/-24 consensus sequence that

characterizes a σ^{54} -dependent promoter (2) (Fig. 1B). Two additional extension products (Fig. 1C) might account for alternative start sites or processing of the large mRNA transcript. As shown in Fig. 2B, while the wild-type *E. coli* YMC10 strain showed a *p*-hydroxybenzoate-inducible expression of the *Py::lacZ* fusion, almost no β -galactosidase was detected in its isogenic *rpoN* mutant strain, TH1 (7), suggesting that *Py* is a σ^{54} -dependent promoter.

Orthologous *bamVW* genes are found in the genomes of other *Geobacter* strains, e.g., *Geobacter* sp. strain FRC-32, *Geobacter bemidjensis*, and *Geobacter* sp. strain M21, and putative *bamW* homologs, ACP50612 and YP_463092, have been detected in the vicinity of the *bcl* and Syn_1638 genes encoding benzoate-CoA ligases in other strict anaerobes that degrade aromatic compounds (11, 16). Whether two-component regulatory systems controlling the expression of aromatic catabolic clusters in strict anaerobes might have recruited a periplasm-sensing histidine kinase and a σ^{54} -

FIG. 2. Expression of the *Py::lacZ* fusion. (A) β -Galactosidase activity of *E. coli* AFMCPy cells, which contain a chromosomal insertion of the mini-Tn5 transposon expressing the *Py::lacZ* translational fusion, harboring plasmid pIZ1016 and plasmid pCK01BamVW (VW), pCK01BamV (V) or pBBR5BamW (W). Km^r , kanamycin resistance; $T7$, T7 phage transcriptional terminator. Cells were grown anaerobically until stationary phase in 30 mM glycerol-containing M63 minimal medium in the absence (white bar) or presence (black bars) of 0.5 mM IPTG. Benzoate (Bz; 1 mM), 1 mM *p*-hydroxybenzoate (*p*Bz), 0.5 mM *p*-cresol (*p*Cr), or 0.5 mM either toluene, *m*-xylene, phenylacetate, benzylalcohol, phenol, benzaldehyde, *p*-toluate, or *p*-xylene (indicated as Ar) was added to the culture medium from the start of the growth. (B) β -Galactosidase activity of *E. coli* YMC10 (YMC10) and its isogenic *E. coli* TH1 *rpoN* (TH1) strains harboring plasmids pSJ3Py (*Py::lacZ*) and pCK01BamVW. Ap^r , ampicillin resistance; *ori*, ColE1 replication origin. Cells were grown anaerobically as indicated above in the absence (white bars) or presence (black bars) of 1 mM 4-hydroxybenzoate. β -Galactosidase assays with permeabilized cells were performed as reported previously (1).

dependent transcriptional activator is a tempting speculation that requires further studies.

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