Microbial Sulfur Metabolism

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Chapter 1
Genetics and Genomics of Sulfate Respiration in *Desulfovibrio*

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Abstract Bacteria that have evolved to use sulfate as a terminal electron acceptor must commit to spending energy for sulfate activation before there is a return on the investment allowing net energy gain. How sulfate is used and how electron flow is controlled have provided challenging topics for research for many years. Having the complete genome sequences of several of these bacteria is a monumental step in the elucidation of these questions. This information has provided the tools for determining the quantity of transcripts for genes under defined growth conditions, not just the relative changes in transcripts in two growth conditions. A comparison of the hybridization signal of messenger RNA with that of genomic DNA with oligonucleotide microarrays of all open reading frames reveals the differences in steady-state levels of transcripts for each gene. Growth of *Desulfovibrio vulgaris* Hildenborough on defined medium with lactate as a carbon and reductant source and with sulfate as the electron acceptor has been examined by this procedure for levels of gene expression. Relative functional importance was inferred from the levels of gene transcription, in spite of the recognized limitations of this interpretation. Not surprisingly, genes encoding established functions for sulfate reduction were highly expressed. However, the high molecular mass *c*-type cytochrome genes thought to encode a most important transmembrane electron conduit for sulfate reduction were expressed at quite low levels.

1.1 Introduction

Sulfate-reducing bacteria (SRB) are Gram-negative deltaproteobacteria, ubiquitously present in soils, that are able to obtain energy by the dissimilatory reduction of sulfate. These bacteria are considered anaerobes, although their genome sequences have revealed multiple genes putatively encoding enzymes that reduce oxygen or detoxify its products (Klenk et al. 1997; Heidelberg et al. 2004; Rabus et al. 2004). The ever-evident and offensive end product of sulfate reduction, sulfide, has brought much attention to the activities of the SRB in the environment. The black precipitates in sediments and shorelines, discoloration of paper during the milling processes (Postgate 1984) and the corrosion of ferrous metals (Hamilton

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2003) are a few of the less desirable effects. On the other hand, the low redox potentials achieved by these bacteria also provide them with the capacity of reducing a number of toxic metals, thereby changing their solubilities, and offering a potential method for remediation of metal-contaminated environments (Lovley et al. 1991; Gorby and Lovley 1992).

Members of the genus *Desulfovibrio* are perhaps the most easily and rapidly cultured of the SRB and, therefore, have been the subject of the most intensive biochemical and molecular research (Postgate 1984; Peck 1993; Voordouw 1993). Still, there are gaps in understanding energy generation by these anaerobes. For example, what is the role of hydrogen, formate, carbon monoxide, or ethanol during the respiration of sulfate with lactate or pyruvate as an electron donor? The genome sequences available for a few of the SRB are offering us the boundaries, the "parts list," for our inquiries into these questions. Of course, it does not help that all the parts are not definitively labeled.

Hydrogen metabolism has played a prominent role in the metabolism of many anaerobes and the SRB are no exception. Hydrogen can support sulfate respiration, is produced during fermentative growth, and is apparently also involved in the metabolism of a number of organic acids. A hydrogen transient is observed upon inoculation of *Desulfovibrio* strains into medium containing organic acids and sulfate (Hatchikian et al. 1976; Tsuji and Yagi 1980). A controversial role for this production and consumption of hydrogen was proposed by Odom and Peck (1981a) to be an obligate chemiosmotic vectorial electron transfer for energy supplementation, called "hydrogen cycling." In this model, the oxidation of organic substrates generates protons and electrons that are substrates for cytoplasmically located hydrogenase(s). Hydrogen produced in the cytoplasm then diffuses across the cytoplasmic membrane, where the periplasmic hydrogenases oxidize the hydrogen, recapturing the electrons for transfer back to the cytoplasm for sulfate reduction and liberating the protons to contribute to the proton motive force. Alternative explanations for this burst of hydrogen have been offered, such as a necessary redox adjustment of electron transport components (Tsuji and Yagi 1980) or the need for fermentative ATP production to initiate sulfate activation by ATP sulfurylase (Lupton et al. 1984). The complete genome sequence of several SRB allows a closer examination of this model.

1.2 Approach

As a part of a collaborative effort to understand how the SRB reduce toxic metals and how environmental stresses impact this ability, a number of experiments have been undertaken to examine the changes in transcription and protein expression during stress in *Desulfovibrio vulgaris* Hildenborough (Virtual Institute for Microbial Stress and Survival 2002). To examine the differentially expressed genes, microarray analysis of transcripts of putative open reading frames (ORFs) have been used (Li et al. 2005). To normalize the data for comparison of expression

levels across stresses, the transcript hybridization to microarrays for all experiments has been compared with genomic DNA hybridization. As a result, a sizeable data set has been obtained that provides the expression level of all genes in the microarray.

Experiments for stress analyses were performed with defined medium containing sodium lactate (60 mM) as an electron donor and sodium sulfate (50 mM) as an electron acceptor (LS4D medium; Mukhopadhyay et al. 2006). *D. vulgaris* was grown from freezer stocks to an optical density at 600 nm of 0.3 (approximately 1×10^8 cells per milliliter) and the stress was imposed. Table 1.1 lists the various treatments for which data were collected. Triplicates for the control and the treated cultures were sampled at the initiation of the treatment and at specified intervals, usually not exceeding 4 h, following the treatment. The average of the triplicates was a single data point.

In all, 173 data points comparing transcript and genomic DNA hybridization to ORF probes have been analyzed. From these data, the relative abundance of transcripts present for a given gene in an exponentially growing culture of *D. vulgaris* respiring sulfate with lactate at 30°C was determined. Figure 1.1 illustrates the distribution of $\log_2$ of the hydridization signal for transcripts divided by that of genomic DNA for two different genes. Data points that were not significantly above the experimental noise were not included in the average calculation. It should be pointed out that at least 50% of the data points were from untreated control cultures. In addition, any given treatment or stress resulted in the differential expression of only a few hundred genes out of about 3,600 ORFs in the genome. Thus, for regulated genes, the average expression would not expect to be biased by the various stresses, but regulation would be evident in an increased standard deviation of the average.

In the following discussion, expression levels of genes involved in various aspects of metabolism are presented. However, to obtain a reference for the meaning of the data, Table 1.2 provides expression levels for comparison genes (operon predictions

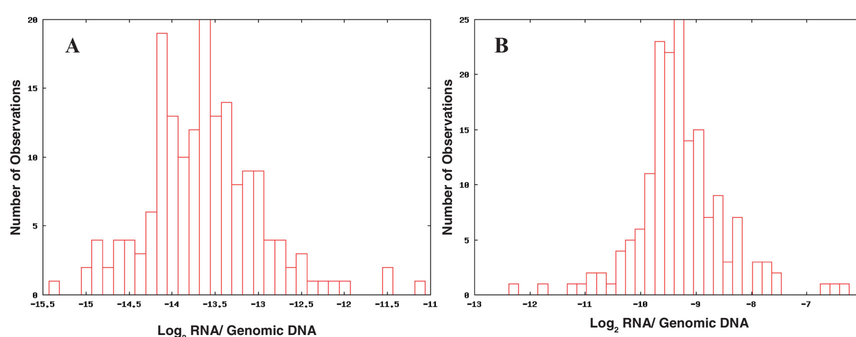

Fig. 1.1 Histogram of transcripts of **a** *hisD*, DVU0796, average $\log_2$(RNA/genomic DNA) = –13.6 ± 0.7 (173 total observations) and **b** *sat*, DVU1295, average $\log_2$(RNA/genomic DNA) =–9.2 ± 0.8 (171 total observations). Cultures were grown as described (Table 1.1) for RNA preparation and hybridization to microarrays (Mukhopadhyay et al. 2006)

Table 1.1 Treatments examined for transcriptional responses in *Desulfovibrio vulgaris* Hildenborough

Treatment	Concentration or condition	Comparison culture
Cold	8°C	30°C
Heat	50°C	37°C
Oxygen	0.1%	No O_2
Alkaline pH[a]	pH 10	pH 7
Acid pH[b]	pH 5.5	pH 7
Nitrite[c]	2.5 mM	No NO_2^-
Nitrate	105 mM	No NO_3^-
Sodium[d]	250 mM	No added Na^+
Potassium	250 mM	No added K^+
Chromate	0.45 µM	No CrO_4^{2-}
Stationary phase	0.8 OD_{600}	Mid exponential phase 0.3 OD_{600}

[a] pH was adjusted by addition of KOH.
[b] pH was adjusted by addition of H_2SO_4.
[c] Growth occurred after cells reduced the nitrite concentration below 0.5 mM.
[d] Total concentration of sodium in the treatment was about 462 mM; other components were present as sodium salts.

Table 1.2 Expression levels of *D. vulgaris* Hildenborough reference genes during exponential growth phase of cells respiring sulfate with lactate as an electron donor

Operon	DVU numbers[a]	Putative gene name	Average log exp[b] ± SD
Ribosomal proteins	DVU1302	*rpsJ*	−10.1 ± 0.8
	DVU1303	*rplC*	−10.8 ± 0.8
	DVU1304	*rplD*	−10.4 ± 0.8
	DVU1305	*rplW*	−11.5 ± 0.8
	DVU1306	*rplB*	−11.1 ± 0.8
	DVU1307	*rpsS*	−11.0 ± 0.8
	DVU1308	*rplV*	−11.2 ± 0.7
Tryptophan biosynthesis	DVU0465	*trpE*	−14.6 ± 0.9
	DVU0466	*trpG*	−15.0 ± 0.9
	DVU0467	*trpD*	−14.2 ± 0.9
	DVU0468	*trpC*	−14.4 ± 1.0
	DVU0469	*trpF-1*	−14.3 ± 1.1
	DVU0470	*trpB-2*	−13.7 ± 1.1
	DVU0471	*trpA*	−13.7 ± 0.9
High molecular mass cytochrome *c*	DVU0529	*rrf2*	−15.1 ± 1.4
	DVU0530	*rrf1*	−15.0 ± 1.4
	DVU0531	*hmcF*	−15.6 ± 1.5
	DVU0532	*hmcE*	−15.2 ± 1.4
	DVU0533	*hmcD*	−15.6 ± 1.4
	DVU0534	*hmcC*	−14.7 ± 1.2
	DVU0535	*hmcB*	−14.9 ± 1.5[c]
	DVU0536	*hmcA*	−15.1 ± 1.8

SD standard deviation.
[a] DVU numbers from TIGR annotation (Heidelberg et al. 2004).
[b] Average log exp is the average $\log_2$ of the RNA to genomic DNA signal from whole genome transcript microarrays from cultures treated as in Table 1.1. In calculating average expressions, fewer than 10% of the 173 data points available for each gene were eliminated because of poor signal-to-noise ratio unless otherwise indicated.
[c] For DVU0535, 27 data points were below the cutoff criterion.

were as described by Price et al. 2005). Ribosomal protein genes are expected to be rather highly expressed during exponential growth and the $\log_2$ of the ratio of the messenger RNA to DNA (a large negative number because of the greater quantity of DNA used for hybridization) was in the range of −10.9. That for the tryptophan operon was −14.3, an operon that must function in medium lacking tryptophan, yet, because large quantities of this amino acid are not needed, would not be expected to be highly expressed. Finally the operon for the high molecular mass cytochrome *c* was expressed at a still lower level, −15.2. The latter was an unexpectedly low value for transcription of this operon thought to encode an important conduit for electrons for sulfate reduction.

1.3 Sulfate Metabolism

The enzymology of sulfate reduction by *Desulfovibrio* strains is rather mature (Peck and LeGall 1982; Peck 1993) and enzymes involved were reported to be constitutively present in sulfate-respiring cells (Odom and Peck 1981b). Four cytoplasmic enzymes are sufficient for conversion of sulfate to sulfide in an eight electron reduction pathway. Annotated in the *D. vulgaris* genome, ATP sulfurylase (DVU1295, *sat*) activates the sulfate-generating adenosine 5′-phosphosulfate (APS) in preparation for the first two-electron reduction. Inorganic pyrophosphate is released which is cleaved by an inorganic pyrophosphatase (DVU1636, *ppaC*) to "pull" the reaction. APS is then reduced by APS reductase, a two-subunit enzyme (DVU0846/0847, *apsBA*). Sulfite is the reduced product that becomes the substrate for the six-electron reduction by sulfite reductase, also known as desulfoviridin in the *Desulfovibrio* strains (DVU0402–0404, *dsrABD*; DVU2776, *dsrC*). In vitro, this enzyme is capable of producing sulfide as the final end product of sulfate reduction.

Table 1.3 shows the remarkably high level of expression of the genes for sulfate respiration that exceeds that of ribosomal protein genes. Of the candidate ORFs for

Table 1.3 Expression levels of putative genes coding for enzymes of sulfate reduction in *D. vulgaris* Hildenborough

Protein function	DVU number	Putative gene name	Average log exp ± SD
Sulfate adenylyltransferase	DVU1295	*sat*	−9.2 ± 0.8
Adenosine 5′-phosphosulfate reductase	DVU0846	*apsB*	−8.8 ± 0.7
	DVU0847	*apsA*	−8.9 ± 0.8
Sulfite reductase	DVU0402	*dsrA*	−9.3 ± 0.9
	DVU0403	*dvsB*	−9.5 ± 0.7
	DVU0404	*dsrD*	−8.8 ± 1.0
	DVU2776	*dsrC*	−10.3 ± 1.1[a]
Inorganic pyrophosphatase	DVU1636	*ppaC*	−11.1 ± 1.0

[a] Of the 173 data points available, 50 were below the cutoff for the signal-to-noise ratio.