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A Survey of Protein Post-Translational Modifications Found in the Sulfate-Reducing Bacterium *Desulfovibrio vulgaris* Hildenborough

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ESPP Environmental Stress Pathway Project

VIMSS Virtual Institute for Microbial Stress and Survival

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT — OFFICE OF SCIENCE, U.S. DEPARTMENT OF ENERGY

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INTRODUCTION

Sulfate reducing bacteria (SRB), found widely in nature, use sulfate as the terminal electron acceptor in their respiratory cycle, leading to the production of hydrogen sulfide. These bacteria have both ecological and economic importance. SRB play a role in various biogeochemical cycles including the sulfur and carbon cycles. They have a negative economic impact on the oil industry, where their metabolism causes corrosion and clogging of machinery, and fouling of oil wells. However, they have also been shown to reduce and/or immobilize toxic water-soluble metals such as copper (II), chromium (IV) and uranium (VI), and thus are candidates for bioremediation applications.

Desulfovibrio vulgaris Hildenborough (DvH) is a member of the most well studied genus of SRBs. A goal of the Environmental Stress Pathway Project (ESPP) in the Virtual Institute for Microbial Stress and Survival (VIMSS) is to understand the regulatory networks in DvH for applications to bioremediation. One aspect of this is the elucidation of protein post-translational modifications (PTMs) in DvH.

PTMs play various roles in the cell. Some modifications play a role in protein structure, such as lipid anchors or some disulfide bonds. Others are directly involved in regulation of protein function such as phosphorylation and glycosylation. Still others arise through cellular damage such as irreversible oxidation events. Whatever the role these PTMs play, they must be characterized at the protein level because they are not directly coded for in the genome. Furthermore, DvH may be particularly likely to use PTMs as a regulatory mechanism: Evidence for this includes the observation that the DvH genome encodes an abnormal number of histidine kinases. Our goal is to determine the types of protein modifications that arise in DvH and how these modifications affect the ability of DvH to survive or adapt to its environment.

EXPERIMENTAL METHODS

Samples Used and Data Generation

Batch cultures of *Desulfovibrio vulgaris* Hildenborough (DvH) were grown in the laboratory of Dr. Terry Hazen. Three separate experiments were conducted in which WT culture was subjected to either nitrate, air or oxygen stress. A separate DvH culture was grown in the laboratory of Dr. Mathew Fields where the cells were grown on a bed of glass beads, forming a biofilm. In all 4 experiments (nitrate, air, oxygen, biofilm) a WT DvH batch culture was grown in parallel which served as the control.

Each set of samples (control vs. experimental) was labeled with the iTRAQ reagent (Applied Biosystems, Foster City) according to the manufacturer's protocol to enable relative quantitation of protein expression, reported elsewhere.

Each of the 4 complex protein mixtures was digested with trypsin, and the resulting peptides were separated by strong cation exchange chromatography into ~20 fractions. Each fraction was analyzed by LC/MS/MS on an Applied Biosystems QStar.

Data Analysis for PTM Survey

Peaklists (m/z vs. intensity) were generated for each individual LC/MS/MS file using the Distiller program (Matrix Science Inc, Boston, MA). Once the peaklists were generated for each LC/MS/MS file individually, all lists from the same experiment (nitrate, biofilm, oxygen, air) were merged for subsequent searches.

The merged peaklists were submitted to Mascot searches against the Desulfovibrio vulgaris Hildenborough protein database (database version 882). Searches were conducted for the following modification types: oxidation, acetylation, methylation, sulfation/phosphorylation, and N-terminal modification.

Two filters were applied to the resulting matches to generate the results table:
1) Only the top ranked sequence match for the given spectrum was accepted.
2) Only matches with an expectation value of less than 0.05 were accepted. This corresponds to the 95% confidence limit of the Mascot scoring scheme.

RESULTS

Putative Modification	Amino Acid	Delta Mass (Da)	Comment	# Unique Sequences Matched at 95% Confidence, Grouped By Sample (% of obs unmodified) Nitrate	Biofilm	Air	O_2
unmodified	--	--		6308	4629	2820	1756
oxidation	M	16	sulfoxide	104 (1.6)	512 (11.1)	153 (5.4)	71 (4.0)
	M	32	sulfone	7 (0.11)	18 (0.39)	10 (0.35)	3 (0.17)
	C	16	sulfenic acid	1	5	2	0
	C	32	sulfinic acid	12	20	0	0
	C	48	sulfonic acid	2	14	6	1
	HW	16		7 (0.11)	26 (0.56)	6 (0.21)	9 (0.51)
methylation	QE	14		8	16		
	H	14		13	9		
	K	14		11	10		
	K	28	di-methylation	20	28		
acetylation	K	42	mass shift is equivalent to tri-methylation	18	16		
sulfation	STY	80	mass shift is equivalent to phosphorylation	10	12		
N-terminal processing	protein N-term	42	acetylation	5	6		
	protein N-term	28	formylation	3	13		

nitrate	GGEK**TACMYICPNDLMILDPEEMR	4.8E-03
	TACMYICPNDLMILDPEEMR	4.6E-08

nitrate	FKFPIR	3.2E-02
biofilm	FKFPIR	3.9E-02
	FK**FPIR	2.0E-02
	FK§FPIR	1.4E-03

>VIMSS206272 adenylylsulphate reductase, beta subunit (TIGR) [Desulfovibrio vulgaris Hildenborough] ORF00336 DVU0846 ApsB 46579259

MPTYVDPSKCDGCKGGEKTACMYICPNDLMILDPEEMRAYNQEPEACWECYSCV
KICPQGAITARPYADFAPMGGTCIPMRSADSIMWTVKERNGNVKRFKFPIRTTPEG
SIKPFEGKPEAGDLESELLFTETALTAPKEALGKKFDVTDAETSQCWYETGCQGG
NR

nitrate	SADSIMWTVK**FR	7.6E-04
	SADSIMWTVK§FR	7.7E-03
biofilm	SADSIMWTVK**FR	2.1E-05
	SADSIMWTVK§FR	8.3E-03

* = +14 (methylation)
** = +28 (di-methylation)
§ = +42 (tri-methylation, acetylation)

nitrate	ESEGISDISPDHQK	4.9E-08
	ESEGISDISPDH*QK	1.0E-04
	ESEGISDISPDHQK**IIDFLQDYYK	1.8E-07
	ESEGISDISPDHQK§IIDFLQDYYK	2.3E-06
	ESEGISDISPDHQK*IIDFLQDYYK**	1.8E-03
biofilm	ESEGISDISPDHQK§IIDFLQDYYK	0.00037

>VIMSS208282 dissimilatory sulfite reductase, gamma subunit (TIGR) [Desulfovibrio vulgaris Hildenborough] ORF03581 DVU2776 dsrC 46581180

MAEVTYKGKSFEVDEDGFLLRFDDWCPEWVEYVKESEGISDISPDHQKIIDFLQDY
YKKNGIAPMVRILSKNTGFKLKEVYELFPSGPGK**GACK**MAGLPKPTGCV

nitrate	LK§EVYELFPSGPGK	7.5E-03
	LK*EVYELFPSGPGK	1.3E-03
biofilm	LK§EVYELFPSGPGK	4.0E-04

CONCLUSIONS

The purpose of the current research is to conduct a broad survey for possible post-translational modifications observed in *Desulfovibrio vulgaris* Hildenborough, leveraging data acquired during the course of the ESPP project.

When the resulting data was filtered for modified sequence matches at the 95% confidence threshold, 296 unique sequences from 229 unique proteins were identified with one or more modifications (other than methionine sulfoxide). Evidence was found for oxidation of methionine, cysteine, histidine, and tryptophane; methylation of glutamate, histidine and lysine; acetylation of lysine; and sulfation (or phosphorylation) of serine/threonine/tyrosine.

Protein N-terminal processing was also investigated: From the spectra acquired for the nitrate stressed sample, 114 different protein N-termini were matched. Of these, 53 (~46%) were observed to have the initial methionine cleaved. Three of these were observed to have further processing by acetylation of serine or threonine now at the N-terminus. A few N-termini with the initial methionine intact were also observed as acetylated or formylated.

Interestingly, several members of the sulfate reduction pathway proteins were observed to be extensively methylated/acetylated. Two examples are shown here. In general, peptides with modified lysine residues were observed as tryptic peptides with a missed cleavage at the modified residue. This conforms to the known reactivity of trypsin, and lends credibility to the assignments. Also, the same peptide with the same modification often appeared in multiple samples, giving further confidence to these assignments.

FUTURE DIRECTIONS

Data mining for additional PTMs is ongoing. In particular, algorithms that allow "blind" search of MS/MS data such as Mascot "Error Tolerant" search and Popitam will be used to uncover unexpected modifications.

We will also focus on validation of our findings and determining which, if any, of these modifications play a regulatory role in DvH. Validation will require selective isolation of the proteins of interest for further characterization. Here, protein isolation is made possible through the work being performed at LBL and the University of Missouri to generate DvH mutants containing tagged versions of DvH proteins.

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