Quantification of Reductive Dehalogenase Peptides Using Multiple Reaction Monitoring Proteomics

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Background/Objectives. Mass spectrometry-based proteomic strategies are currently being developed as next-generation monitoring tools for environmental applications, including assessing reductive dechlorination at field sites. Many organism- and a number of process-specific biomarker genes for monitoring reductive dechlorination have been identified, and the vinyl chloride reductive dehalogenase (RDase) genes *bvcA* and *vcrA* of *Dehalococcoides mccartyi* (*Dhc*) serve as main biomarkers for ethene formation at sites impacted with chlorinated ethenes. Whereas the abundance of RDase genes alone provides a measure of reductive dechlorination potential, the quantitative assessment of RDase gene transcripts and proteins can provide information about actual reductive dechlorination activity. A multiple reaction monitoring (SRM) proteomics assay was therefore developed to identify and quantify these biomarkers specific to the degradation of chlorinated ethenes.

Approach/Activities.
LC-MS-targeted proteomics approaches, based on multiple reaction monitoring (MRM) proved to be effective for the analysis of biomarker RDases related to degradation of chlorinated ethenes. In an MRM experiment, the two mass analyzers of a triple quadrupole mass spectrometer are used to isolate the peptide ions of interest and the derived fragment ion(s). This enables the precise quantification of peptides in diverse biological backgrounds, and thus the quantification of the abundance of the corresponding protein(s) present in the samples. The applicability of the method to multiplex analytes allows monitoring of panels of proteins across multiple samples by monitoring the signals of each specific transition (i.e., precursor – fragment ion pairs) during a predefined elution window. The favorable duty cycle and the selective mass filtering of the SRM technique results in a high degree of specificity and low limit of detection (LOD) and limit of quantification (LOQ) (i.e., in the low attomole range).

Results/Lessons Learned.
In the present study, the SRM approach was applied to determine LOD and LOQ of selected RDase peptides in consortium SDC-9™, a well characterized bioaugmentation culture capable of degrading chlorinated ethenes, as well as in microcosms that received different quantities of *Dehalococcoides mccartyi*. After optimization of the sample preparation protocol in terms of reproducibility and sensitivity using spiked isotopically labeled proteins, quantitative measurements were carried out for four selected RDase proteins (PceA, TceA, VcrA and FdhA) and 28 unique peptides. This approach verifies the robustness of the MRM approach in terms of sensitivity and selectivity and establishes the basis for quantitative analysis of RDase marker candidates in environmental samples.