Molecular investigation of electron transfer mechanisms involved in microbial selenate reduction

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Background

Microorganisms inhabiting selenium contaminated sediments are able to reduce dissolved selenate oxyanions to sparingly soluble elemental selenium. The reductive precipitation of selenium by Se-reducing bacteria has been proposed as a potential bioremediation strategy. However, in order to employ Se-reducing bacteria for practical bioremediation purposes, reliable models must be developed to predict microbial behavior, including when they are active, what mechanisms are involved, and under what conditions the mechanisms function. In this study, we demonstrate that anaerobic electron carriers play an essential role in governing the activity of a Se-reducing bacterium.

Methods

Transposon mutagenesis was used to generate mutant strains of *Enterobacter cloacae* SLD1a-1 that have lost the ability to reduce selenate. We characterized a mutant strain designated as 4E6 that was defective in selenate reduction activity. Genomic libraries of SLD1a-1 and 4E6 were constructed to clone the mutated operon that resulted in the loss of selenate reduction activity. Sequencing of the operon containing the transposon was obtained by restriction mapping and primer walking. Selenate reduction activity of wild-type and mutant strains were quantified using batch kinetic experiments. Finally, the ability of menaquinone analogues and precursor compounds to rescue the selenate reduction activity was tested.

Results and Discussion

Mutagenesis experiments showed that mutation of the *menD* gene in the menaquinone biosynthesis operon produces derivative strains that are deficient in selenate reduction activity. Complementation by the wild-type sequence and menaquinone analogues restored the ability of mutant strains to reduce selenate. Menaquinones are small lipid-soluble molecules that act as electron shuttles in the bacterial electron transport chain. The biosynthesis of menaquinone occurs under oxygen-limiting conditions, and is known to mediate anaerobic respiration of alternate electron acceptors. The results of this study indicate that anaerobic electron carriers are required for microbial selenate reduction.

Atmospheric noble gas signatures in deep Michigan Basin brines as indicators of a past thermal event

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Atmospheric noble gases in crustal fluids are introduced into the subsurface by recharge water in solubility equilibrium with the atmosphere (air saturated water - ASW). Because ^{22}Ne, ^{36}Ar, ^{84}Kr and ^{130}Xe are conservative in nature and are not produced in significant amounts by natural nuclear reactions in the crust, these isotopes in groundwater are only sensitive to subsurface physical processes. These include oil-gas-water phase interactions, as well as the occurrence of boiling and steam separation. Atmospheric noble gases can thus provide precious information with respect to the origin and evolution of subsurface fluids and thus, to the tectonic and thermal evolution of particular areas.

Here, we present ^{22}Ne, ^{36}Ar, ^{84}Kr and ^{130}Xe concentrations of 38 deep (down to ~3.6 km depth) brines from the Michigan Basin. These show a strong depletion pattern with respect to ASW. Depletion of lighter gases (^{22}Ne and ^{36}Ar) is stronger as compared to the heavier ones (^{84}Kr and ^{130}Xe). To understand the mechanisms responsible for this overall atmospheric noble gas depletion, conceptual phase interaction models were tested. While oil-water and gas-water interaction models could not, under reasonable assumptions, explain both the extent and the observed depletion pattern, the opposite is true with a model involving subsurface boiling and steam separation. Indeed, the latter not only explains the overall observed atmospheric noble gas depletion pattern, it also points strongly to the presence of a past thermal event. This finding is consistent with the presence of primordial solar-like He and Ne signatures in the basin previously identified in these same brines, and suggest a mantle origin for the occurrence of this thermal event. Such a boiling and steam separation model is also consistent with the presence of past elevated basin temperatures (e.g., >80-260°C) at shallow depths as suggested by numerous previous studies in the basin. We suggest that recent reactivation of the ancient mid-continent rift system underneath the Michigan Basin is likely responsible for the release of both heat and mantle noble gases into the basin via deep-seated faults and fracture zones. While heat has already escaped the system, such a thermal event can be still traced by the presence of the observed atmospheric noble gas depletion.