Breathing chlorinated compounds: unravelling the biochemistry underpinning (de)halorespiration, an exciting bacterial metabolism with significant bioremediation potential

Sprawozdania

Informacje na temat projektu

DEHALORES

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Ten projekt został przedstawiony w...

Final Report Summary - DEHALORES (Breathing chlorinated compounds: unravelling the biochemistry underpinning (de)halorespiration, an exciting bacterial metabolism with significant bioremediation potential)

Bacterial (de)halorespiration or organohalide respiration is a microbial respiratory process that uses halogenated hydrocarbons (from natural or anthropogenic origin) as terminal electron acceptors. This project seeks to study representatives of the distinct biological components (reductive dehalogenases as well as key elements of the associated regulatory systems) to understand and apply the biochemistry behind this process. Using an interdisciplinary, biophysical approach focused around X-ray crystallography, enzymology and molecular biology, combined with novel reductive dehalogenase production methods, we aim to provide identification and a detailed understanding of the structural elements crucial to reductive dehalogenase mechanism and regulation. In conjunction, we aim to study the feasibility of generating improved halorespiratory components for biosensing or bioremediation applications through laboratory-assisted evolution.

We have made significant progress in the study of key transcriptional regulators, as well as the production of the reductive dehalogenase itself. The transcriptional regulators involved in halorespiration belong to various distinct classes, with CprK (a member of the CRP-FNR family) the best characterized to date. We have been able to study CprK function in vivo, establishing direct evidence for positive cooperative behaviour as predicted through our in vitro studies. Surprisingly, key mutants studied revealed the mechanism of sensing halogenated phenolic compounds to be more robust than anticipated, but also established that CrpK is limited to phenolic organohalides. We have constructed a biosensor using CprK than displays high sensitivity for such compounds. Unfortunately, most environmentally relevant organohalides do not contain a phenolic group. We have therefore focussed attention on distinct regulators. We have determined the crystal structure of a MarR-type regulator, in complex with polychlorinated aromatics. In vitro DNA-binding properties have been established for the MarR regulator, although we have yet to identify the physiological ligand. In addition, we have obtain the structure of the regulator component of a two-component system, revealing large scale domain reorientation occurs upon phosphorylation by the histidine kinase. Determination of the binding properties of the associated His-kinase component are ongoing, but our data suggests chlorinated ethylene compounds function as

physiological ligands. We will seek to use both these systems to develop novel biosensors for organohalides in future, focussed on detected either aromatic organohalides (such as dioxins, PCBs) or smaller, aliphatic compounds.

Following extensive trials to produce soluble and active reductive dehalogenase in a standard heterologous host such as E. coli, we have managed to obtain soluble protein that is able to bind the essential B12-cofactor using a new protein fusion construct. Unfortunately, we encountered ongoing problems with the stability of the associated iron-sulphur clusters and could not obtain active protein. Following transfer of this system to the alternative host Bacillus megaterium, a source of active protein was obtained. Furthermore, diffraction quality crystals were obtained for one of the enzymes studied. Both of these present significant achievements in the field, were progress has been hampered by a lack of a suitable source of enzyme and the absence of any structural insights into this system. We are presently in the process of solving the dehalogenase crystal structure, and will focus on resolving the mechanism behind the reductive dehalogenation catalysed. This will allow us to drive studies that seek to apply this enzyme or its variants in bioremedation.

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