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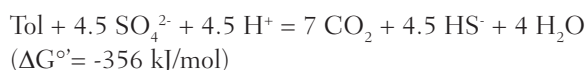
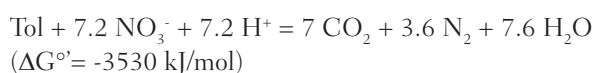
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Anaerobic bacterial hydrocarbon metabolism

Hydrocarbons are chemically very inert compounds, whose biological degradation by bacteria and fungi under aerobic conditions always involves initial reactions by oxygen-dependent mono- or dioxygenases. Therefore, it was a major surprise since ca. 1990, when many bacterial hydrocarbon degrading species were isolated that do not require molecular oxygen for growth. These bacteria are ubiquitous in the environment and belong mostly to the denitrifying, metal ion or sulfate reducing bacteria. The hydrocarbon substrates used under anaerobic conditions include aliphatic hydrocarbons, such as alkanes and alkenes, and aromatic hydrocarbons, e. g. benzene, toluene, ethylbenzene, xylenes or naphthalene. It is obvious that the pathways of hydrocarbon degradation under anaerobic conditions must be fundamentally different than those known from aerobic organisms. Initial studies on the biochemical basis of anaerobic metabolism of a few model hydrocarbons revealed that bacteria have indeed developed several new biochemical strategies to attack these recalcitrant substrates. Our research focuses on the biochemical mechanisms of enzymes involved in the pathways of anaerobic catabolism of alkylbenzenes, such as toluene and ethylbenzene, as well as alkenes and related phenolic or carbonylated compounds.

Model organisms studied for their hydrocarbon metabolic pathways are the denitrifying Betaproteobacteria *Thauera aromatica* and *Aromatoleum aromaticum*, and the iron(III)- resp. sulfate-reducing Deltaproteobacteria *Geobacter metallireducens* and *Desulfobacula toluolica*.

Whereas denitrifying bacteria gain high amounts of energy from hydrocarbon degradation, considerably less energy can be obtained by sulfate reducers:



(Tol = Toluene)

The difference in energy gains between denitrifying and sulfate-reducing bacteria is likely associated with different bioenergetical coupling of some reactions in the respective degradation pathways.

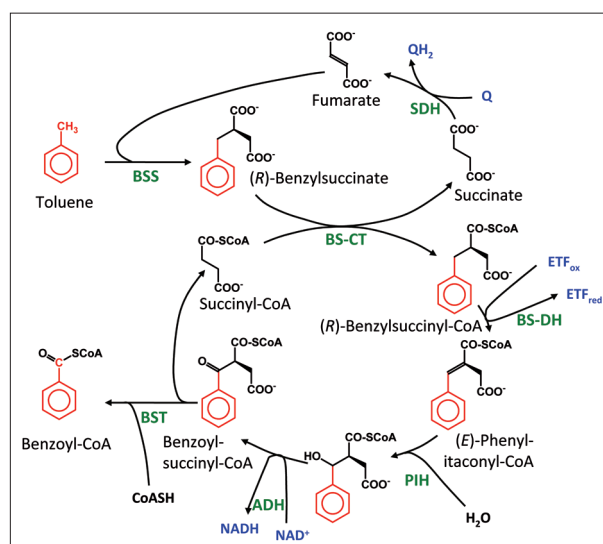


Fig. 1. Anaerobic metabolism of toluene

1. Enzymes of anaerobic toluene metabolism.

A novel biochemical reaction was identified as initial step of anaerobic toluene metabolism, namely the enzyme-catalysed stereospecific addition of the methyl group to the double bond of a fumarate cosubstrate. The product formed from toluene by this reaction, (R)-benzylsuccinyl-

nate, is further degraded to benzoyl-CoA and succinate via specific enzymes of a β -oxidation pathway (Fig. 1)

The pathway is initiated by benzylsuccinate synthase (BSS), which catalyses the addition of the methyl group of toluene to a fumarate cosubstrate. The first intermediate (R)-benzylsuccinate is then activated to the CoA-thioester by a succinyl-CoA-dependent CoA-transferase (BS-CT), and benzylsuccinyl-CoA is oxidised via beta-oxidation to benzoyl-CoA and succinyl-CoA. This involves the consecutive action of benzylsuccinyl-CoA dehydrogenase (BS-DH), an enoyl-CoA hydratase (PIH), alcohol dehydrogenase (ADH), and a benzoylsuccinyl-CoA thiolase (BST). We characterise all enzymes of this pathway from *T. aromatica* biochemically and our goal is to study their catalytic mechanisms and structure/function relationships. The key enzyme of anaerobic toluene metabolism, (R)-benzylsuccinate synthase, is a novel glyceryl radical enzyme, and a possible radical-based reaction mechanism has been postulated based on that feature. Similar fumarate addition reactions have since been recognised in anoxic metabolic pathways of other hydrocarbons and appear to be widespread in nature. In addition to several aromatic compounds (e. g. cresols, ethylbenzene or 2-methylnaphthalene), even alkanes seem to be degraded anaerobically via fumarate addition. Therefore, our results provide foundations to understand a significant part of processes in anaerobic environments and to develop more effective methods in bioremediation of hydrocarbon-contaminations.

2. Enzymes of anaerobic ethylbenzene metabolism

Although the aromatic hydrocarbon ethylbenzene is chemically very similar to toluene, a completely different biochemical pathway is employed to degrade it anaerobically in denitrifying bacteria. The initial metabolic step is catalysed by the periplasmic ethylbenzene dehydrogenase, a molybdenum enzyme of the DMSO reductase family containing a Mo-bisMGD cofactor, 5 FeS clusters and a heme *b*. It catalyses an oxygen-independent stereospecific hydroxylation of the methylene group of ethylbenzene, generating (S)-1-phenylethanol as first intermediate (Fig. 2).

The enzyme oxidizes a wide range of substrate analogs and appears to operate via two successive one-electron transfer steps from the hydrocarbon to the Mo cofactor, as inferred by structure-based modeling studies. Further metabolism of (S)-1-phenylethanol proceeds via oxidation to acetophenone by a stereospecific and highly regulated alcohol dehydrogenase. Acetophenone is then

carboxylated at the methyl group to benzoylacetate by a complex ATP-dependent carboxylase (Fig. 3)

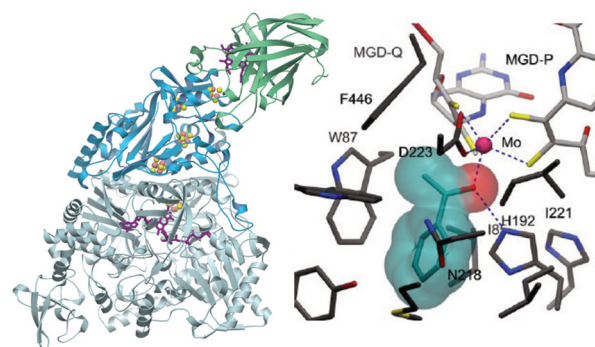


Fig 2. Structure of ethylbenzene dehydrogenase: left: ribbon model with cofactors; right: active site with bound reaction product (modelled). The enzyme consists of three subunits (α in gray, β in blue, γ in green) and contains a molybdenum-bis-molybdopterin-guanine-dinucleotide (MGD) cofactor, five Fe-S clusters and a heme *b*

Further metabolic steps are activation to a CoA-thioester and thiolytic cleavage, leading to formation of benzoyl-CoA as central intermediate of anaerobic aromatic catabolism. We are characterising the enzymes involved in the pathway to identify the biochemical principles and reaction mechanisms of these reactions and introduce the enzymes to possible applications, e.g. in the synthesis of fine chemicals.

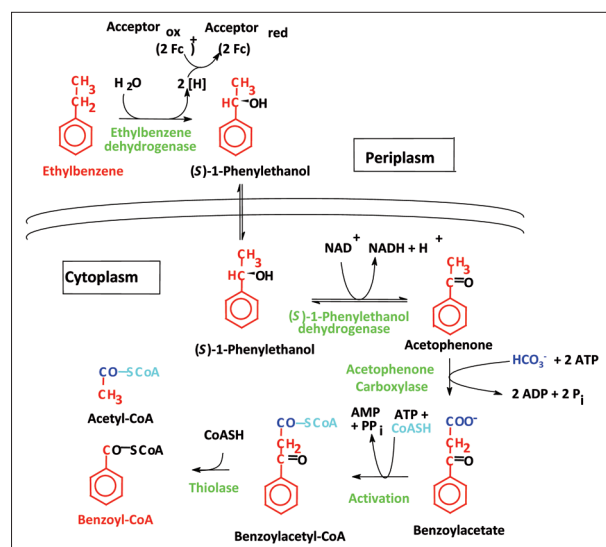


Fig 3. Anaerobic metabolism of ethylbenzene

3. Regulation of anaerobic hydrocarbon metabolism

Anaerobic toluene and ethylbenzene metabolism seems to be regulated by closely related two-component systems in denitrifying bacteria. The enzymes involved in hydrocarbon degradation are strongly induced in cells grown on the respective hydrocarbon and are encoded in two operons for either of the pathways. In case of toluene metabolism, the operons are coordinately regulated by the same two-component system, whereas ethylbenzene metabolism is sequentially regulated by two separate two-component systems responsive to ethylbenzene and the intermediate acetophenone, respectively. We are interested in the functionality of the sensory compounds of these regulatory systems and study the molecular basis of discrimination of the highly similar substrates and the mechanisms of signal integration.

4. Molybdenum- or tungsten-enzymes involved in anaerobic aromatic metabolism

As apparent from the genome sequence of *A. aromaticum*, there are almost 20 operons coding for molybdenum or tungsten enzymes in this organism. Whereas many of them can be readily attributed to already known functions in the metabolism of aromatic compounds or other roles in anaerobic metabolism, a putative tungsten-containing enzyme was recently identified as an aldehyde:ferredoxin oxidoreductase involved in anaerobic phenylalanine metabolism. As this type of enzyme is normally only known from (hyper) thermophilic Archaea and Bacteria, we are interested to learn about its biochemical properties and functions in a mesophilic bacterium. Moreover, we intend to identify the biochemical roles of several other putative molybdenum enzymes of *A. aromaticum* and hydrocarbon-degrading sulfate reducers that we can not associate with known functions yet.

5. Enzymes of anaerobic alkene metabolism

Besides aromatic hydrocarbons, alkanes and alkenes are readily degraded by many anaerobic bacteria. Whereas fumarate addition has been shown to be a possible initial reaction for alkane degradation, no enzyme was characterized for the first step of anaerobic alkene metabolism, which is postulated to consist in addition of water to the double bond. Therefore, we are studying the pathway of alkene degradation in sulfate-reducing bacteria in order to identify and characterize the enzymes involved. In addition, we also investigate an artificial side activity detected for ethylbenzene dehydrogenase, which consists in apparent water addition reactions to alkenes when the enzyme is exposed to these unnatural substrates.

Publications

Jobst B., Schühle K., Linne U., and Heider J. (2010) ATP-dependent carboxylation of acetophenone by a novel type of carboxylase. *J. Bacteriol.*, 192: 1387–1394.

Szaleniec M., Borowski T., Schühle K., Witko M., and Heider J. (2010) *Ab initio* modelling of ethylbenzene dehydrogenase reaction mechanism. *J. Amer. Chem. Soc.*, submitted

Boll M., and Heider J. (2009) Anaerobic Degradation of Hydrocarbons: Mechanisms of C-H-bond activation in the absence of oxygen. In: *Handbook of Hydrocarbon and Lipid Microbiology* (ed. K. N. Timmis), Springer-Verlag, Heidelberg

Heider J. (2008) Bakterieller Kohlenwasserstoff-Abbau - es geht auch ohne Sauerstoff. *Biospektrum*, 14, 28-31

Heider J., and Rabus R. (2008) Genomic insights in the anaerobic biodegradation of organic pollutants. In: *Microbial biodegradation: Genomics and molecular biology* (E. Diaz ed.), pp. 25-54, Caister academic press, Norfolk, UK

Szaleniec, M., Witko, M., and Heider J. (2008) Quantum chemical modelling of C-H cleavage mechanism in oxidation of ethylbenzene and its derivatives by ethylbenzene dehydrogenase. *J. Molec. Catal. A*, 286, 128-136

Grundmann O., Behrends A., Rabus R., Amann J., Halder T., Heider J., and Widdel F. (2008) Genes encoding a tentative enzyme for anaerobic activation of n-alkanes in the denitrifying bacterium, strain HxN1. *Environ. Microbiol.*, 10, 376-385

Breuer M., Rabus R., and Heider J. (2008) Method for production of optically active alcohols using *Azoarcus* sp. EbN1 dehydrogenase. Pub. No. WO/2008/1545302; Intl. Appl. No. PCT/EP2008/057522

Finished theses

PhD thesis

Marie-Luise Lippert (2009) Biochemie von Enzymen des anaeroben Stoffwechsels von Toluol in *Thauera aromatica* (TU Darmstadt)

Diploma thesis

Regina Fischer (2009) Chloramphenicol Acetyltransferase als direkt selektierbares Reportersystem für die Insertion von Selenocystein in bakterielle Proteine

MSc thesis

Daniel Horst Knack (2009) Die Zweikomponenten-Sensorkinasen TdiS, EdiS und AdiS des anaeroben Toluol- und Ethylbenzolabbaus in *Aromatoleum aromaticum* Stamm EbN1

Lisa Lena Carlotta Debnar-Daumler (2009) Anaerober Abbau von Phenylalanin in *Aromatoleum aromaticum* Stamm EbN1: Oxidation von Phenylacetaldehyd zu Phenylacetat

BSc thesis

Janina Stephanie Kölschbach (2009) Biochemische Charakterisierung der Benzoylsuccinyl-CoA Thiolase, eines Enzyms des anaeroben Toluolabbaus

Structure of the group (12/2009)

Group leader: Prof. Dr. Johann Heider

Secretary: Patricia Wagner

Postdoctoral fellows: Dr. Ashraf Alhapel, Dr. Karola Schühle

PhD students: Carlotta Debnar-Daumler, Markus Hilberg, Daniel Knack, Sebastian Kölzer

BSc students: Timo Kraushaar, Svenja Schäfer

Technical assistants: Elke Eckel, Gabriele Höff, Iris Schall

Trainee: Lukas Burk

External fundings

Deutsche Forschungsgemeinschaft: Support for 3 PhD students (part of the time reported)

Invited lectures

MEOR workshop (BASF), Deidesheim, 24/25 March 2009; University of Georgia, Athens, GA (USA), 04 September 2009

Lab retreats

10–15 August 2008 in Hirschegg, Kleinwalsertal, together with the groups of Ulrich Ermler, MPI for Biophysics in Frankfurt, Bernhard Jaun, ETH Zürich, Georg Fuchs, University of Freiburg, Rolf Thauer, MPI Marburg, and Wolfgang Buckel, Philipps-Universität Marburg (see p. 111).

23–28 August 2009 in Hirschegg, together with the groups of Ulrich Ermler, MPI for Biophysics in Frankfurt, Bernhard Jaun, ETH Zürich, Georg Fuchs, University of Freiburg, Rolf Thauer, MPI Marburg, and Wolfgang Buckel, Philipps-Universität Marburg including several guests from the DFG-funded priority program: “Anaerobic hydrocarbon degradation” (see p. 111).

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