Elucidation of a dearomatising reductase reaction involved in the anaerobic degradation of naphthalene

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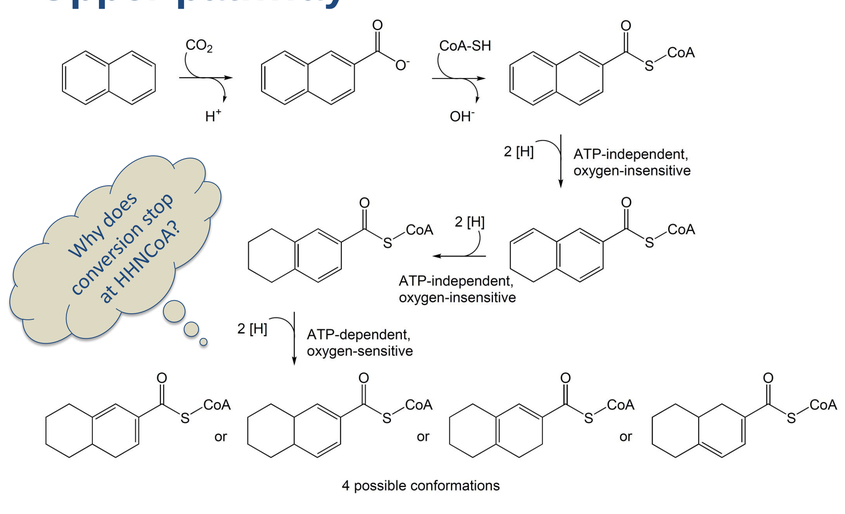
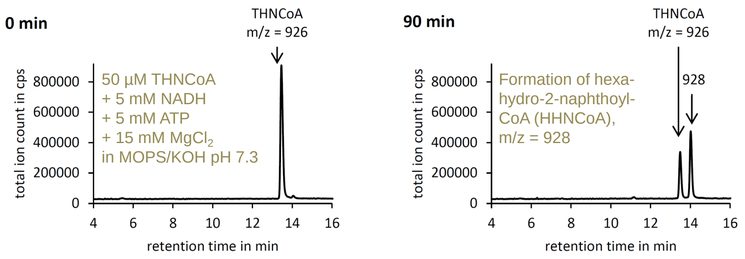
Offen im Denken

Background: Naphthalene degradation by sulphate-reducing bacteria proceeds via 5,6,7,8-tetrahydro-2-naphthoyl-CoA (THNCoA), which gets reduced to a hexahydro-2-naphthoyl-CoA (HHNCoA) with unknown conformation by an enzyme similar to class I benzoyl-CoA reductases (Eberlein *et al.*, 2013). For the downstream pathway, β-oxidation-like reactions were proposed as indicated by metabolites identified in culture extracts (Annweiler *et al.*, 2002). In previous studies, the THNCoA reductase reaction was measured with NADH as electron donor, but neither could a complete conversion of THNCoA be achieved, nor could further metabolites be detected.

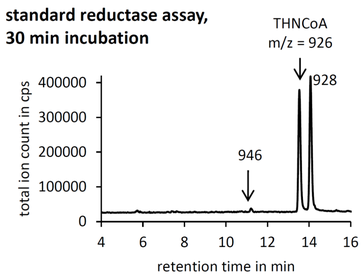
Upper pathway

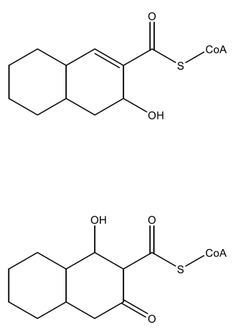


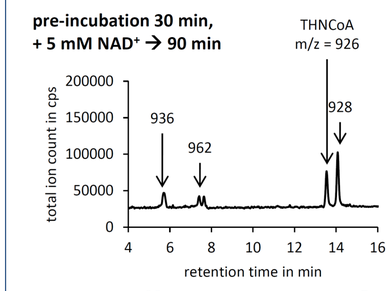
THNCoA reductase reaction

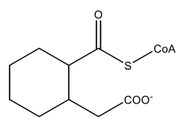


THNCoA reductase assays in N47-cfe



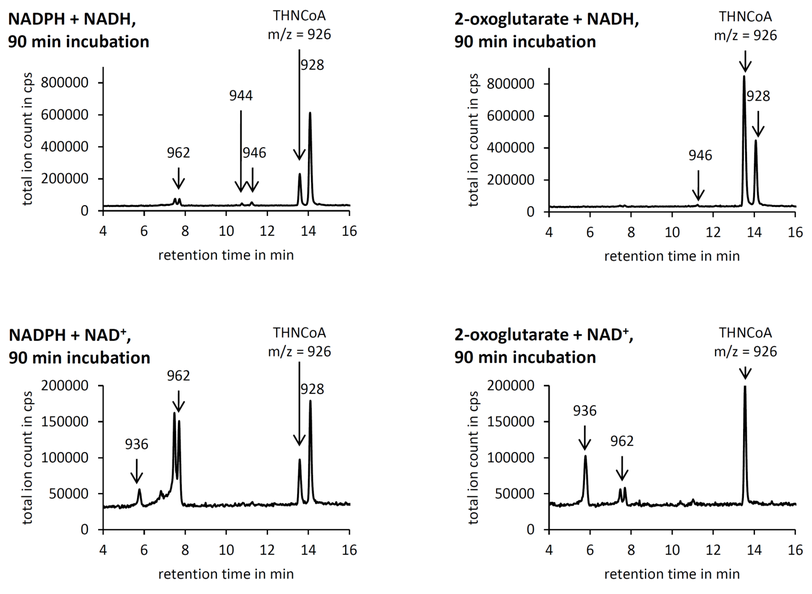






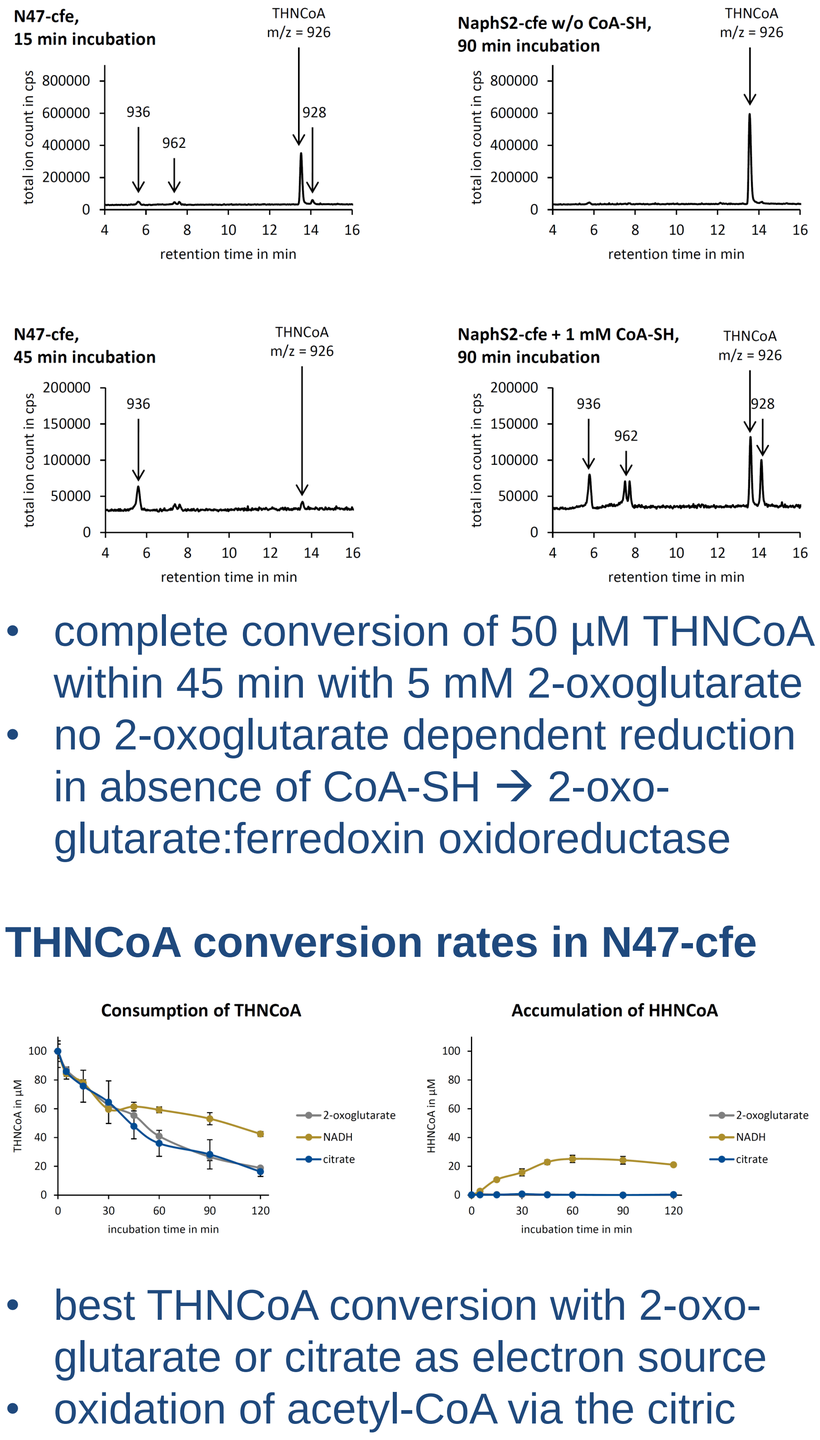
- small amount of HHNCoA hydratase product formed (m/z = 946)
- NADH inhibits downstream reactions

Assays with alternative electron donors and NaphS2-cfe



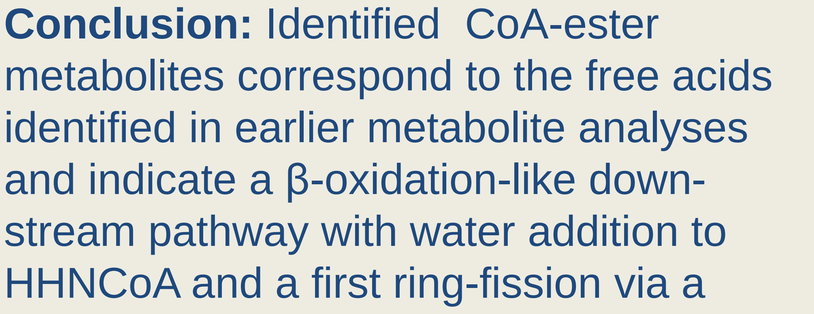
- β-hydroxyacyl-CoA dehydrogenase requires high NAD^+/NADH ratio

Assays in with 2-oxoglutarate as electron donor



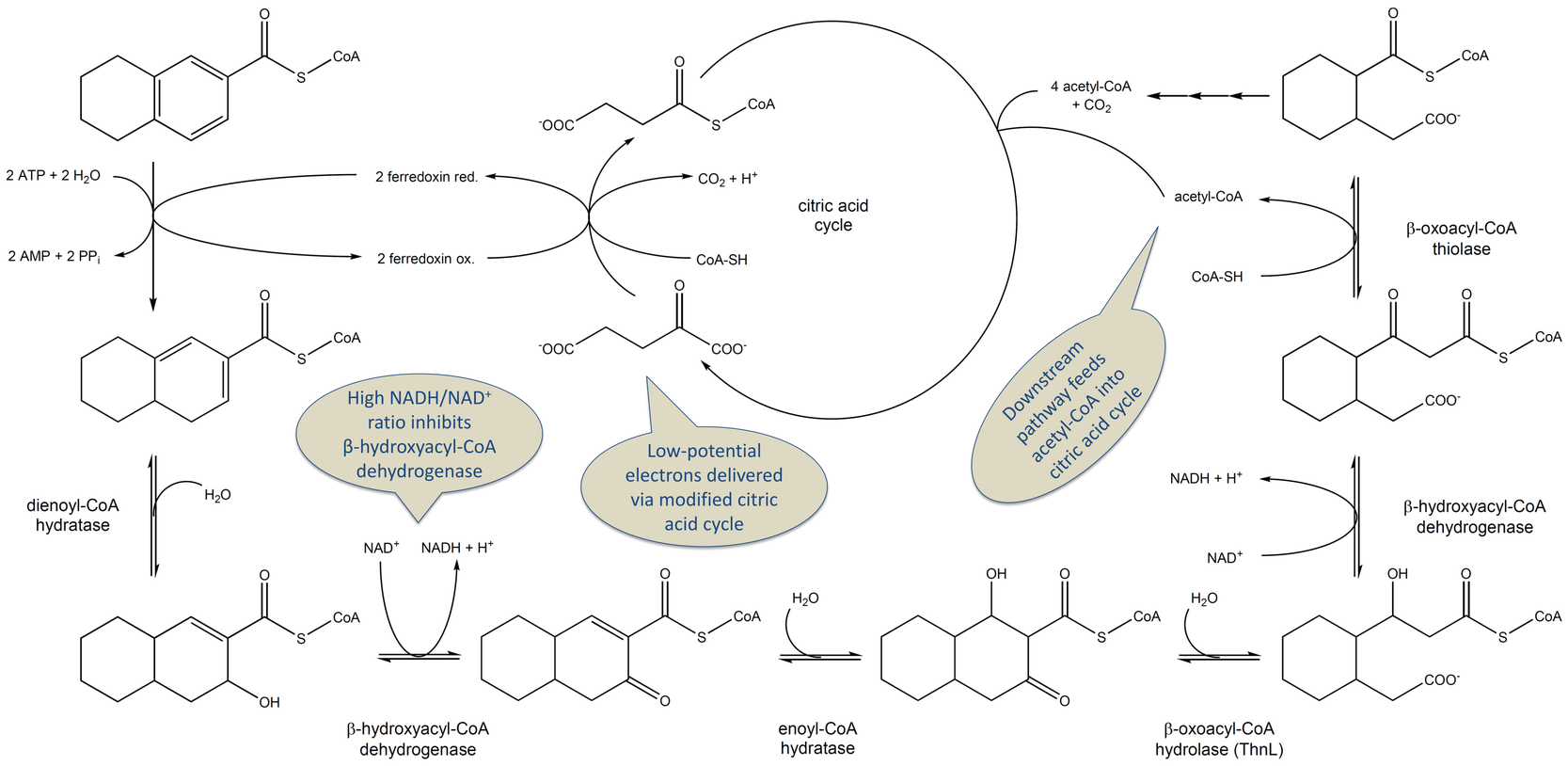
- complete conversion of 50 µM THNCoA within 45 min with 5 mM 2-oxoglutarate
- no 2-oxoglutarate dependent reduction in absence of CoA-SH → 2-oxo-glutarate:ferredoxin oxidoreductase

THNCoA conversion rates in N47-cfe



- best THNCoA conversion with 2-oxo-glutarate or citrate as electron source
- oxidation of acetyl-CoA via the citric acid cycle provides reduced ferredoxin as co-substrate for THNCoA reductase

Proposal for the downstream degradation pathway → β-oxidation like reactions



Conclusion: Identified CoA-ester metabolites correspond to the free acids identified in earlier metabolite analyses and indicate a β-oxidation-like down-stream pathway with water addition to HHNCoA and a first ring-fission via a hydrolase acting on a β'-hydroxy-β-oxo-decahydro-2-naphthoyl-CoA intermediate.

References

Annweiler, E., Michaelis, W., and Meckenstock, R.U. (2002) Identical ring cleavage products during anaerobic degradation of naphthalene, 2-methylnaphthalene, and tetralin indicate a new metabolic pathway. Appl Env Microbiol 68: 852-858.

Eberlein, C., Johannes, J., Mouttaki, H., Sadeghi, M., Golding, B.T., Boll, M., and Meckenstock, R.U. (2013) ATP-dependent/-independent enzymatic ring reductions involved in the anaerobic catabolism of naphthalene. Env Microbiol 15: 1832-1841.

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