A chemoenzymatic route from carboxylic acids to nitriles

Margit Winkler,[1,2] Melissa Horvat[1], Victoria Weilch[1], Robert Rädisch[3], Sebastian Hecko[4], Astrid Schiefer[4], Florian Rudroff[4], Miroslav Pátek[3], Ludmila Martínková[3]

[1] *Institute of Molecular Biotechnology, Graz University of Technology, Graz, Austria,*
[2] *acib GmbH, Graz, Austria,*
[3] *Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic,*
[4] *Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria,*
*margit.winkler@tugraz.at

The direct conversion of a carboxylic acid to the respective nitrile was described in biosynthesis pathways towards deazapurine-containing compounds.[1] 7-Cyano-7-deazaguanine synthases activate the carboxylate with ATP and ammonia acts as a nucleophile. The intermediate amide reacts with a second ATP to form the nitrile.[2] The conceptual elegance of this enzymatic reaction cannot be exploited for synthesis, because the responsible enzymes are strictly specific for their natural substrates.[3] Other biogenic nitriles are derived from amino acids in an enzymatic cascade involving three enzymes and carbon chain shortening.[2]

In this communication, we report an artificial pathway from carboxylic acid to nitrile, in which the carbon atom of the acid eventually becomes the nitrile carbon and the carbon chain length is retained. In the first step, a carboxylic acid reductase (EC 1.2.1.30, CAR) reduces the carboxylate to the respective aldehyde, which is trapped with hydroxylamine. The resulting oximes undergo enzymatic dehydration catalyzed by aldoxime dehydratase (EC 4.99.1.5 -7, Oxd). The first step in the cascade is CAR-mediated acid reduction of carboxylic acid to the respective aldehyde in a whole cell system. In this setup, oxygen is required for cell viability and constant ATP formation, the co-factor that is needed for carboxylate activation. The presence of hydroxylamine in the reaction medium leads to aldehyde trapping as aldoxime, the substrate for the second enzymatic step. Oxds are heme-dependent enzymes that transform primary oximes to the respective nitriles. Oxds were reported to be most active under anaerobic conditions, which maintained the catalytically essential ferrous state of the heme.[4] OxdBr1 from *Bradyrhizobium* sp. LTSPM299 does not require strictly anaerobic conditions,[5] which was a key asset in light of the oxygen requirement of the CAR-containing living cell biocatalyst.[6,7] We report the scope and limitations of nitrile formation via this sustainable chemoenzymatic one-pot route.

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