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Xenobiotic Reductase A in the Degradation of Quinoline by *Pseudomonas putida* 86: Physiological Function, Structure and Mechanism of 8-Hydroxycoumarin Reduction

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A continuous evolutionary pressure of the biotic and abiotic world has led to the development of a diversity of microbial pathways to degrade and biomineralize aromatic and heteroaromatic compounds. The heterogeneity of compounds metabolized by bacteria like *Pseudomonas putida* indicates the large variety of enzymes necessary to catalyse the required reactions. Quinoline, a *N*-heterocyclic aromatic compound, can be degraded by microbes along different pathways. For *P. putida* 86 quinoline degradation by the 8-hydroxycoumarin pathway has been described and several intermediates were identified. To select enzymes catalysing the later stages of the 8-hydroxycoumarin pathway *P. putida* 86 was grown with quinoline. The FMN-containing enzyme xenobiotic reductase A (XenA) was isolated and analysed for its reactivity with intermediates of the 8-hydroxycoumarin pathway. XenA catalyses the NADPH-dependent reduction of 8-hydroxycoumarin and coumarin to produce 8-hydroxy-3,4-dihydrocoumarin and 3,4-dihydrocoumarin, respectively. Crystallographic analysis of XenA alone and in complex with the two substrates revealed insights into the mechanism. XenA shows a dimeric arrangement of two $(\beta/\alpha)_8$ barrel domains each binding one FMN cofactor. High resolution crystal structures of complexes with 8-hydroxycoumarin and coumarin show different modes of binding for these molecules in the active site. While coumarin is ideally positioned for hydride transfer from N-5 of the isoalloxazine ring to C-4 of coumarin, 8-hydroxycoumarin forms a non-productive complex with oxidised XenA. Orientation of 8-hydroxycoumarin in the active site appears to be dependent on the electronic state of the flavin.

We postulate that XenA catalyses the last step of the 8-hydroxycoumarin pathway before the heterocyclic ring is hydrolysed to yield 3-(2,3-dihydroxyphenyl)-propionic acid. As XenA is also found in *P. putida* strains unable to degrade quinoline, it appears to have more than one physiological function and is an example of how enzymes with low substrate specificity can help to explain the variety of degradation pathways possible.

Keywords: xenobiotic reductase; flavin; quinoline; *Pseudomonas putida* 86; Old Yellow Enzyme

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Abbreviations used: XenA, xenobiotic reductase A; OYE, Old Yellow Enzyme; NG, nitroglycerin; PETN, pentaerythritol tetranitrate.

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Introduction

Approximately 15×10^6 tons of coal tar are produced worldwide every year and are the source for condensed aromatics and *N*-heteroaromatics like quinoline (2,3-benzopyridine).[1] Microorganisms have developed strategies to metabolize naturally occurring aromatic compounds and xenobiotics and as they are able to biomineralize potentially toxic compounds, they can be exploited in the bioremediation of polluted soils and waters.[1] Quinoline is a ubiquitous, soluble, heteroaromatic pollutant with cancerogenic properties.[2] Several bacterial species are capable of degrading quinoline and can grow with quinoline or its derivatives as sole sources of carbon, nitrogen and energy. A unique pathway exists in *Pseudomonas putida* 86 that was isolated from soil near a coal tar factory (Rütgerswerke, Castrop-Rauxel, Germany) by quinoline enrichment culture (Figure 1).[3] Within the 8-hydroxycoumarin pathway of *P. putida* 86 the *N*-heterocyclic ring of quinoline **1** is cleaved preferentially to form 8-hydroxycoumarin **4**. Four intermediates (**2**–**5**) have been identified in the 8-hydroxycoumarin pathway,[2,4] and the enzymes that catalyse the first two steps have been investigated (Figure 1). The molybdo-iron-sulfur flavoprotein quinoline oxidoreductase (QOR) hydroxylates quinoline **1** at C-2 to yield 1*H*-2-oxoquinoline **2**.[5–7] The multicomponent enzyme 1*H*-2-oxoquinoline 8-monooxygenase catalyses the NADH-dependent second hydroxylation reaction at C-8 to yield 1*H*-8-hydroxy-2-oxoquinoline **3**.[8,9] The next two intermediates are 8-hydroxycoumarin **4** and 3-(2,3-dihydroxyphenyl)-propionic acid **5** (Figure 1).[4] The enzymes producing and converting these two compounds are not known. Quinoline degrading *P. putida* strains do not grow with coumarin as a sole source of carbon and energy. However, when bacteria that were grown with quinoline are incubated with coumarin **6**, 3-(2-hydroxyphenyl)-propionic acid **7** accumulates.[4] Characteristic reactions of coumarins are additions to the C-3/C-4 double bond and the nucleophilic opening of the lactone group.[10] Addition of a hydride and a proton to the C-3/C-4 double bond and hydrolysis of the lactone explains the observed products 3-(2,3-dihydroxyphenyl)-propionic acid **5** and 3-(2-hydroxyphenyl)-propionic acid **7** and defines the activity of the enzymes able to convert 8-hydroxycoumarin **4** and coumarin **6** (Figure 1). While 3-(2,3-dihydroxyphenyl)-propionic acid **5** can be further degraded to intermediates of the tricarboxylic acid cycle,[11] 3-(2-hydroxyphenyl)-propionic acid **7** cannot, explaining why *P. putida* 86 does not grow with coumarin as carbon source.[4]

Bacterial enzymes known to catalyse the reduction of the olefinic bond of α,β-unsaturated carbonyl compounds including ketones and esters are a group of enzymes called xenobiotic reductases.[12–18] These enzymes constitute a bacterial subgroup of the Old Yellow Enzyme (OYE) family and are monomeric, homodi- or tetrameric, NAD(P)H-dependent, FMN-containing oxidoreductases with a subunit size of ~40 kDa. Like for OYE itself, the physiological oxidant and with it the physiological function is unknown for almost all members of the OYE family.

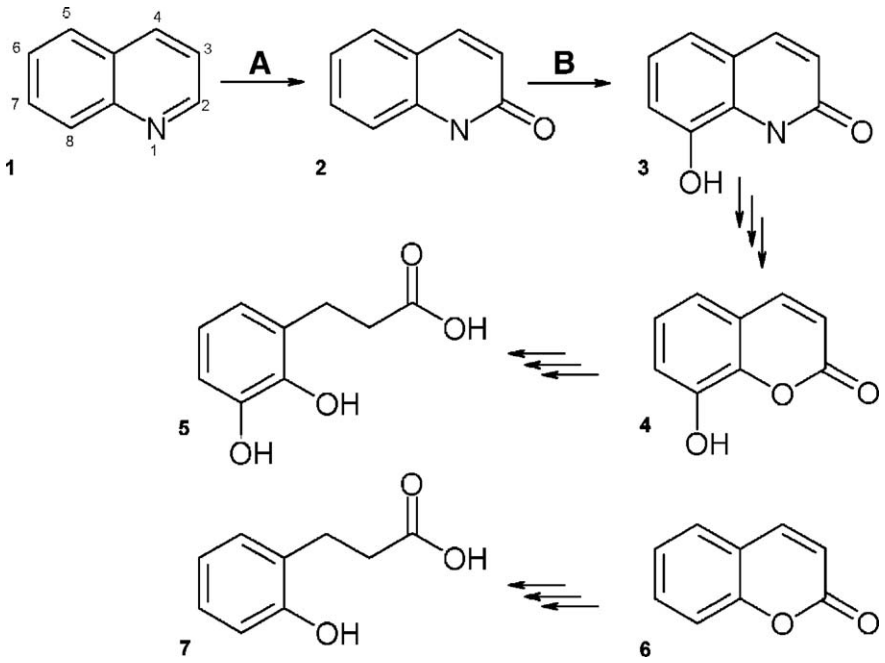

Figure 1. Degradation of quinoline and coumarin by *P. putida* 86. Intermediates of the 8-hydroxycoumarin pathway are depicted.[4] The first reaction of the pathway is labelled with A and is catalysed by quinoline 2-oxidoreductase.[6,7] The second reaction, labelled with B, is catalysed by 2-oxoquinoline 8-monooxygenase.[8,9] Triple arrows indicate that the reaction sequence between the different intermediates is unclear and multiple steps could be involved. In the following the names of all substances depicted are given: (1) quinoline, (2) 1*H*-2-oxoquinoline, (3) 1*H*-8-hydroxy-2-oxoquinoline, (4) 8-hydroxycoumarin, (5) 3-(2,3-dihydroxyphenyl)-propionic acid, (6) coumarin, and (7) 3-(2-hydroxyphenyl)-propionic acid.