Title: Glutathione *S*-transferases for the synthesis of chiral aromatic compounds

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State of the art

We discovered and described a new pathway for the degradation of styrene leading to phenylacetic acid (Fig. 1) (1). It comprises activities of styrene monooxygenases (SMO; 2) and phenylacetaldehyde dehydrogenases (PAD; 3), but also involves two novel glutathione *S*-transferases (GST), namely StyI and StyJ, which are related to enzymes from isoprene degradation (1,4-6). Recently, we were able to demonstrate that StyI is the actual epoxide converting enzyme and that it may represent a new sub-class of GSTs (6). This work was achieved in collaboration with **Hofmann** group, and we moved on to biochemically study the enzymes (unpublished). Besides kinetic and biotransformation data we are interested in solving the crystal structure of StyI and related enzymes from the pathway. The results obtained demonstrate that StyI is highly promiscuous and accepts all kinds of epoxides. We also reported earlier that this glutathione dependent styrene route allows to produce ibuprofen (7). This is only possible with GST attacking respective epoxides, but at low concentrations only.

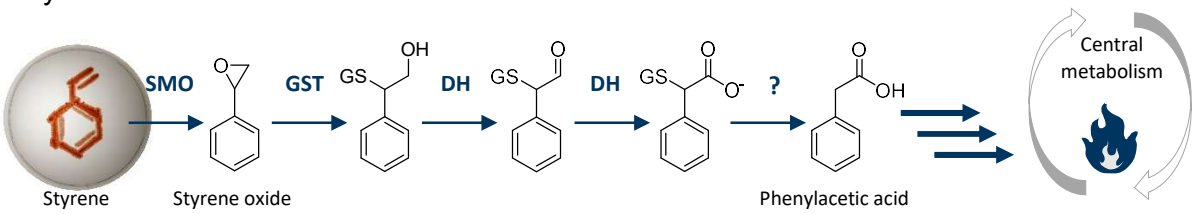

Figure 1 Upper styrene metabolic pathway for styrene degradation present in *Gordonia rubripertincta* CWB2 (orange-red biomass shown as inset on a agar plate in the form of styrene substrate) comprises a styrene monooxygenase leading (SMO) to *S*-styrene epoxide (2,8-10), a glutathione *S*-transferase (GST: StyI) to form a GS-conjugate (1,4-6) which is further converted by dehydrogenases (DH) towards the central metabolite phenylacetic acid (3). The second GST (StyJ) is supposed to release the glutathione from the conjugate to be elucidated, hence not included here (6).

As a model organism serves the soil actinobacterium *Gordonia rubripertincta* CWB2 which was isolated with styrene as sole carbon and energy source (1), and its capability to degrade/convert styrene as well as chemical analogous compounds (7). The respective styrene pathway is encoded on a plasmid which seems transferable between Actinobacteria. We identified the same plasmid comprising this metabolic capability in another *Gordonia* strain (unpublished). A detailed biochemical and biocatalytical characterization of the native enzymes involved will be carried out; with focus on glutathione *S*-transferases; StyI and StyJ. As mentioned above, for StyI we achieved already a set of data in the first part of MiCon which we plan to extend. But the role of StyJ in the pathway needs to be clarified. Also, we need molecular genetic tools for these Actinobacteria to manipulate them to uncover pathway steps as well as to manipulate them towards powerful whole-cell biocatalysts. Those tools we will develop within an international cooperation (Prof. Dr. L. Eltis, UBC, Canada) on base of the molecular genetic tools to modify rhodococci (11).

Specific aims

(1) The role of glutathione in degradative pathways is not well documented which is true for the GSTs as well, and their potential to function as chiral biocatalysts is not explored yet. This aspect we want to study from a biochemical, structural, and biocatalytic point of view.

(2) The presence of two different glutathione *S*-transferases in the isoprene and the styrene degradation pathways remains to be described. This we want to clarify by manipulating the respective genes in the native host.

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