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Aromatic hydroxylation catalyzed by an unspecific peroxygenase from Aspergillus brasiliensis

AUTHORS

Fabian SCHMITZ / INSTITUTE OF BIOCHEMISTRY HEINRICH-HEINE-UNIVER, UNIVERSITÄTSSTRASSE 1, DÜSSELDORF
Katja KOSCHORRECK / INSTITUTE OF BIOCHEMISTRY HEINRICH-HEINE-UNIVER, UNIVERSITÄTSSTRASSSSE 1, DÜSSELDORF
Frank HOLLMANN / DEPARTMENT OF BIOTECHNOLOGY, DELFT UNIVERSITY OF TECHNOLOGY, JULIANALAAN 136, DELFT
Vlada B URLACHER / INSTITUTE OF BIOCHEMISTRY HEINRICH-HEINE-UNIVER, UNIVERSITÄTSSTRASSE 1, DÜSSELDORF

PURPOSE OF THE ABSTRACT

Unspecific peroxygenases (UPOs, EC 1.11.2.1) are heme-thiolate enzymes secreted by fungi [1]. These enzymes use hydrogen peroxide to form a highly reactive iron-oxo species, Compound I, to perform various oxidation reactions like hydroxylation, epoxidation, and O-dealkylation [2]. Nevertheless, despite of an increasing number of identified UPOs and more than hundreds of identified substrates, aromatic hydroxylation catalyzed by these enzymes is rarely reported while aliphatic hydroxylation reactions have been often described in literature [2, 3]. Aromatic hydroxylation of substituted benzenes gives access to versatile phenolic synthons in the synthesis of dyes, pharmaceuticals, and agrochemicals.

In attempt to identify a UPO enabling aromatic hydroxylation we cloned in Pichia pastoris a number putative UPOs. Out of seven UPOs successfully expressed in P. pastoris we focused on an UPO from Aspergillus brasiliensis (AbrUPO). With expression yields of up to 0.75 g/l culture medium, this UPO fulfils a requirement for the production of valuable chemicals at a high scale. Unsimilar to most reported UPOs, AbrUPO was found to catalyze aromatic hydroxylation of substituted benzenes. The chemoselectivity of AbrUPO and its preference for aromatic or benzylic hydroxylation was found to depend on chemical properties and length of the alkyl chain. A comparative analysis of the active site of AbrUPO revealed that it is very different from the active sites of the UPOs unable of aromatic hydroxylation. These observations render AbrUPO not only an interesting biocatalyst for synthetic chemistry but also a good candidate for protein engineering studies to achieve a better insight on the molecular factors governing the chemoselectivity of heme-thiolate enzymes.

FIGURES

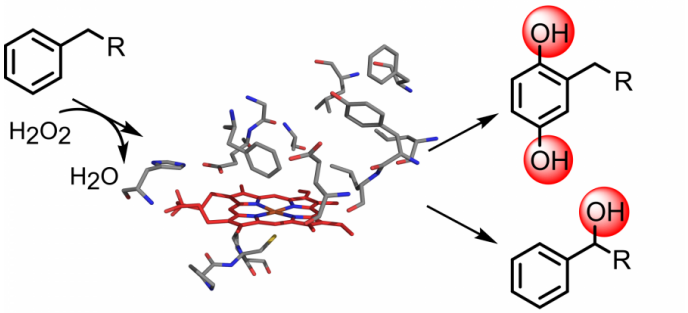

FIGURE 1

Reaction scheme for AbrUPO

An unspecific peroxygenase from Aspergillus brasiliensis highly expressed in Pichia pastoris catalyses aromatic hydroxylation of benzylic compounds.

FIGURE 2

KEYWORDS

unspecific peroxygenase | Pichia pastoris | aromatic hydroxylation | heterologous expression

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