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A Magic Piece of Unspecific Peroxigenases? - A Surface Alpha Helix as Key Part for Catalytic Specificity

AUTHORS

Carsten PICHLER / GRAZ UNIVERSITY OF TECHNOLOGY, PETERSGASSE 14, GRAZ
Vanessa HOLZSCHUSTER / GRAZ UNIVERSITY OF TECHNOLOGY, PETERSGASSE 14, GRAZ
Anton GLIEDER / GRAZ UNIVERSITY OF TECHNOLOGY, PETERSGASSE 14, RAZ

PURPOSE OF THE ABSTRACT

Unspecific peroxygenases (UPOs, EC 1.11.2.1) are heme thiolate enzymes categorized in two classes - long and short UPOs - that combine peroxidase (one electron transfer) and peroxygenase (two electron transfer) activity [1]. UPOs are capable of catalyzing oxyfunctionalization reactions using hydrogen peroxide as a co-substrate to activate C-H bonds [2] and beyond that they are naturally secreted and stable enzymes. These properties make them interesting for industrial applications, including e.g. the production of agrochemicals, insecticides, dye precursors or pharmaceuticals [3]. But until now these enzymes only have been found in Ascomycota, Basidiomycota and Oomycota [1][4]. Recently, several long and short UPO sequences were successfully expressed by Pichia pastoris and helped expanding the amount of recombinant expressed enzymes but still there is a huge interest to find new or even evolved variants with attractive activities [1][5]. In this study, two novel (Aspergillus brasiliensis, Podospora anserina) and one already described (Hypoxylon sp. E38) [6] short UPO were investigated by random as well as site-directed mutagenesis (SDM). There, a surface located alpha helix close to the heme entrance tunnel turned out to be of particular interest. Replacement of amino acids at certain positions within this alpha helix with polar or nonpolar residues, promote either activity on typical peroxidase (ABTS, 2-6-DMP) or peroxygenase (indole, naphthalene, NBD) substrates. Our results help to expand the repertoire of recombinant expressed UPOs, to clarify mechanistic differences between the two different catalytic reactions (peroxidase vs. peroxygenase), and to support the structure/function relationship in respect to these specific catalytic properties.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

UPO | Pichia pastoris | surface | rational design

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