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Comparative Analysis of Fungal Pyranose Dehydrogenases and Pyranose 2-Oxidases Reveals Aminoacid Residues Responsible for Regioselectivity of Monosaccharide Oxidation

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Pyranose dehydrogenase (PDH) is a fungal flavindependent sugar oxidoreductase which is structurally and catalytically related to fungal pyranose 2-oxidase (P2O) and probably fulfills similar biological functions in lignocellulose degradation [1]. P2O oxidizes D-glucose at the second carbon (C2) to 2-keto-D-glucose by a highly regioselective mechanism [2] while PDH displays broader substrate specificity and targets both C2 and C3 for oxidation. Bioinformatic analysis was used to understand structural basis of functional variability and different regioselectivity between PDHs and P2Os in the absence of PDHs' three-dimensional crystallographic structures. All detectable sequences and structural homologs of PDH and P2O were identified in Swiss-Prot protein sequence and PDB protein structures databanks. Analysis revealed 34 sequence-based homologs (29 of those were selected for further study as most informative) and 24 structure-based homologs of P2O. Corresponding sequence alignments were made and studied using phylogeny approach. Comparative analysis and homology modeling revealed a major similarity between active sites of P2Os and PDHs. P2Os have either two catalytic histidines or an asparagine in place of the second histidine. PDHs possess two histidines, as do several P2Os. Bioinformatic analysis disclosed subfamily-specific positions (SSPs) that define functional variability between enzymes with different properties, *i.e.* positions with residues tending to be conserved within groups of PDHs and P2Os, but different between them. The most statistically significant seven SSPs were selected as templates for mutagenesis in order to generate *in silico* library of P2O variants that contain corresponding PDHs' amino acid residues in the SSPs. Molecular docking was used to evaluate the efficiency of mutated P2O variants in binding of monosaccharide substrates (glucose, aldose and galactose) for C2/C3 regioselective oxidation. Structural filtration was implemented as a post-docking tool to select reactive enzyme-substrate complexes that satisfy knowledge-based criteria of near-to-attack conformation. Two sets of filters were used to evaluate: i) substrate interaction with the catalytic histidine (distance and angle); ii) substrate interaction with FAD cofactor. Molecular docking of monosaccharides into active site of wild type P2O showed that substrates were oriented to facilitate oxidation at C2 that is in agreement with previously published crystallographic and kinetic studies. *In silico* screening of enzyme-substrate complexes formed by mutant forms of P2O with predicted substitutions in specific positions (R>N, Q>L and Y>D) revealed two alternative binding conformations of the glucose substrate each promoting C2 or C3 regioselective oxidation activity. Orientation pluralism among so close homological enzymes P2Os and PDHs is mainly due to diffrent network of hydrogen bonds with glucose hydroxyls. Thus, double and triple mutants introducing PDH amino acid states into specific positions of P2O

were suggested to extend regiospecificity of the wild type enzyme P2O towards both C2 and C3 oxidation of D-glucose and other monosaccharides. The selected mutations were recommended for experimental evaluation of regioselectivity and catalytic activity.

Литература

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