Setting the stage for electron transfer: Molecular basis of ABTS-binding to four laccases from Trametes versicolor at variable pH and protein oxidation state - DTU Orbit (18/11/2019)

Laccases are multi-copper oxidases having exquisite oxidation power, high stability, and multiple industrial applications. Although Km varies ~1000-fold across laccases, the molecular basis of substrate binding is poorly understood. Furthermore, laccase isoenzymes vary substantially in stability and activity for unknown reasons, and are thus useful probes of stability-function trade-offs relevant to protein engineering. We report here the first systematic comparison of ABTS-binding to different proteins, i.e. the four isoforms of Trametes versicolor, using a combination of sequence clustering, density functional theory calculations, homology modeling, and multiple induced-fit docking protocols at variable pH-dependent protonation states and T1-copper oxidation state. Clustering analysis provided a systematic overview of laccases across Trametes and revealed distinct isoenzyme classes (A–J) with the four T. versicolor isoforms belonging to separate classes. The T1 oxidation state had minor effect on ABTS binding, whereas the protonation state of Asp206 was important, consistent with site-directed mutagenesis studies. The absence of active poses for the δ-isoform agrees with its large Km, whereas the α-isoform with the smallest Km also had most active poses with protonated Asp206 corresponding to its pH opt ~2. Protonated Asp can bind to ABTS to form strong, active conformations partially explaining the low pH opt of fungal laccases toward ABTS. We identified several laccase properties optimal for ABTS binding. Notably, very high (R2=0.99) correlation was observed between logKm (ABTS) and binding-pocket charge due to sites 157, 161, 269, 271, and 333, i.e. laccases optimal for ABTS turnover have positively charged anchor points in their pockets. Our work also demonstrates how activity-constraints can markedly improve docking to reduce inactive false positives.

General information
Publication status: Published
Organisations: Department of Chemistry, Physical and Biophysical Chemistry
Contributors: Christensen, N. J., Kepp, K. P.
Pages: 68-77
Publication date: 2014
Peer-reviewed: Yes

Publication information
Journal: Journal of Molecular Catalysis. B, Enzymatic
Volume: 100
ISSN (Print): 1381-1177
Ratings:
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.5 SJR 0.744 SNIP 1.036
Web of Science (2014): Impact factor 2.128
Web of Science (2014): Indexed yes
Original language: English
Keywords: Laccase, Substrate, Sequence analysis, Docking, DFT
DOIs: 10.1016/j.molcatb.2013.11.017
Source: dtu
Source ID: n::oai:DTIC-ART:compendex/429988685::37109
Research output: Contribution to journal › Journal article – Annual report year: 2014 › Research › peer-review