Biodegradation of Lignin by Laccase for Conversion of Biomass to Fuel: Analysis of Substrate Binding

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Conversion of renewable energy resources such as lignocellulose to fuel is hampered by lignin, which is recalcitrant to degradation.[1] At the same time, multicopper oxidase (MCO) laccases were shown to be useful to mitigate biomass recalcitrance by mediation with easily oxidizable phenolic compounds through a radical-catalysed reaction,[2] whose efficiency is primarily dependent on the redox potential difference between the enzyme and the substrate.[3] However, the relative propensity towards binding the phenolic compounds could also assist in improved catalytic efficiency, as has been examined in some cases by Madzak et al.[4] and more recently, for example, for the *Melanocarpus albomyces* (MaL)[5] and *Trametes Versicolor* (TvL) laccases.[6]

In this work, we examined the binding propensity of six phenolic compounds (listed in Table I) for wild type and mutated MaL, TvL (shown in Fig. 1) and eight other laccases, including *Coprinus cinereus* (CcL), *Coriolopsis gallica* (CgL), *Cerrena maxima* (CmL), *Lentinus tigrinus* (LtL), *Rigidoporus lignosus* (RlL), *Thielavia arenaria* (TaL), *Trametes hirsuta* (ThL), and *Trametes trogii* (TtL**)**. Alignment of the sequences of these selected laccases for detection of conserved motifs in the binding site was carried out using COBALT.[7] Docking calculations with Autodock,[8] followed in some cases by geometry optimization with empirical force fields, were used to identify candidates for mutation. For validation, calculations of the free energy of binding were performed for selected cases.

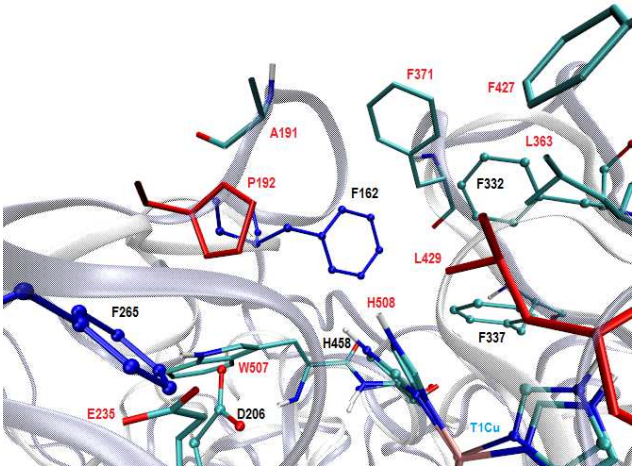

Fig. 1. Binding pocket in Mal and TvL. Mal: Amino acids in bond form and mutated side-chains in red, backbone in ice-blue; TvL: Amino acids in sphere and stick form and mutated residue in blue color, backbone in white.

Upon sequence alignment, we note that the position of the amino acids in the binding pockets was considerably conserved, mostly only one or two amino acid away from each other. Binding affinities of the phenolic substrates for wild-type and single-point mutated variants of TvL are shown in Table I as an example, including the experimental oxidation efficiency. A lower efficiency due to mutation was well correlated with weaker binding of the substrate by the enzyme, and vice versa. For instance, the higher efficiency of TvL-F162A for the trimeric substrate could be ascribed to stronger binding. Binding free energy calculations by all-atom MD simulations based on umbrella sampling and PDLD/S-LRA demonstrated a trend consistent with the docking result. Based on these results, suggestions for mutated laccases for improved biocatalytic efficiency could be provided.

TABLE I. Calculated Binding Affinity (BA, kcal/mol) for Phenolic Substrates

Substrate	Enzyme	BA	O_{phenol} - H_{His} distance (Å)	Catalytic efficiency[a]
Thymol	TvL-wt	-5.2	2.00	99
	TvL-F265A	-4.8	1.95	79
	TvL-L164K	-5.7	2.20	
2-t-Bu-phenol	TvL-wt	-4.8	2.01	69
	TvL-F162A	-4.7	1.90	99
	Mal-P192A	-5.7	2.06	
3,5-di-t-Bu-phenol	TvL-wt	-6.0	2.16	42
	TvL-F162A	-5.6	2.25	63
	TvL-F265A	-5.6	3.93	14
	Mal-P192A	-6.5	2.45	
2,6-di-t-Bu-phenol	TvL-wt	-5.7	2.31	41
	TvL-F265A	-5.0	2.45	19
	ThL-G164P	-6.1	2.25	
Bis-phenol	TvL-wt	-5.3	2.06	72
	TvL-F265A	-5.5	2.02	49
	TaL, LtL	-6.5, -6.0	3.04, 2.31	
Trimeric substrate	TvL-wt	-6.6	2.94	20
	TvL-F162A	-8.6	2.59	45

[a]Consumption % after 24 hours[6]

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