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Molecular basis of Laccase bound to lignin: Insight from comparative studies on the interaction of *Trametes versicolor* laccase with various lignin model compounds

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Abstract

Laccase, a type of multicopper oxidase, is capable of efficiently degrading lignin. Until now, molecular basis of laccase interacting with lignin is still poorly known. Here, five lignin model compounds (2,6-dimethoxyphenol, ferulic acid, guaiacol, sinapic acid and vanillyl alcohol) were selected to demonstrate the key binding mechanisms between *Trametes versicolor* laccase and lignin. The results showed that the interaction energies of lignin model compounds with laccase varied widely, which suggested different molecular efficiency of laccase in degrading various components of lignin. This was in full agreement with experimental reports. Hydrophobic interactions seemed to be necessary to the interaction of lignin/lignin model compounds with laccase, while H-bonds were alternative. Molecular basis produced by this study was helpful in designing highly efficient laccases again lignin waste to achieve the environmental protection.

Introduction

Lignin is the second largest number of natural organic polymer after cellulose, constituting about 20% of lignocellulosic material [1-3]. Agricultural, industrial and urban activities have accumulated huge amounts of lignin waste [4] and other types of pollutants [5-8], causing serious environmental problems. Thus, lignin degradation is required for environmental protection and its efficient utilization [9]. The major difficulty in exploring the molecular basis for lignin biodegradation is the high complexity of lignin structure. Until now, there is still a lack of complete lignin 3D structure. To address this problem, lignin model compounds were often selected as substitutes for the studies on lignin degradation [10, 11]. Several lignin model compounds including 2-methoxyphenol, 2,6-dimethoxyphenol, syringyl alcohol, syringic acid, p-coumaric acid, ferulic acid, coniferyl alcohol and vanillyl alcohol have been employed as the representatives for lignin degradation studies [12-14]. Thus, consistent with previous studies, we also employed lignin model compounds to investigate the interaction of laccase with lignin in this study.

Laccase, one of main ligninolytic enzymes, has already been used as a biocatalyst in the fields of industry and environmental protection today [15], and can efficiently degrade lignin [16], polycyclic aromatic hydrocarbons [17], and other pollutants [15]. Laccases are presented in microbes, plants and animals [18]. Generally, laccases are composed of three domains with three copper-binding sites (T1, T2 and T3). Electron transfer occurs between them. Laccase can either transform contaminants into less toxic compounds or change the nature of the pollutants to degrade easily [19]. Among all analyzed microbial laccases, *Trametes versicolor* laccase has received wide attention within the past few decades. The adsorption progress of *T. versicolor* laccase onto alkali lignin was investigated, showing the presence of a mediator enhanced the binding affinity between laccase and lignin [20]. Kolb et al. [21] examined the effect of *T. versicolor* laccase on the variation in phenolic monomers, and found that almost all

types of monomer phenolic compounds could be removed under the proposed conditions. Another study showed that *T. versicolor* laccase could completely oxide micropollutants diclofenac, mefenamic acid, bisphenol A and triclosan [19].

It was suggested that lignin-degrading efficiency was affected by the interaction between laccase and lignin [22]. Also, the structures of lignin model compounds had impact on laccase' oxidation rate [12]. Thus, understanding how laccase interacts with lignin is helpful in increasing lignin-degrading efficiency and broadening our knowledge about laccase catalysis mechanism. The molecular basis of laccases for lignin biodegradation in relation to the interaction details between laccases and lignin is not well-understood until now. In this regard, molecular docking and molecular dynamics (MD) simulations have proved to be a reliable technique for the exploration of protein-ligand interaction at the atomic and molecular level [15, 23, 24]. Moreover, it was shown that MD simulations were a useful method to obtain the structural and dynamic information on *Pleurotus ostreatus* laccase [25] and CotA laccase [26].

In the present study, molecular docking and molecular dynamics simulation have been used to study and compare the interaction between various lignin model compounds (2,6-dimethoxyphenol, ferulic acid, guaiacol, sinapic acid and vanillyl alcohol) and *T. versicolor* laccase. Based on the interaction information from the present study, a proposed molecular basis of laccase bound to lignin has been deduced, which might be helpful in the understanding of lignin-degrading reaction.

Materials and methods

Laccase structure

The 3D structure of *T. versicolor* laccase created by Piontek et al. [27] at 1.90 Å resolution, was retrieved from the Protein Data Bank [28] (http://www.rcsb.org/pdb/home/home.do) under the code 1GYC. All ligands and water molecules were deleted.

Lignin model compounds studied

The structures of lignin model compounds were downloaded from ChemSpider [29] (http://www.chemspider.com/). A total of five lignin model compounds were selected for the present study: 2,6-dimethoxyphenol, ferulic acid, guaiacol, sinapic acid and vanillyl alcohol.

Docking

The binding site of lignin model compounds inside laccase was identified by a grid-based cavity prediction algorithm of Molegro Virtual Docker (MVD) [30]. Then, lignin model compounds were docked into the binding site. Docking analyses were performed between all analyzed lignin model compounds and laccase. The maximum population size was set to 50 individuals, and 1500 maximum interactions were allowed. The MolDock scoring function (MolDock Score) and Moldock SE algorithm were used to rank the docking poses. To avoid the appearance of the stochastic errors, five runs were carried out for each ligand. The best ranking pose was selected as the optimal binding mode between laccase and lignin model compound. MD simulations were used to analyze the best binding mode.

Interaction analysis

The interactions of laccase with lignin model compounds were analyzed and depicted using LigPlot+ [31].

MD simulations

Lignin model compounds were parameterized using Automated Topology Builder (ATB) [32] (http://compbio.biosci.uq.edu.au/atb/). Complexes of laccase with 2,6-dimethoxyphenol, ferulic acid, guaiacol, sinapic acid and vanillyl alcohol were referred as LAC-DI, LAC-FE, LAC-GU, LAC-SI and LAC-VA, respectively. Each complex was then subjected to MD simulation by Gromacs 4.5.6 package [33]. The parameters used for protein and ligand were from gromos53a6 force field. SPC water molecules were applied to solvate these complexes under a periodic boundary condition. The system was neutralized by adding 11 Na^+. We performed energy minimization by steepest-decent algorithm. These minimized systems were

then submitted to NVT (1.4 nm for short-range electrostatic cutoff and van der Waals cutoff, a time step of 2 fs, 300 K for Maxwell distribution and -1 for the generation of a random seed) and NPT (no velocity generation, LINCS algorithm [34] for constraints and Parrinello-Rahman [35] for pressure coupling). Finally, MD simulations were carried out up to 10 ns using V-rescale [36] for temperature coupling. Long range electrostatic interactions were calculated according to Particle Mesh Ewald [37].

Results

Binding mode

Validation of docking accuracy. Docking analyses were performed between all analyzed lignin model compounds and laccase. We selected the best docking poses for further study. Fig. 1 shows the binding conformations of lignin model compounds in the active site of laccase. Docking accuracy was validated by re-docking protocol. We used the best poses of lignin model compounds as reference ligands. Root-mean-square deviation (RMSD) between re-docked poses and reference ligands were 0.043 Å for 2,6-dimethoxyphenol, 0.015 Å for ferulic acid, 0.009 Å for guaiacol, 0.463 Å for sinapic acid and 0.014 Å for vanillyl alcohol (Table 1). All these RMSD values were lower than 2.0 Å that was considered as acceptable and correct [38], meaning that the present docking protocol could accurately reveal the conformation and binding mode of lignin model compounds into the binding pocket of laccase.

Binding orientation and interaction. The orientation of ligand is important for acceptor-binding activity. Clearly, binding orientation of lignin model compounds inside laccase highly varied as can be seen from Fig. 1, which suggested the performance of laccase in catalysis for the degradation of lignin model compounds was different. The interaction energies of LAC-DI, LAC-FE, LAC-GU, LAC-SI and LAC-VA were analyzed in detail (Table 1). When making comparisons between these six complexes, the most noticeable

difference was the interaction energy. Their interaction energy changed in a wide range. Sinapic acid showed a lowest interaction energy with laccase (-112.174 kcal mol^{-1}), which suggested better interaction with the binding site of laccase than other lignin model compounds. It was showed that 2,6-dimethoxyphenol interacted by H-bonds with residues Gln442 and His402 (Fig. 2). For guaiacol, we found that its interaction energy with laccase was highest (-78.992 kcal mol^{-1}), lacking H-bonds with laccase. Ferulic acid presented H-bonds with His111, Ala80, Leu459, Glu460, and Gly462 of laccase with interaction energy of -111.765 kcal mol^{-1}. We further observed that Pro394 participated in the formation of H-bond with sinapic acid. No H-bonds were found between laccase and vanillyl alcohol. This means that H-bonds were an alternative way to determine the interaction of laccase with lignin model compounds or lignin.

Hydrophobic interaction seemed to be a more important factor for the binding of laccase to lignin model compounds than H-bonds, because all lignin model compounds formed hydrophobic interactions with laccase (Fig. 2). We observed local differences in the types of amino acid residues participated in hydrophobic interactions. 2,6-dimethoxyphenol presented hydrophobic interaction with seven amino acid residues of laccase: Phe441, Pro314, Phe422, Ala403, Gly401, Asp444 and Phe69. The involved residue number for ferulic acid was the same with those for 2,6-dimethoxyphenol, including Leu112, Ser113, Pro79, Pro346, Phe344, Phe450 and Ala461. As can be seen in Fig. 2, guaiacol presented hydrophobic interaction with Asp214, Ile213, Ser212, Arg260, Asp234, Gln293, Thr294 and Ser296. In addition, ten amino acid residues took part in the hydrophobic contact with sinapic acid, including Pro163, Gly392, Asp206, Asn208, Phe239, Ile455, Pro207, Phe265, Ala393 and Asn264. In LA-VA, residues Gln293, Arg260, Ile213, Ser212, Asp214, Thr294, Asp234, Ser296 and Thr295 participated in hydrophobic interaction with vanillyl alcohol. These results showed that hydrophobic interactions were necessary for the binding of laccase to lignin model

compounds, and thus were potentially important to lignin degradation, similar to previous report that hydrophobic interactions were crucial for determining the binding modes between ligninolytic enzymes and lignin [22].

MD simulations

MD simulations have been used for the study of laccase in previous studies [15]. For example, 3D models of wild-type enzyme and mutant for *Pleurotus ostreatus* laccase were constructed, and then were compared by MD simulations [25]. Consistent with previous studies, in the present study, MD simulations were conducted for providing the insights into the dynamic behavior of lignin model compounds inside laccase, giving new information on the molecular basis of laccase for lignin degradation.

In previous studies, root-mean-square deviation (RMSD) was often employed to measure the stability of a structure during simulation [39, 40]. Backbone RMSD calculations were carried out on each complex at every 10 ps during whole simulation, and results were showed in Fig. 3. The RMSD values of all analyzed complexes quickly tended to reach equilibrium, with maximum deviation being less than 2.9 Å (RMSD values: <2.6 Å for LAC-DI, <2.5 Å for LAC-FE, <2.9 Å for LAC-GU, <2.3 Å for LAC-SI and <2.8 Å for LAC-VA). This indicated that laccase could maintain stability when binding to lignin model compounds. RMSD for LAC-DI became stable around 2400 ps, and remained for the rest of simulation time, while RMSD for LAC-FE initially increased to about 0.18 nm, and then was at stable level. From Fig. 3, it was found that for LAC-GU, the RMSD value was 0.21 nm at 4000 ps, and hold steady until the end. RMSD values for LAC-SI and LAC-VA became stable after 7000 ps and 3200 ps, respectively.

The radius of gyration (Rg) reflects the compaction of a structure [41]. Rg of any an analyzed complex did not fluctuate sharply during whole simulation excerpt for the initial stage with a relatively fast increase (Fig. 4). The average Rg for LAC-DI, LAC-FE, LAC-GU, LAC-SI

and LAC-VA was 2.213 nm, 2.216 nm, 2.217 nm, 2.218 nm and 2.222 nm, respectively.

RMSF was calculated to analyze the dynamics of C_α atoms from laccase, and was shown in Fig. 5. The C_α-RMSF value for LAC-DI was highest in residues 497. LAC-DI exhibited higher flexibility in regions composed of residues 68-78, 353-366, 424-439 and 487-500. Fluctuation in residues 171-198 261-300, 350-370, 424-439 and 487-500 for LAC-GU was greater than other regions. The largest fluctuation for LAC-GU was found in residues 169-186, 260-275, 425-440 and 484-500. As seen in the C_α-RMSF plot, key fluctuations for LAC-SI occurred around the residues 148-187, 427-439 and 488-500. The maximum fluctuations for LAC-VA were found in residues 463-500.

Discussion

Biodegradation technology is becoming more and more attractive for environmental remediation due to its environmentally friendly nature [42]. Laccase-based applications for lignin waste degradation is a good example [3, 43, 44]. To increase the lignin-degrading efficiency of laccase, previous studies investigated the impact of substrate structure on laccase-mediated oxidation rate [12], the stability of five fungal laccases in organic solvents [13] and bioconversion of substrates by *Rhodococcus opacus* DSM 1069 and PD630 [14], using lignin model compounds. The lignin-degrading efficiency of laccase was largely related to the properties of enzyme and substrates [12], such as their binding property [22]. The stability and catalytic activities of laccase was potentially influenced by the binding modes between it and its substrates. However, the detailed interaction mechanism between laccase and lignin is still unclear, limiting laccase application in lignin degradation to some extent. Thus, the illustration of interaction between laccase and lignin model compounds is important. Molecular simulations such as molecular docking and MD simulation have proved to be a robust technology for the analyses of intermolecular interactions [41, 45-49]. This article performed an investigation of the molecular basis of laccases for lignin degradation, using

molecular docking and molecular dynamics simulations. We showed that the present protocol was capable of giving a molecular insight into interaction of laccase with lignin model compounds, and in this way we found several rules that may be important to lignin degradation.

It was showed that lignin model compounds bound to laccase by a wide range of interaction energies. Guaiacol had the worst interaction with laccase, whereas sinapic acid had the best interaction with laccase, because lower interaction energy often represents better interaction [38].

On the one hand, laccase formed H-bonds with 2,6-dimethoxyphenol, ferulic acid and sinapic acid, but not with vanillyl alcohol and guaiacol. On the other hand, laccase had hydrophobic contacts with all analyzed lignin model compounds. Therefore, we proposed that H-bonds were alternative, but hydrophobic contacts were necessary to the interaction of laccase with lignin model compounds or lignin.

Mean backbone RMSD values for different complexes varied (LAC-DI: 0.199 nm; LAC-FE: 0.190 nm; LAC-GU: 0.211 nm; LAC-SI: 0.178 nm; LAC-VA: 0.220 nm). It not only meant stable behavior of these complexes, but also indicated that the stability was different between various complexes. The stability of each complex was also confirmed by Rg and C_α-RMSF. The fluctuation of Rg was not very sharp, reflecting well the compaction of analyzed structures [23]. The dominant fluctuated regions were often inconsistent between these surveyed complexes as shown in C_α-RMSF plot. Overall MD simulation results showed that LAC-DI, LAC-FE, LAC-GU, LAC-PC, LAC-SI and LAC-VA were reasonably stable during simulation.

Noteworthy, steric hindrance might occur in the lignin-degrading process mediated by laccase when the substrate was real lignin due to the fact that lignin was bulky and high molecular weight aromatic compounds. Fortunately, it was suggested that the introduction of

mediators could solve this steric hindrance problems [50]. We will simulate the effect of mediators on the interaction of laccase with lignin by MD simulations in the future.

Conclusion

This study explored the molecular basis of laccase for lignin degradation by comparing the interaction of *T. versicolor* laccase with lignin based on five lignin model compounds, using molecular docking and MD simulations. The best poses were adopted on the basis of MolDock score and Re-Rank score. Hydrophobic contacts were necessary, but H-bonds were alternative, to the interaction of laccase with lignin model compounds or lignin. The interaction energies varied in a wide range. MD simulation results from RMSD, C_α-RMSF and Rg showed that the systems composed of laccase and lignin model compounds were highly stable. The present study provides dynamic and structural information on the interaction mechanism between laccase and lignin, being useful to develop new laccases with high lignin-degrading ability in the field of environmental protection and industrial applications.

Conflicts of interest

Authors declare that they have no competing interests.

Acknowledgments

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References

1. T. D. Bugg, M. Ahmad, E. M. Hardiman and R. Rahmanpour, *Nat. Prod. Rep.*, 2011, 28, 1883-1896.
2. A. J. Ragauskas, G. T. Beckham, M. J. Biddy, R. Chandra, F. Chen, M. F. Davis, B. H. Davison, R. A. Dixon, P. Gilna and M. Keller, *Science*, 2014, 344, 1246843.
3. S. Majumdar, T. Lukk, J. O. Solbiati, S. Bauer, S. K. Nair, J. E. Cronan and J. A. Gerlt, *Biochemistry*, 2014, 53, 4047-4058.
4. C. Feng, G. Zeng, D. Huang, S. Hu, M. Zhao, C. Lai, C. Huang, Z. Wei and N. Li, *Process Biochem.*, 2011, 46, 1515-1520.
5. G. Zeng, M. Chen and Z. Zeng, *Nature*, 2013, 499, 154-154.
6. G. Zeng, M. Chen and Z. Zeng, *Science*, 2013, 340, 1403-1403.
7. J.-L. Gong, B. Wang, G.-M. Zeng, C.-P. Yang, C.-G. Niu, Q.-Y. Niu, W.-J. Zhou and Y. Liang, *J. Hazard. Mater.*, 2009, 164, 1517-1522.
8. P. Xu, G. M. Zeng, D. L. Huang, C. L. Feng, S. Hu, M. H. Zhao, C. Lai, Z. Wei, C. Huang and G. X. Xie, *Sci. Total Environ.*, 2012, 424, 1-10.
9. V. Mendu, T. Shearin, J. E. Campbell, J. Stork, J. Jae, M. Crocker, G. Huber and S. DeBolt, *Proc. Natl. Acad. Sci. U.S.A*, 2012, 109, 4014-4019.
10. Q. Bu, H. Lei, A. H. Zacher, L. Wang, S. Ren, J. Liang, Y. Wei, Y. Liu, J. Tang and Q. Zhang, *Bioresour. Technol.*, 2012, 124, 470-477.
11. P. J. Deuss and K. Barta, *Coord. Chem. Rev.*, 2015, http://dx.doi.org/10.1016/j.ccr.2015.1002.1004.
12. M. Lahtinen, K. Kruus, H. Boer, M. Kemell, M. Andberg, L. Viikari and J. Sipila, *J. Mol. Catal. B: Enzym.*, 2009, 57, 204-210.
13. P. Maijala, M. L. Mattinen, P. Nousiainen, J. Kontro, J. Asikkala, J. Sipila and L. Viikari, *J. Mol. Catal. B: Enzym.*, 2012, 76, 59-67.
14. M. Kosa and A. J. Ragauskas, *Appl. Microbiol. Biotechnol.*, 2012, 93, 891-900.
15. N. J. Christensen and K. P. Kepp, *PLoS ONE*, 2013, 8, e61985.
16. T. D. Bugg, M. Ahmad, E. M. Hardiman and R. Singh, *Curr. Opin. Biotechnol.*, 2011, 22, 394-400.
17. A. I. Cañas, M. Alcalde, F. Plou, M. J. Martínez, Á. T. Martínez and S. Camarero, *Environ. Sci. Technol.*, 2007, 41, 2964-2971.
18. P. Strong and H. Claus, *Crit. Rev. Environ. Sci. Technol.*, 2011, 41, 373-434.
19. J. Margot, J. Maillard, L. Rossi, D. A. Barry and C. Holliger, *New Biotechnol.*, 2013, 30, 803-813.

20. C. Lai, G. M. Zeng, D. L. Huang, M. H. Zhao, H. L. Huang, C. Huang, Z. Wei, N. J. Li, P. Xu, C. Zhang and G. X. Xie, *Int. Biodeterior. Biodegrad.*, 2013, 82, 180-186.
21. M. Kolb, V. Sieber, M. Amann, M. Faulstich and D. Schieder, *Bioresour. Technol.*, 2012, 104, 298-304.
22. M. Chen, G. Zeng, Z. Tan, M. Jiang, H. Li, L. Liu, Y. Zhu, Z. Yu, Z. Wei, Y. Liu and G. Xie, *PLoS ONE*, 2011, 6, e25647.
23. C. B. Jalkute, S. H. Barage, M. J. Dhanavade and K. D. Sonawane, *Protein J.*, 2013, 32, 356-364.
24. H. N. Banavath, O. P. Sharma, M. S. Kumar and R. Baskaran, *Sci. Rep.*, 2014, 4.
25. G. Festa, F. Autore, F. Fraternali, P. Giardina and G. Sannia, *Proteins*, 2008, 72, 25-34.
26. N. Gupta, F. S. Lee and E. T. Farinas, *J. Mol. Catal. B: Enzym.*, 2010, 62, 230-234.
27. K. Piontek, M. Antorini and T. Choinowski, *J. Biol. Chem.*, 2002, 277, 37663-37669.
28. P. W. Rose, A. Prlić, C. Bi, W. F. Bluhm, C. H. Christie, S. Dutta, R. K. Green, D. S. Goodsell, J. D. Westbrook and J. Woo, *Nucleic Acids Res.*, 2015, 43, D345-D356.
29. H. E. Pence and A. Williams, *J. Chem. Educ.*, 2010.
30. R. Thomsen and M. H. Christensen, *J. Med. Chem.*, 2006, 49, 3315-3321.
31. R. A. Laskowski and M. B. Swindells, *J. Chem. Inf. Model.*, 2011, 51, 2778-2786.
32. A. K. Malde, L. Zuo, M. Breeze, M. Stroet, D. Poger, P. C. Nair, C. Oostenbrink and A. E. Mark, *J. Chem. Theory Comput.*, 2011, 7, 4026-4037.
33. B. Hess, C. Kutzner, D. van der Spoel and E. Lindahl, *J. Chem. Theory Comput.*, 2008, 4, 435-447.
34. B. Hess, H. Bekker, H. J. Berendsen and J. G. Fraaije, *J. Comput. Chem.*, 1997, 18, 1463-1472.
35. M. Parrinello and A. Rahman, *J. Appl. Phys.*, 1981, 52, 7182-7190.
36. G. Bussi, D. Donadio and M. Parrinello, *J. Chem. Phys.*, 2007, 126, 014101.
37. T. Darden, D. York and L. Pedersen, *J. Chem. Phys.*, 1993, 98, 10089.
38. J. O. Giacoppo, D. T. Mancini, A. P. Guimaraes, A. S. Goncalves, E. F. da Cunha, T. C. Franca and T. C. Ramalho, *Eur. J. Med. Chem.*, 2015, 91, 63-71.
39. X. Wang, H. Yang, X. Hu, X. Zhang, Q. Zhang, H. Jiang, W. Shi and H. Yu, *Environ. Sci. Technol.*, 2013, 47, 11802-11809.
40. J. Jayakumar and S. Anishetty, *Bioorg. Med. Chem. Lett.*, 2014, 24, 2098-2104.
41. C. B. Jalkute, S. H. Barage and K. D. Sonawane, *RSC Adv.*, 2015, 5, 10488-10496.
42. C. Sánchez, *Biotechnol. Adv.*, 2009, 27, 185-194.
43. L. Munk, A. K. Sitarz, D. C. Kalyani, J. D. Mikkelsen and A. S. Meyer, *Biotechnol. Adv.*, 2015, 33, 13-

24.

44. S. R. Couto and J. L. T. Herrera, *Biotechnol. Adv.*, 2006, 24, 500-513.

45. K. M. Kumar, A. Anbarasu and S. Ramaiah, *Mol. Biosyst.*, 2014, 10, 891-900.

46. M. Rajasekaran and C. P. Chen, *Int. J. Biol. Macromol.*, 2012, 51, 305-313.

47. M. H. Fazil, S. Kumar, R. Farmer, H. P. Pandey and D. V. Singh, *J. Mol. Model.*, 2012, 18, 1-10.

48. M. T. Cambria, D. Di Marino, M. Falconi, S. Garavaglia and A. Cambria, *J. Biomol. Struct. Dyn.*, 2010, 27, 501-509.

49. S. Kannan, A. Poulsen, H. Y. Yang, M. Ho, S. H. Ang, T. S. Eldwin, D. A. Jeyaraj, L. R. Chennamaneni, B. Liu, J. Hill, C. S. Verma and K. Nacro, *Biochemistry*, 2015, 54, 32-46.

50. M. A. Tadesse, A. D'Annibale, C. Galli, P. Gentili and F. Sergi, *Org. Biomol. Chem.*, 2008, 6, 868-878.

Figure legend

Fig. 1. Binding orientation of lignin model compounds in laccase. A, LAC-DI; B, LAC-FE; C, LAC-GU, D, LAC-SI; E, LAC-VA.

Fig. 2. Interaction of laccase with (A) 2,6-dimethoxyphenol, (B) ferulic acid, (C) guaiacol, (D) sinapic acid and (E) vanillyl alcohol.

Fig. 3. Backbone RMSD for LAC-DI, LAC-FE, LAC-GU, LAC-SI and LAC-VA.

Fig. 4. Radius of gyration for LAC-DI, LAC-FE, LAC-GU, LAC-SI and LAC-VA.

Fig. 5. C_{α}-RMSF for LAC-DI, LAC-FE, LAC-GU, LAC-SI and LAC-VA.

Table 1. Interaction energies, docking accuracy, MolDock score and Re-Rank score for laccase with lignin model compounds.

Complex	Interaction energy (kcal mol^{-1})	Re-docking RMSD (Å)	MolDock score	Re-Rank score
LAC-DI	-89.931	0.043	-75.925	-69.628
LAC-FE	-111.765	0.015	-103.472	-90.888
LAC-GU	-78.992	0.009	-67.442	-60.550
LAC-SI	-112.174	0.463	-101.184	-91.124
LAC-VA	-93.467	0.014	-83.321	-71.087

LAC-DI, complex composed of laccase and 2,6-dimethoxyphenol; LAC-FE, complex composed of laccase and ferulic acid; LAC-GU, complex composed of laccase and guaiacol; LAC-SI, complex composed of laccase and sinapic acid; LAC-VA, complex composed of laccase and vanillyl alcohol.

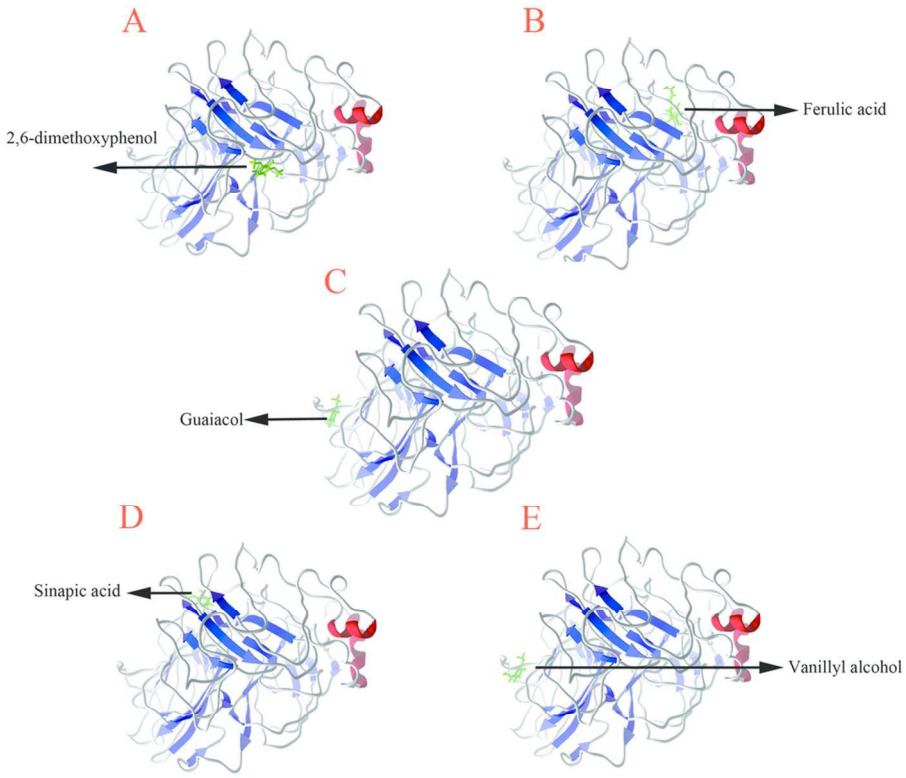

Fig. 1. Binding orientation of lignin model compounds in laccase. A, LAC-DI; B, LAC-FE; C, LAC-GU, D, LAC-SI; E, LAC-VA.
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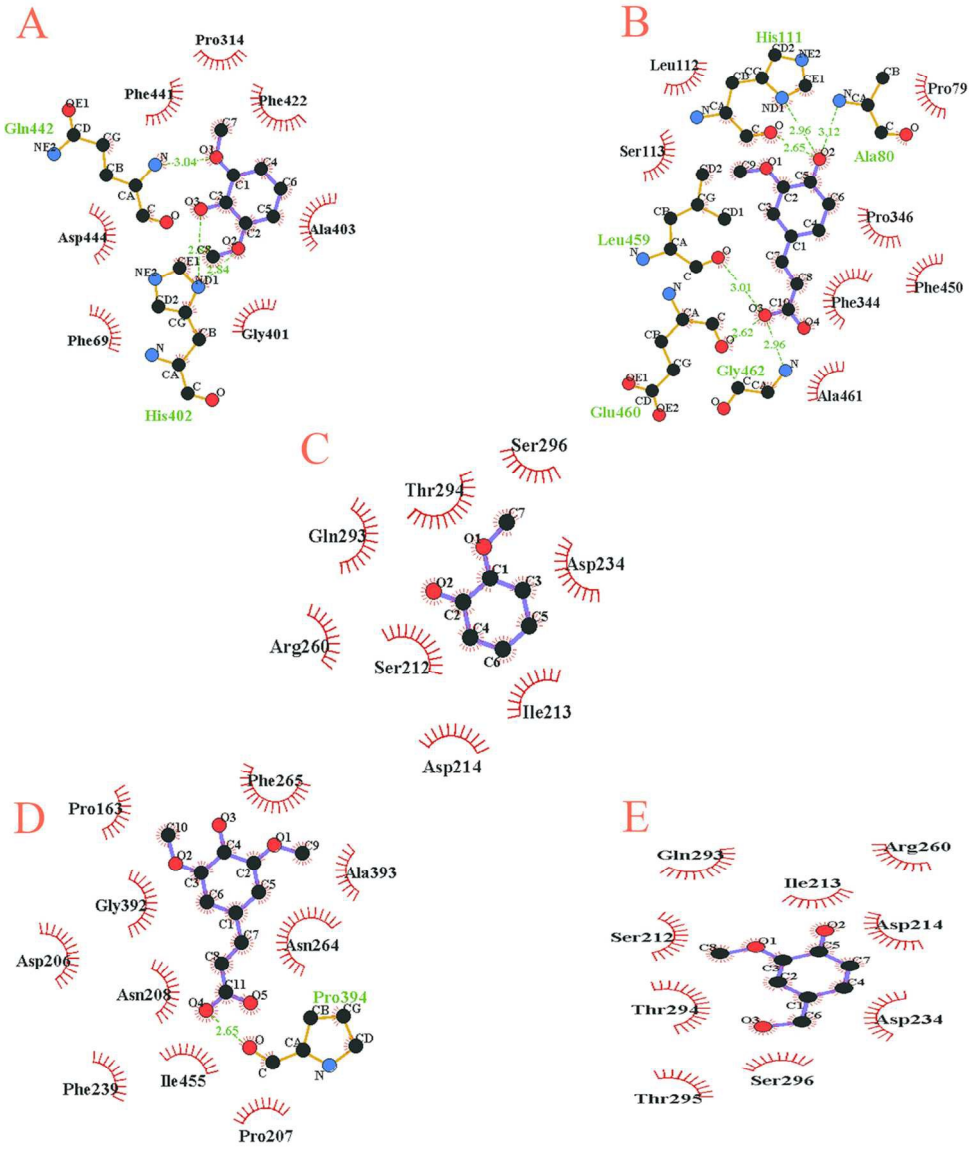

Fig. 2. Interaction of laccase with (A) 2,6-dimethoxyphenol, (B) ferulic acid, (C) guaiacol, (D) sinapic acid and (E) vanillyl alcohol.
111x131mm (300 x 300 DPI)

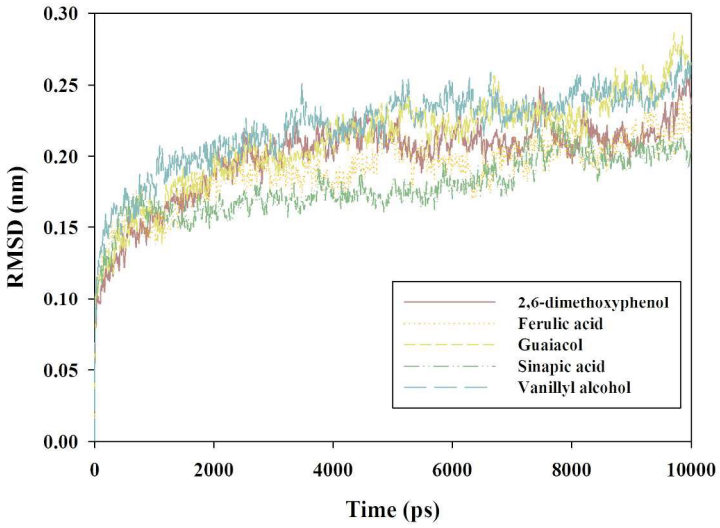

Fig. 3. Backbone RMSD for complexes of laccase with lignin model compounds. LAC-DI, complex composed of laccase and 2,6-dimethoxyphenol; LAC-FE, complex composed of laccase and ferulic acid; LAC-GU, complex composed of laccase and guaiacol; LAC-SI, complex composed of laccase and sinapic acid; LAC-VA, complex composed of laccase and vanillyl alcohol.
209x186mm (300 x 300 DPI)

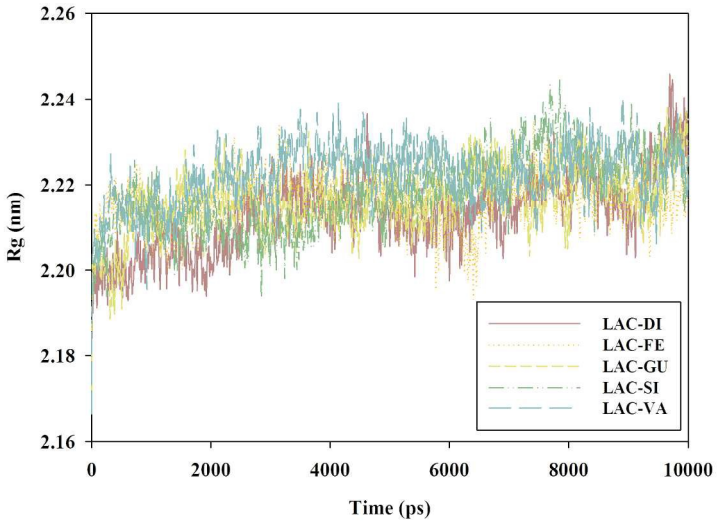

Fig. 4. Radius of gyration for complexes of laccase with lignin model compounds. Rg, radius of gyration; LAC-DI, complex composed of laccase and 2,6-dimethoxyphenol; LAC-FE, complex composed of laccase and ferulic acid; LAC-GU, complex composed of laccase and guaiacol; LAC-SI, complex composed of laccase and sinapic acid; LAC-VA, complex composed of laccase and vanillyl alcohol.
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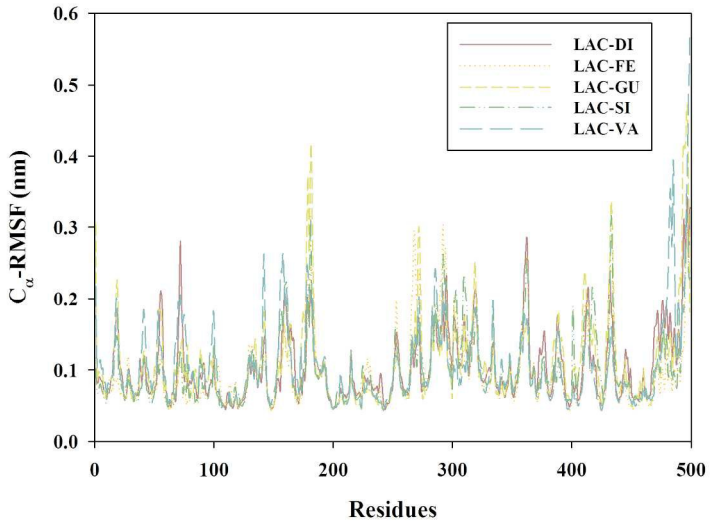

Fig. 5. Cα-RMSF for complexes of laccase with lignin model compounds. LAC-DI, complex composed of laccase and 2,6-dimethoxyphenol; LAC-FE, complex composed of laccase and ferulic acid; LAC-GU, complex composed of laccase and guaiacol; LAC-SI, complex composed of laccase and sinapic acid; LAC-VA, complex composed of laccase and vanillyl alcohol.
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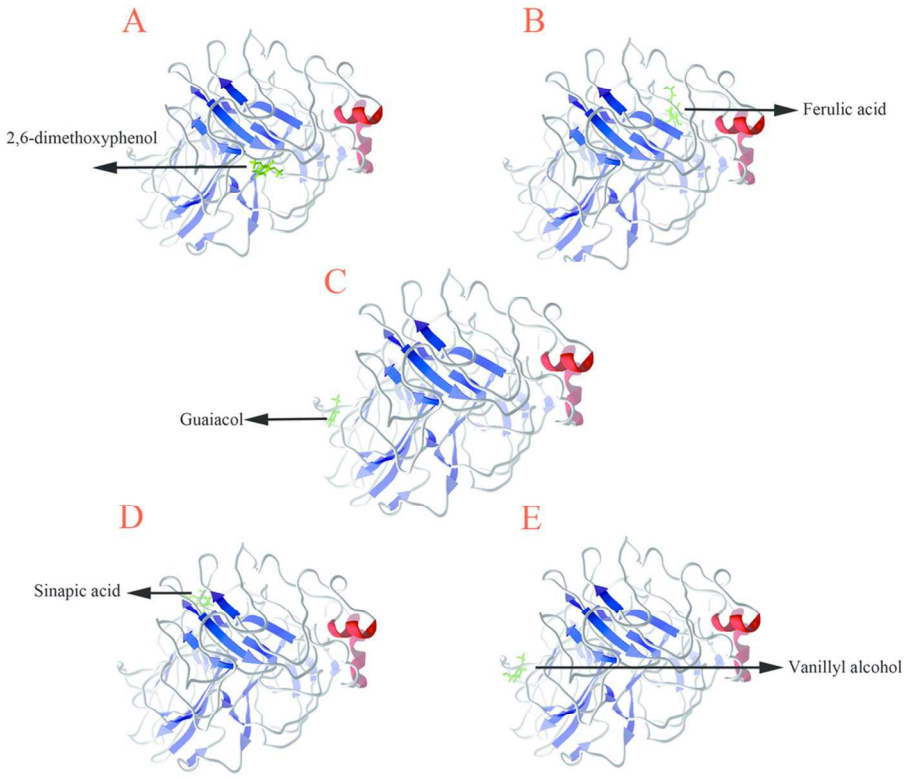

Graphic Abstract
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