Supporting information for

Degradation of acenaphthylene and anthracene by chemical modified laccase from Trametes versicolor

Yulong Liu[a], Xiufu Hua [b,c,*]

[a] Department of Basic Teaching, Yancheng Institute of Technology, Yancheng, 224051, China

[b] Department of Scientific Research and Development, Tsinghua University, Beijing ,100084, China

[c] Nanjing Ruhan Biotech Co., Ltd. Nanjing, 210012, China

*Corresponding author

Department of Scientific Research and Development, Tsinghua University, Beijing, 100084, China. E-mail address: hua_xiufu@163.com, TEL.: 86-10-6279-2750

Enzyme Activity Assay. The activity of free laccase and MA-Lac was determined by using ABTS as substrate in a 1ml reaction mixture containing 20μl of 50mM ABTS in 0.05M phosphate buffer, pH 3.0 and 50μl of enzyme. The assay was performed

spectrophotometrically by measuring the absorbance increase at 420nm at room temperature [1].

Chemical Modification. The procedure for the chemical modification of maleic anhydride was as report [2, 3]. During a run, 1.0ml of 5 g/l laccase in 50 mM phosphate buffer (pH 7.0) and 0.15ml of 10 g/l maleic anhydride in DMSO were mixed and the reaction was preceded at 4°C for 1h. The product solution was dialyzed against 25mM Tris-HCl buffer (pH 7.5) at 4°C to removal excess reagent and prepare for purifying by anion-exchange chromatography.

Eluting Process. The dialyzed solution was loaded on a HiTrap DEAE FF (1ml column) which was preequilibrated with the same buffer (25mM Tris-HCl buffer with pH 7.5). Then washing the bound material out of the column was by eluting with the buffer added 1M NaCl solution with the elution rate of 1 ml/min. Each collected fraction (1ml) of the free laccase and MA-Lac was analyzed for enzyme activity, respectively.

Free Amino Group Determination. Determination of the numbers of the free amino group of laccasse after chemical modification was performed using trinitrobenzenesulfonic acid (TNBS) method, as described in reference [4, 5].

Fluorescence emission spectrum Assay. Fluorescence emission spectrum was performed using a Hitachi F-2500 spectroflurimeter, in which the excitement wavelength was 280nm and the emission spectra was recorded in a wavelength range of

250-500nm. CD measurements (190-250nm) were performed in a quartz cuvette with a 2mm path length on a JASCO-715 spectropolarimeter (Japan). The CD data were expressed in terms of mean residue ellipticity, [θ], in deg·cm^2/dmol.

Stabilization Assay. Stabilization of laccasse at an acidic condition of free and chemical modified laccases was examined by incubating samples in the pH 3.5 using 20mM di-sodium hydrogen phosphate-citric acid as buffer at 30°C. At predetermined time intervals, appropriate aliquots of the enzyme were withdrawn and their residual activity was assayed as mentioned method. Thermal stability of the free and chemical modified laccase was compared at 55°C at pH 7.0. The structure changes of the free and chemical modified laccases were illuminated by the fluorescence intensity to detect the changes in tertiary.

PAH Oxidation. Acenaphthylene and anthracene were powdered in a mortar and pestle respectively, and 100mg of each PAH was dissolved in 10ml of acetone and added to a solution of 5ml Tween 80, for increasing PAH bioavailability, in 50ml of distilled water. The homogenization of each PAH was obtained with the help of an ultrasonic bath and diluted to a final volume of 200ml with distilled water. 1ml of this solution, containing 0.5mg of each PAH, was added to the reactor [6].

During a run, 1.5ml of enzyme solution 0.1g/l dissolved in 20mM di-sodium hydrogen phosphate-citric acid buffer (pH 4.5), was mixed with a 1.0ml aliquot of PAH stock solution. The influence of ABTS on PAHs degradation was determined by adding

it to some treatments to a final concentration of 1.0mM. The reactors were incubated on a horizontal shaker at 30°C (150rpm) in dark for 72 h.

The sample was prepared by injecting 0.1ml reaction solution into 0.8ml solution of methanol/water (4:1, v/v) and analyzed by HPLC with a reverse phase C-18 column. Elution was methanol/water (4:1, v/v) at 40°C, at 1.0ml·min-1. And the eluate was monitored at 270nm. The retention times of acenaphthylene and anthracene were 12.2 and 14.6 min, respectively. The percentage of each PAH oxidized was calculated from the difference between each PAH level in the experimental assay and the corresponding control. All treatments, including controls, were replicated three times.

Kinetic studies. As for acenaphthylene, kinetic parameters Km, Kcat and Kcat/Km were 8.2 $mmol \cdot l^{-1}$, 1.26×10^6 s^{-1} and, 1.54×10^5 s^{-1} $mmol \cdot l^{-1}$ for the free enzyme and 5.4 $mmol \cdot l^{-1}$, 1.02×10^6 s^{-1} and, 1.94×10^5 s^{-1} $mmol \cdot l^{-1}$ for the modified enzyme. As for anthracene, kinetic parameters Km, Kcat and Kcat/Km were 23.5 $mmol \cdot l^{-1}$, 3.84×10^6 s^{-1} and, 1.63×10^5 s^{-1} $mmol \cdot l^{-1}$ for the free enzyme and 14.8 $mmol \cdot l^{-1}$, 4.02×10^6 s^{-1} and, 2.72×10^5 s^{-1} $mmol \cdot l^{-1}$ for the modified enzyme. The larger value of Kcat/Km indicates the higher substrate affinity. Additional, in the redox mediator, the kinetic parameters could not be determined because of the PAH was not a single substrate.

References

1. C. Johannes, A. Majcherczyk, J. Biotechnol. 2000, 78, 193.
2. R.K. Bund, R.S. Singhal, Carbohydr. Polym. 2002, 47, 137.
3. Y. Shin-ya, H.N. Aye, K.J. Hong, T. Kajiuchi, Enzyme Microb. Technol. 2005, 36, 147.

4. L. Banci, S. Ciofi-Baffoni, M. Tien, Biochem. 1999, 38, 3205.

5. A. Majcherczyk, C. Johannes, A. Huttermann, Enzyme Microb. Technol. 1998, 22, 335.

6. A. Schützendübel, A. Majcherczyk, C. Johannes, A. Hüttermann, Int. Biodeter. Biodegr. 1999, 43, 93.