Electronic Supplementary Material (ESI) for Catalysis Science & Technology

Supplementary Information

Construction of biocatalysts using the myoglobin scaffold for the synthesis of indigo from indole

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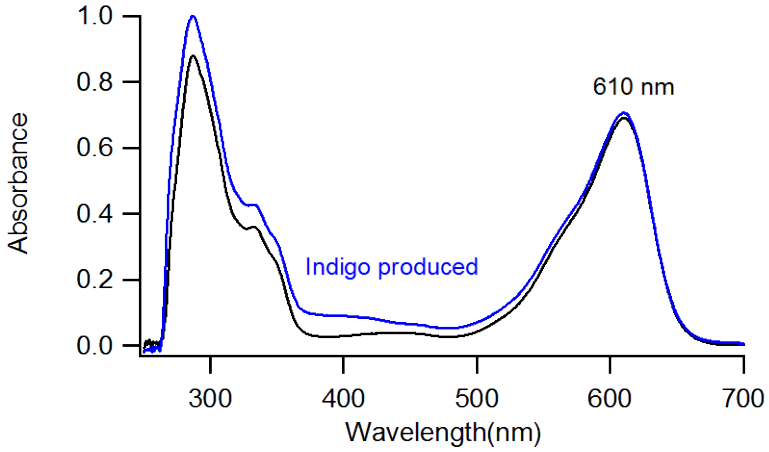

Figure S1. UV–visible spectrum of the purified product with a retention time 16.0 min on HPLC. The product was obtained by indole oxidation using H64D/V68I Mb mutant. The UV–visible spectrum was recorded in DMF at 25°C. The concentration of indigo for taking the reference curve is 43 μM.

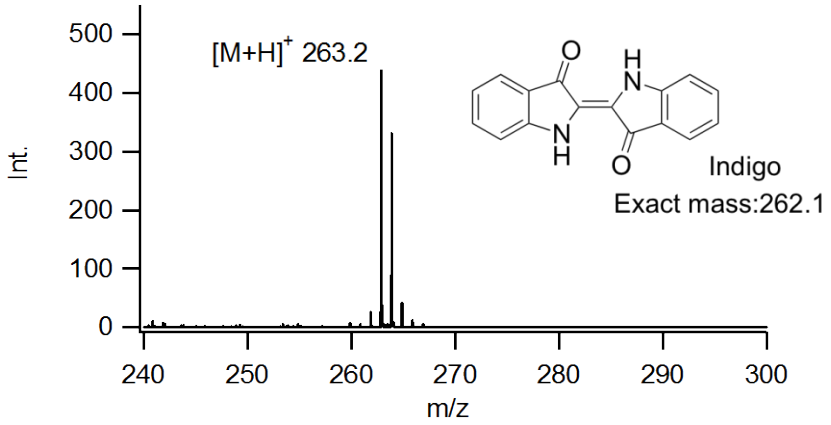

Figure S2. The MALDI-TOF mass spectrum of the purified product with a retention time 16.0 min on HPLC. The product was obtained by indole oxidation using H64D/V68I Mb mutant. Mass spectrometric analysis was carried out using dithranol (1, 8, 9-anthracenetriol) as a matrix.

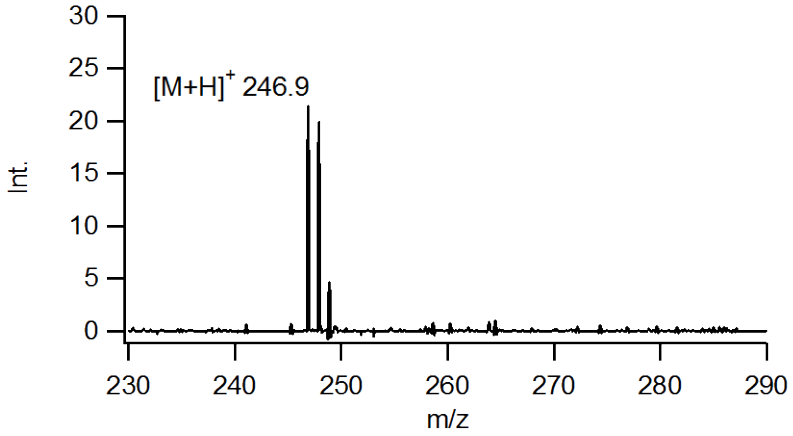

Figure S3. The MALDI-TOF mass spectrum of the purified product with a retention time 21.7 min on HPLC. Mass spectrometric analysis was carried out using dithranol (1, 8, 9-anthracenetriol) as a matrix.

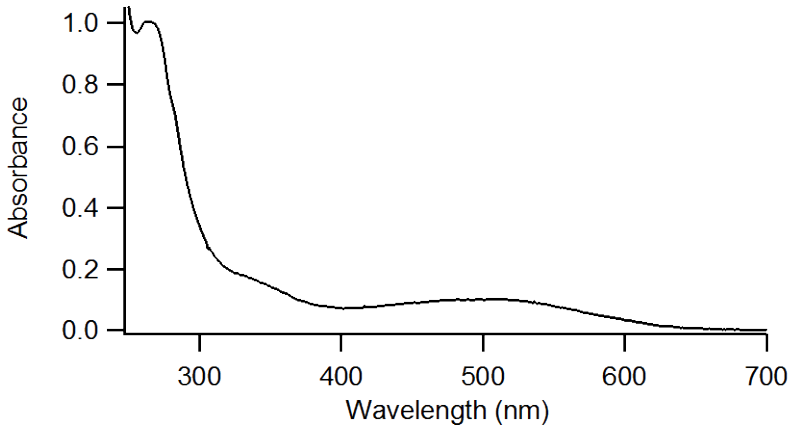

Figure S4. UV–visible spectrum of the purified product with a retention time 21.7 min on HPLC. The UV–visible spectrum was recorded in a 1:1 mixture of acetonitrile and 20 mM potassium phosphate buffer solution (pH 7.0) at 25°C.

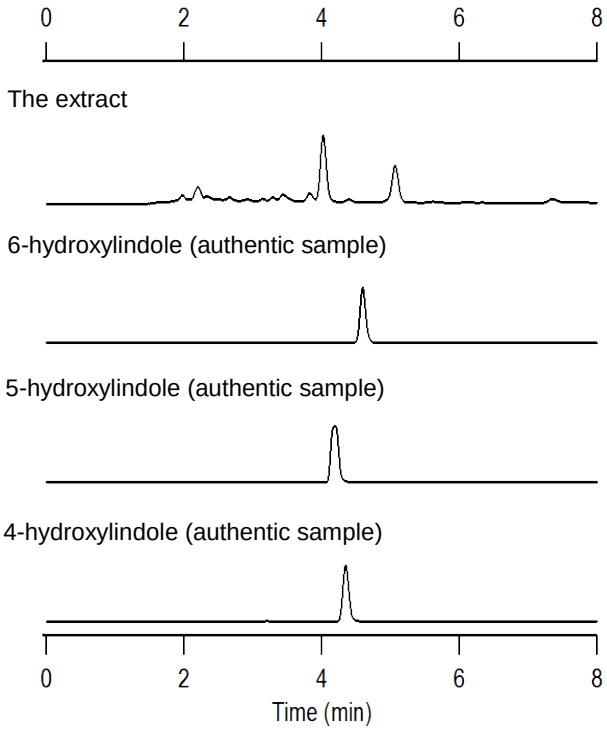

Figure S5. HPLC chart of 4-hydroxyindole, 5-hydroxyindole, and 6-hydroxyindole (monitored at 280 nm). HPLC analysis of extract of reaction mixture catalyzed by H64D/V68I mutant is also shown (only first 8 mins). HPLC analysis was carried out on a Shimadzu LC-10AD equipped with a SPD-10A UV-visible detector and a 4.6 mmΦ× 250 mm intersil ODS 3 reversed-phase HPLC column. The column was eluted for 25 min isocratically using a 1:1 mixture of acetonitrile and 20 mM potassium phosphate buffer solution (pH 7.0) at a flow rate of 1 ml/min at 30°C. The retention time for the authentic samples is: 4-hydroxyindole (4.3 min), 5-hydroxyindole (4.2 min), and 6-hydroxyindole (4.6 min).

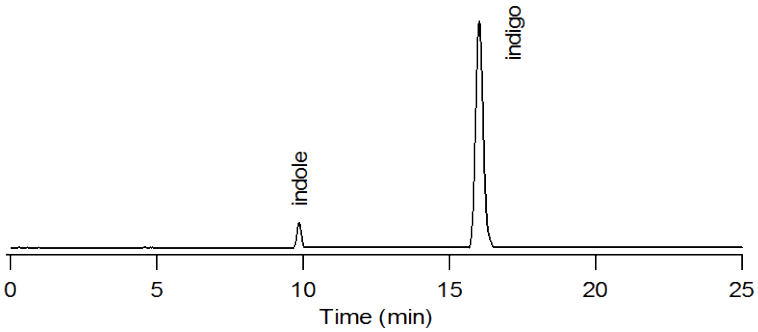

Figure S6. HPLC chart of the precipitate in the indole oxidation catalyzed by H64D/V68I mutant (monitored at 280 nm). The elution conditions are the same as above.

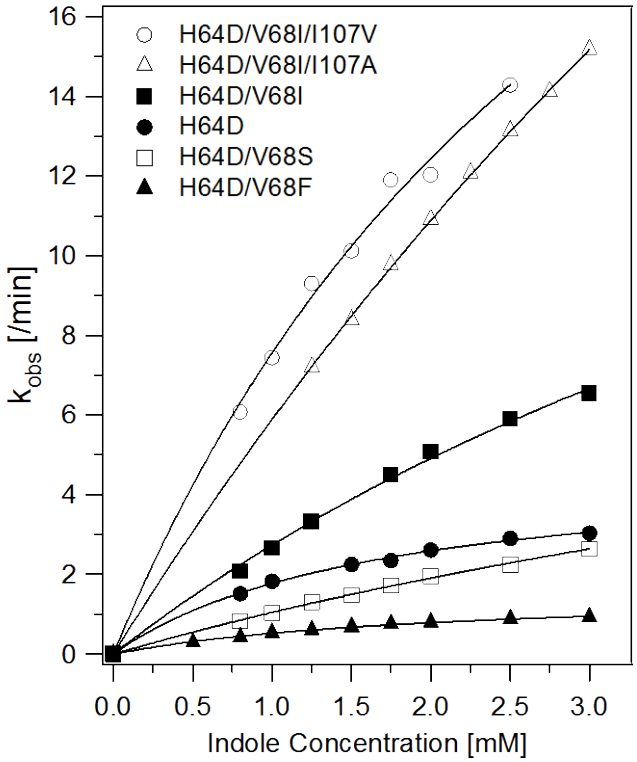

Figure S7. The plots of catalytic reaction rate of the indole formation by a series of Mb mutants against the concentration of indole.