Laccase-catalyzed synthesis of aniline oligomers and their application for the protection of copper against corrosion

Galina P. Shumakovich[a], Grigory V. Otrokhov[a], Maria E. Khlupova[a], Irina S. Vasil'eva[a], Elena A. Zaitseva[b], Olga V. Morozova[a] and Alexander I. Yaropolov[a*]

[a] *Laboratory of Chemical Enzymology, A. N. Bach Institute of Biochemistry, Russian Academy of Sciences, Leninskii pr. 33, 119071 Moscow, Russia*
E-mail: yaropolov@inbi.ras.ru; Tel: +7 (495) 954 4477; Fax: +7 (495) 954 2732
[b] *Department of Chemistry, Moscow State University, 119991 Moscow, Russia*

Electronic Supplementary Information

1. General remarks

Aniline (Labtech, Russia) was twice distilled under a reduced pressure before use. Sodium dodecylbenzenesulfonate (SDBS), sodium hydroxide, Na_2HPO_4, citric acid (Riedel-deHaen, Germany), tetrahydrofuran (VWR, Austria) were used without further purification. Copper foil (99.99 %) and S-10-camphorsulfonic acid were purchased from Aldrich. Laccase was isolated from the culture liquid of the fungus *Trametes hirsuta* (Wulfen Pilat CF-28) as described in [Gorshina et al., 2006].

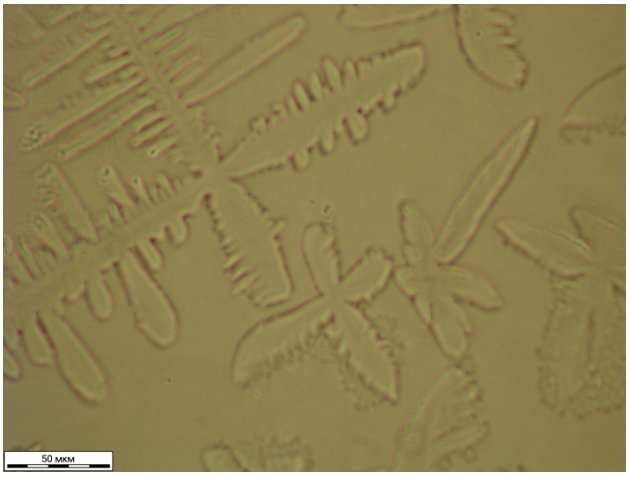
2. Aniline polymerization

a. A reaction solution containing equimolar concentrations of aniline (AN) monomer and SDBS (10 mM) in 0.1M sodium-citrate phosphate buffer (pH 3.5) was stirred for 1 h. The enzymatic synthesis of polyaniline was initiated by adding laccase to a final concentration of 0.4 U/ml. Atmospheric oxygen served as oxidant. The pH value of the reaction solution was constant.

b. A reaction solution containing aniline (150 mM) and S-camphorsulfonic acid (155 mM) in deionized water, pH 2.8 (without any buffer compounds) was stirred for 30 min. The polymerization was initiated by adding laccase to a final concentration of 0.4 U/ml. The enzymatic synthesis was carried out for 10 h under air saturated conditions with continuous

stirring and then stopped by adding 3% aqueous ammonia. The water insoluble aniline oligomers were separated by the centrifugation. The precipitate was washed several times with deionized water and dried.

3. SDBS/AN^+ complexes in buffer solution imaged with phase contrast microscopy using an Olympus BX41 Microscope.



Conditions: 0.1 M sodium citrate-phosphate buffer (pH 3.5), [AN]=[SDBS]=10 мМ.

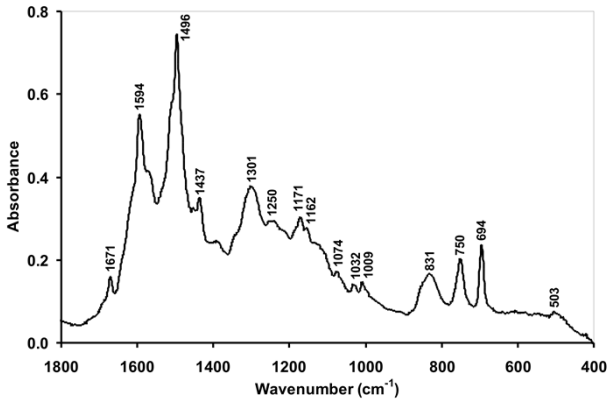
4. UV-vis and electrochemical measurements of aniline polymerization

The guide-template aniline polymerization was studied by UV-vis spectroscopy using a Shimadzu UV1240 mini spectrophotometer and by measuring the redox potential of the reaction mixture using a BAS CV-50W analyzer. A gold wire and Ag/AgCl electrode (BAS) were used as the working electrode and the reference electrode, respectively.

5. MALDI-TOF and FTIR analyses

MALDI-TOF spectra of dedoped aniline oligomers were recorded in the reflection mode using a Brucker Daltonocs Micriflex mass spectrometer, which was calibrated with peptides with known molecular masses from 700 to 3500 Da.

FTIR spectrum of dedoped products of the enzymatic aniline polymerization in KBr pellet was recorded with IR Prestige Fourier spectrophotometer (Shimadzu).



FTIR absorption spectrum of dedoped aniline oligomers prepared in SDBS micellar solution by laccase-catalyzed method.

6. Electrochemical measurements of copper corrosion

Copper corrosion was studied by linear voltammetry. The potential was scanned from -0.5 to 0.5 V *vs*. Ag/AgCl at a scan rate of 50 mV/s. Copper foil strips (2×10 mm) used as working electrode were first rubbed with emery paper, polished with AP-A alumina suspension (Struers), washed with deionized water, acetone and again with water, and then dried. The solution of aniline oligomers in THF (20 µl, 3 mg/ml) was continuously being poured on the pretreated copper electrode, which was then air dried. All the experiments were performed in aerated test solutions of 0.1 M HCl and 1 M NaCl, prepared with deionized water.

7. Weight- loss experiments

Weight loss was measured for copper strips (1x1 cm) for exposure period of 5 days in 10 ml of aerated 0.1 M HCl in the absence and presence of 1.5 mg/ml aniline oligomers. Weight loss (W_t) per unit area (S, mg·cm^{-2}), the corrosion rate (V) and the percentage of the inhibition efficiency (IC%) over the exposure time were calculated according to the following equations:

$$W_t = \frac{W_0 - W_5}{S} \qquad (1)$$

$$V = \frac{W_t}{T} \qquad (2)$$

$$IC\% = \left[\frac{V_2 - V_1}{V_2}\right] \times 100\,, \qquad (3)$$

where W_0 is the initial weight of the copper strip (mg); W_5 – the weight after immersion in the test electrolyte for 5 days; V_1 and V_2 – the corrosion rates ($mg \cdot cm^{-2} \cdot day^{-1}$) with and without corrosion inhibitor, respectively.

8. Determination of the laccase activity

The activity of laccase was measured spectrophotometrically, using 10 mM catechol as a chromogenic substrate ($\lambda = 410$ nm, ε = 740 $M^{-1}cm^{-1}$), at 24°C in 0.1M Na-citrate-phosphate buffer, pH 4.5. One unit of activity is defined as the amount of laccase oxidizing 1μm of substrate per min. The specific activity of the enzyme preparation was about 130 U/mg of protein.

9. References

E.S. Gorshina, T.V. Rusinova, V.V. Biryukov, O.V. Morozova, S.V. Shleev and A.I. Yaropolov, *Appl. Biochem. Microbiol.*, 2006, **42**, 558.