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

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A new method for the enzymatic synthesis of oligoaniline soluble in organic solutions is developed. The high-redox potential laccase from the fungus *Trametes hirsuta* is used as a biocatalyst and atmospheric oxygen serves as oxidant. Aniline oligomers showed a high inhibition of copper corrosion in aqueous HCl and NaCl solutions.

Biocatalysis is an environmentally friendly alternative to chemical production of different compounds, which meets the requirement of "white" biotechnology. Recently, enzyme-catalyzed polymerization has received much consideration, for this method enables the synthesis of products with new properties.[1]

Polyaniline (PANI) is an electrically conducting polymer, used to protect iron and steel against corrosion.[2] PANI has a great potential for practical applications, as it is environmentally stable and easily prepared. The low cost of aniline is another advantage.

What is more, the physico-chemical properties of PANI vary with the oxidation state and the degree of protonation. The polymer can be applied to separate physiologically active chiral substances, in chemical sensors and biosensors, energy accumulation and energy storage devices, electrochromic displays and antistatic coating.[3]

PANI is usually prepared by chemical or electrochemical oxidation of aniline.[4] Chemical polymerization is more widely used as the conducting polymer is deposited on the surface by *in situ* method. However, the chemical synthesis has serious drawbacks, since the reaction requires high amounts of oxidants, such as ammonium peroxydisulfate, ferric chloride or potassium dichromate,[5] proceeds in highly acidic solutions and has an autocatalytic character.[6] Besides, benzidine is a toxic by-product of polyaniline synthesis in highly acidic media.[7]

Electrochemical oxidation of aniline also has some limitations. The polymer cannot be deposited on nonconducting surfaces, the electrode surface is limited, and the synthesis should be performed in highly acidic solution.

Biocatalysis is an alternative method for polymerization of conducting PANI. In contrast to traditional chemical and electrochemical oxidation of aniline, the enzyme-catalyzed reaction proceeds under environmentally friendly and rather mild conditions without any toxic by-products.

Oxidoreductases are usually used as biocatalysts in this reaction,[8] and among them, laccases and peroxidases seem to be the most attractive enzymes for aniline polymerization. Water is the final product of oxidant reduction in the enzyme-catalyzed reactions.

It is noteworthy that chemically synthesized PANI is poorly processable since it is insoluble in common polar and nonpolar solvents. Therefore, PANI in the emeraldine oxidation state (base or salt) is used as a component of various paints or blends to prevent iron or steel against corrosion. At the same time, aniline oligomers with polymerization degree from 2 to 14 are soluble in certain organic solvents, e.g. tetrahydrofuran,[9] which enables their even depositing on metallic surfaces. Another shortcoming of the chemical synthesis of aniline is impossibility of producing great amounts of aniline oligomers in the emeraldine oxidation state, since the reaction proceeds *via* the stage at which aniline oligomers exist in pernigraniline oxidation state.[10]

Copper is considered to be one of the most preferred materials applicable to different industries due to its high electrical and thermal conductivity and mechanical processability.[11] Copper is widely used in electronic industry and communications, as well as in household appliances. However, the metal subjects to corrosion in aggressive media, in particular in the presence of chloride anions. Thus, the search for new efficient inhibitors for copper corrosion is a rather pressing problem. Inhibitors for copper corrosion comprise inorganic compounds, various classes of organic compounds and their derivatives, including azoles, amino acids, amines and others.[12] Aniline oligomers can find their use in preventing copper corrosion. Aniline oligomers with polymerization degree higher than 3 are hardly soluble in aqueous media, but they are soluble in certain organic solvents, which enables their depositing on the surface without using any binders.

The aim of the present work is the study of laccase-catalyzed synthesis of aniline oligomers soluble in organic solvents and demonstration of their efficiency in the protection of copper against corrosion.

The mechanism of chemical aniline polymerization is rather well studied. The chemical process monitored with the open-circuit potential (OCP) technique and by UV-vis spectroscopy is described in [10,13]. The polymerization may proceed upon pernigraniline chains to afford polymer chains of higher molecular weights. In the presence of peroxidisulfate these new polymer chains may be oxidized to pernigraniline again. The process proceeds until all the

oxidant is consumed. After that, the unreacted aniline reduces pernigraniline to the emeraldine oxidation state.

In contrast to chemical polymerization, the enzymatic reaction of aniline polymerization has hardly been investigated. In this study we used high-redox potential laccase from the fungus *Trametes hirsuta* as catalyst to synthesize PANI in an aqueous micellar solution of SDBS. In water solutions, SDBS can form stable micelles at concentrations higher than 1.6 mM, which is the critical micelle concentration. The formation of SDBS/AN^+ complexes (see Electronic Supplementary Information) occurs with the solution turbidity. As was shown[14] by Y.Haba and coworkes, mixing aniline and SDBS in an aqueous medium creates an insoluble crystalline SDBS/AN^+ complex. We have also found that without aniline laccase showed no catalytic activity in 10 mM SDBS micellar solution, when using catechol as substrate. Protonated aniline molecules may penetrate into the micelles and interact with negatively charged sulfo-groups of the surfactant, while the benzene rings of aniline may incorporate in the micelle hydrophobic pocket forming AN-SDBS complex, which prevents laccase denaturation by the surfactant.

Aniline polymerization was carried out at 25^{O}C for 12 h under aerobic conditions. As the reaction progressed, the dispersion changed from white *via* bluish green to deep green. Thus we prepared a stable water dispersion of oligoaniline/SDBS. The corresponding UV-vis spectrum is shown in Fig. 1 (curve 1). It includes three characteristic absorption bands observed at 325 - 360, 400 - 430 and 780 - 826 nm. The first absorption band arises from electron transitions within the aromatic rings. The second adsorption band is only found for pH<7 and represents the protonation stage of the PANI chains. The absorption around 750 - 780 nm is assigned to the presence of polaron resulting from the doping process.[15] In order to obtain aniline oligomers, the enzymatic reaction was stopped after 12 h by adding ethanol (*ca*. 50 v. %). The precipitate was separated by centrifugation, treated with 3% aqueous ammonia, washed with deionized water and dried for 48 h at 70^{O}C. Then the dried precipitate was dissolved in THF. A UV-vis absorption spectrum of enzymatically synthesized aniline oligomers in THF in the emeraldine oxidation state (dedoped form) is shown in Fig. 1 (curve 2). FTIR spectrum was recorded to characterize the final product in detail (see Electronic Supplementary Information). The spectrum of the dedoped oligoanilines has bands at 1594 cm^{-1} and 1496 cm^{-1} characteristic of C-C stretching vibration in quinoid diimine and benzenoid diamine units of the oligomers, respectively. The absorption bands at 1032 cm^{-1} and 1009 cm^{-1} (asymmetric and symmetric stretching of the sulfonate group of SDBS) are due to SDBS which could not be completely removed.[16]

It is essential that in contrast to chemical polymerization the laccase-catalyzed reaction of aniline polymerization can be stopped at the stage when aniline oligomers are in the emeraldine oxidation state.

The redox potential of the reaction solution increased by 7-10 mV within the first 5 min of laccase-catalysed reaction of aniline polymerization in aerobic conditions (Fig. 2, curve 1). After that the redox potential gradually decreased to ~ 290 mV for 25-30 min. The increase in the optical density of the reaction solution at 750 nm indicates the formation of an electroconducting polymer (Fig. 2, curve 2). Thus, in laccase-catalyzed polyaniline synthesis conducting chains of the polymer form in the emeraldine oxidation state without an induction period.

The analysis of the THF-dissolved products of laccase-catalysed anyline polymerization by MALDI-TOF method showed the presence of 2-7-unit aniline oligomers (Fig. 3). The peaks combined in multiplets, which seem to correspond to different end groups of the oligomers.

Thus, there is a main difference between chemical and laccase-catalyzed aniline polymerization: the growth of chemically synthesized polyaniline chain occurs *via* pernigraniline intermediate, which is finally reduced by unreacted aniline to the emeraldine oxidation state, while in laccase-catalyzed polymerization, PANI chains in the emeraldine oxidation state form immediately after adding the enzyme, i.e. the enzymatic reaction proceeds without an induction period.

The solution of aniline oligomers in THF was continuously being poured on copper foil electrodes, which were then allowed to dry. The coat formed on the electrode surface without any binders showed good adhesion and high stability. The inhibition of copper corrosion by aniline oligomers was studied in 0.1 M HCl and 3 % NaCl by linear voltammetry (Fig. 4). The anodic dissolution of copper in aerated 0.1 M HCl solution dramatically decreased when the copper electrode was modified with aniline oligomers as compared with the control electrode (Fig. 4A). The efficiency of inhibition calculated from the anodic current at a potential of 0.45V *vs.* Ag/AgCl was about 89 %. The anodic current of the copper electrode protected with aniline oligomers remained constant for 22 h.

The inhibiting effect of enzymatically synthesized aniline oligomers was also observed in the aerated sodium chloride solution (Fig. 4B). The anodic dissolution of copper on the electrode modified with aniline oligomers decreased by 96% as compared with the control electrode.

In our experiments, we observed an increase in the anodic current at the potential range -0.1 – +0.1 V (*vs.* Ag/AgCl) due to dissolution of copper and formation of Cu^{+} ions. Then CuCl is formed on the copper surface and serves as a protective layer preventing further dissolution of copper. At an overpotential of 0.2V the anodic current increased again due to the anodic

dissolution of copper and formation of $CuCl_2$. These results are in good agreement with those reported in [17].

To make the enzymatic-catalyzed synthesis of inhibitors for copper corrosion economically sound, one should use high concentration of aniline and a low molecular weight dopant, as well as the culture liquid of basidial fungus instead of the purified enzyme. We have produced aniline oligomers using high concentration of aniline (150 mM) and S-camphorsulfonic acid (150 mM) as dopant. Our experiments, in which we measured the copper strip weight loss in aerated 0.1 M HCl solutions have shown that the dissolution rate of copper in the presence of aniline oligomers decreases by about 90% when compared with control without the inhibitor (see Electronic Supplementary Information).

Conclusions

We have demonstrated that laccase-catalysed polymerization of aniline proceeds with the formation of aniline oligomers in the emeraldine oxidation state. Furtermore, the enzymatic reaction can be stopped at the step of formation of low molecular products which, unlike PANI, are soluble in organic solvents, e.g. THF. Finally, the enzymatically synthesized aniline oligomers show a high inhibition efficiency of copper corrosion in aqueous 1M HCL and 3 % NaCl solutions (89 and 96 % respectively).

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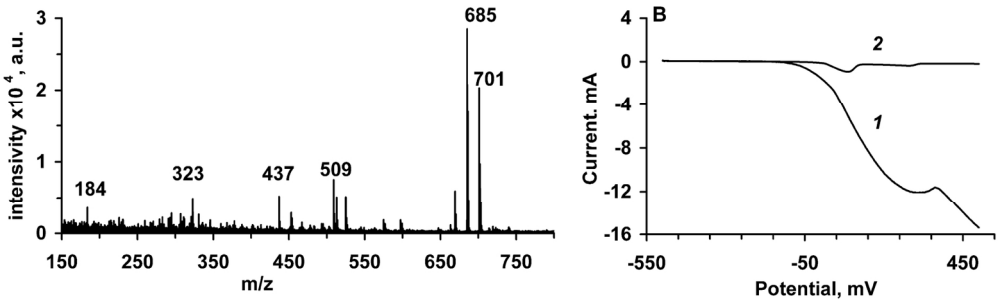
Legends

Fig. 1. UV-vis absorption spectra of aniline oligomers in the emeraldine oxidation state: in the reaction medium in the form of emeraldine salt (1) and in THF in the form of emeraldine base.

Fig. 2. The OCP – time profile of the template-guide laccase-catalyzed aniline polymerization in 0.1 M Na-citrate phosphate buffer (pH 3.5) containing 10 mM SDBS (1). The absorbance – time profile of the reaction solution during enzymatic aniline polymerization recorded at 750 nm (2).

Fig. 3. MALDI-TOF spectrum of low molecular products of laccase-catalyzed aniline polymerization in THF.

Fig. 4. Linear voltammograms for the control copper electrode (1) and the copper electrode modified with aniline oligomers (2) in aerated 0.1 M HCl (A) and 3 % NaCl (B) solutions. The potential scan rate is 50 mV/s.



A new method for the enzymatic synthesis of oligoaniline soluble in organic solutions is developed. Aniline oligomers showed a high inhibition of copper corrosion in aqueous HCl and NaCl solutions.
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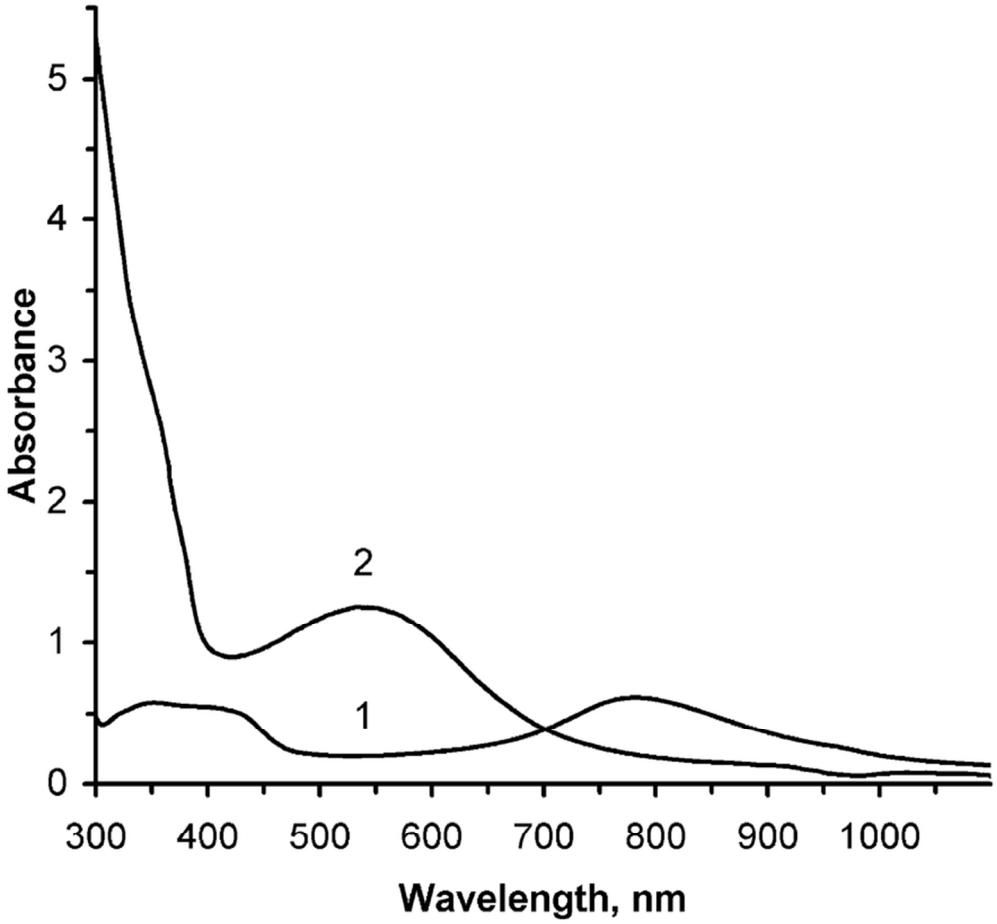

Fig. 1. UV-vis absorption spectra of aniline oligomers in the emeraldine oxidation state: in the reaction medium in the form of emeraldine salt (1) and in THF in the form of emeraldine base.
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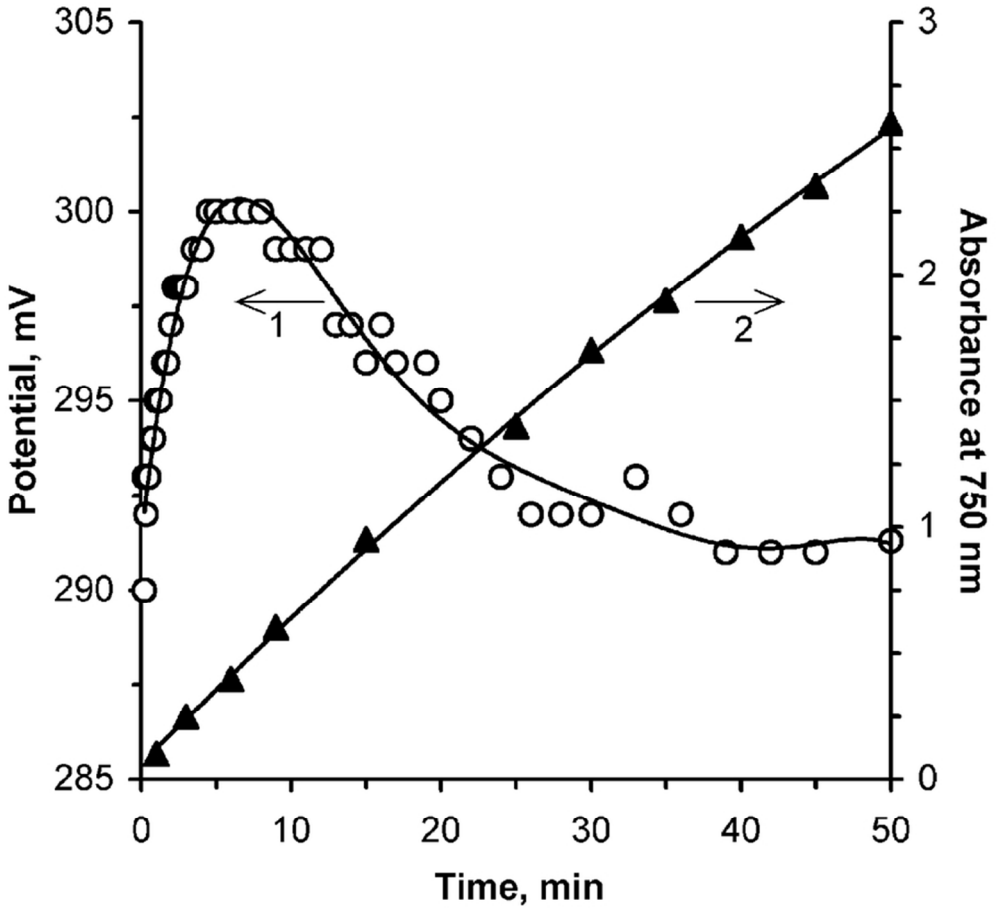

Fig. 2. The OCP – time profile of the template-guide laccase-catalyzed aniline polymerization in 0.1 M Na-citrate phosphate buffer (pH 3.5) containing 10 mM SDBS (1). The absorbance – time profile of the reaction solution during enzymatic aniline polymerization recorded at 750 nm (2).

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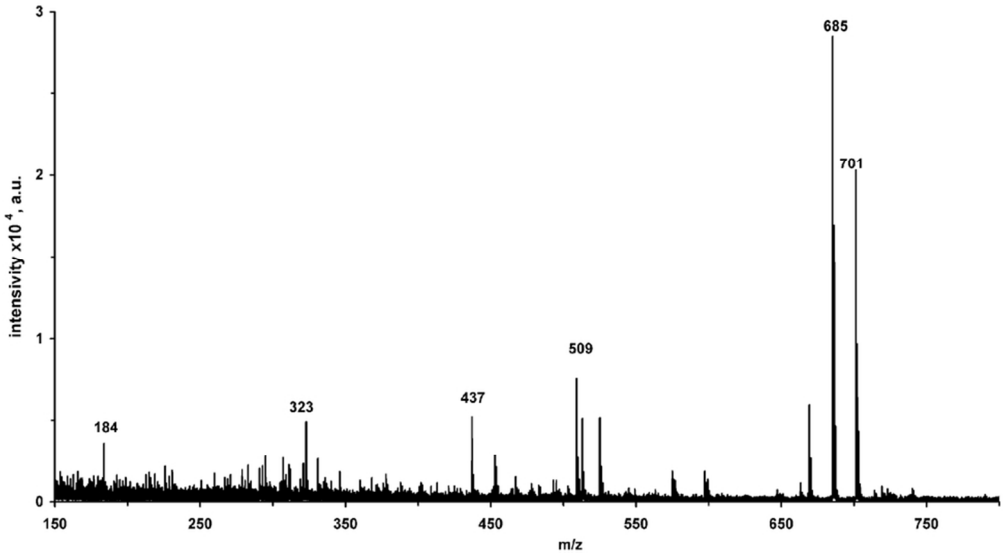

Fig. 3. MALDI-TOF spectrum of low molecular products of laccase-catalyzed aniline polymerization in THF.
41x24mm (600 x 600 DPI)

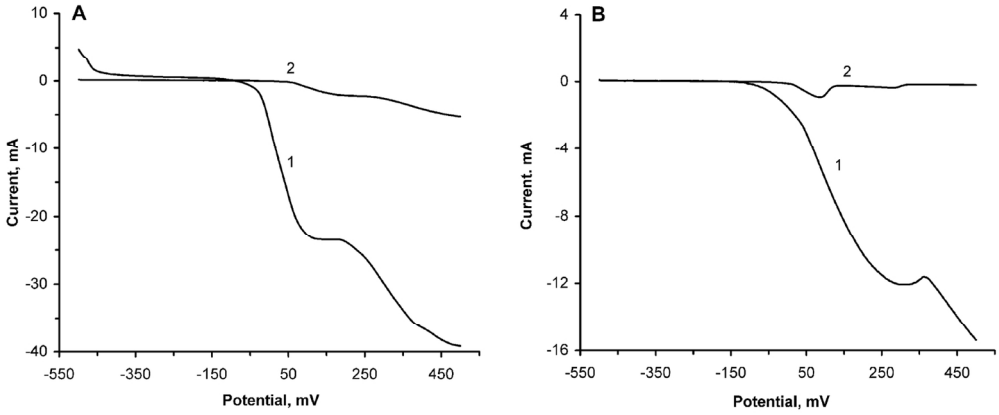

Fig. 4. Linear voltammograms for the control copper electrode (1) and the copper electrode modified with aniline oligomers (2) in aerated 0.1 M HCl (A) and 3 % NaCl (B) solutions. The potential scan rate is 50 mV/s.
75x33mm (600 x 600 DPI)