Appl Biochem Biotechnol (2010) 162:927–934
DOI 10.1007/s12010-009-8805-7

Enzymatic Synthesis of Semiconductor Polymers by Chloroperoxidase of *Caldariomyces fumago*

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Received: 24 July 2009 / Accepted: 28 September 2009 /

Abstract Among intrinsically conducting polymers, polyaniline is traditionally synthesized by chemical or electrochemical methods. Recently enzymatic synthesis of conducting polymers has been explored. In this work, polymers were synthesized using chloroperoxidase from *Caldariomyces fumago* and substituted anilines such as 2,6-dimethylaniline, 2,6-dichloroaniline, and 2,3,5,6-tetrachloroaniline (TCA), in order to promote a linear polymerization. These polymers were doped with (1*S*)-(+)-10-camphorsulfonic acid, dodecylbenzenesulfonic acid, and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA) with molar ratios of 1:0.25 and 1:0.5. Doped polymers showed conductivity corresponding to the semiconductors. TCA polymer doped with AMPSA showed the higher conductivity values. Different AMPSA concentrations have been tested, and the highest conductivity value of $1.6\times10^{-2}\text{S}\bullet\text{m}^{-1}$ was obtained for the complex with molar ratio of 1:0.5. This value is similar to those found with other substituted anilines with sulfonic groups. In addition, the enzymatically synthesized polymeric film showed combined transparency and semiconducting properties.

Keywords Chloroperoxidase · Intrinsically conducting polymers · Substituted anilines · Transparent semiconductor

Introduction

Plastics are widely used polymeric materials, and they are fundamental for the modern society. Traditionally, we have associated plastics with insulating properties, and many of their applications are related to this characteristic. In fact, they are used to insulate electric

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cables. This perception has been changed after one of the most important discoveries of the last century. Under certain synthesis conditions, the polyacetylene is able to conduct electricity at the same levels that the metals [1]. This discovery originated a materials revolution and generated a large variety of applications such as light emission diodes (LEDs), microelectronics, and materials for electromagnetic protection. The importance of this discovery has been recognized by the 2000 Nobel Prize earned by Hideki Shirakawa, Alan McDiarmid, and Alan Heeger.

The metallic atoms contain an electron placed in the last orbital that freely move when an electrical potential is applied. In order to have a similar process in a polymer, the polymer should contain alternate double and single bonds in a conjugate bond system. In addition, to increase the conducting properties, electrons should be added by reduction or removed by oxidation, thus when an electrical potential is applied, the electrons from the π bonds could move through the polymeric chain. The reduction or oxidation processes is known as “doping” and a doped polymer contain a delocalized π system through the molecular structure. Because intrinsically conducting polymers (ICPs) are P-type semiconductors, there is one incomplete covalent bond due to deficiency of an electron. Therefore, the holes move in the semiconductor structure, and the conductivity here is mainly due to motion of holes or positive charge.

Thus, an ICP or synthetic metal is a doped polymer that has metal-like electric, electronic, and magnetic properties and in addition, has specific optical properties, conserving the plastic mechanical properties [2].

After the polyacetylene (the first ICP synthesized), a variety of ICP have been synthesized such as poly(*p*-phenylene), polypirrole, polyaniline, polythiophene, and poly (*p*-phenylvinylene). The polyaniline (PANI) is considered a good ICP because of their electrical, electrochemical, optical properties, and stability. Nevertheless, PANI is not soluble in common solvents making its processing difficult. To improve the PANI solubility, different strategies have been implemented such as the use of substituted anilines and anionic dopants to obtain the polymer in the conducting form. These strategies reduce the maximal conductivity of the PANI, but increase its mechanical properties for processing and conserving adequate levels of conductivity for many applications.

PANI is usually obtained by chemical or electrochemical oxidation of aniline. However, in the last years, the enzymatic-mediated synthesis has been explored. The use of enzymes seems attractive because of several advantages such as mild synthesis conditions and environmental compatibility. Different oxidoreductases have been employed to synthesize PANI; bilirubine oxidase, laccases, and peroxidases from horseradish, palm, and soybean [3]. Independently of the synthesis method, the free radical generation and their coupling is the mechanism for the polymer production. These free radicals tend to polymerize in the *ortho* and *para* positions forming branched polymers with poor conductivity. To improve the conducting capacity of PANI, the polymerization should be at the *para* position producing a linear polymer containing the conjugated bond system.

A linear PANI has been enzymatically obtained by a polymerization of aniline catalyzed by horseradish peroxidase in the presence of a negatively charged template, which line up the aniline radicals to obtain *para* orientation coupling and also to increase the water solubility of the formed polymer [4–6].

As alternative to the use of template, the aim of this work is to induce the monomer coupling in *para* position by using substituted anilines in order to stabilize the aniline radical in *para* position. Substituted anilines were polymerized by chloroperoxidase from *Caldariomyces fumago*, which has been previously reported as able to transform highly substituted anilines and phenols [7].

Materials and Methods

Enzyme and Reactants Chloroperoxidase (CPO) from *C. fumago* 98362 was kindly donated by Dr. Michael A. Pickard, University of Alberta, Canada. Purified enzyme preparation showed a Reinheitzahl coefficient (Rz, A_{403} nm/A_{280} nm) of 1.41. The Reinheitzahl is a purity indicator, and from pure CPO, an Rz of 1.44 has been reported [8]. The substrates 2,6-dimethylaniline (DMA), 2,6-dichloroaniline (DCA), and 2,3,5,6-tetrachloroaniline (TCA), and the dopants; (1*S*)-(+)-10-camphorsulfonic acid (CSA), dodecylbenzenesulfonic acid (DBSA), and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). High-performance liquid chromatography (HPLC) grade organic solvents were obtained from Fisher Scientific (Springfield, NJ, USA). Buffer salts and other chemicals were obtained from J.T. Baker (Phillipsburg, NJ, USA).

Polymer Synthesis Polymers were synthesized in a conical flask with 1 L of reaction medium containing aniline monomer (4 mM DMA or 4 mM DCA or 0.14 mM TCA) and the enzyme CPO (75 nM for DCA and DMA or 330 nM for TCA) in an acetate buffer 60 mM, pH3, with 20 mM KCl and 20% *v*/*v* of isopropanol. The monomer concentration has been selected according their solubility in the reaction mixture and the CPO concentration according the substrate reactivity. Reaction was started by adding 0.5 mM H_2O_2. Reaction mixture was softly stirred, and successive additions of CPO and H_2O_2 every 2 h were performed until the respective substrate was exhausted as indicated by HPLC monitoring. At the end of the reactions, the polymer formed was recovered by centrifugation. The pellet was intensively washed with water and then with methanol.

Linear polyaniline (PANI-SPS) was synthesized with sulfonated polystyrene, as template, according to Samuelson [4] and Liu et al. [5, 6], but using CPO instead of horseradish peroxidase.

Formation of Thin Films from Doped and Undoped Polymers Polymer solutions were prepared at a concentration of 5 mg/mL. Generous drop of TCA polymer dissolution was deposited on a glass plate. DMA and DCA polymer dissolutions were deposited in acrylic plates. Drops were let to dry under controlled temperature of 40 °C, forming the polymer film. The same procedure was followed in the case of doped polymers. Doping of polymers was carried out mixing the dopant into the polymer solution at different aniline monomer:dopant molar ratios.

TCA was dissolved on NH_4OH 10 mM, and dopants were dissolved in deionized water. DMA and DCA polymers and their dopants were dissolved in methanol.

Film Thickness and Silver Electrode Dimensions Determination Thickness of films was determined using a profilometer from Tencor Instruments Alpha-Step 100. Alternatively, film thickness was measured by image analysis. The deposited films in glass plates were halved using a diamond tip. Samples were placed vertically in the optical microscope in order to observe the films thickness. Values were obtained by analyzing the images obtained with a CCD camera (Hitachi Color, KP-D50) coupled to a microscope (Nikon Optiphot-2) connected to a computer. The image acquisition and thickness determination were realized with the commercial software Image Pro Plus 5.1 (Media Cybernetics, MD, EUA). A calibration curve (pixels per micrometer) was constructed by acquiring images of a standard micrometer at each of the magnifications of the microscope. With this calibration curve, it was possible to convert pixels to micrometers in accordance to the magnification at which every image was acquired. Silver electrode dimensions were determined using an electronic Vernier caliper Ultra Cal Mark III (Sylvac-Fowler) with precision of 0.02 mm.

Electrical Response Two parallel silver electrodes with a separation of about 4 mm and length of about 4 mm were drawn over the polymer thin film surface using silver paste and a fine bristle brush. Electrical response of the film samples was determined by applying a voltage (V) at regular intervals between −100 and 100 V and measuring the resulting current (I) using a Keithley 236 current and voltage source unit. All tested samples were prepared and characterized by triplicate.

Polymer Conductivity Determination The electrical conductivity (σ) of the enzymatically synthesized polymers was obtained by measuring the sheet resistance (R_s) of their thin film samples, following the next deduction (Scheme 1):

The resistance (R) of a three-dimensional conductor can be written as follows:

$$R = \rho \frac{S}{A}$$

where:

ρ resistivity of the conductor (Ω meter)
A cross-sectional area (square meter)
S separation of the two parallel electrodes of equal length (meter)

The cross-sectional area can be split into the length of the electrodes (L) and the film thickness (d) (Scheme 1).

By grouping the resistivity with the thickness, the resistance can then be written as a function of the sheet resistance (R_s) of the film sample and the geometric dimension of the electrodes (S and L):

$$R = \frac{\rho}{d}\frac{S}{L} = R_s \frac{S}{L}$$

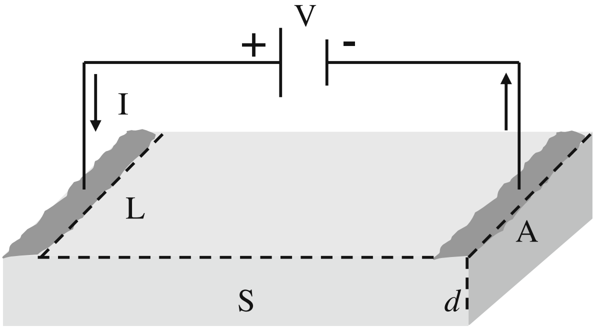
where:

$$R_s = \frac{\rho}{d}$$

Reorganizing the previous equality and knowing that conductivity, σ ($S \cdot m^{-1}$), is the reciprocal of resistivity, it is possible to write:

$$\sigma = \frac{1}{\rho} = \frac{1}{R_s d}$$

Scheme 1 Schematic representation of the polymeric film and the dimensions needed to obtain the conductivity values



Since the *I–V* curves of all tested film samples exhibited a linear behavior in the applied voltage interval, the resistance of each sample was determined then by the Ohm's law ($V=I•R$).

Results and Discussion

Polymers of substituted anilines, DCA, TCA, and DMA were synthesized in an enzymatically catalyzed reaction with chloroperoxidase from *C. fumago* (CPO). Chloroperoxidase was chosen because as previously reported [6], from several tested peroxidases, only CPO was able to transform highly halogenated anilines. In addition, the specific activity of CPO on pentachlorophenol was one order of magnitude higher than that of versatile peroxidase, and two orders of magnitude was higher than those of lignin peroxidase and horseradish peroxidase, confirming that CPO is the fastest peroxidase known so far [9]. CPO is an extracellular glycoprotein produced by the filamentous fungi *C. fumago* with a molecular weight of approximately 42 kDa [10]. This versatile enzyme shows halogenase-, peroxidase-, catalase-, and monooxygenase-like activities [11].

In all the cases, the main reaction product was a polymer which represented more than 80% of monomeric substrate. For TCA, from 87% to 95% of the substrate was integrated in the polymeric product. The mass spectrometric analysis of the resting dimers and trimers showed the presence of head-to-tail monomer coupling (*para* coupling) [7]. No oligomers in *ortho* nor *meta* positions were found. This is important because the rational of this work was to induce the lineal monomer coupling by using substituted anilines in order to stabilize the aniline radical in *para* position. Based on the characteristics of identified products, we suggest that obtained polymers are linear and keep a conjugated bond system. A conjugated bond system is the principal characteristic for electron conduction across the polymer backbone.

The conductivity values of different enzymatically synthesized polymers with and without doping processes were resumed in Table 1. Doping can increase the conductivity up to two orders of magnitude in all polymeric films. σ varies 10^{-8} to $10^{-3}\,\mathrm{S•m^{-1}}$, and the highest conductivity values were obtained with the films of tetrachloroaniline polymers doped with AMPSA. Insulator materials are those showing conductivity values between 10^{-16} and $10^{-7}\,\mathrm{S•m^{-1}}$, and semiconducting materials show conductivity values from 10^{-7} to $10^{2}\,\mathrm{S•m^{-1}}$. Thus, most of the polymers enzymatically synthesized from substituted anilines are semiconductors, and few of them are in the transition zone between insulators and semiconductors.

As observed in Table 1, the electrical conductivity values of the polymer films are susceptible to the molar ratio between the polymer and dopant. In order to explore the effect of the dopant proportion in the film preparation, σ values of thin films prepared from polymeric TCA doped with AMPSA were plotted in Fig. 1 as a function of the molar ratio of polymer:dopant in the film from 1:0 to 1:2. It is important to point out that the polymeric TCA is insoluble in methanol or in different water-miscible organic solvents tested. This polymeric preparation showed water solubility in alkaline medium. In order to improve the contact between the polymer of TCA (alkaline soluble) and AMPSA molecules (acidic nature), a reduction of the NH_4OH concentration to 5 mM was made. With this change in the film production procedure, a significant increase in the conductivity was obtained. For example, the conductivity of the polymeric TCA doped with AMPSA at 1:0.5 molar ratio increased from $1.11\times10^{-3}\,\mathrm{S•m^{-1}}$, when polymeric TCA was dissolved in 10 mM NH_4OH (Table 1), to $1.6\times10^{-3}\,\mathrm{S•m^{-1}}$, when it was dissolved in 0.5 mM NH_4OH (Fig. 1).The conductivity increases with the dopant up to a molar ratio of 1:0.5; a higher concentration

Table 1 Sheet resistance and conductivity values of enzymatically synthesized polymers from substituted anilines.

Polymer obtained from:	Dopant	Molar ratio (polymer:dopant)	Sheet resistance (R_s; Ω)	Conductivity (σ; S/m)
DMA	–	–	1.05×10^{13}	6.79×10^{-8}
	CSA	1:0.25	1.87×10^{10}	1.74×10^{-5}
		1:0.50	1.88×10^{13}	2.20×10^{-8}
	DBSA	1:0.25	3.29×10^{10}	8.06×10^{-6}
		1:0.50	5.02×10^{10}	3.99×10^{-6}
	AMPSA	1:0.25	8.92×10^{11}	5.44×10^{-7}
		1:0.50	1.09×10^{13}	1.93×10^{-8}
DCA	–	–	7.35×10^{11}	1.21×10^{-6}
	CSA	1:0.25	2.08×10^{13}	1.99×10^{-8}
		1:0.50	3.95×10^{12}	1.07×10^{-7}
	DBSA	1:0.25	3.76×10^{10}	1.46×10^{-5}
		1:0.50	4.15×10^{9}	3.82×10^{-4}
	AMPSA	1:0.25	1.46×10^{13}	6.56×10^{-8}
		1:0.50	2.06×10^{12}	1.15×10^{-6}
TCA[a]	–	–	8.14×10^{12}	4.69×10^{-6}
	CSA	1:0.25	2.31×10^{10}	8.86×10^{-6}
		1:0.50	6.99×10^{10}	2.32×10^{-6}
	DBSA	1:0.25	1.62×10^{13}	5.72×10^{-7}
		1:0.50	4.49×10^{12}	1.58×10^{-7}
	AMPSA	1:0.25	1.84×10^{9}	1.58×10^{-4}
		1:0.50	1.58×10^{8}	1.11×10^{-3}
PANI-SPS[b]	Nad	Nad	8.67×10^{7}	2.45×10^{-3}

DMA 2,3-dimethylaniline, *DCA* 2,3-dichloroaniline, *TCA* 2,3,5,6-tetrachloroaniline, *CSA* (1*S*)-(+)-10-camphorsulfonic acid, *DBSA* dodecylbenzenesulfonic acid, *AMPSA* 2-acrylamido-2-methyl-1-propane-sulfonic acid, *Nad* not additional dopant

[a] TCA was dissolved on NH_4OH 10 mM DMA, and DCA polymers and their dopants were dissolved in methanol

[b] Polyaniline synthesized with sulfonated polystyrene as template, according Samuelson [4] and Liu et al. [5, 6], using CPO instead of horseradish peroxidase

of AMPSA does not improve anymore the electrical properties of the film, moreover, the plastic properties of the film are compromised.

As mentioned above, TCA–AMPSA film doped in a molar ratio of 1:0.5 showed the higher conductivity value (1.6×10^{-3} S•m^{-1}), corresponding to semiconductor range. As hypothesized, the tetra-substituted aniline, having only free the *para* position, seems to be polymerized preferentially lineal form with conjugated bond system. Semiconductors have also been obtained by the copolymerization of aniline and sodium diphenylamine-4-sulfonate [11], polymerization of *o*-aminobenzylphosphonic acid [12], polyaniline chemically modified with 1,3-propanesulfonate [13], and the enzymatic polymerization of 2,5-diaminobenzenesulfonate [14] with σ values varied between 10^{-3} and 100 S•m^{-1}. These values are also close to the conductivity values of 10^{-2}S•m^{-1} showed by the composite polyaniline–polymethyl methacrylate [15] and to the 10^{1}S•m^{-1} obtained from the polyaniline–sulfonated polystyrene

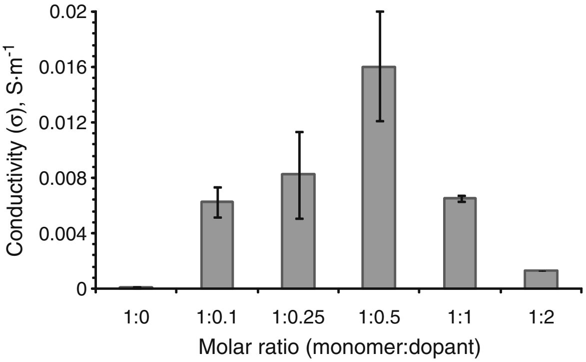


Fig. 1 Effect of the dopant proportion on the conductivity of tetrachloroaniline (TCA) polymer films doped with 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA). TCA was dissolved in NH_4OH 5 mM NH_4OH

synthesized using the horseradish peroxidase [4]. The conductivity TCA–AMPSA film is also similar to values obtained with other modified anilines.

An important characteristic of the films obtained from TCA polymer doped with AMPSA is the transparency and flexibility (Fig. 2). Transparent electronic devices formed on flexible substrates are expected to meet emerging technological demands where silicon-based electronics cannot provide a solution. An example of potential application refers to a diversity of displays, such as car windshields, eye goggles, glasses, billboards, wearable computers, or other forms where digital information might be desirable to have [16]. These transparent displays allow for information to appear where it was previously impossible to display information. Many research efforts have been addressed to obtain transparent and flexible semiconductors, mainly based on hydrogenated amorphous silicon [17–20], organic semiconductors [18–25], and novel semiconducting materials, such as the In–Ga–Zn–O system deposited on polyethylene terephthalate [26].

Conclusions

Chloroperoxidase from *C. fumago* catalyzes the polymerization of substituted anilines. As hypothesized, substituted anilines stabilize the aniline radical in *para* position and induce

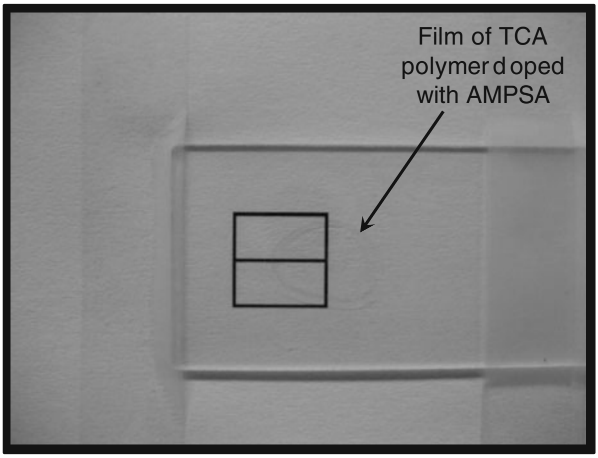


Fig. 2 Transparency of the film of tetrachloroaniline (TCA) polymer doped with AMPSA. The film was dried on a glass slide photographed on a white paper with a halved square drawing

the monomer coupling in this position to form lineal polymers. Undoped polymers showed conductivity values close to transition zone between insulator and semiconductor materials. However, doped polymers showed conductivities two orders of magnitude higher than the undoped films, crossing the semiconducting barrier. Enzymatically synthesized TCA polymer doped with AMPSA showed the highest conductivity (1.60×10^{-2} S•m^{-1}). This semiconductor film is transparent and flexible, with several potential applications.

Our results support the potential use of enzymes to synthesize new semiconducting and conducting polymeric materials, which combine properties of plastics and conducting materials.

Acknowledgments Authors thank Ma. Concepción Arenas Arrocena, Soledad Córdova Aguilar, and Raunel Tinoco for their technical assistance. This work has been funded by the Mexican Council of Science and Technology (CONACYT).

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