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Conductivity measurements of laccase for various concentrations, pH and ionic liquid solutions

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ABSTRACT

This study investigates the effects of concentrations, pH, the Pyrrolidinium Formate ([pyrr][Fo]) and the Morpholinium Formate ([morph][Fo]) ionic liquids (ILs) on the electrical conductivity of laccase for a wide range of temperatures. We have shown from the effects of concentrations that the higher values of the electrical conductivity are due essentially to cooper ions even the laccase is in its isoelectric point. From Arrhenius law, we have determined the energy of activation to follow the conformational changes. From the variation of the reduced electrical conductivity, we have studied the effects of temperature on the critical concentration c^{**} separating the various concentrations regimes. We have expended by analogy the two-state mechanism model at low and high temperatures to follow the unfolding process and to determine the thermodynamic parameters. The effects of [pyrr][Fo] and [morph][Fo] on the electrical conductivity of laccase are also investigated. We have demonstrated that the electrical conductivity and the energy of activation are intimately related to the ILs characteristics and the laccase conformation.

1. Introduction

Electrical conductivity is one of the transport properties more frequently request by chemists, biologists and engineers due to its great interest in various fields of research [1–3]. The electrical conductivity gives information on the strength of materials and the movement of electrons in molecules [1,4]. This propriety remains one of the important transport proprieties of polymers, electrolytes and proteins in aqueous solutions [5–8]. It depends on the wide range of interactions as solvent-solvent, ion-solvent and ion-ions [1,4,9].

For enzyme (or protein) solutions, measurements of electrical conductivity have become crucial to the control and the optimization of the electrolysis processes and the production of the electrochemical energy sources [10–12]. Therefore, understanding the mechanism of electron transduction through biological macromolecules has become one of fundamental tools due to the development of bioelectronic devices [13–15]. The metallo-enzymes, which are an important class offer several specific advantages more than other biopolymers. By definition, metallo-enzymes are proteins containing metal ions directly related to their internal structure. These metal centers are redox-active which qualifies the metallo-enzymes as good electrical conductors where the molecular electronic structure can be controlled the change in the oxidation of the metal centers [2]. The metallo-enzymes are usually used to understand the properties of electrolyte solutions and to evaluate the electron transport characteristics by photochemical or electrochemical methods [16–19].

In this context, we will study the electrical conductivity of fungal laccase from Trametes versicolor as metallo-enzyme. Laccases (EC 1.10.3.2) are blue multicopper oxidases that catalyze the oxidation of aromatic substrates by reducing the molecular oxygen to water [20]. This enzyme has 4 copper atoms distributed in type 1, type 2 and type 3 sites [21–23]. The copper ions placed in T1 site is responsible of the reduction of the substrate binds [24–26]. The other are grouped in T2/T3 sites. The distribution of copper ions controlled the laccase conduction properties in different environments knowing that the fungal laccases have a higher redox potential. Fungal laccases are used in many industrial applications

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such as textiles, food, pharmaceutical industries and it used in the degradation of aromatic pollutants causing environmental problems [27]. In the same way, various studies are interested to the ILs as a solvent media for enzymatic reactions [28–31]. They have proved the influence of anions and cations structure of ILs on the conformational stability, the resistance of laccase and the development of the catalyst of the biocathodes in enzymatic biofuel cells with a high biocompatibility [32–34].

Therefore, the present study focuses the investigation of the effects of concentrations, pH solutions and various mass fractions of ionic liquids (ILs) solvent as function of temperature on the electrical conductivity and thermodynamic parameters of laccase. We have extrapolated the two-state mechanism model to investigate the denaturation process of laccase in various environments via the electrical conductivity. The thermodynamic parameters as the free energy, the enthalpy and the entropy are also determined using Arrhenius law are also calculated.

2. Material and methods

All the chemicals and synthesized sample desctiption are given in Table 1. We have chosen a laccase from fungus Trametes versicolor (EC 1.14.18.1/CASRN = [80498-15-3]) and has a molecular weight of 57 KDa. The laccase is of the highest purity grade available from Sigma-Aldrich and used without further purification. The used laccase is powder and its activity is superior to 0.8 U/mg. This enzyme contains 4 copper atoms distributed in type 1, type 2 and type 3 sites [20–26]. Ultra-pure water which has a specific conductivity of about 0.65 μS/cm was used to prepare all concentrations. The solution preparations were based on the “Cold Method” technique [35]. The Cold Method was expanded from the preparation polymer aqueous solutions. It consists in the dissolving the enzyme in cold water where the temperature does not exceed 4 °C. All concentrations are prepared from a stock enzyme solution ($x = 1$, $C = 8$ mg/mL). The solutions were stirred for 3 h. After agitation, the solutions are equilibrated for 24 h at 4 °C [35]. To prepare solutions for different pH, we have used the Sodium acetate of 0.1 M to obtain solutions with pH 3, 4 and 5. The Phosphate buffer of 0.1 M is used to prepare solutions of pH 6 and 7. The pH of solutions was adjusted by using the HCl (0.1 M) and NaOH (0.1 M) to obtain a desired degree of acidity or basicity. The constancy of the pH of the solutions was verified by measuring it before and after measurements. All pH measurements were made at room temperature using a consort C862 pH meter. The electrical conductivity is immediately measured for each mixture. The concentration of laccase is fixed at $x = 0.208$. The Pyrrolidinium Formate ([pyrr][Fo]) and the Morpholinium Formate ([morph][Fo]), which have the chemical formula ($C_5H_{11}NO_2$) and ($C_5H_{11}NO_3$) respectively are the two ionic liquids used in this work. The [pyrr][Fo] and the [morph][Fo] are in their liquid state and have a hydrophilic character. They are used for mass fractions ranging between 0.110 and 0.817 of the total mass of the laccase/water mixture. It is important to be certain that the reaction is at equilibrium before measurements are made. Each measurement is made on a separate solution. The electrical conductivity measurements were performed using a conductimeter type consort C862 with fixed spacing circular electrodes and an accuracy of 0.01 mS/cm. The calibration of the cell was made using KCl solutions of known electrical conductivity. The cell containing solutions was immersed inside a temperature controlled water bath, type TC-502D-230, (Brookfield Engineering Laboratories Inc., USA), with an accuracy of 0.1 °C to maintain the sample at the desired temperature. Measurements of the relative temperature in the cell can be verified with the use of a second electrode which detects the temperature value in solution with an accuracy of 0.02 °C. The variation of the temperature during the experiment was carried out between 5 and 60 °C. All studied systems are reported at atmospheric pressure. All curves are treated using the software OriginPro (OriginLab/OriginPro 8.5, USA).

3. Results and discussion

3.1. Effect of temperature and concentration

The laccase from Trametes versicolor was dissolved in sodium acetate around its isoelectric point which is around 5.68 in the aim to investigate the effect of copper ions on the condumetric

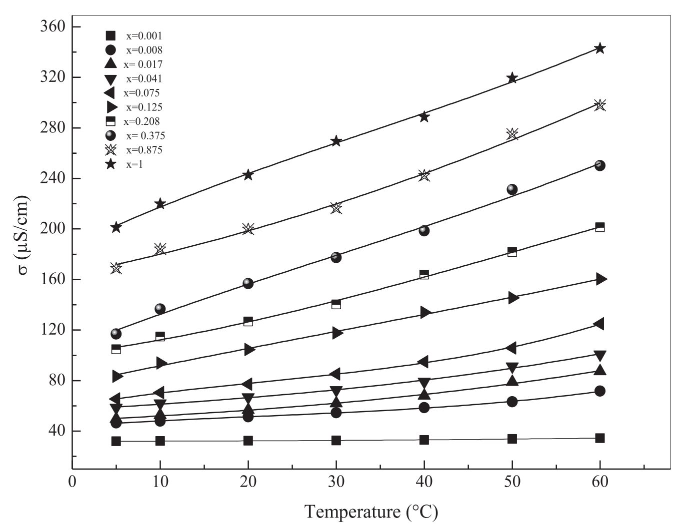

Fig. 1. Electrical conductivity of laccase from Trametes versicolor as function of temperature for various mass fractions.

Table 1
Sample description.

Chemical name	Source	Final mole fraction purity	Purification method
Laccase	Sigma Aldrich	–	None
Sodium acetate	Sigma Aldrich	0.990	None
Phosphate buffer	Sigma Aldrich	0.990	None
[pyrr][Fo]	Synthesized	0.990	DURP
[morph][Fo]	Synthesized	0.990	DURP
NaOH	Sigma Aldrich	0.980	None
Hcl	Sigma Aldrich	0.370	None

[pyrr][Fo]: pyrrolidinium Formate.
[morph][Fo]: morphilinium Formate.
NaOH: Sodium hydroxide.
Hcl: Hydrochloric acid.
DURP: Distillation Under Reduced Pressure.