



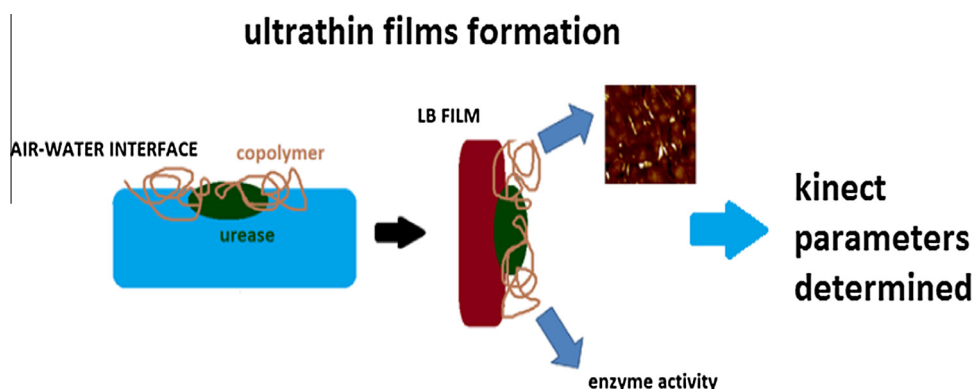
Conjugated polymers nanostructured as smart interfaces for controlling the catalytic properties of enzymes



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GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 15 April 2016

Revised 17 May 2016

Accepted 18 May 2016

Available online 19 May 2016

Keywords:

Conjugated polymer

Urease

Langmuir-Blodgett

Enzyme activity

Biosensor

ABSTRACT

The search for new molecular architectures to improve the efficiency of enzymes entrapped in ultrathin films is useful to enhance the effectiveness of biosensors. In this present work, conjugated polymers, based on thiophene and fluorine, were investigated to verify their suitability as matrices for the immobilization of urease. The copolymer poly[(9,9-dioctylfluorene)-*co*-thiophene], PDOF-*co*-Th was spread on the air-water interface forming stable Langmuir monolayers as determined by surface pressure-area isotherms, polarization-modulation reflection-absorption infrared spectroscopy (PM-IRRAS), and Brewster angle microscopy (BAM). Urease was incorporated in the floating monolayers being further transferred to solid supports as mixed Langmuir-Blodgett (LB) films. These films were then characterized with transfer ratio, fluorescence spectroscopy, PM-IRRAS and atomic force microscopy, confirming the co-transfer of the enzyme as well as its structuring in β -sheets. The catalytic activity was detected for urease, with a lower reaction rate than that encountered for the homogeneous environment. This was attributed to conformational constraints imposed to the biomacromolecule entrapped in the polymeric matrix.

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1. Introduction

Conjugated polymers (CPs) are widely used in the field of biosensors because of their electric, electronic, magnetic and

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optical properties [1,2]. The immobilization of biomolecules in CPs matrices has been extensively explored in order to obtain an effective contact between these two elements, maintaining the activity and stability of the molecules, as well as ensuring the identification of the analyte of interest [3].

Polythiophenes are a class of conjugated polymers that have been exploited for the manufacture of biosensors because of their

good solubility, processability, electrochemical stability, and the possibility to assemble new structures to adjust their physical properties [4,5]. However, polythiophenes have low photoluminescence quantum efficiency [6]. The combination of thiophene and fluorene groups, which have a high quantum yield, enables the formation of stable copolymers whose optical and electrical properties are useful for the application in optoelectronic devices and sensors [7–9].

The structuring of these polymers in nanostructured films is a suitable strategy to control the molecular architecture allowing for easy manipulation of the device properties. Among the kinds of procedures to form ultrathin films, Langmuir–Blodgett (LB) films not only allow for the formation of structures with control over the thickness and molecular architecture, but also serve to immobilize enzymes for the production of biosensors. For that, the enhancement of the activity properties of the immobilized biomolecule is desirable [10–12].

Some studies report the immobilization of the enzyme urease in LB films [13–17] and in conjugated polymer matrices [18–22]. Park et al. [18] developed a voltammetric biosensor immobilizing urease in poly(3-methylthiophene)-coated platinum electrodes by the chronoamperometric method. Other studies report the electrochemical immobilization of urease onto the PANi-Nafion/Au/ceramic plate composite film by the casting method, obtaining a sensor with high sensitivity and selectivity [19]. Furthermore, it is reported the covalent immobilization of this enzyme in a pyrrole copolymer film, producing a biosensor with a linear response to urea [20]. Vieira et al. developed a biosensor based on field-effect by means of films of polyaniline/urease constructed through the layer-by-layer technique [21]. Films of polypyrrole and urease were also fabricated by electropolymerization, and the effect of different dopant anions could be investigated [22].

In these works, it is clear that not only the structure of conjugated polymers may enhance the electrical and optical signals given by the enzymatic reaction, but also that these polymers serve as matrices for the immobilization of enzymes. The function and catalytic activity of the enzyme may depend on its conformation, which may be influenced by its interaction with the polymeric matrix. The structure of the matrices reported in these papers presents different levels of molecular organization, but none of them presents urease immobilized in structures layered as LB films.

The present work aims to study if a copolymer of thiophene and fluorene immobilized in solid supports as LB films is appropriate as matrix for the incorporation of urease. The relative enzyme activity and the catalytic constants were determined, proving the feasibility of such assemblies for the construction of biosensors for urea in a proof-of-concept experiment.

2. Materials and methods

2.1. Copolymer synthesis

The copolymer poly[(9,9-dioctylfluorene)-*co*-thiophene], denominated here as PDOF-*co*-Th, was synthesized according to the literature [23,24], using the Suzuki reaction method, as seen in Scheme 1. In a reflux system without oxygen, 2.62 mmol of 9,9-dioctylfluorene-2,7-diboric acid (Aldrich, 96%), and 2.12 mmol of 2,5-dibromothiophene (Aldrich, 95%) were added, and

0.013 mmol of tetrakis-(triphenylphosphine) palladium (P(Ph₃)₄Pd) (Aldrich, 99%) was employed as catalyst. Also, 120 mL of xylene, and 10 mL of 2 mol/L of potassium carbonate were added. This solution was stirred for 72 h at 100 °C under nitrogen flow (step 1). Thereafter, 0.5 mmol of 2-bromothiophene (Aldrich, 98%) was added and then kept under reflux for more 24 h (step 2). After this period, 20 mL of hydrogen peroxide were slowly added with the mixture kept at room temperature. The polymer was extracted with toluene, and recrystallized with methanol (yield 97%). Purity was near 100% as characterized with ¹H NMR (400 MHz, CDCl₃, δ, ppm): 7.64, 7.61, 7.55, 7.35, 7.28, 7.19, 7.05, 2.55, 2.25, 2.00, 1.51, 1.18, 1.03, 0.73. ¹³C NMR (400 MHz, CDCl₃, δ, ppm): 124.64, 123.92, 120.15, 119.82, 40.49, 31.81, 30.04, 29.23, 23.78, 22.61, 14.09. ¹³C NMR (400 MHz, solid state) δ: 152.9, 145.3, 142.1, 134.6, 125.4, 122.8, 56.9, 42.8, 31.6, 24.6 and 15.82.

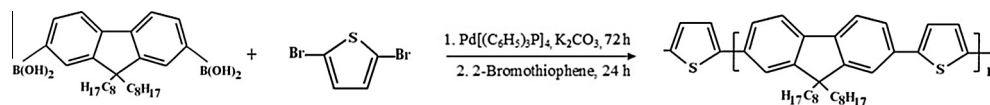
2.2. Characterization of PDOF-*co*-Th

UV–vis absorption spectra were collected by using a Shimadzu 1501 spectrophotometer. The measurements were performed in a solution using chloroform as solvent into 10 mm square quartz cells, in the λ = 200–800 nm range. The emission spectra (fluorescence) of the solutions in chloroform solution were obtained by using a Shimadzu 5301PC spectrophotometer with a slit width of 5 mm. The wavelengths of the maximum absorption according to the UV–vis spectra were used as excitation wavelengths.

2.3. Langmuir films

Ultrapure water was supplied by a Millipore system (resistivity of 18.2 MΩ cm, pH of 5.5–6.0, and surface tension of 72.5 mN/m at 25 °C) and employed as the subphase for the monolayers. PDOF-*co*-Th was dissolved in chloroform to yield a concentration of 20 mg/mL. Urease was dissolved in phosphate buffer solution (pH 7.5) to yield a concentration of 0.5 mg/mL. Aliquots of 25 μL of PDOF-*co*-Th were spread carefully, drop by drop, on the surface of the aqueous subphase. Aliquots of 25 μL of urease were inserted in the aqueous subphase close to the air-water interface and at least 30 min was allowed for enzyme adsorption and lateral diffusion. Longer waiting times were also employed and no significant difference was observed for the isotherms. The amount of polymer or enzyme incorporated at the air-water interface was low enough to initialize the curve in surface pressures close to zero. For the mixed monolayer, the surface pressure stabilized at 1.3 mN/m and then the interface was compressed.

The compression of the air-water interface was carried out with two movable barriers at a rate of 25 cm²/min, using a mini-model Langmuir trough (KSV instruments). The surface pressure was measured during the compression using a Wilhelmy plate. All monolayers were produced at a constant temperature of 25 ± 1 °C. The Langmuir monolayers were characterized using surface pressure-area isotherms, Brewster angle microscopy (micro-BAM, KSV Instruments) and polarization-modulation infrared reflection absorption spectroscopy (PM-IRRAS, KSV Instruments). BAM presented a resolution of 12 μm, and a light source power of 50 mW and a wavelength of 659 nm. The incident beam was p-polarized and reached the air-water interface at a fixed angle



Scheme 1. Synthesis of poly[(9,9-dioctylfluorene)-*co*-thiophene] (PDOF-*co*-Th).

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