Pyrolyzed Photoresist Carbon Electrodes in Aprotic Solvent: Bilirubin Electrochemistry and Interaction with Electrogenerated Superoxide

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ABSTRACT

Pyrolyzed photoresist carbon electrodes (PPCEs) are fabricated by the photopatterning of a negative tone epoxy-based photoresist, SU-8, through optimized standard UV photolithography. The electrochemical characteristics of PPCEs are investigated in dimethyl sulfoxide (DMSO), observing a wider accessible potential window and a smaller capacitance with respect to glassy carbon electrodes. PPCEs are used to study the cyclic voltammetric behavior of bilirubin (BR) in DMSO. Detailed information is obtained on the multiple steps involved both in the electrochemical oxidation and reduction of BR. Interesting points concerning the electrochemical oxidation of BR to biliverdin are clarified, identifying the formation of an intermediate whose fate depends on the time scale of the electrochemical experiment.

PPCEs are also used to electrogenerate the superoxide anion $O_2^{-\bullet}$ in DMSO for studying possible reactions between BR and $O_2^{-\bullet}$. The results obtained demonstrate that BR is an efficient superoxide scavenger and that a concentration 2 mM of BR is high enough to consume all the $O_2^{-\bullet}$ generated by oxygen reduction at the PPCE/DMSO interface.

1. Introduction

Carbon electrodes possess many advantages including low fabrication cost, wide accessible potential window, chemical and electrochemical stability [1] so that, in many cases, these electrodes are superior with respect to noble metal electrodes [2]. Among common carbon electrode materials, such as pyrolytic graphite, carbon fibers or carbon paste [3–6], glassy carbon (GC) is the most widely used [7,8]. Glassy carbon is impermeable to gases and liquids, has a very small porosity and can be polished to a mirror-like finish an almost infinitive number of times. Moreover, it is very resistant both to high oxidizing and reducing potentials thereby allowing one to perform electrochemistry in a 4–5 V wide potential window [9], when suitable electrolytes are used.

Recently, a new procedure to fabricate carbon electrodes based on the controlled pyrolysis of polymeric photoresist has been introduced, obtaining so-called pyrolyzed photoresist carbon electrodes (PPCEs) [10]. This is a very promising approach since, by using simple UV photo-lithography it is possible to pattern high-performance carbon electrodes with complex geometries, such as interdigitated arrays of microelectrodes [11,12]. The optimization of the pyrolysis conditions allows one to obtain PPCEs in which the carbon is mostly amorphous GC [13]. Studies performed in aqueous media demonstrated that the electrochemical behavior of PPCEs compares with that of classical electrodes prepared from bulk glassy carbon rods, with the advantage of being cheaper and with the possibility to customize their design [13]. In this paper we report, for the first time, the application of PPCEs for voltammetric measurements in an aprotic solvent, namely dimethyl sulfoxide (DMSO), used here as medium to dissolve bilirubin (BR) and to study its electrochemical behavior together with its reactivity towards electrogenerated superoxide radical anions.

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M (methyl) = -CH$_3$

V (vinyl) = -CH=CH$_2$

P (propionic) = -CH$_2$-CH$_2$-COOH
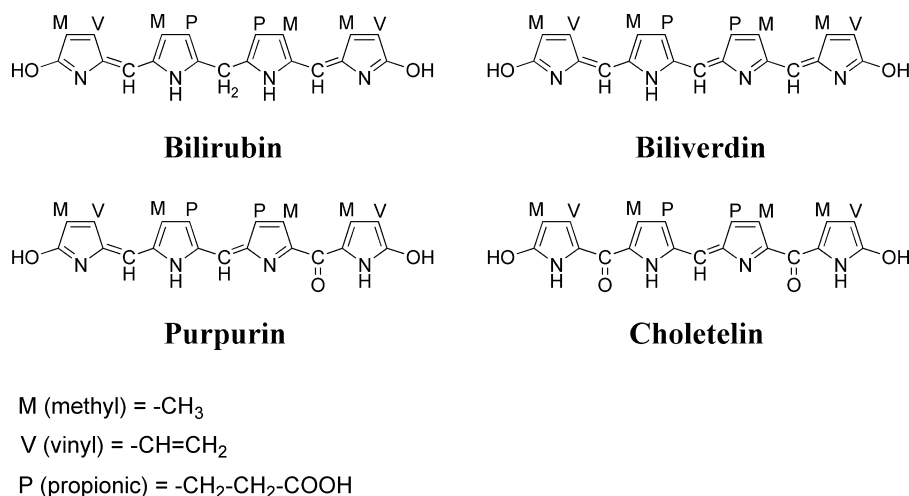
Scheme 1. Structural formulas of bilirubin and its oxidation products, namely biliverdin, purpurin and choletelin.

Bilirubin (BR) is the yellow-orange bile pigment found in blood, mostly bound to the plasma protein albumin. It is a linear tetrapyrrole (see scheme 1), insoluble in water at neutral pH, but very soluble in organic solvents. BR is an important serum biomarker used in clinical medicine for assessing hemolysis, hepatic function and cardiovascular risk [14]. This molecule originates from the degradation of the heme moiety in hemoglobin, other hemoproteins, such as cytochromes, catalase, peroxidase and tryptophan pyrrolase, and free heme [15]. In human body BR is present mainly as conjugated and unconjugated BR; the first forms a complex with gluconic acid, which makes it water soluble. Because of the important role played by this redox compound in several physiological processes and diseases [16], BR has been the subject of several previous electrochemical studies performed both in aqueous [17–21] and non aqueous media [22–29]. The results of these studies indicate that the electrochemical oxidation of BR is a multistep process in which, depending on the applied potential, BR is electrochemically oxidized to biliverdin (BV), purpurin or choletelin (see Scheme 1) [22].

Because of the poor water solubility of unconjugated BR, the use of non aqueous media is to be preferred for gathering fundamental information on its electrochemical behavior. This notwithstanding, even for measurements performed in aprotic media like DMF [22–27] and DMSO [28,29], in the literature there is no agreement on the oxidation mechanisms or even on the number of electrons involved in the oxidation steps, since different authors report different results. For instance, concerning the first oxidation process, coulometric data support a two-electrons oxidation [28,29]. However, voltammetric data gathered at the corresponding oxidation peak do not agree with a two-electron process; for instance, $|E_p - E_{p/2}|$ values are significantly larger than the 28.5 mV value expected for a reversible two-electron oxidation [30]. Moreover, Ribo et al. [26] reported voltammograms in which the first oxidation peak of bilirubin presented the same peak current as the one-electron oxidation of similar molecules. Similarly, for the BR reduction, it is not clear which peaks are ascribed to the direct reduction of BR and which are related to the electroactivity of BV electrogenerated in the anodic portion of the cyclic voltammetric experiments. These controversial results prompted us to undertake the present study with the goal of studying the electrochemical performance of PPCEs in DMSO, and then revisiting the electrochemical behavior of BR in this aprotic solvent. The findings enabled us to use the PPCE for the electrochemical generation of $O_2^{-\bullet}$ (by reduction of dissolved oxygen) and to study the reactivity of BR towards this radical as a proof of the radical scavenging capabilities of BR.

2. Experimental section

2.1. Electrochemical apparatus

All voltammetric measurements were carried out at room temperature ($22 \pm 1\,°C$) with a CHI1222A potentiostat controlled via a personal computer with its own software. A three-electrode single-compartment cell made of dark glass and equipped with a PPCE (geometric area = 0.031 cm^2) or a glassy carbon working electrode (GCE; geometric area = 0.071 cm^2), a platinum counter electrode and a platinum pseudo - reference electrode (Pt-pseudo). All potential values are finally referred to the $E_{1/2}$ of the ferrocene/ferricinium (Fc/Fc$^+$) redox couple recorded in the same experimental conditions as the data of interest [31,32]. For the latter aim, 1 mM Fc was added to the electrolyte and the relevant $E_{1/2}$ was evaluated as $E_{1/2} = (Ep_f + Ep_b)/2$ where Ep_f and Ep_b are the forward and backward peak potentials, respectively; in all these measurements the peak-to-peak separation ($\Delta Ep = Ep_f - Ep_b$) for Fc/Fc$^+$ was 60 ± 5 mV and $E_{1/2} = +0.37$ V vs Pt-pseudo. PPCEs were used as obtained after the pyrolysis procedure. Before use, GCEs were mirror polished with a 0.3 and a 0.05 μm alumina slurry on microcloth pads, rinsed with water and dried.

Coulometric measurements were carried out at room temperature with an AMEL 552 potentiostat with the associated AMEL integrator 731. In order to avoid the generation of products derived from higher oxidation potential process (see below), the potentials applied during electrolysis were kept approximately 30 mV below the peak potential recorded voltammetrically for the process of interest. When required, electrochemical measurements were performed under a nitrogen atmosphere, after purging the electrolyte solution for 15 minutes. Nitrogen was dried by bubbling through concentrated sulfuric acid and pre-saturated with DMSO vapors. Measurements involving the $O_2/O_2^{-\bullet}$ couple were performed in solutions equilibrated with atmospheric oxygen.

The supporting electrolyte tetrabutylammonium tetrafluoroborate (TBABF$_4$) was dried overnight in a vacuum oven at $40\,°C$ and stored in a desiccator before use. All laboratory glassware was carefully dried with a heat-gun before use in order to remove water.