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Modification of glassy carbon electrodes by 4-chloromethylphenyl units and D-glucosaminic acid

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ABSTRACT

The present work is dealing with the attachment of D-glucosaminic acid (D-GA) on glassy carbon electrode by two different methods. Firstly, the electrode was modified by chloromethylphenyl groups by reduction of 4-chloromethylphenyldiazonium cations followed by the nucleophilic substitution of the chlorine by the amine functionality of D-GA and secondly by the direct immobilization of the amine terminated molecule. The generality of the nucleophilic substitution reaction and the direct immobilization of an amine were also demonstrated with reactants bearing an electroactive ferrocene moiety; 4-nitrophenylferrocene (NFc) and 4-ferrocenylaniline (FcA). The surfaces modified with FcA and NFc were investigated by cyclic voltammetry, and the D-GA modified electrodes were characterized by X-ray photoelectron spectroscopy. A preliminary evaluation of the efficiency of these surface modifiers to prevent protein adsorption was realized by scanning electron microscopy.

1. Introduction

The control of the surface properties of materials by chemical modification is of great interest in several areas. In this context, electrochemistry is a powerful technique since it allows a rapid, simple and efficient method to modify and attach specific molecular moieties at an electrode surface. The grafted layer can promote the change or the improvement of various properties such as biocompatibility [1] or electronic properties [2]. The surface can be modified by formation of a conducting polymer [3], oxidation of an amine [4] or an alcohol [5] and reduction of a diazonium cation [6–9]. Among these, the reduction of a diazonium salt presents the advantage to form a robust organic layer covalently bonded to the electrode. This technique is applicable on numerous substrates such as carbon (glassy carbon [10], carbon powder [11], carbon nanotubes [12]...), silicon [13] and also to several metals [14,15]. Most of all, the method is compatible with the attachment of complex functional groups enabling the subsequent immobilization of various molecules.

The aim of the present work was to modify glassy carbon electrode with an hydrophilic molecule: D-glucosaminic acid (D-GA). It was motivated by its potential and never tested properties to prevent the non-specific adsorption of protein on a surface. This phenomenon, which constitutes a real drawback for electrochemical measurements of biological samples, or biocompatibility of medical implants [16], seems to operate through Van der Waals, electrostatic and hydrophobic interactions [17,18]. Most of the studies reported in the literature aimed at increasing the hydrophilicity of the surface by grafting polyethylene glycol to prevent the adsorption [19–22].

In this work, two methods were used to immobilize D-GA at the glassy carbon electrode surface. The first one involves the grafting on the electrode surface by means of an alkyl chloride functionality present on the glassy carbon surface by the electrochemical reduction of 4-chloromethylphenyldiazonium cations. Modified electrodes obtained by this method have already been studied by Pinson and co-workers [23–25] and Holm et al. [26]. They have shown that chloromethylphenyl (ClMP) terminated electrode can be used as a Merrifield resin analogue and allows the formation of covalent links with a primary amine. The second modification method consists in the immobilization of D-GA directly on an unmodified glassy carbon electrode. The generality of the substitution reaction was initially tested with two molecules bearing an electroactive ferrocene unit that can be conveniently detected electrochemically. The reaction should occur with the first one, a primary amine, 4-ferrocenylaniline, which was chosen as nucleophilic model molecule, whereas 4-nitrophenylferrocene should not react (Scheme 1).

The electrodes modified with D-GA were immersed in a BSA solution and the quantity of residual protein aggregates on the substrates was evaluated by scanning electron microscopy (SEM). Several techniques have already been used to evaluate the protein

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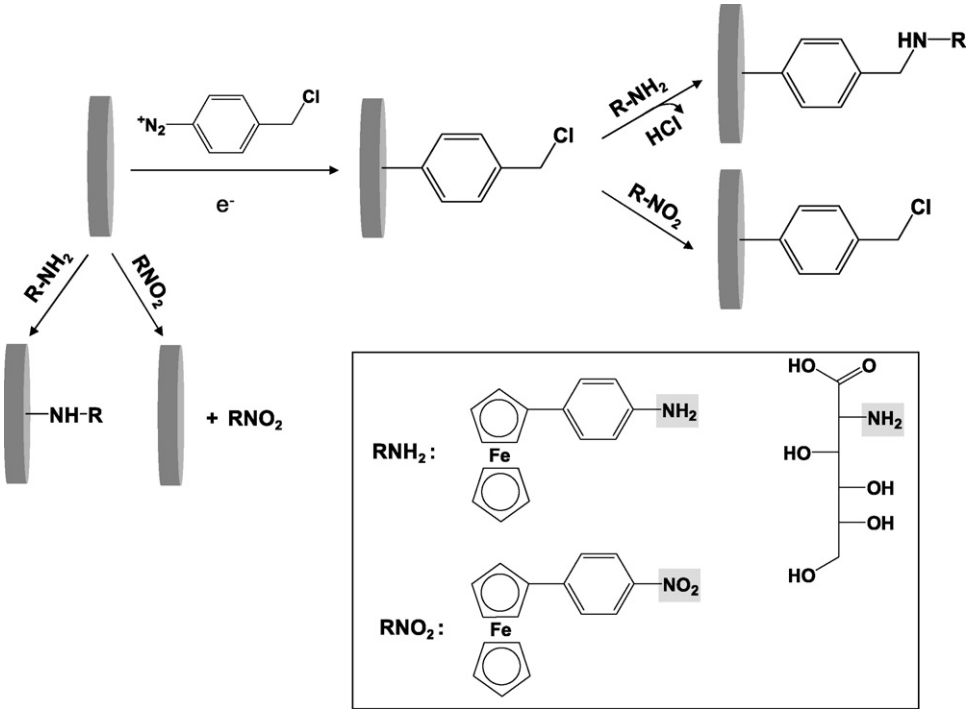

Scheme 1. Reaction of bare and chloromethylphenyl modified glassy carbon electrodes with various molecules.

adsorption on surfaces, such as fluorescence microscopy [27], X-ray photoelectron spectroscopy [28], ^{125}I radiolabeling, electrochemical quartz crystal microbalance [29], cyclic voltammetry [30,31] and electrochemical impedance spectroscopy [32]. For a preliminary study, SEM presents the advantage of being easy to use, rapid and not requiring the chemical modification of the proteins.

2. Experimental

2.1. Chemicals

Acetonitrile ACS grade (EMD, $H_2O < 0.3\%$), potassium ferrocyanide (Aldrich), potassium ferricyanide (Aldrich), potassium chloride (Aldrich), 4-aminobenzyl alcohol (Aldrich), 4-nitroaniline (Aldrich), p-toluidine (Aldrich), t-butyl nitrite (Aldrich), ferrocene (Aldrich), D-glucosaminic acid (TRC), Bovine Serum Albumin (BSA, Biobasic), were used as received without further purification. Tetrabutylammonium hexafluorophosphate (Bu_4NPF_6, Alfa Aesar) was dried at 80 °C under active vacuum for 15 h prior to use.

The 4-chloromethylphenyl diazonium tetrafluoroborate salt was synthesized from 4-aminobenzyl chloride hydrochloride, itself obtained from 4-aminobenzyl alcohol, according to a published procedure [23]. The synthesis of 4-nitrophenylferrocene (NFc) was performed by arylation of ferrocene by the 4-nitrobenzene diazonium salt [33] and 4-ferrocenylaniline (FcA) was obtained by reduction of the nitro functionality of NFc with tin in acidic condition [34].

2.2. Electrode preparation and modification

Glassy carbon electrodes were obtained from Bioanalytical Systems Inc. (Model MF-2012, diameter 3 mm) and were used as working electrodes. Glassy carbon electrodes (Electrosynthesis Co., area about 2 cm^2) were used for SEM experiments and for XPS measurements. The working electrodes were polished with 1 μm and 0.05 μm alumina (Buehler) and ultrasonicated in de-ionized water (18 MΩ, obtained from a Sybron/Barnstead de-ionized system) before each experiment. All electrochemical experiments were performed under dried argon atmosphere in a three-electrode cell. The counter and reference electrodes were a platinum gauze and an Ag/AgCl (saturated KCl) electrode, respectively. All potentials were reported versus this electrode. The electrochemical modification of the glassy carbon electrodes was carried out in an acetonitrile solution containing 0.1 M Bu_4NPF_6 as support salt and 1 mM 4-chloromethylphenyl diazonium tetrafluoroborate. The electrochemical reduction of the diazonium salt was performed by applying a potential of −0.3 V (50 mV after the reduction wave of diazonium cations) during 120 s. Glassy carbon electrodes were also functionalized with methylphenyl (MP) groups by electrochemical reduction at −0.5 V during 15 min of a solution containing 10 mM of the corresponding in situ generated diazonium cations and 0.1 M Bu_4NPF_6 in acetonitrile. The in situ synthesis of the p-toluidinediazonium cation (diazonium cation bearing a methyl group on the para position) was performed directly in the electrochemical cell from p-toluidine and 3 equivalents of t-butyl nitrite.

Following electrodeposition, the working electrodes were washed in acetonitrile and ultrasonicated during 10 min. For the reactions with FcA and NFc, the ClMP-modified, the MP-tethered and the bare glassy carbon electrodes were immersed in a 10 mM solution in acetonitrile at 70 °C during 16 h, rinsed and ultrasonicated during 1 h in acetonitrile. The supported nucleophilic substitution of the chlorine by the terminal primary amine of D-GA was performed by soaking the electrode modified by ClMP groups, in an aqueous solution containing 10 mM D-GA at 70 °C during 16 h or at room temperature during 45 h. For the reaction between bare glassy carbon and D-GA, the electrodes was dipped in an aqueous solution containing 10 mM D-GA at 70 °C during 16 h. After washing in de-ionized water and ultrasonication during 1 h, the functionalized electrodes were dipped for 4 h in a sodium borate buffer solution (3.8%, w/w $Na_2B_4O_7{\cdot}10H_2O$ and 0.02%, w/w NaN_3, pH 8.4) containing 100 g/L BSA. A final rinsing was made in de-ionized water and the electrodes were dried and stored under vacuum before characterization by SEM.