Carbon 112 (2017) 230–237

Contents lists available at ScienceDirect

Carbon

journal homepage: www.elsevier.com/locate/carbon

The influence of carbon-oxygen surface functional groups of carbon electrodes on the electrochemical reduction of hemoglobin

Justin Tom, Heather A. Andreas*

Department of Chemistry, Dalhousie University, Halifax, NS B3H 4R2, Canada

ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form
16 October 2016
Accepted 29 October 2016
Available online 1 November 2016

ABSTRACT

The electron transfer between a carbon electrode and hemoglobin in solution is known to be a slow and difficult process, likely due to the difficulty in accessing the redox active heme groups of hemoglobin. Literature data are inconsistent regarding electrochemical activity of hemoglobin on carbon electrodes and whether carbon-oxygen-containing surface functional groups enhance or inhibit the electroactivity of hemoglobin. Herein we examine the role of carbon surface functionalities in hemoglobin-electroactivity through cyclic voltammetry and differential pulse voltammetry in a neutral phosphate buffered electrolyte. Oxygen-containing surface functionalities were tracked *ex situ* using temperature programmed desorption, X-ray photoelectron spectroscopy and attenuated total reflectance Fourier transform infrared spectroscopy. Hemoglobin electroactivity is inhibited by ether and carbonyl surface groups present on the carbon electrode. A carbon material which showed little hemoglobin electroactivity was made more active through ultrasonication and electrochemical reduction, related to the removal of ether and carbonyl groups from the carbon surface. This known surface modification is quick, easy to implement, and avoids contamination from the use of additional reagents. The knowledge of carbon-oxygen surface functionalities is essential to better understand hemoglobin's electroactivity on carbon and influences the choice of carbon electrode materials for further development of hemoglobin electrochemical biosensors.

1. Introduction

Electrochemical biosensors are promising devices for providing point-of-care diagnostics due to their relatively fast detection, portability and low-cost [1]. Obtaining an electrochemical signal for hemoglobin (Hb) may provide a means for an alternative direct Hb detection, which is important due to the role Hb plays in various diseases [2,3]. The electroactivity of Hb is believed to be related to the oxidation and reduction of the iron within the heme prosthetic group [4–7], similar to those seen with other heme-containing proteins such as cytochrome *c* [8], myoglobin [9,10], and horseradish peroxidase [10]. Direct electron transfer between Hb and an electrode is generally considered to be difficult because the four redox active heme groups are buried in the interior hydrophobic regions of the protein [11,12].

The low cost and environmental sustainability of carbon makes it a promising material for biosensor electrodes. However, the electroactivity of Hb is inconsistent on different carbon electrode materials [5,10,13–16]. Hb has been shown to be electroactive on carbon nanotubes [7,10,15,17,18], glassy carbon [14], carbon black [19], and pyrolytic graphite [16]; yet inactive on pyrolytic graphite [13], and glassy carbon [15]. Often the inconsistency between electroactivity is due to whether the hemoglobin is immobilized on the electrode or is in solution, as highlighted by the conflicting results for pyrolytic graphite where the immobilized form is electroactive [16], whereas this carbon exhibits no Hb electroactivity with solution-based Hb [13]. Clearly, for a point-of-care diagnostic, it is vital to be able to measure the Hb concentrations within a liquid phase, and therefore a carbon material which evidences Hb-electroactivity is required.

For carbons which demonstrate Hb electroactivity, there is disagreement whether carbon-oxygen surface functional groups improve [10,14] or inhibit [5] its electroactivity. Previous literature suggests that the presence of more C-O groups such as phenols or alcohol groups are responsible for favouring the electron transfer process possibly due to changing polypeptide conformations [14]. Whereas, more recent experimental evidence from the literature

* Corresponding author.
E-mail address: heather.andreas@dal.ca (H.A. Andreas).

http://dx.doi.org/10.1016/j.carbon.2016.10.090
0008-6223/

suggests less oxidized carbon surfaces have a larger electrochemical response to Hb [5]. Knowledge of the presence of carbon-oxygen surface functional groups and their modification is clearly important in changing the electroactivity of Hb. Although not dealt with in this paper, exploration of this idea may give insight into the carbon-Hb mechanism.

In this paper, we evaluated the observed electroactivity of Hb with respect to the presence and identity of carbon-oxygen surface functional groups. The electroactivity of Hb was tracked using cyclic voltammetry and differential pulse voltammetry. Carbon-oxygen surface functionalities were characterized using X-ray photoelectron spectroscopy (XPS), temperature programmed desorption (TPD) and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR).

2. Experimental

2.1. Carbon materials

Four carbon powders used were tested for Hb electroactivity: graphite (Sigma-Aldrich, > 99.99%, -325 mesh), glassy carbon (Aldrich, 99.95%, 2–12 μm, spherical), Vulcan XC-72 (Cabot) and Black Pearls 2000 (Cabot). For most experiments, carbon powders were used as-received.

The carbon surface functionalities were modified on the Black Pearls 2000 and Vulcan XC-72 carbons, to alter their Hb electroactivity. Black Pearls 2000 was heat treated at 1000 °C under Ar atmosphere for two hours to preferentially remove all COOR surface groups while only removing some C-O and C=O surface groups [20]. Vulcan XC-72 was electrochemically reduced using a procedure similar to that used by Guo et al., shown to preferentially reduce C=O groups [21]. 4 mg of Vulcan XC-72 was ultrasonicated (Branson 1510 Ultrasonic Cleaner) in 4 mL of 18.2 MΩ cm water two times, 5 min each, to create a suspension. 10 μL of the suspension was air-dried at room temperature onto thoroughly cleaned and diamond polished (3 μm and 1 μm) glassy carbon electrodes (CHI104 and BASi-MF2012, 3 mm diameter, mirror-like finish). The dried Vulcan XC-72 was then reduced at -1.95 V for 15 min in a stirred 10 mM phosphate electrolyte (pH 5.03) purged with N_2.

A Hitachi S-4700 FEG scanning electron microscope was used to collect images of the Black Pearls 2000 and Vulcan XC-72 carbon blacks. An accelerating voltage of 5000 V was used for imaging the Black Pearls 2000 before and after the heat treatment. A lower accelerating voltage of 2000 V was used for the Vulcan XC-72 as-received, after sonication and after sonication plus electrochemical reduction. These carbon materials were imaged at 80,000× magnification.

2.2. Electrochemistry

Cyclic voltammograms (CVs) and differential pulse voltammograms (DPVs) of the carbon powders were recorded using a microcavity electrode consisting of a 250 μm diameter platinum wire sealed in glass with a cavity depth of 193 μm measured by optical coherence tomography. The counter electrode was a platinum mesh and the reference electrode was a Hg/Hg_2SO_4 (saturated K_2SO_4) (measured as 0.692 V vs. the standard hydrogen electrode). All potentials are reported versus this reference electrode. The Hg/Hg_2SO_4 reference electrode was used to avoid possible chloride contamination in the electrolyte since chloride has a well-known allosteric effect on Hb [22,23].

The 0.1 M phosphate buffer (PB) electrolyte (pH 7.08) consisted of K_2HPO_4 (ACS Reagent, Sigma Aldrich, ≥98%) and KH_2PO_4 (Sigma Life Science, ≥99.0%) in a 64.5%–35.5% mole ratio. Bovine hemoglobin (BHb) (lyophilized powder, from bovine blood, Sigma Life Science) stock solution was injected into the electrochemical glass cell to achieve a range of concentrations: 0.2, 0.1, 0.05, 0.01, 0.005, and 0.001 g L^{-1}, with a total electrolyte volume of 5 mL. All solutions were made with 18.2 MΩ cm water.

Differential pulse voltammetry was conducted between 300 and -900 mV with a scan rate of -20 mV s^{-1}. The initial potential was held for 2 s, all subsequent steps had a pulse height of 50 mV, pulse width of 250 ms, step height of -25 mV, and a step time of 1250 ms. However, the Black Pearls 2000 samples used a step time of 25 s and an effective sweep rate of -1 mV s^{-1}, because of its high surface area and resultant slow pore response. The same potential limits and a scan rate of 20 mV s^{-1} was used for all cyclic voltammetry experiments. A Bio-Logic VMP3 multipotentiostat was used for electrochemical measurements.

Prior to each measurement, the single compartment 5-mL glass electrochemical cell was thoroughly washed, wiped, soaked in 1 M NaOH for 5 min and washed with 18.2 MΩ cm water. The microcavity working electrode was cleaned by ultrasonication in a distilled water bath (Branson 1510 Ultrasonic Cleaner) for 3 min to physically remove the carbon powder from the cavity. The electrode was then cycled in 1 M H_2SO_4 from −0.750 to 1.300 V (1 V s^{-1}) for 100 cycles and then -0.670 to 0.930 V (1 V s^{-1}) for an additional 100 cycles to ensure no residual carbon. Then the electrode was rinsed and was tested for cleanliness by a CV or DPV scan in 0.1 M PB immediately prior to experiments.

2.3. X-ray photoelectron spectroscopy

A Thermo VG Scientific Multilab ESCA 2000 spectrometer was used to collect X-ray photoelectron spectroscopy (XPS) data with a Mg K_α X-ray source (1253.6 eV, 0.6 mm diameter spot size) and a CLAM4 MCD electron energy analyzer with a pass energy of 30 eV. The spectra of the carbon samples were collected at room temperature and a pressure of 1×10^{-9} Torr. The data were then analyzed using CasaXPS software. The counts per second were normalized relative to the graphitic peak for all spectra. Additionally, only the XPS spectra for Vulcan XC-72 on glassy carbon plates required shifting relative to a graphitic peak position of 284.3 eV. Fitted C_{1s} XPS peaks were positioned based on known literature values: C=C 284.3 eV [24–27], C-O 285.5 eV [26–30], C=O 286.8 to 287.1 eV [25,26,29,31–33], COOR 288.8 eV [25,26,31–34], π-π* shakeup 290.3 to 290.4 eV [24,31,34,35], and plasmon processes 292.6 eV [31,35]. Monte Carlo simulations were performed using CasaXPS software to provide error estimates to determine whether the surface concentration percentages calculated from fitted peaks of different samples were considered significant.

2.4. Temperature programmed desorption

Temperature programmed desorption (TPD) was conducted on a TA Instruments SDT Q600 system connected to a TA Instruments Discovery quadrupole mass spectrometer. The TPD system was purged with Ar gas at a rate of 400 mL min^{-1} and then the flow was adjusted to 100 mL min^{-1} for the measurement. Each carbon sample (*ca.* 2 mg) was held in an alumina crucible and equilibrated at 25 °C and further held at that temperature for 5 min to ensure stable measurements. The temperature was programmed to change at a linear rate of 5 °C min^{-1} from 25 to 1000 °C. Each carbon sample was tested 3 times to ensure reproducibility. TPD data were processed using TA Universal Analysis Software.

The desorbed CO and CO_2 gases were sampled through a 300 °C heated capillary leading to the quadrupole mass spectrometer. The internal pressure of the mass spectrometer was 27 Torr. The dual Faraday detector gathered data up to 50 *m/z* with particular attention on *m/z* of 28 (CO) and 44 (CO_2).