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Full Length Article

Electrochemical behavior of immobilized hemoglobin in alkaline solution

Nataša Jović-Jovičić[a], Zorica Mojović[a,*], Miloš Mojović[b], Predrag Banković[a], Marija Ajduković[a], Aleksandra Milutinović-Nikolić[a], Dušan Jovanović[a]

[a] University of Belgrade – Insititute of Chemistry, Technology and Metallurgy, Center for Catalysis and Chemical Engineering, Njegoševa 12, 11000 Belgrade, Serbia
[b] Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, P.O. Box 47, 11158 Belgrade, Serbia

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ABSTRACT

Glassy carbon electrode was modified with different synthesized hybrid clay-based materials and tested in alkaline solution with and without H_2O_2. The hybrid materials were obtained by immobilizing hemoglobin (Hb) on acid activated (AA) clay, or on AA clay modified with different sodium dodecyl sulfate (SDS) loadings. The obtained materials were characterized using DR UV–vis and ESR spectroscopy, elemental analysis, and SEM. The characterization confirmed higher degree of hemoglobin incorporation in the presence of SDS. The presence of SDS on the surface of clay particles resulted in the partial oxidation/denaturation of hemoglobin and formation of hemichrome. Cyclic voltammetry was used for the investigation of the electrochemical behavior of immobilized hemoglobin in alkaline solution. Two cathodic peaks at −0.45 V and −0.70 V were recorded and ascribed to the reduction of heme Fe(III)/Fe(II), and formation of HbFe(I) – highly reduced form of hemoglobin – respectively. The latter peak reflects hemoglobin denaturation. The presence of H_2O_2 in the alkaline solution increased current intensities corresponding to both peaks (−0.45 V and −0.7 V). Linear response of peak current intensity vs. H_2O_2 concentration was monitored for all investigated samples within different H_2O_2 concentration ranges. The AA-SDS1.0-Hb electrode exhibited the highest current response with linear regression equation in the following form: $I(\mu A) = 7.99 + 1.056 \times [H_2O_2]$ (mM) ($R = 0.996$). The limit of detection of 28 μM was estimated using the 3 sigma method.

Different modified electrodes exhibited different degrees of denaturation resistance. The obtained values of Michaelis-Menten constant indicated that prolonged cycling in the presence of SDS increases protein denaturation.

1. Introduction

Hemoglobin is a protein consisted of four polypeptide chains. Each chain contains an iron-bearing heme group, positioned near the surface of the protein and accessible to oxygen molecules. However, these heme groups are more deeply buried comparing with similar groups in cytochrome c, which results in slower electron transfer between hemoglobin molecule and electrode surface [1]. Heme groups in hemoglobin have to be more accessible in order for the rate of electron transfer to be improved. Surfactant compounds might combine with the protein in such manner that the accessibility of the electroactive section is improved [2]. It has previously been found that the presence of sodium dodecyl sulfate (SDS) enhances the electrochemical response of hemoglobin in solution [3].

Electron transfer between heme protein and electrode surface can also be enhanced by the use of electrode modifiers such as clays. Smectites are the class of clay minerals that consist of negatively charged 2:1 aluminosilicate layers with intercalated exchangeable cations and water molecules. They are easy to modify, thus becoming efficient materials designed for various applications, including electrode modification. The interaction of proteins and modified clays has been studied in a number of studies [4,5]. It has been established that clays provide favorable microenvironment for proteins [6] and facilitate electron transfer between heme protein and electrode surface [7]. Besides, the native structure of proteins is usually preserved in clay films [8].

* Corresponding author.
E-mail address: zoricam@nanosys.ihtm.bg.ac.rs (Z. Mojović).

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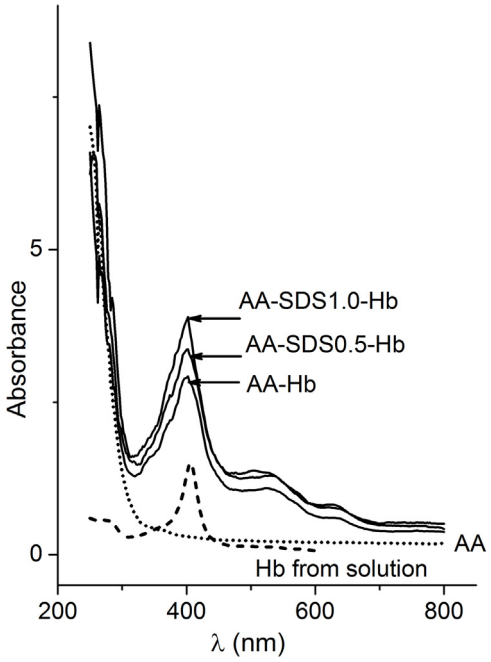

Fig. 1. DR-UV–vis spectra of investigated samples and UV–vis spectra of hemoglobin from solution.

Most of electrochemical investigations are performed in a buffer solution i.e. at pH close to physiological pH [9,10]. The use of hemoglobin as sensor relies on its ability to retain the native structure, which enables conformational shifts. At extreme pH values protein denaturation occurs, resulting in conformation loss. Therefore, the electrochemical investigation of hemoglobin response at extreme pH values has not drawn attention. In this work the investigation was carried out in highly alkaline solutions with the goal of obtaining hemoglobin in denaturized forms.

The preparation of the electrodes used for the investigation of the electrochemical behavior of smectite-supported hemoglobin in alkaline media consisted of several steps. Sodium-dodecyl sulfate (SDS) was attached to the clay surface with the goal of further enhancing electron transfer from hemoglobin to electrode. Prior to the addition of SDS, the acid modification of montmorillonite was performed in order to enable its bonding to the clay surface. The acid treatment changes surface charge of smectite into positive, leading to electrostatic interaction with negative sulfate ions of SDS. Glassy carbon electrode was modified with complex monmorillonite-SDS-hemoglobin layer. The testing of hemoglobin in alkaline solution was performed using H_2O_2 as test probe since it was shown that hemoglobin exhibits enzyme-like catalytic activity toward H_2O_2 [11]. Different hemoglobin types have different denaturation resistivity [12] and the concept of this research was to obtain the results that might be the first step in the initiation of a new electrochemical detection method that enables distinguishing between various types of hemoglobin.

2. Material and methods

Montmorillonite was purchased from The Source Clays Repository – The Clay Minerals Society, New Castle formation, Country of Crook, State of Wyoming, USA. According to the supplier, the ideal chemical formula is:

(Ca0,12 Na0,32 K0,05)[Al3,01 Fe(III)0,41 Mn0,01 Mg0,54 Ti0,02][Si7,98 Al0,02]O20(OH)4.

Sodium dodecyl sulfate (SDS) with 99% purity was purchased from Acros Organics, and it was used as received.

Human hemoglobin was supplied as lyophilized powder by Sigma Aldrich.

Hydrogen-peroxide (30% purity) used for the electrochemical tests was obtained from Fluka.

Sample preparation

2.1. Acid activation of montmorillonite

Acid activation was performed in order to enhance specific surface area of sample as well as surface acidity. The procedure was adopted according to Vuković et al. [13] and the reaction conditions were as follows. Dried sample (5 g) was treated with 22.50 cm^3 of 4.5 M hydrochloric acid solution (solid to liquid phase ratio was 1:4.5). The dispersion was stirred at 90 °C for 2 h with constant stirring rate of 120 rpm in glass reactor equipped with a reflux condenser. After acid activation, the sample was cooled to room temperature, collected over a filter funnel under vacuum and the filtration cake was dialyzed against demineralized water until the filtrate was free of Cl^- ions (tested using 0.1 M $AgNO_3$). The sample was dried at 110 °C to constant mass, and denoted as AA.

2.2. Synthesis of acid-activated montmorillonite/SDS nanocomposites

The AA-SDS nanocomposites were prepared by the drop-wise addition of SDS solutions into AA dispersion at room temperature. Two samples, with different SDS loading, were prepared. The dispersion contained 1 g of AA in 50.0 cm^3 of distilled water. The SDS solutions containing either 50 mM or 25 mM of SDS were added into 20.0 cm^3 of the dispersion. Thus, nanocomposites with the SDS loading of 1 mmol g^{-1} and 0.5 mmol g^{-1}, respectively, were obtained. The dispersion was stirred for 2 h. The solid phase was separated by centrifugation at 6000 rpm for 30 min (model Heittech Eva 21). The samples were rinsed with demineralized water, dried at 60 °C for 12 h, and denoted as AA-SDS0.5 and AA- SDS1.0. The adopted denotation does not imply that the entire introduced amount of SDS was adsorbed. The actual SDS content was determined by means of elemental analysis.

2.3. Preparation of hemoglobin saturated samples

The preparation of hemoglobin saturated samples was performed in aqueous solution in a batch system by the adsorption of hemoglobin (Hb) on the acid activated clay (AA) and both nanocomposites (AA-SDS0.5 and AA-SDS1.0). The experiments were conducted at room temperature (25 °C) using the same volume of the solution (v) = 75.0 cm^3, and equal mass of each sample (m_{ads}) = 100.0 mg. The adsorption was carried out in a thermostated shaker (Memmert WNE 14 and SV 1422). The initial concentration of hemoglobin solution was 0.25 mg cm^{-3}. The dispersions were centrifuged (EBA 21 by Hettich) at 6000 rpm for 10 min. The absorbance of the supernatant solution was measured in the UV and visible wavelength range. The spectra of Hb were obtained using a Thermo Electron Nicolet Evolution 500 UV–vis spectrophotometer. Hemoglobin concentration was monitored at the characteristic wavelength (λ_{max} = 406 nm).

The adsorption was monitored with respect to contact time, in order for the equilibrium time to be estimated. The equilibrium time was 120 min for all investigated adsorbents. After that time, further adsorption was insignificant.

The saturated samples obtained after 120 min of adsorption were designated as AA-Hb, AA-SDS0.5-Hb and AA-SDS1.0-Hb.