Journal of Electroanalytical Chemistry 707 (2013) 102–109

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Adsorption of Euplotes octocarinatus centrin on glassy carbon electrodes as substrates to study europium–protein interactions

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ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 26 August 2013
Accepted 31 August 2013
Available online 7 September 2013

Keywords:
Eu^{3+}
Centrin
$Fe(CN)_6^{3-/4-}$
Cyclic voltammetry
Ac impedance spectroscopy

ABSTRACT

The adsorption of N-terminal domain of ciliate Euplotes octocarinatus centrin (N-EoCen) onto a glassy carbon (GC) electrode was studied by cyclic voltammetry and electrochemical impedance spectroscopy. The adsorption process obeys Langmuir isotherm adsorption equation. Based on the adsorption, direct immobilization of N-EoCen onto the glassy carbon surface was used for construction of an N-EoCen-modified GC electrode. Then the electrode was used to probe the binding mode of europium ions with N-EoCen. The results show that with the increasing concentration of europium ions, the redox peaks of probe increase gradually. Simultaneously, the peak separation decrease and peak currents of the redox reaction increase. In addition, two binding sites in N-EoCen show no-equiv signal of CV change: the signal is slightly changed by the binding of the first europium ions and largely did by the binding of the second europium ions. It can be attributed to the change of conformation or aggregation of protein after binding of europium ions. It offers a viable model for illustrating the interaction of lanthanides with EoCen. Through the titration curve the process of combination can be explored.

1. Introduction

Centrin belongs to the EF-hand calmodulin superfamily of calcium-modulated proteins and play a fundamental role in centrosome duplication and contraction of centrin-based fiber systems [1–3]. It can coordinate four Ca^{2+} with two high affinity sites in C-terminal and two low affinity sites in N-terminal [4], upon Ca^{2+} binding, triggered proteins undergo a large conformational change and in turn regulate a vast number of target proteins [5–7].

Ciliate Euplotes Octocarinatus centrin (EoCen) was firstly reported by our laboratory [8]. Lanthanide ions (Ln^{3+}) can react with EoCen occupying Ca^{2+} binding sites and induce EoCen to undergo conformational changes from closed state to open state, resulting in exposing hydrophobic patches to external environments [9,10]. The binding properties of Ln^{3+} (La^{3+}, Nd^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Tm^{3+}, Lu^{3+}) with EoCen were measured by the UV–Vis spectra [11,12], fluorescence spectra [13], circular dichroism spectra (CD) [14], resonance light scattering measurements (RLS) [15] and Cyclic voltammetry (CV) [16]. Self-assembly or namely aggregation, is an important characteristic of centrin in many life process [17]. Previous work proved that lanthanides also lead to the self-assembly of EoCen by spectroscopic measurement. Even though these studies have demonstrated the interaction of Ln^{3+} with EoCen to some extent, they are still to the disadvantage of the study of the lanthanides which have no sensitized fluorescence such as Eu^{3+}. Based on our knowledge, few work concerned with using electrochemical methods to investigate the interaction between Ln^{3+} and EoCen has been reported.

The interaction of proteins with solid conducting surfaces has long been an intensely investigated phenomenon [18–21]. Electrochemical methods, such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), reveal the changes in the electrochemical properties of the electrode due to adsorbed protein [22–25]. In all cases it was found that upon adsorption of protein, the electron transfer rates between the electrode surface and electrolyte are severely hindered. This effect was clearly demonstrated by the work performed by Guo et al. [26], using cyclic voltammetry at a gold, platinum and glassy carbon electrode in potassium ferricyanide before and after adsorption of serum albumin. They found that as protein was adsorbed, the formation of the inert protein layer caused a decrease in anodic peak current and an associated oxidation/reduction potential shift to more and less positive values, respectively, resulting in a decrease in the electron transfer in the redox reaction of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions at the electrode surface. Similar studies have been performed, such as haemoglobin, lactalbumin at a platinum electrode [27,28].

In this paper N-EoCen (about 101 residues and a molecular mass of 10 kDa) was gotten by using biological engineering method [8], which is the N-terminal domain of EoCen including the first and

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http://dx.doi.org/10.1016/j.jelechem.2013.08.035

second EF-hand domain (site I and site II). We constructed N-EoCen film on glassy carbon electrodes by adsorption as interfacial films, with Eu^{3+} regulating the permeation of $Fe(CN)_6^{3-/4-}$ to the N-EoCen film to investigate the interaction of Eu^{3+} with the N-EoCen.

2. Experimental

2.1. Reagents

N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (Hepes) was purchased from Sigma and used without further purification. All other chemicals are of analytical grade. The Eu^{3+} stock solutions were prepared by dissolving the appropriate mass of the Eu_2O_3, in hydrochloric acid, which was then standardized with EDTA in 0.1 M HAc–NaAc buffer at pH 5.5. In the electrochemical measurements, the supporting electrolyte was usually 0.01 M buffers of Hepes containing 0.1 M or 0.02 M KCl. Solutions were prepared with twice distilled water.

2.2. Electrochemical study

Electrochemical studies were carried out in a 5 mL cell incorporating three-electrode configuration containing supporting electrolyte, powered by the CHI 660C electrochemical analyzer (CHEN HUA Instrumental Co., Shanghai). A potassium chloride saturated calomel electrode (SCE) and a platinum wire were used as reference and counter electrodes, respectively. A glassy carbon disk electrode (GC) of 3 mm diameter was employed as the working electrode. Prior to each experiment, the GC electrodes were cleaned by polishing with an alumina–water slurry (high-purity Al_2O_3, particle size 0.3 and 0.05 μm, BDH) and sonicated briefly, followed by thorough rinsing with water. The potential sweep rate used was 50 mV s^{-1} in CV experiments, using a potential window of 0.5 to −0.2 V. As expected, EIS measurement was performed at amplitude of 10 mV and potential of 0.175 V (formal potential of ferro/ferricyanide redox) in the presence of 1.0 mM potassium ferricyanide solution containing 20 mM KCl. The frequency range was from 100 kHz to 0.1 Hz. Impedance data were fitted to appropriate model using the ZSimpWin software (Ametek).

2.3. Protein expression and purification

A truncated ciliate EoCen, N-EoCen, including the first and the second EF-hand domain, was obtained using biological engineering methods (see Supporting information). The protein concentration was measured spectrophotometrically at 280 nm using molar extinction coefficients of 4350 M^{-1} cm^{-1} for N-EoCen. The extinction coefficient of N-EoCen was estimated from the Tyr content as described by Pace et al. [29].

2.4. Resonance light scattering

Resonance light scattering (RLS) of samples was monitored by fluorescence in quartz cells of 1 cm optical path at 25 °C. The RLS was performed in 0.01 M Hepes at pH 7.4, 0.1 M KCl with a fluorescence spectrometer (F-2500, Hitachi, Japan) using the same excitation and emission wavelengths. Samples were prepared by gradually adding Eu^{3+} into solution of proteins. An equilibrium time of 5 min was used between each titration.

3. Results and discussion

3.1. The adsorption of N-EoCen onto a glassy carbon electrode

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used for monitoring the effect of exposing the GC electrode to a solution containing N-EoCen on heterogeneous electron transfer. The ferricyanide ions were used as the redox probe. The cyclic voltammograms of a 0.01 M Hepes buffer solution (pH7.4) containing 4.0 μM N-EoCen, 0.1 M KCl supporting electrolyte and 1.0 mM potassium ferricyanide at various exposure times are represented in Fig. 1. The potential sweep rate used was 50 mV s^{-1}, using a potential window of 0.5 to −0.2 V. As expected, $Fe(CN)_6^{3-/4-}$ shows the reversible behavior on a bare glassy carbon electrode (Fig. 1, curve a). In all voltammograms, when the GC electrode is exposed to N-EoCen in the solution, there is a decrease in peak currents and an increase in the peak potential separation over time. In the presence of N-EoCen, the reduction peak potential in the voltammogram was, for example, shifted 96 mV more negative after 280th cycle (~130 min) and the corresponding peak current was reduced by ~59%, as compared to the reduction peak current at the electrode exposed to the N-EoCen-free solution. A peak current profile for N-EoCen adsorption during 170 min is represented in Fig. 2 (curve b). It was constructed by dividing the background-corrected time-varying cathodic peak current for ferricyanide in the N-EoCen-contained solution ($I_{p,c}(t)$) by the cathodic peak current measured at time zero (the cyclic voltammogram recorded immediately after immersion of the GC electrode in N-EoCen-contained solution, ($I_{p,c}(0)$). A steady state current was reached at about 130 min later. Therefore, we chose 130 min as protein adsorption time on the GC electrode in this paper. This profile indicates that as the adsorption time increase, the cathodic peak current decrease. The insulating layer blocks the electron transfer between the redox probe and electrode.

For comparition, a peak current profile of ferricyanide at bare GC electrodes with 300 scan cycles is shown in Fig. 2 (curve a). There is a small decrease in peak currents upon cycling due to the electrode fouling by polymerized ferricyanide. However, the degree of peak currents decrease in the presence of N-EoCen is greater than that without protein. It is almost negligible for the adsorption effect of potassium ferricyanide compared with protein adsorption. Moreover, considering the isoelectric point of N-EoCen is 4.8, the N-EoCen is negatively charged at pH 7.4. Therefore, the mutually exclusive electrostatic interaction forces are expected between the N-EoCen and ferricynide. So, co-adsorption of protein with ferricyanide is expected to be very small. In order to clarify

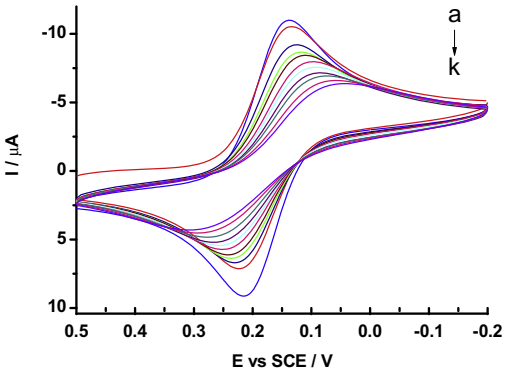

Fig. 1. Selected consecutive cyclic voltammograms of 1.0 mM $Fe(CN)_6^{3-}$ in 10 mM Hepes, 0.1 M KCl buffer (pH 7.4) in the absence (a) and presence of 4.0 μM N-EoCen (from b to k), using a potential sweep rate of 50 mV·s^{-1}. The cycle numbers are b: 1; c: 2; d: 3; e: 4; f: 10; g: 20; h: 50; i: 100; j: 200; k: 280.