Biosensors and Bioelectronics 103 (2018) 19–25

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ferritin based bionanocages as novel biomemory device concept

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ARTICLE INFO

Keywords:
Ferritin
Cage protein
Bionanocage
ANADOLUCA
Apoferritin
Biomemory

ABSTRACT

Ferritin is an iron cage having protein, capable of extracting metal ions in their cages and a consequence of the electron transfer of metal ions in their cage by reduction and oxidation processes, electrochemical information storage devices can be designed. In this work, ferritin based protein biomemory substrate has been synthesized by using Amino Acid (monomer) Decorated and Light Underpinning Conjugation Approach (ANADOLUCA) method, which utilizes photosensitive electron transfer based microemulsion co-polymerization as nanobead form of ferritin. Protein substrate contains metal ions such as silver and copper or metal ion pairs namely, silver-copper (Janus bionanocage) and co-polymeric shell of the photosensitive crosslinker protein. The redox behavior of bionanocages differentiates electrochemical "writing" and "erase" states depending on these metal ions (silver or copper) or metal ion pairs. The bionanocages based biomemory substrates have been immobilized using graphene modified glassy carbon electrodes and the memory functions of ferritin based bionanocages have been confirmed by chronoamperometry (CA) and open circuit potential amperometry (OCPA). The stability and durability of multi-state memory devices represent promising properties for future bioelectronic information technologies.

1. Introduction

Biomemory systems have been recently attracted because of some of the limitations in silicon memory devices as silicon based electronic device shift registers memory function based on electron transfer. Biomaterial based memory devices are used for energy storage and information transfer applications due to their outstanding functional and operational redox properties (Lee et al., 2011; Wang et al., 2015; Yagati et al., 2013; Güzel et al., 2017).

The metalloproteins are inspiring biomolecules that exhibit memory properties due to their redox behavior and electron transfer capabilities. The redox behavior of the metalloproteins is a consequence of the electron transfer of metal ions in the core by reduction and oxidation processes. Metalloproteins represent positive and negative charges in the presence of external voltage. In recent years, the design of metalloproteins has been widely used in nanoscience including biotechnology, electronics, medicine and sensing etc. (Ueno et al., 2007; Armstrong et al., 1997; Liu et al., 2014; Artés et al., 2012; Waldron and Robinson, 2009). In applications, when positive charge is applied up to oxidation potential level, electrons move from the protein monolayer to the electrode. This positive charge process corresponds to the function of information storage, which is the writing process. When the reduction potential is applied, electrons return to the protein monolayer, this behavior is called the erase state. Under the open circuit potential, electrons move freely in the material medium, which is the reading process. Because of "write", "read" and "erase" functions, metalloprotein biomaterials display memory properties like silicon based memory devices (Yagati et al., 2009; Yuan et al., 2013; Chung et al., 2010; Lee et al., 2010).

Graphene is an allotrope of carbon in the form of a two-dimensional honeycomb crystal lattice and hexagonal network in which one atom forms each vertex. It is the basic structural element of other allotropes, including graphite, charcoal, carbon nanotubes and fullerenes. Graphene has a considerable attention in the recent years due to its unique optical, chemical and electronic properties. In biosensing, graphene modified electrode is preferred because of its biocompatibility, ease for wiring up biomaterials to its high surface area and superior electrochemical properties. Other applications of graphene include in

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https://doi.org/10.1016/j.bios.2017.12.011
Received 4 October 2017; Received in revised form 4 December 2017; Accepted 6 December 2017
Available online 07 December 2017
0956-5663/

sensor technology, opto-electronic devices, high electron mobility transistors, supercapacitors, catalysis, photovoltaic (nanocrystal solar cell) and desalination (Notley et al., 2013; Champavert et al., 2015; Zuhang et al., 2012; Garacci et al., 2017; Xie et al., 2016; Parlak and Turner, 2016; Parlak et al., 2015; Liu and Long, 2015; Fan et al., 2010; Pumera et al., 2010; Kang et al., 2009; Güzel et al., 2018; Kuzum et al., 2014).

Ferritins are in the class of iron storage and mineralization proteins that can be extracted from the animals, plant and microbial agent. Ferritin is a protein, which stores iron and delivers it to the body by binding to a carrier called “transferrin”. Only two sizes of ferritin cages are known, 12 subunits (mini ferritins) and 24 subunits (maxi ferritins). Cage diameters are 12 nm and 8 nm with interior (inner) diameter of approximately 8 and 5 nm. Apoferritin (ApoFt) is a protein component of the iron storage ferritin consisting of twenty polypeptide chains, each of molecular weight about 23,000. ApoFt has an iron content of up to 4500 atoms, arranged symmetrically to form a hollow shell and carries nano-metal properties such as surface plasma, paramagnetic properties, electrical conductivity and drug carrier. In addition to that, it helps to reduce agglomeration of nanometal (Chasteen and Harrison, 1999; Harrison and Arosio, 1996; Zhang et al., 2007; Banyard et al., 1978; Massover, 1993).

Here, we fabricated ferritin based bionanocage based memory devices that have extractable metal ions in cages and represent stable multi-state memory behaviors. These single and Janus bionanostructures were evaluated as a new biomemory concept, and the bionanostructures were synthesized to replace metal ions in the cage of ApoFt protein using ANADOLUCA (Say et.al., 2011), which utilizes photosensitive electron transfer based microemulsion co-polymerization as nanobead form of ferritin. Ferritin based protein nanosubstrate contains metal ion extractable protein cage and co-polymeric shell of the ruthenium based photosensitive cross-linker to the protein (Fig. 1). The bionanocage based biomemory substrates were immobilized using graphene on glassy carbon electrodes (GCEs) to obtain a bionanomemory device for the first time. Size and shape of the bionanocage based bionanostructures were characterized by transmission electron microscopy (TEM) and scanning electron microscope (SEM). The memory functions of ferritin based bionanocages (FBNCs) were demonstrated by chronoamperometry (CA) and open circuit potential chronoamperometry (OPCA).

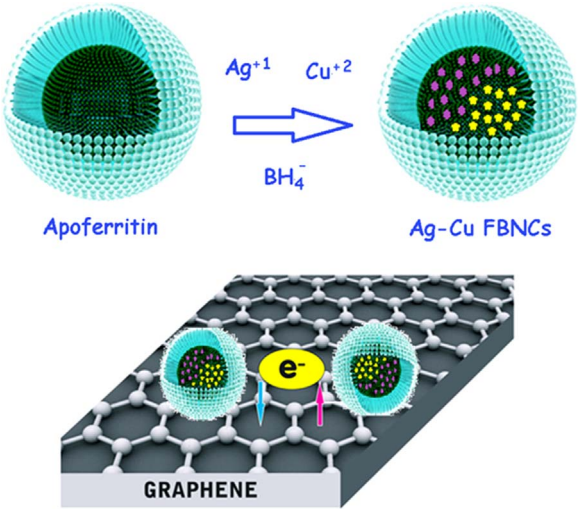

Fig. 1. Schematic representation of the oxidation potential; electron is transported from metal atoms in cages of ferritin bionanostructure “bionanocages” substrate to graphene electrode (write function), the reduction potential makes the electron returns backs to the bionanocages (erase function).

2. Materials and methods

2.1. Materials

$Cu(NO_3)_2{\cdot}3H_2O$, $AgNO_3$, KH_2PO_4, K_2HPO_4, NaOH, HCl, $NaBH_4$, acetonitrile and isopropile alcohol were purchased from Merck and glycine, polyvinylalcohol (PVA), tartaric acid and 3,3′,5,5′-tetramethylbenzidine (TMB) were provided from Sigma-Aldrich. ApoFt was obtained from Sigma-Aldrich at a concentration of 25 $mgmL^{-1}$ in 50% glycerol and 0.075 M NaCl. Amoniumpersulfate (APS) was purchased from local sources. Bis (2-2′-bipyridyl)-methacryloylamido tyrosine ruthenium(II) (MATyr-Ru$(bipyr)_2$-MATyr) was synthesized using previously published procedures (Say et al., 2011; Say, 2011).

2.2. Electrochemical analysis of different metal ions having bionanocage based biomemory substrates

Electrochemical measurements were performed by Gamry Reference 3000 Workstation (Gamry, USA) equipped with C3 cell stand. The electrodes consisting of silver, copper and mixture of silver-copper ions that are bound to ApoFt bionanocage on graphene modified GC was used as a working electrode) (Fig. 1). Platinum wire and Ag/$AgCl/KCl_{(sat)}$ electrodes were used as the counter and reference electrodes, respectively. Pt counter electrode, GCE and Ag/AgCl reference electrode were purchased from BAS (USA). Before modification, electrodes were dried under argon gas stream. The cyclic voltammetric (CV) measurements were performed in 10 mM phosphate buffer (pH 7.2) solution.

Electrochemical Impedance Spectroscopy (EIS) experiments were carried out using Gamry Reference 3000 workstation with EIS 3000 software. Graphene-based ferritine bionanocages electrodes were characterized in 1 mM ferrocyanide/1 mM ferricyanide redox couple via EIS methods. A sinusoidal potential modulation of 10 mV amplitude was superimposed on a fixed DC potential, amplitude and phase angle of the resulting current were recorded at frequencies ranging from 100 kHz to 0.1 Hz. Experimental data of EIS plot were analyzed by non-linear least squares (NLLS) fitting to the theoretical model represented by an equivalent electrical circuit.

SEM (FEI Quanta FEG250, Czech Republic) operated at 30 kV was used for the characterization of the morphology of different metal nanocage ApoFt structures. The ferritin-cage based bionanostructure was characterized by placing a drop of the suspension in a TEM copper grid with a holey carbon film and then images were taken using a FEI TECNAI TEM at an accelerating voltage of 200 kV.

2.3. Preparation of nanoapoferritin beads (NApoFPs)

ApoFt based bionanoparticles were synthesized according to ANADOLUCA method (Say et.al., 2011) Firstly, the microemulsion media was prepared by dispersing 0.5 g PVA in 45 mL of deionized water. 1000 ppm ApoFt solution was mixed with 10 mL of MATyr-Ru$(bipyr)_2$-MATyr for 20 min as aminoacid monomer cross-linker and the mixture was added into 15 mL of PVA. Initiator solution was prepared by dissolving 0.02 g APS in 45 mL of deionized water and then, 5 mL of this solution was added into the reaction mixture. The reaction was stirred for 48 h under nitrogen atmosphere, at room temperature. ApoFt nanoparticles (NApoFPs) were separated from the solution, the mixture was homogenized by centrifugation at 12,000 rpm for 10 min and washed twice with deionized water to remove unreacted substances.

2.4. Preparation of Ferritin Based Bionanocages as Bionanomemories (NFCages)

Ferritin Based Bionanocages *(NFCages)* were synthesized using NApoFPs as main bionanostructure and various metal atoms can be embedded into lattice of this bionanostructure. Primarily, solutions of