Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Functional interfaces for biomimetic energy harvesting: CNTs-DNA matrix for enzyme assembly☆

Rachel M.E. Hjelm [a], Kristen E. Garcia [c], Sofia Babanova [b], Kateryna Artyushkova [b], Ivana Matanovic [d], Scott Banta [c], Plamen Atanassov [b,*]

[a] Nanoscience and Microsystems, MSC01 1120, 1 University of New Mexico, Albuquerque, NM 87131-0001, USA
[b] Department of Chemical and Biological Engineering, Farris Engineering Center 209, MSC01 1120, 1 University of New Mexico, Albuquerque, NM 87131-0001, USA
[c] Department of Chemical Engineering, Columbia University, 500 W 120th St, New York City, NY 10027, USA
[d] Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545, United States

ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form 4 December 2015
Accepted 23 December 2015
Available online xxxx

Keywords:
Biomimetic
Enzymatic fuel cell
Enzymatic cascade
DNA-CNT matrix
Small laccase
Zinc finger

ABSTRACT

The development of 3D structures exploring the properties of nano-materials and biological molecules has been shown through the years as an effective path forward for the design of advanced bio-nano architectures for enzymatic fuel cells, photo-bio energy harvesting devices, nano-biosensors and bio-actuators and other bio-nano-interfacial architectures. In this study we demonstrate a scaffold design utilizing carbon nanotubes, deoxyribose nucleic acid (DNA) and a specific DNA binding transcription factor that allows for directed immobilization of a single enzyme.

Functionalized carbon nanotubes were covalently bonded to a diazonium salt modified gold surface through carbodiimide chemistry creating a brush-type nanotube alignment. The aligned nanotubes created a highly ordered structure with high surface area that allowed for the attachment of a protein assembly through a designed DNA scaffold. The enzyme immobilization was controlled by a zinc finger (ZNF) protein domain that binds to a specific dsDNA sequence. ZNF 268 was genetically fused to the small laccase (SLAC) from *Streptomyces coelicolor*, an enzyme belonging to the family of multi-copper oxidases, and used to demonstrate the applicability of the developed approach. Analytical techniques such as X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and enzymatic activity analysis, allowed characterization at each stage of development of the bio-nano architecture.

This article is part of a Special Issue entitled Biodesign for Bioenergetics — the design and engineering of electronic transfer cofactors, proteins and protein networks, edited by Ronald Koder and J.L. Ross Anderson.

1. Introduction

Biomimetics or bio-inspired design is a term that can be seen very often currently in almost any field of scientific research. Facing the rapid technological development and the increasing engineering demands, scientists have looked for new technologies inspired by biological solutions at both the macro- and especially at the nanoscale [1–4].

A key component in the design of enzyme-based technologies, such as enzymatic fuel cells, photo-bio energy harvesting devices, nano-biosensors, bio-actuators and other bio-nano-interfacial architectures is the effectiveness, specificity and stability of the enzyme immobilization [5,6]. Over the years researchers have developed methods for integration of enzymes and nanomaterials. Some of these methods include direct protein adsorption [7,8], enzyme tethering [9–11], entrapment in polymers [12], cross-linking [13], chemical bonding [14], protein engineering to form hydrogels [15–17] or fusion of proteins to create novel bioactive materials [18]. Proteins have high affinity for direct absorption onto carbon materials, such as carbon nanotubes (CNTs), however, the hydrophobic nature of the CNTs surface has resulted in lowered catalytic activity and shortened lifetimes likely due to altered tertiary structure and protein denaturing. The use of surfactants, ssDNA and polymers are approaches that have been developed to overcome this problem. These methods are non-covalent and thereby do not directly interfere with the intrinsic properties and structure of either proteins or CNTs [19]. Polymer entrapment of enzymes is one of the most common methods for constraining enzymes on support materials [6,12,20–24]. Unfortunately, enzyme distribution throughout the polymer matrix can be heterogeneous [6]. On the other hand, protein engineering strategies have enabled proteins to self-assemble while ither retaining or enhancing their catalytic performance [15].

☆ This article is part of a Special Issue entitled Biodesign for Bioenergetics — the design and engineering of electronic transfer cofactors, proteins and protein networks, edited by Ronald Koder and J.L. Ross Anderson.

* Corresponding author.
E-mail addresses: rhjelm@unm.edu (R.M.E. Hjelm), keg2135@columbia.edu (K.E. Garcia), babanova@unm.edu (S. Babanova), kartyush@unm.edu (K. Artyushkova), igonzales@unm.edu (I. Matanovic), sb2373@columbia.edu (S. Banta), plamen@unm.edu (P. Atanassov).

http://dx.doi.org/10.1016/j.bbabio.2015.12.012
0005-2728/

An example of an advanced bio-nano-interfacial architecture has been demonstrated by Rawson et al., who vertically aligned single walled carbon nanotubes (VASWCNTs) on an indium tin oxide (ITO) surface and explored them for intracellular electrochemical sensing in eukaryotic cells [25]. To achieve uptake of the CNTs into the cells, the VASWCNTs were wrapped with ssDNA. The resulting DNA-VASWCTNs assemblies were taken up naturally by a mouse macrophage cells and used to electrochemically investigate the intracellular environment and activity.

This architecture was further developed in this study and combined with a protein engineering approach for controlled attachment of enzymes. The specificity of zinc finger-DNA interactions was explored for controlled enzyme immobilization. As proof of concept, a zinc finger (ZNF268) was genetically fused to the small laccase (SLAC) from *Streptomyces coelicolor* and attached to a three-dimensional carbon nanotube-DNA assembly. In our previous studies we demonstrated that a multifunctional SLAC-3ZNF fusion protein could be engineered, expressed and successfully immobilized on dsDNA [26]. Here this finding was expanded toward the incorporation of the SLAC-3ZNF protein into a more sophisticated design of the supporting assembly. For the creation of the supporting scaffold, carbon nanotubes aligned in a brush-type formation were wrapped with DNA engineered to be recognized by the zinc finger, acting as a docking place for its attachment.

2. Materials and methods

2.1. Reagents and stock solutions

Dibasic potassium phosphate (K_2HPO_4, Lot # 2014091787), monobasic potassium phosphate (KH_2PO_4, Lot # 2012022368), dimethyl sulfoxide (DMSO, Lot # SHBC2756V), 1 N hydrochloric acid (HCl, Lot # 49279) and HPLC Omnisolv water (Lot # 57184) were purchased from EMD Millipore (Billerica, MA). Single walled carbon nanotubes 99 were purchased from cheaptubes.com. Carboxylic acid functionalized single walled carbon nanotubes (f-SWNT, Lot # 03619LD), 2,6-dimethoxyphenol (DMP, Prod. No. D135550-25G), p-phenylenediamine (PPD, Low # MKBJ5024V), N,N-dicyclohexylcarbodiimide (DCC, Lot # SHBC2756V) and sodium nitrite 97+% ($NaNO_2$, Lot # 08316DJ) were purchased from Sigma-Aldrich. Acetone (Lot # 050713E) was provided by BHD chemicals. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was from Gold Biotechnology (St. Louis, MO). HALT protease inhibitor, precast NuPAGE SDS-PAGE gels, NuPAGE SDS MES running buffer and Novex Sharp Pre-stained Protein Standard were from Thermo Fisher Scientific (Waltham, MA). HisTrap HP columns and the ÄKTA FPLC system were purchased from GE Healthcare (Piscataway, NJ). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) at the highest purity unless otherwise specified.

2.2. Gold modification

Gold deposition on glass support was carried out through spray evaporation. Glass covered slides were cleaned using ozone prior to deposition. A 3 nm layer of chromium and 100 nm layer of gold were deposited subsequently. The gold surface was further modified through electrochemical grafting. Three-electrode set-up with a gold covered slide used as working electrode; Ag/AgCl as reference and Pt-wire as counter electrodes, respectively was used for the electrochemical grafting. 10 ml solution of 7 mM p-phenyleneamine diazonium salt was used as electrolyte and modifying solution. The arylamine diazonium salt was synthesized when 7 mM p-phenylenediamine (PPD) interacted with 1 mM $NaNO_2$ in 0.5 M HCl for 5 min under ice [27]. Cyclic voltammetry (CV) was utilized as electrochemical technique for the grafting procedure. CVs at 100 mV/s in three potential windows starting from 0.4 V to −0.6, −0.4 and −0.2 V vs. Ag/AgCl, respectively was carried out to form an arylamine layer on the gold surface [27,28].

2.3. Nanotube attachment

The next step was covalent attachment of carbon nanotubes onto the modified gold slides. Carboxyl-functionalized single walled carbon nanotubes (f-SWNT, 3 to 5 μm) were explored due to the presence of carboxyl functional groups at the mouth of the nanotubes necessary for the chemical bonding with the amine groups of the arylamine layer deposited onto the support surface. 0.4 mg/mL f-SWNTs were suspended in dimethyl sulfoxide (DMSO), sonicated for 15 min and then combined with a solution of 0.4 mg/mL N,N′-dicyclohexylcarbodiimide (DCC) in DMSO. The modified gold samples were submerged in the solution and sonicated for 15 min. The submerged samples were then transferred into an oven and heated in a closed cell for 24 h at 55 °C. Samples were then washed with a series of polar solvents starting with 2 min sonication in acetone followed by 10 s sonication in isopropyl alcohol and rinsed with HPLC grade water.

2.4. DNA scaffold

The ssDNA sequence 5′-TTTTTTTTTTTTTTTTTTTTCGCCCACGCTTTTTTTTTTTTTTTTT-3′ and ssDNA of complimentary ZNF specific binding base sequence 5′-GCGTGGGCG-3′ were ordered as custom oligomers from Sigma-Aldrich. The complementary DNA (5′-GCGTGGGCG-3′) was marked with Thiol-SS-C3, a disulfide-containing oligo modifier, at the 5′ terminus and with Thiol-SS-C6 at the 3′ terminus. DNA hybridization took place in solution. dsDNA was allowed to hybridize through 15-minute sonication prior to submerging Au-Aryl-SWNT samples or f-SWNTs (1:1 M ratio), depending on the experiment. Samples underwent sonication for 15 min and were left in solution for 24 h before being removed, rinsed with HPLC water and dried with N_2.

2.5. SLAC-3ZNF complex expression and purification

Expression and purification of SLAC-3ZNF with the plasmid pSLAC-3ZF were performed as described in Szilvay et al. 2011 (Supplementary Methods) [26] with minor modifications. Cells were grown in 2xYT media at 37 °C until the OD_{600} reached approximately 1.5. Protein expression was induced with 0.4 mM IPTG, and protein expression continued for 20 h at 25 °C before cells were collected by centrifugation and stored at −20 °C. Pellets corresponding to 1 L of culture were thawed and suspended in 50 mL of binding buffer (20 mM Na Pi pH 7.3, 50 mM NaCl, 40 mM imidazole) supplemented with HALT EDTA-free protease inhibitor. Cells were sonicated on ice for 6 min with a microtip probe, and lysates were clarified by centrifugation. Lysates were then purified using a HisTrap HP column equilibrated with binding buffer. A gradient of elution buffer (20 mM NaPi pH 7.3, 500 mM NaCl, 500 mM imidazole) was applied, and fractions were collected and analyzed with SDS-PAGE. Fractions containing SLAC-3ZF-His shown at 50 kDa were pooled together. The protein was then dialyzed against binding buffer containing 1 mM $CuSO_4$ and 1 mM $ZnSO_4$ at 4 °C overnight. The sample was then dialyzed against ammonium bicarbonate buffer and concentrated by ultrafiltration. They were then frozen overnight in micro-centrifuge tubes at −80 °C, followed by a minimum of 24 h of lyophilization.

2.6. Enzyme immobilization

A solution of 1 mg/ml SLAC-3ZNF in HPLC water was prepared. SWNT-dsDNA complexes aligned on the gold support were submerged in the enzyme solution and left for 24 h at 4 °C for enzyme immobilization. The sample was removed from the SLAC-3ZNF solution, washed three times and dried under N_2.

Please cite this article as: R.M.E. Hjelm, et al., Functional interfaces for biomimetic energy harvesting: CNTs-DNA matrix for enzyme assembly, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbabio.2015.12.012