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Interactions between avidin and graphene for development of a biosensing platform

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ABSTRACT

Fundamental understanding of interactions at the interface of biological molecules, such as proteins, and nanomaterials is crucial for developing various biocompatible hybrid materials and biosensing platforms. Biosensors comprising of graphene-based conductive nanomaterials offer the advantage of higher sensitivity and reliable diagnosis mainly due to their superior specific surface area and ballistic conductivity. Furthermore, conductive nanocomposite structures that immobilize proteins can synergize the properties of both transducers and molecular recognition elements improving the performance of the biosensing device. Here we report for the first time, using a combined molecular dynamics simulations and experimental approach, the interactions between avidin and graphene for the development of a sensing platform that can be used for the detection of biological macromolecules such as mismatch repair proteins through biotinylated DNA substrates. We find that the interactive forces between avidin and graphene are mainly hydrophobic, along with some van der Waals, electrostatic and hydrogen bonding interactions. Notably, the structure and function of the avidin molecule are largely preserved after its adsorption on the graphene surface. The MD results agree well with scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS) analysis of avidin immobilized on a graphenated polypyrrole (G-PPy) conductive nanocomposite confirming the adsorption of avidin on graphene nanoplatelets as observed from the Fourier-transform infrared spectroscopy (FTIR).

1. Introduction

With the advance of nanobiotechnology, the investigation of biological macromolecules and nanomaterials has progressed to molecular and atomic levels with a focus on understanding and controlling the interfaces between them (Sarikaya et al., 2003; Zhao et al., 2008; Nel et al., 2009; Kostarelos and Novoselov, 2014). Research on the interface between protein and conducting nanomaterials, such as graphene and carbon nanotubes, has revolutionized industries particularly interested in developing hybrid biomaterials and biosensors (Zhang et al., 2013; Georgakilas et al., 2012; Hu et al., 2011; Lu et al., 2009; Gräslund et al., 2008; Xue et al., 2014).

Biosensors are now ubiquitous in medical diagnostics and analysis systems in order to detect and quantify macromolecules such as oligonucleotides and proteins. One of the primary performance matrices of a biosensor is its sensitivity, which depends on the design and synthesis of the interface between the biological recognition element and the transducer. Graphene, due to its superior mechanical and electrical properties, has been the center of interest in the development of biosensors (Rodrigo et al., 2015; Zhang et al., 2015; Kuila et al., 2011; Shao et al., 2010). Advances in DNA immobilization techniques also point to the use of conductive polymers such as electropolymerized polypyrrole in avidin/biotin systems as a means of irreversibly immobilizing oligonucleotides to form DNA-based biosensors (Dupont-Filliard et al., 2001; Ouerghi et al., 2002; Dupont-Filliard et al., 2004). These methodologies typically utilize impedance measurement for label-free detection of target molecules (Daniels and Pourmand, 2007; Drummond et al., 2003; Sadik et al., 2009). Combining these concepts, the development of a biotinylated-DNA-avidin-graphene based biosensor is a plausible approach for functional detection of DNA-binding protein

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biomarkers (for example, mutant DNA mismatch repair proteins associated with Lynch Syndrome (Tiwari et al., 2015)).

Development of such sensitive biosensors demands a thorough understanding of the interactions at the interface of single-layer graphene and avidin molecules. It has been previously reported that protein adsorption on a graphene surface is minimal, mainly due to hydrophobic interactions (Russo and Passmore, 2014). It is also known that chemical modifications or the presence of support substrates can substantively improve the adsorption of biomolecules on the surface of graphene/graphene oxide (Lee et al., 2011; Yokota et al., 2011; Alava et al., 2013). More recently, it has been shown that avidin can selectively deposit on graphene flakes in the presence of a chemically modified SiO_2/Si support substrate (Kamiya et al., 2014). However, the process of self-assembly between a graphene surface and avidin molecule through strong non-bonding interactions is not understood in detail. This knowledge is particularly important in the case of proteins because their rapidly changing conformations can alter the interactions within a matter of few femtoseconds, making physical quantification extremely difficult. Molecular Dynamics (MD) simulations can be used effectively to address this problem. Previous MD simulation data on avidin are mostly concerned with the formation or dissociation of the avidin-biotin complex and the energy changes involved therein (Wilchek and Bayer, 1988; Evans and Ritchie, 1997; Izrailev et al., 1997; Isralewitz et al., 2001); however, to our knowledge there are no such data available on interactions between avidin and other macromolecules, especially nanoparticles. In contrast, there is a plethora of MD simulations on graphene alone to determine its various physical properties such as thermal and electrical conductivity as well as mechanical properties such as stress, strain and Young's modulus (Hu et al., 2009; Jiang et al., 2009; Pei et al., 2010; Zhong et al., 2011). However, even in this case, only limited data are available on integrated atomic MD simulations of graphene and avidin. For instance, Haddad and colleagues have reported the only MD simulation study of the interactions of avidin and single-walled carbon nanotubes wherein avidin was deposited on biotinylated carbon nanotubes, the latter being assembled via pyrrole electro-polymerization (Haddad et al., 2009). Hence, a survey of recent literature indicates that limited information on direct interactions between graphene and avidin is currently available.

Given that additional details on the interface between avidin and nanomaterials could significantly improve the design of biosensing platforms, we conducted a MD simulation study of the adsorption of avidin on the surface of non-functionalized graphene. We found that the interaction forces between avidin and graphene are mainly hydrophobic, along with some van der Waals, electrostatic, and hydrogen bonding interactions. Importantly, the structure and function of avidin is preserved even after its adsorption on the graphene surface. Furthermore, the results from the MD simulations are supported by scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS) experiments examining the morphological features of avidin immobilized on a graphenated polypyrrole (G-PPy) conductive substrate. The findings offer new insights into graphene-avidin interactions that can be applied towards a novel 'DNA-avidin-G-PPy' biosensing platform for the detection of DNA binding proteins.

2. Materials and methods

2.1. Molecular dynamics simulations and analysis of the interactions between avidin and graphene

Interactive forces between graphene and avidin were set up and analyzed using Visual MD (VMD) (Humphrey et al., 1996) and the simulations were carried out using NAMD (Phillips et al., 2005). Note that since avidin can selectively deposit on exposed graphene flakes in the presence of other substrates as reported by Kamiya and colleagues (Kamiya et al., 2014), pyrrole was excluded from the simulations. The avidin pdb file (2AVI) was obtained from the protein data bank and an inbuilt graphene sheet builder plugin in the VMD was used to create a single layer graphene sheet of dimensions 88 Å × 121.44 Å. All-atom simulations containing a single-barrel avidin monomer (120 residues) and a single layer graphene sheet (4292 atoms) were carried out for a time period of 100 ns. All simulations used the CHARMM (MacKerell, et al., 1998) force field and TIP3 (Jorgensen et al., 1983) water model with a neutralizing salt concentration of NaCl for effective polarization of the water molecules. A cluster of four HP Z230 systems with a total of 32 cores each using Intel Xeon processor and Quadro K620 CUDA acceleration capability were used to perform all simulations.

In each simulation, the temperature was maintained at 300 K by Langevin thermostat and a pressure of 1 atm through Nose-Hoover Langevin-Piston barostat with a period of 100 ps and a decay rate of 50 ps assuming the periodic boundary conditions. A 10,000-step energy minimization of the entire system (containing graphene, avidin, water, and NaCl ions) was performed first to reach a stable state. All atom-simulations employed an integrated time step of 2 fs. A cut-off of 10–12 Å designated the short-range forces while long-range forces were calculated using Particle Mesh Ewald (PME) algorithm. Root Mean Square Deviation (RMSD) and NAMD energy extensions were used to determine interaction energy between avidin and graphene. VMD Timeline tool was used to determine the secondary structure of avidin and Root Mean Square Fluctuations (RMSF) as well as RMSD for the adsorbed residues. TCL scripting was used to determine the number of atoms per cut off distance from the graphene surface and interaction energy per number of atoms.

2.2. Reagents and apparatus

The chromium-plated nickel chips were purchased from ExpressPCB (Santa Monica, CA, USA), each consisting of a 6 finger, incomplete circuit embedded on a 4 cm × 2 cm plastic frame. Avidin (Egg white, unconjugated, 10 mg) and HEPES (1 M, 100 ml, pH 7.2–7.5) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Pyrrole (100 ml, reagent grade 98%, vapor density 2.31, density 0.967 g/ml at 298 K) and sodium sulfate (Na_2SO_4, anhydrous, reagent grade, 500 g) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Carolina Biological Supply (Burlington, NC, USA) respectively. Graphene nanoplatelets were purchased from Cheap Tubes Inc. (Cambridgeport, VT, USA).

Electrochemical polymerization was performed using a conventional 3-electrode cyclic voltammetry setup (eDaq USA, Model number ER466): a working electrode (the chip) onto which the G-PPy substrate is formed, a platinum wire as the counter electrode (CE) and Ag/AgCl (saturated KCl) as the reference electrode (RE). EIS was performed using an electrochemical impedance analyzer (eDaq USA, ERZ 100). The morphology of the surface was analyzed using SEM (SEM, S-4800, Hitachi, Japan) with an acceleration voltage of 20 kV. Exposure time was fairly limited due to the denaturation of avidin under prolonged radiation.

2.3. Preparation of graphene-polypyrrole solution and its in-situ polymerization

A modification of the method described previously by Aphale and colleagues was used for preparation of the G-PPy nanocomposite film (Aphale et al., 2015). The initial pyrrole-graphene homogenized mixture was prepared by dissolving 0.2 ml of pyrrole, 0.355 g of sodium sulfate (Na_2SO_4) and 0.01% (w/v) of