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Molecular modeling of fibronectin adsorption on topographically nanostructured rutile (110) surfaces

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ABSTRACT

To investigate the topographical dependency of protein adsorption, molecular dynamics simulations were employed to describe the adsorption behavior of the tenth type-III module of fibronectin (FN–III_{10}) on nanostructured rutile (110) surfaces. The results indicated that the residence time of adsorbed FN–III_{10} largely relied on its binding mode (direct or indirect) with the substrate and the region for protein migration on the periphery (protrusion) or in the interior (cavity or groove) of nanostructures. In the direct binding mode, FN–III_{10} molecules were found to be 'trapped' at the anchoring sites of rutile surface, or even penetrate deep into the interior of nanostructures, regardless of the presented geometrical features. In the indirect binding mode, FN–III_{10} molecules were indirectly connected to the substrate via a hydrogen–bond network (linking FN–III_{10} and interfacial hydrations). The facets created by nanostructures, which exerted restraints on protein migration, were suggested to play an important role in the stability of indirect FN–III_{10}–rutile binding. However, a doubly unfavorable situation – indirect FN–III_{10}–rutile connections bridged by a handful of mediating waters and few constraints on movement of protein provided by nanostructures – would result in an early desorption of protein.

1. Introduction

Adsorption of proteins at a solid–liquid interface is an important phenomenon in biology and highly relevant for the design of implant with excellent biocompatibility [1,2]. When a biomaterial comes into contact with a biological milieu containing cells, the soluble proteins adsorb onto the surface of biomaterial rapidly, saturating it within a time frame of seconds to minutes [3]. Therefore, living cells may never see the native biomaterial, and the cell–surface interplay is ultimately an interaction between cells and surface–bound protein layer [4]. That is, the cellular response to the biomaterials largely depends on the speciation, conformation and orientation of the bound protein layers, whereas the geometrical properties of surfaces can be conveyed indirectly through the adsorbed proteins [5]. Hence, the local changes, especially in the nanoscale surface topographies, may lead to changes in quantity, density and orientation of the adsorbed proteins; thus attention needs to be paid to understand how the protein perceives its substrate.

Many experimental approaches have been applied to figure out whether the existence of nano–scale geometrical characteristics of substrates can enhance protein adsorption, or which kind of nanostructure may act as a preferred site for protein deposition. De Luca et al. [6] found that fibronectin was adsorbed onto the microgrooved metallic surfaces at low concentrations, and the protein molecules were observed to selectively aggregate at the groove/ridge boundaries. Huang et al. [7] prepared the TiO_2/silicate hierarchical coatings with various nanostructures on titanium substrates, finding that the improvement of fibronectin adsorption was morphological dependent according to the trend: nanoleaf coating > nanoplate coating > nanorod coating. Giamblanco et al. [8] fabricated chemically homogeneous nanostructured surfaces of variable local curvature, concluding that the local curvature was strongly correlated with the kinetic adsorption phase and rate of laminin adsorption on the nanostructured surfaces. The

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dependence of protein adsorption on nano–scale patterned surface was also confirmed in the AFM–based force spectroscopy experiments [9] and the radiolabeling experiments [10]. The fibronection tended to adsorb preferentially in the concave grooves on the surface of boron-doped silicon wafers [9]. Similarly, the adsorbed fibrinogen was shown to be regularly distributed most on the flanks and valleys of the dot–like protrusions, but sprinkled throughout the flat surface of poly(dimethylsiloxane) [10].

The current researches suggest that proteins are sensitive to the nanoscale environment of anchoring surface, probably even showing special affinity for certain type of nanostructures. That is, although the protein-surface systems cover a huge diversity, a detailed understanding of how the individual proteins attach to artificially nanostructured surfaces on a molecular level is urgently required. Compared to the experimental approaches, computational methods, which could eliminate occasional influences of experiments and create plausible structural models of proteins on solid surfaces, seems to be a feasible option to reveal the interaction mechanism between the surface of biomaterials and proteins. Monti [11] and Skelton et al. [12] conducted molecular dynamics (MD) simulations to understand the adsorption of polypeptides on the strongly polar surfaces with explicit water, observing that binding is not to the surface itself, but rather to a structured water layer on the surface. Yang et al. [13] believed that the driving force for protein binding on the titania nanotubes derived from the physical adsorption on the basis of MD simulations. Raffaini and Ganazzoli [14] adopted MD simulations to assess the effect of surface curvature of single-walled carbon nanotubes (SWNT) on protein adsorption, finding that the adsorption strength was slightly weaker on the outer convex surfaces of SWNT and was conversely enhanced on the inner concave surface of SWNT. Our group performed MD simulations to figure out the influence of cation mediation in peptide-surface interaction, observing that the divalent cations seemed more effective than the monovalent cations in bridging the negatively charged adsorbate to the negatively charged adsorbent, [15–17] as reported in some experimental studies [18,19].

MD simulations were also adopted in the present work to describe early adsorption of a fragment of fibronectin (FN) onto the nanostructured surfaces. FN is a major component of the extra-cellular matrix in tissue, which influences cell adhesion and migration through interactions with other extra-cellular components as well as with cell surface receptors [20]. FN comprises three different kinds of homology units referred to as types I–III, and the 10th Type III module (FN-III_{10}) contains the important cell–binding sequence—arginine–glycine–aspartic acid (Arg–Gly–Asp, RGD) [21]. The RGD sequence serves as a primary cell attachment cue and modulates the cell adhesion by being linked to integrin receptors located at the cell membrane, i.e., $\alpha_{5\beta1}$, $\alpha_{x\beta1}$ and $\alpha_{3\beta1}$ [21]. Therefore, FN-III_{10} was selected as the adsorbate and the most stable phase of TiO_2 polymorphs at ambient temperature—rutile [22] was chosen to be the adsorbent. As is well known, the hydroxyl coverage of a native oxide film formed on a Ti-surface depends on the adsorption state of water; however, whether water dissociates upon adsorption on the TiO_2 surface remains controversial. The common view based on experiments [23–25] is that water adsorbs molecularly on the ideal TiO_2 (110) surface and dissociation prefers to take place at defect sites, however, several recent experimental studies have presented evidences for the presence of a dissociated species on the defect-free surfaces [26,27]. In theoretical studies, a delicate balance between dissociated, partial dissociated, and molecular adsorption of water is suggested [28–30]. In light of this, it is hard to gain a clear-cut answer to whether water adsorbs associatively or there is dissociation of water to produce hydroxyl sites on the TiO_2 surface on the basis of the available information. Moreover, the focus of this work is to assess the influence of geometrical features of substrate on the adsorption behavior of protein, thus three nonhydroxylated rutile (110) surfaces characterized with different types of nanostructures (i.e., protrusion, cavity or groove) were introduced here. Meanwhile, the evaluation and structural optimization of hydrated/hydroxylated step edges of rutile (110) surface, which could be applied to the protein/rutile/aqueous solution system, will be covered in another paper of ours [31].

2. Simulation methods

The MD simulations of FN-III_{10}–rutile assembly in aqueous solution were carried out in the NVT ensemble, where the number of particles (N) and volume (V) of the system are held constant and the temperature (T) is in equilibrium with that of its heat bath. The rutile (110) surface was reproduced through the Matsui and Akaogi parametrization [32]. Since a single FN-III_{10} molecule can be enveloped in an ellipsoid with an approximate dimension 43 Å × 19 Å × 17 Å, [33] the nanostructures in this work were built with dimensions in the same order as the protein segment, in order to get a detailed understanding of the influence of surface nanotopographies on protein adsorption. The dimensional details of protrusion, cavity and groove are shown in Fig. 1. The Amber force field [34] was utilized to describe the protein and the SPC/E water model [35] was chosen to represent the solvent. The interaction potentials between amino acid residues/water and rutile atoms were obtained from the literature, [36] and the parameters of Lennard-Jones potential for the cross interactions between nonbonded atoms followed the Lorentz-Berthelot rule [37]. The simulation box with the size roughly equal to 142 × 130 × 80 $Å^3$ was filled with water molecules, the density of which was set to the value corresponding to 1 atm of solution pressure. Periodic boundary conditions were applied in the *x*– and *y*–direction, and a reflecting boundary condition was applied in the *z*–direction. The Nosé–Hoover thermostat [38] was used to control the temperature and the particle–particle particle–mesh (PPPM) solver [39] was employed to handle the long–rang electrostatic interactions.

The FN-III_{10} molecules were put close to the nanostructured rutile surfaces with different arrangement, maintaining the initial distances from any atom of FN-III_{10} to the rutile atoms larger than 4.5 Å. The solute (FN-III_{10} and rutile) was kept frozen first to randomize the position of water molecules, but the constraints were removed after an energy minimization (corresponding to approximate 0 K), only keeping the lowest layer of rutile fixed till the end of simulations. The system temperature was then increased gradually from 0 K to 300 K in increments of 50 K every 250 ps, with the FN-III_{10} treated as an independent rigid body, which moved and rotated as a single entity (i.e., body movement). After that, the systems were relaxed at T = 310 K for 1 ns with position restraints on the backbone atoms of amino acid residues (i.e., restraining movement), and the resulting configurations were equilibrated without any constraint for 8 ∼ 16 ns, depending on the specific state of different molecular assemblies.

3. Results

In the MD simulations, FN-III_{10} molecules were initially put close to the rutile surface without any direct connection. The proteins remained in the immediate vicinity of nanostrucures after the 'body' and 'restraining' movements were the focus of this work, and seven typical configurations shown in Fig. 2 were selected to proceed to the production run. Proteins can 'sense' the topography of surfaces at the nanometer scale; [40–43] but the research to gain a detailed understanding of how different surface nanostructures affect the binding affinity of proteins is still in its infancy. Therefore,