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Effect of nano-scale constraint on the mechanical behaviour of osteopontin–hydroxyapatite interfaces

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ABSTRACT

In many biological materials, the interfacial behaviour between constituents such as protein and minerals greatly contributes to the overall mechanical behaviour. However, the governing mechanisms, in particular at small material length scales, have not been clearly understood. This paper presents a molecular dynamics (MD) study of the mechanical behaviour of osteopontin (OPN) and hydroxyapatite (HA) interfaces under different geometrical constraints. The results indicate that some OPN residues are attracted to the HA layers during loading. The formation of new interfacial bonds leads to a stick-slip type motion of the OPN peptides along the HA surfaces, resulting in high pulling force and energy dissipation. The attractive interaction energy and energy dissipation generally increase with reducing the gap distance between the HA layers, demonstrating a significant nano-scale constraint effect.

1. Introduction

Hard biological materials such as bone and shell possess superior mechanical properties such as combined high stiffness and toughness, although their main components are brittle mineral platelets and soft protein molecules [1,2]. A thorough understanding of the governing mechanisms will help design and develop novel bio-inspired materials with similar or even better mechanical performance. Some examples are nacre-inspired materials introduced previously [3,4].

The tiny sized building blocks in the internal structure of biological materials provide extremely large interfaces between constituents. The interfaces between constituents mainly experience high shear force along the interface direction [5–7]. Therefore, interfacial shearing behaviour greatly affects the mechanical properties at larger length scales (e.g. micro- and macro-length scales). In hard biological materials, some protein molecules are constrained by hard components. For example, thin extra-fibrillar protein matrix is sandwiched between mineralised collagen fibrils (MCF) in bone [8,9]. One of the main components in the extra-fibrillar protein matrix is non-collagenous protein, e.g. osteopontin (OPN), while MCF is mainly made of hydroxyapatite (HA) nanoplatelets. OPN has many acidic amino acid (AA) residues with negative charges [10–12]. OPN is able to dissipate high energy [8,13,14], and regulate the formation of HA nanoplatelets [15].

Interfacial deformation and failure between dissimilar materials have been widely investigated [16–28]. Atomistic simulations, such as molecular dynamics (MD) method, have been used to investigate the material behaviour at nano-length scale [18,19,21–26,28–37]. For instance, Azzopardi et al. [31] discovered that OPN peptide with a lower isoelectric point is closer to HA crystal surface, and the flexibility of OPN peptide is important for the protein–HA interactions. Addison et al. [38] indicated that OPN peptide with a higher number of phosphate groups has higher adsorption energy. These studies provided useful information on the adsorption of OPN peptide on HA surface, but the interfacial shearing behaviour between OPN and HA has not been well understood, especially when the OPN is confined between HA layers. Therefore, it is critical to understand the confinement effect on the interfacial mechanical behaviour.

In this work, the interfacial behaviour between OPN and HA surfaces was investigated using MD simulation, with a focus on the nano-scale constraint effect.

2. Materials and methods

2.1. Models of hydroxyapatite and osteopontin

The hexagonal HA occurs more commonly compared to the monoclinic HA [39–41]. The initial configuration of HA

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($Ca_{10}[PO_4]_6[OH]_2$) mineral layer used in this study has a hexagonal structure of space group $P6_3/m$ and unit cell parameters of $a = b = 9.423$ Å, $c = 6.883$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$ [42]. The HA mineral layer has {100} crystal surfaces created close to the hydroxyl (OH) columns (Supplementary material, Fig. S1). Previous studies suggested that this surface is the main crystal surface found in the HA nanoplatelets [43,44]. The thickness of the HA layer is approximately 2 nm, close to the thickness of HA nanoplatelets observed in bone (1.5–4.5 nm) [9,45–47]. Subsequently, another similar HA layer was placed on top of the existing HA layer, as shown in Fig. 1. The lower half of the lower HA layer and the upper half of the upper HA layer were restrained throughout the MD simulations. Five different gap distances between the two HA layers (1.0–1.4 nm) were adopted to investigate the confinement (constraint) effect at nano-scale. The gaps between the two HA layers were fixed during the MD simulations. The OPN peptide was placed between the two HA layers, and the OPN peptide was oriented with its approximated long axis parallel to the z-axis.

Four types of OPN peptides, namely OPN A, B, C and D, were adopted to represent different types of OPN. As mentioned in the introduction, OPN consists of many acidic AA residues [10–12]. To investigate the effect of acidic residues, these four types of OPN peptides have different numbers of acidic residues. These peptides were obtained from Ref. [38]. The sequence of OPN A (AA 198–215) is LNGAYKAIPVAQDLNAPS, while the sequence of OPN B (AA 115–132) is DDSHQSDESHHSDESDEL. OPN C and D are the phosphorylated forms of OPN B. The sequences of OPN C and D are DDSHQ(pS)DESHH(pS)DE(pS)DEL and DD(pS)HQ(pS)DE(pS)HH(pS)DE(pS)DEL, respectively. The acidic residues include aspartate (D), glutamate (E) and phosphoserine (pS). OPN A has one aspartate residue only. Each OPN B, C and D has five aspartate residues and three glutamate residues. OPN C has additional three phosphoserine residues, whereas OPN D has additional five phosphoserine residues. The net charges of OPN A, B, C and D are 0, −8, −14 and −18, respectively.

The OPN peptide and two HA layers were placed in the simulation cell, with a size of $10.2 \times 7.3 \times 50.0\ nm^3$. Periodic boundary conditions (PBC) were used in all three axes. The empty space of the simulation cell was filled with simple point charge (SPC) water molecules [48] with a density of $\sim 1000\ kg\ m^{-3}$. Previous studies mentioned that extra-fibrillar protein matrix consists of some amounts of calcium ions [8,13,14]. In this study, 0.04 M calcium ions were added in the simulation cell, and some chloride ions were also added to ensure the system neutral.

2.2. Pulling simulation

The MD simulations were performed using software GROMACS [49–52], while Visual Molecular Dynamics (VMD) [53] was used to analyse the results. This study used GROMOS force field [54,55] supplemented with the HA force field parameters developed by Hauptmann et al. [56]. The Lennard-Jones (LJ) parameters converted from the Born-Mayer-Huggins (BMH) parameters in the Hauptmann model were applied in the MD simulations [37]. The HA force field parameters have been widely adopted to describe the structure of HA mineral. They have been successfully used for modelling mineral and protein [18,22,32,33,36]. In this study, the AA residues attracted to the HA surfaces generally have interaction energy higher than 100 kJ/mol, and the obtained interaction energy is in the same order of magnitude as those obtained in previous studies using density functional theory (DFT) [57] and MD [32,36]. Thus, we believe the accuracy of applied force field is adequate for this study of investigating the mechanical behaviour of OPN–HA interfaces and the effect of geometrical constraint.

The interfacial interaction is comprised of electrostatic (elec) and van der Waals (vdw) interactions, with a cut-off distance of 14 Å. The electrostatic interaction was described by Coulomb's law equation, i.e.,

$$V_C = \frac{1}{4\pi\varepsilon_0}\frac{q_i q_j}{\varepsilon_r r_{ij}} \tag{1}$$

where ε_0 is dielectric permittivity of vacuum, ε_r is the relative permittivity of the medium, q is partial charge of atoms, and r is the distance between particles. The van der Waals interaction was described by Lennard-Jones (LJ) potential, i.e.,

$$V_{LJ} = 4\varepsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right) \tag{2}$$

where r is the distance between the particles, ε is the depth of the potential well, and σ is the distance corresponding to zero potential. The LJ parameters for the interfacial interaction were calculated using the Lorentz-Berthelot mixing rule [58], the particle mesh Ewald (PME) method [59,60] was applied for the long-ranged coulomb interactions, i.e.,

$$\sigma_{ij} = \frac{\sigma_i + \sigma_j}{2} \tag{3}$$

$$\varepsilon_{ij} = \sqrt{\varepsilon_i \varepsilon_j} \tag{4}$$

Energy minimization (EM) with steepest descent method was conducted first. It was followed by equilibration simulation to achieve a system with a temperature of 310 K and a pressure of 1 bar. The equilibration simulation includes a 100-ps simulation under canonical (NVT) ensemble and another 100-ps simulation under isothermal-isobaric (NPT) ensemble. MD simulation was subsequently conducted for 5 ns. Although the OPN peptides are quite flexible, the structures of OPN peptides are generally quite

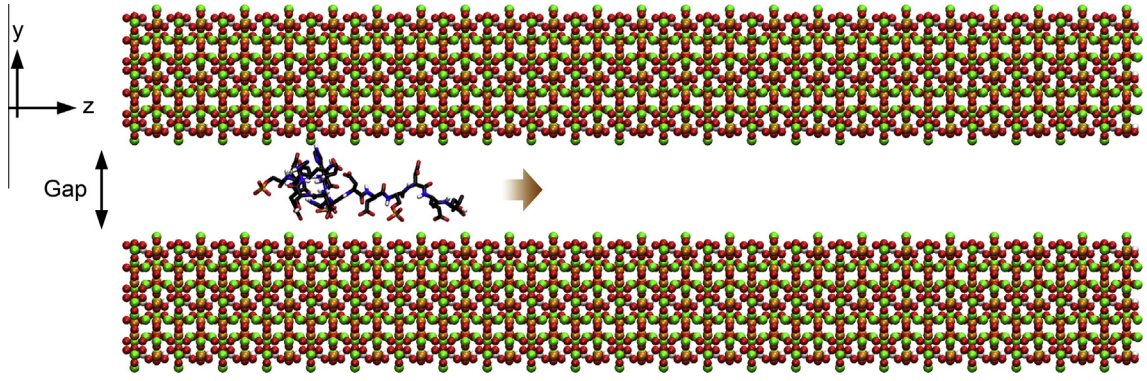

Fig. 1. Computational model. Solvent molecules are not shown for clarity. (Ca – green; P – orange; O – red; H – white; C – black; N – blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)