Molecular Modeling of Cell Adhesion Peptide Adsorption on Hydroxyapatite

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Statement of Purpose: Molecular modeling has proven useful for investigating protein-substrate and peptide-substrate interactions in calcite, calcium oxalate, and apatite systems. Insight pertaining to the interactions of oligopeptides with biominerals can be deduced from this type of modeling, such as adsorption conformation. While most biomolecular modeling has been performed on organic substrates, this initial work strives to model the interaction of an organic molecule with an inorganic lattice. Developing bioactive bone tissue engineered constructs that can recruit and direct cell behavior towards an osteogenic lineage has been increasingly investigated. Strategies of biomaterial-induced cell change include using calcium phosphate substrates and surface modification via peptides adsorption. Peptide sequences for bone tissue have been designed to mimic sections of proteins such as bone sialoprotein (BSP), osteopontin, fibronectin, statherin, collagen, and osteonectin. The goal of this work is to investigate the conformation and adsorption energies of bone specific peptides on hydroxyapatite (HA). An established BSP peptide (EEEEEEEPRGDT), RGD, and RGE were modeled on a [010] step on the HA on a (001) plane using an empirical potential approach.

An empirical force field for HA ($Ca_6(PO_4)_{10}OH$) was derived. A Universal Force Field was applied to investigate molecular modeling of oligopeptide adsorption to a [010] step on a (001) plane of HA to model the presentation of the important integrin binding sequence RGD. Each structure was energy optimized using molecular statics and molecular dynamics.

Methods: A General Lattice Utility Program (GULP) force field for HA was developed using lattice parameters [1] and physical properties of bulk apatite. Two-body interaction potentials between 1) P and O atoms in the phosphate group and 2) O and H atoms in hydroxide are defined by Morse potentials. Lennard-Jones potentials have been used to describe the repulsive part of van-der-Waals interactions between Ca and O. The crystal structure of HA was built using the Cerius[2] Modeling Software. A step in the [010] direction was made by cleaving the original lattice, and charge-neutrality was established while minimizing the dipole moment of the entire crystal structure. Molecular simulations were run in Materials Studio (3.1) under three different simulation conditions: the entire phosphate lattice constrained, the top lattice layer allowed to relax, and the top three layers allowed to relax. The adsorption energy ($E_{binding\ energy}$) required for a given peptide was calculated as $E_{binding\ energy} = \Delta E_{(peptide\text{-}lattice)} - (\Delta E_{peptide} + \Delta E_{lattice})$, where $E_{peptide}$ and $E_{lattice}$ are the minimized energies of the peptide and apatite separately and $E_{(peptide\text{-}lattice)}$ is the minimization energy with the peptide adsorbed to the apatite structure.

Results & Discussion: Both RGD and RGE showed favorable binding energies to the HA surface (Table 1). RGE favored binding where 3 atomic layers were allowed to relax. E_7PRGDT showed greater binding variance between the three models. The starting orientation of the peptide in the [010] step influenced the final binding energy of the peptide on the lattice. The E_7PRGDT peptide was built to maintain a -9 charge at neutral pH.

Table 1: Binding Energies for RGD, RGE, and E_7PRGDT to a [010] Step on a (001) Plane of HA

Peptide	Portion of Lattice Allowed to Relax	Binding Energy kcal/mol
RGD	No layers	-231.9
	Top atomic layer	-164.0
	Top 3 atomic layers	-135.5
RGE	No layers	-180.5
	Top atomic layer	-159.4
	Top 3 atomic layers	-266.2
E_7PRGDT	No layers	45.6
	Top atomic layer	-231.9
	Top 3 atomic layers	25.6

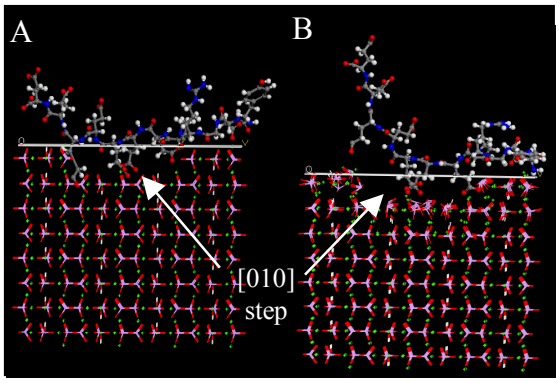

Figure 1: Images showing initial (A) and final (B) conformations of E_7PRGDT (Glutamic tail on left side of peptide). Top 3 atomic layers relaxed.

In observing the final conformation of the peptides on the [010] step, RGE presents itself in a flat manner compared to RGD that has a buckle in the tripeptide structure (images not shown). Surprisingly, the E_7PRGDT peptide orients a portion of the glutamic acid tail away from the lattice structure altogether (Figure 1). The negatively charged phosphate tetrahedra may be responsible for pushing the negatively charged glutamic acid away from the lattice, despite the ability of calcium to move in two of the three modeling simulations.

Both RGD and RGE appear to have similar binding energies to the [010] step of HA; however, their stereological conformations show that RGD adsorbs in a more buckled fashion. Finally, these results show that the glutamic acid tail of E_7PRGDT interacts with the Ca present on the surfaces of the lattice and step.

References: [1] Hauptmann S. et al; Phys. Chem Chem. Phys., 2003; 5; 635-639. This work is supported by NIH R01 DE 015411 and the Tissue Engineering at Michigan Grant T32-DE07057.