Interaction of Peptides with Hydroxyapatite

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Abstract

Bone-related problems have always been a concern in the medical field, particularly how to heal and rebuild bones more rapidly when injuries occur. A path to solving such problems might be to examine the processes when bone develops in nature. This project aims at discovering the chemical reactions that occur when the cell-adhesion peptide arginine-glycine-aspartate (RGD) interacts with hydroxyapatite (HA) in aqueous solution. Associating peptides and biomaterials, which are linked to bone development, is important. Using molecular dynamics to simulate the interactions of RGD with HA in aqueous solution, the scientists were able to imitate the actual process that occurs in the body and achieve a better understanding of how bone development occurs. The research also sought insight into whether RGD inhibits or promotes the formation of new HA seed crystals.

Introduction

This research focused on the relationship between peptides and biomaterials. Peptides are known to either inhibit or promote production of biomaterials in the body. In this case the research aims to observe and characterize atomic-level interactions when cell-adhesive arginine-glycine-aspartate (RGD) is placed on top of crystalline hydroxyapatite (HA) in aqueous solution.

RGD is a tripeptide containing three amino acids joined by peptide bonds. It is a cell-adhesion sequence that interacts with cellular integrin receptor sites, connecting biomaterials to cells of the body.[1] Hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$) is a calcium phosphate–based biomaterial found in bones and other hard structures in the body, such as teeth. HA plays a fundamental role in the process of bone building, as approximately 70 wt% of bone is made up of HA.[2] When the two are combined in a living system, the RGD may either promote the production of HA seed crystals or stop the production process altogether. The seed crystals are important because they spread out across the surface area where bone is to be built and stimulate osteoblasts, the cells responsible for the formation of new bone.[3]

By placing RGD atop HA in aqueous solution, researchers were able to simulate an environment similar to that of the human body. By doing so, it was possible to observe the processes as they might occur in the body. Because of the ethical issues of experimenting on humans, computer simulation by means of molecular dynamics (MD) and visual molecular dynamics (VMD) was used to observe and modify theoretical models. Exploration of simpler model systems also gives insight into basic chemical and physical processes. This project focused on determining whether RGD's interaction with HA would improve bone development.

This research aimed to develop by molecular design a procedure to induce RGD to bond at one end to HA and at another end to an integrin receptor on a cell. In this way an osteoblast can be anchored to HA and perhaps induced to nucleate HA and make bone. See Figure 1 cartoon of RGD attaching to a cell.

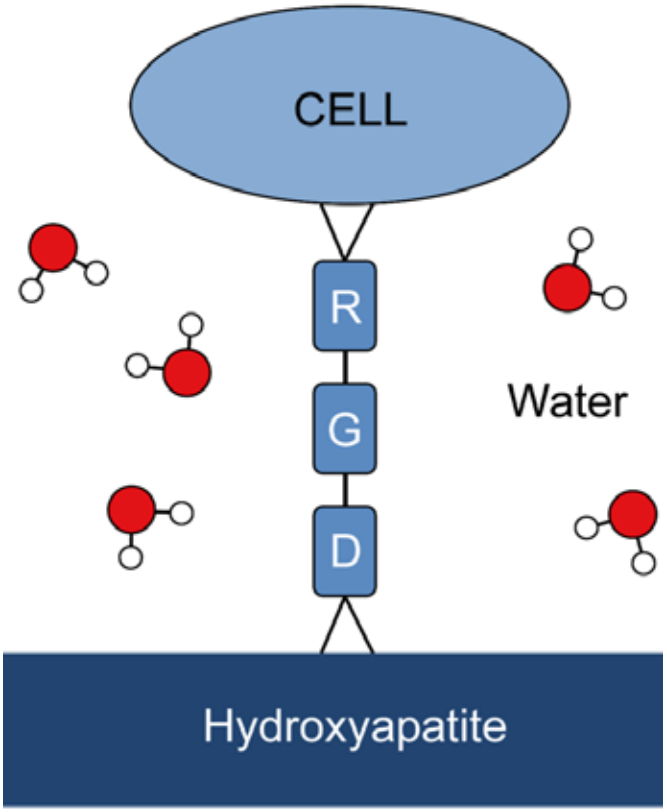

Figure 1. Schematic of RGD attaching HA to cell.

Background

In previous studies peptides containing the RGD sequence were found to mimic the cell-adhesion process in two ways. When in solution they act as an inhibitor preventing adhesion; when coated on a surface, they promoted adhesion. Cell adhesion refers to the binding of one cell to another cell or to a substrate. To date research has been oriented toward developing RGD peptides that mimic cell-adhesion proteins and also bind to integrins. These peptides have a diverse field of application, including angiogenesis and tumor formation, enhancing drug delivery systems, imaging for diagnostic purposes, and coating surfaces for use as biomaterials. It has been shown that integrins, such as fibronectin, play a central role in cell adhesion, cell growth, and tissue repair, among other processes. Hence, a peptide connected to this integrin increases cell attachment to biomaterials, leading to the improvement of the living-nonliving interface.[4]

In biomedical applications HA is synthesized and used to coat implants (such as Ti metal hip implants) and other medical apparatuses placed inside the body. These implants are used to help rebuild broken bones or to replace missing appendages.[5] For example, when a pin used to hold broken bones together is coated with HA, the HA enables the pins to be recognized by the body as another bioparticle. The body does not attack or reject the pins but concentrates on rebuilding the broken bones. The combining of bone material with implants is termed "osseointegration," and it occurs after cell adhesion has taken place.[6]

Research showed that RGD in solution significantly inhibited bone formation by stopping the development of the HA seed crystals. This result demonstrates that even though RGD is known to be a promoter of cell/biomaterial interactions, when used to functionalize HA, it proves to be harmful to the osseointegration process.[7] It is thus important to understand the RGD-HA interaction in detail, at the atomic level.

Bonds are formed when two or more atoms cling together because of some attractive force. Bond forces may be represented by a simple spring potential, such as $V_H = k_H(r - r_0)^2$, where k_H is the spring constant and r_0 is the equilibrium point. This equation approximates the energy associated with vibration about the equilibrium bond length. Angular bonds are represented by another set of springs, such as $VA(\theta) = k_{A,\theta}(\theta - \theta_0)^2$, where $k_{A,\theta}$ controls the stiffness of the angular spring and θ_0 represents the equilibrium state. This is analogous to Hooke's law and is sometimes referred to as the bending energy. Nonbonding van der Waals forces are described by the Lennard-Jones potential (L-J potential), which is described by the equation $V(r) = 4\varepsilon[(\sigma/r)^{12} - (\sigma/r)^6]$, where ε is the depth of the potential well, σ is the distance at which the interparticle potential is zero, and r is the distance between the particles.[8] The L-J potential is basically a simple way to express the van der Waals forces, or the weak attraction and repulsion forces affecting the atoms of the molecule in model form. In addition, a dihedral angle is formed when four atoms connect at a particular angle on the backbone of a protein molecule. Dihedral angular forces are a measure of the torsional forces in molecules and are usually represented by $V_D = k_D[\cos(\theta) - \cos(\theta_0)]^2$, where k_D is the spring constant and θ_0 is the equilibrium dihedral angle. Once the potentials are defined, then the forces are found by differentiation — for example, $F_{x,i} = -(\delta * V_{Total}) / (\delta_{x,i})$. Electric forces either attract or repel atom pairs; this follows the principle that opposite poles attract, whereas like poles repel. Such is the case with Coulomb's law, which states that the electrical force between two charges q_1 and q_2 are proportional to the distance squared between them. This law is represented by the equation $F = k_e * (q_1 q_2 / r_2) / r^2$, where k_e is the Coulomb constant, q is the charge on each atom, and r is the distance between them. Without Coulomb's law there would be no force field governing the attraction or repulsion between molecules, and the different energies of molecules in the atmosphere would be in total disarray. These are the primary principles or interactions used in the molecular model. The model springs used in this study's force fields approximately represent the complex Coulomb forces between electron clouds and nuclei.

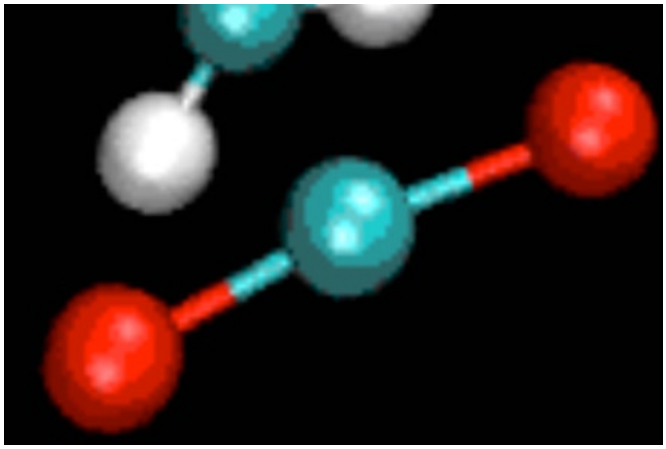

Figure 2. Spheres representing atoms and springs representing bonds.

Approach

For this project, models were built to identify the different attached atoms that make up the RGD sequence. Molecular models imitate the behavior of molecules. They usually depict atoms as point charges with an associated mass. The interactions of the atoms forming bonds are usually represented by springs as described above. Nonbonded interactions are represented here by van der Waals forces, which describe the relatively weak long-range attractive and repulsive forces between molecules. In other words, van der Waals forces are used to maintain the space between nonbonded molecules.

RGD force fields were applied, with the parameters adjusted to reproduce the experimental structure as far as possible. In other words, the force field is what keeps the molecules together in the model and is inclusive of the bond forces.

The program used to model the RGD peptide is MD, a computer technique that allows scientist to simulate different experiments on a micro/nano scale.[9] The properties and conditions under which the simulation is conducted can be controlled by the operator. The RGD trajectories were observed using the model, and the transition states monitored as they occurred. The properties of the RGD peptide model were altered using MD, and simulations were run of the peptide interactions with HA and water. A primary advantage of using MD is that it can slow the speed at which an experiment progresses, so that the stages that occur too quickly for the human eye to see in nature can be observed.

VMD, on the other hand, is a graphical user interface that allows molecules to be manipulated on a 3-dimensional level.[10] VMD is used for modeling, visualization, and analysis of biological systems such as proteins. In this project VMD manipulation tools were used to display, examine, and analyze the results of MD simulations. The CPK style was used to represent the atoms in the form of spheres, while the element style was used to display the different colors representing each atom. The bonds between the atoms were displayed with colored rods, as shown in Figure 2. These styles enabled a better graphical representation of the molecules.

Results

As shown by Figure 3, arginine is the first amino acid to make up the RGD peptide, followed by glycine and aspartate.

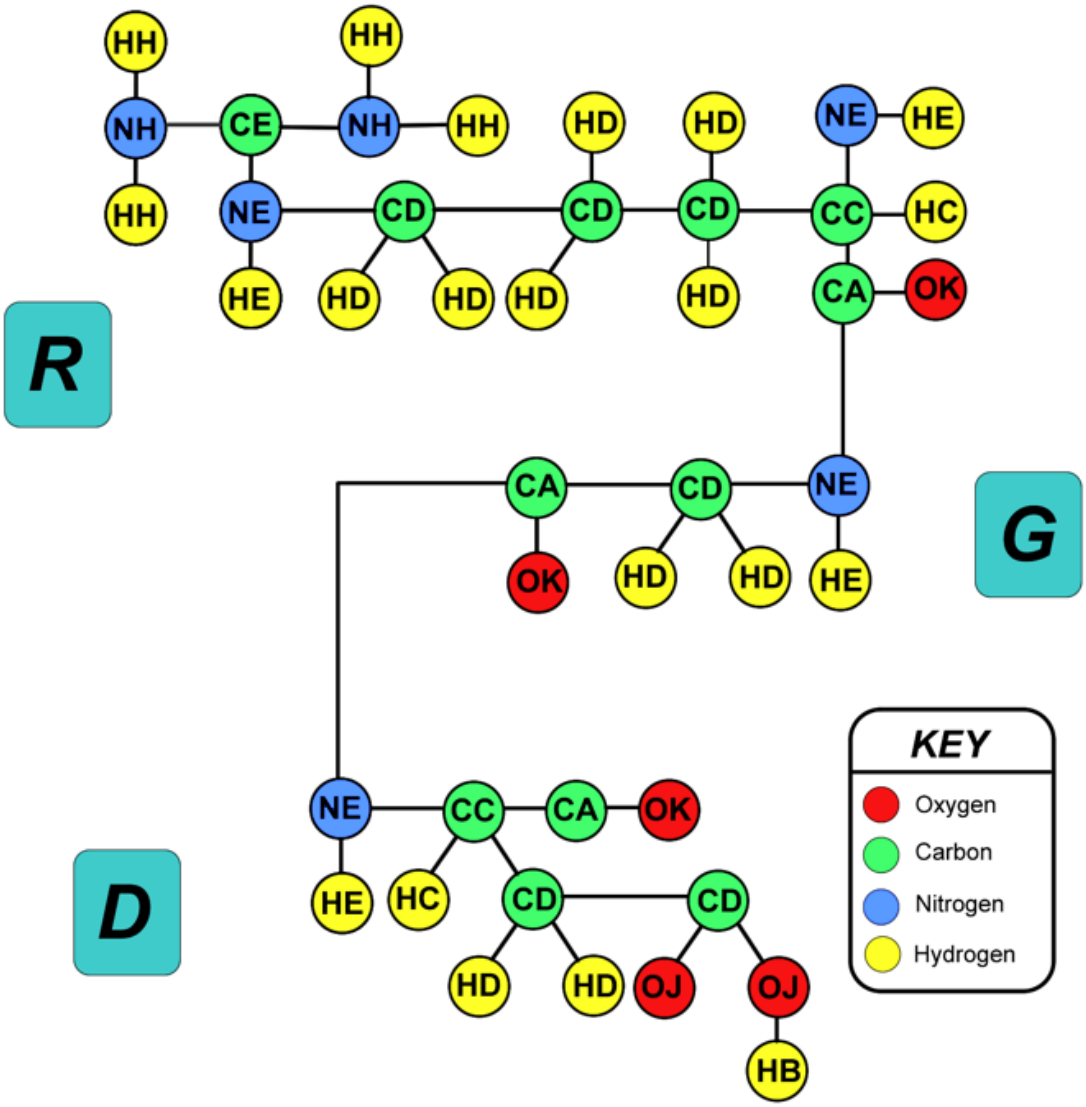

Figure 3. Schematic diagram of arginine-glycine-aspartate (RGD) showing distinctive amino acids making up the peptide.

Types of atoms	Atomic types
NH	*Form bonds with hydrogen (HH) to give NH_2*
HH	*Form bonds with nitrogen (NH)*
NE	*Form bonds with carbon (CE) and nitrogen (NE)*
CE	*Form bonds with nitrogen (NE) while connecting to nitrogen*
CD	*Form bonds with hydrogen (HD) while connected to carbons*
HD	*Form bonds with carbon (CD)*
CE	*Form bonds with nitrogen (NE) while connecting to nitrogen*
CC	*Form bonds with hydrogen (HC)*
HC	*Form bonds with carbon (CC)*
OJ	*Form bonds with carbon (CB)*
HB	*Forms bond with oxygen (OJ)*
CA	*Form bonds with oxygen (OK)*
OK	*Form bonds with carbon (CA)*
CB	*Form bonds with two oxygen (OJ)*

Table 1. Definition of atomic types.

Angular spring	Experimental angles (degrees °)	Spring constants (kcal/mol)
OB-CB-OB	124.05	120
HD-CD-HD	109.11	80
HH-NH-HH	120.07	80
CA-NE-CD	119.71	120

Table 2. Three-body angular interactions.

Molecule pairs	Experimental bond lengths (Å)	Spring constant (kcal/mol)
NH-HH	1.00	895
NE-HE	0.98	895
CE-NE	1.31	900
CD-HD	1.08	800
CC-HC	1.09	800
CA-OA	1.21	1200
CB-OJ	1.22	1200
CA-CA	1.53	800
CC-CD	1.40	800
CD-CD	1.53	800
HB-OJ	1.0	750
CA-CC	1.53	800
NE-CD	1.31	900
NE-CC	1.31	900
NE-CD	1.31	900

Table 3. Two-body radial spring interactions.

Atomic set	Experimental angles (degrees °)	Spring constants (kcal/mol)
CE-NE-CD-CD	94.2	8.0
NE-CA-CA-NE	-95.5	1.4
CA-CA-NE-CD	-179.0	1.4

Table 4. Four-body angular interactions.

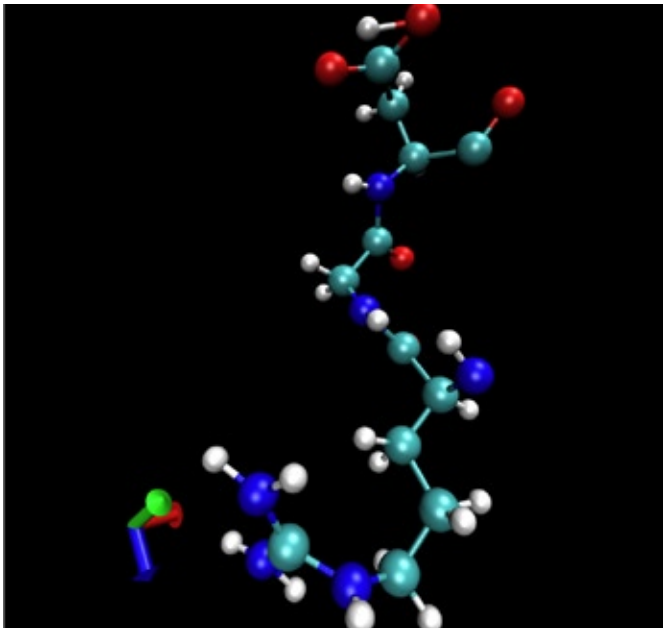

Figure 4: Arginine-glycine-aspartic acid (RGD) in its pure form. Red is oxygen (O), blue is nitrogen (N), aqua is carbon (C), and white is hydrogen (H).

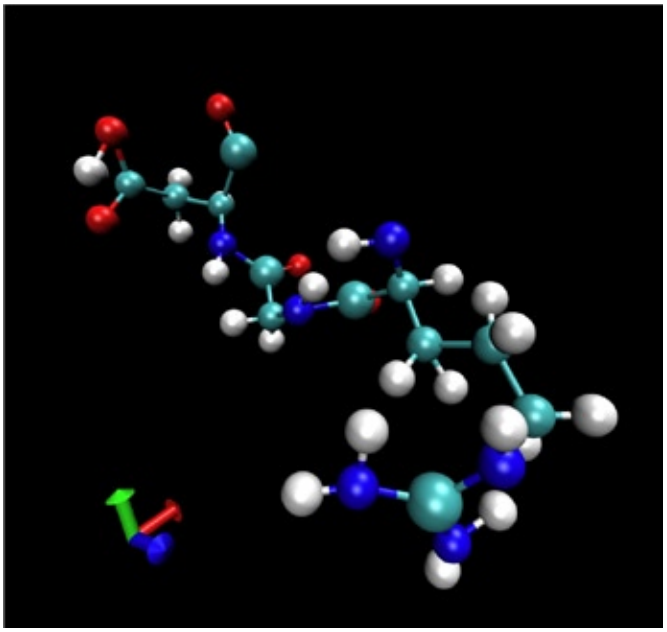

Figure 5. RGD when two-body radial springs were added.

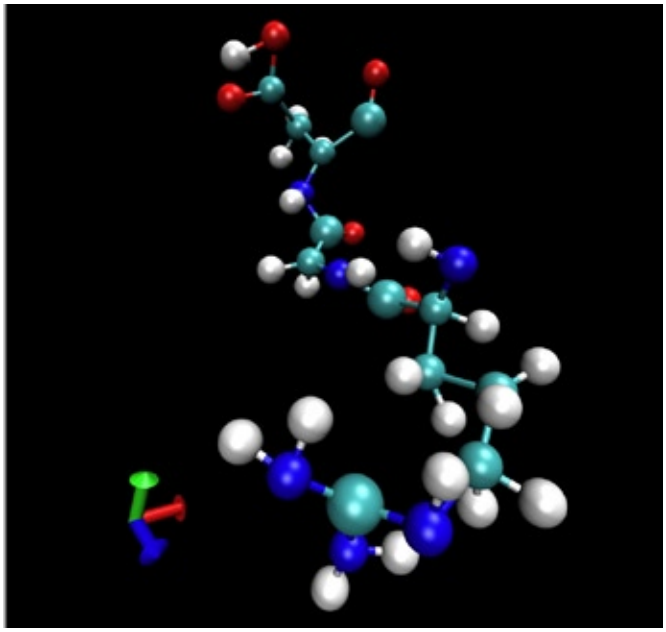

Figure 6. RGD with three-body angular spring interaction.

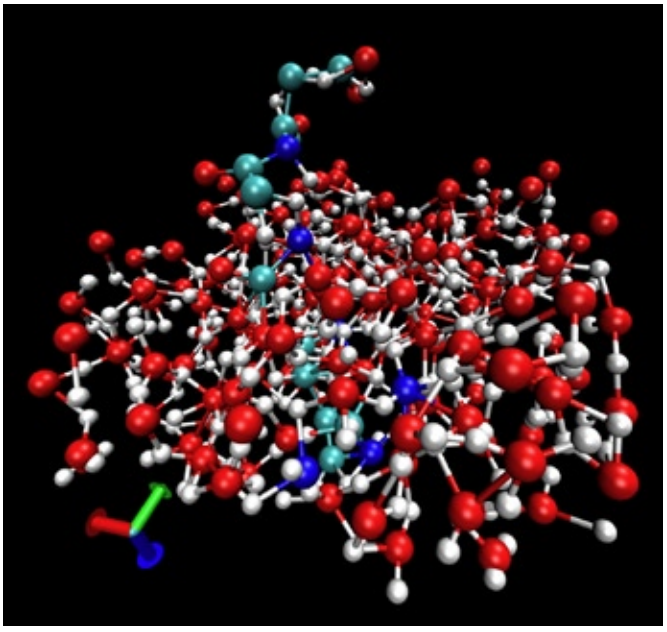

Figure 7. RGD in water. RGD is oriented upwards with the acidic (oxygenated) end sticking out of the water.

Figure 4 shows a modeled version of RGD. In this model, the acidic end of the peptide can be seen where the red atoms (oxygen) are closer together at the top end. The opposing end is termed "basic" because it contains the basic blue atoms (nitrogen). The carbon (green) and nitrogen atoms form the backbone of the peptide.

Figure 5 shows the result when only the two-body radical springs were added to the RGD peptide. As can be seen to the right of the picture, the carbon (green) atom formed bonds with both nitrogen and hydrogen atoms, instead of the nitrogen by itself.

Figure 6 shows the result of the RGD peptide when three-body angular springs were added to the radial spring model. As can be seen in the picture, the backbone of the peptide came apart, breaking the peptide into pieces. Later fine-tuning produced a fully linked RGD similar to that in the experiment.

Figure 7 shows the results when RGD is placed in water. As can be seen in the picture, RGD orients itself vertically.

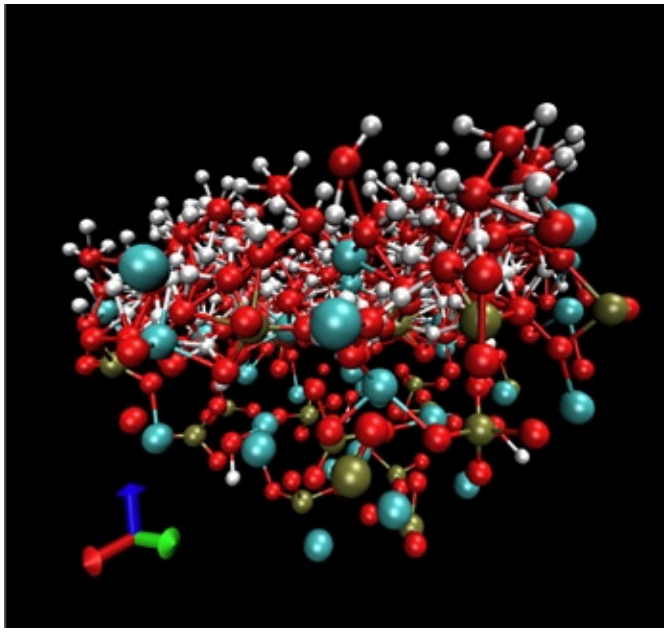

Figure 8. Shows the model of HA placed in water. The HA molecules integrated with the molecules of the water, resulting in a combined molecular structure. The top portion of the picture represents the water molecules, while the bottom portion shows the HA.

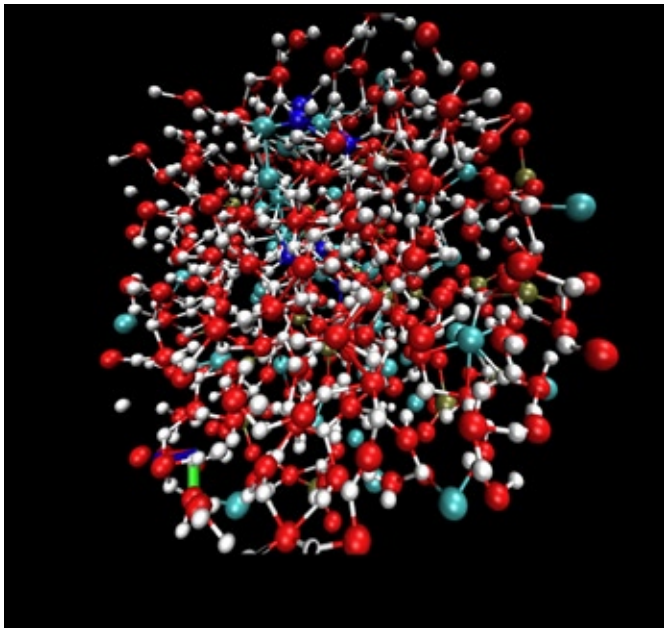

Figure 9. Shows the modeled version of RGD placed atop HA in water. As can be seen in the picture, RGD has completely integrated itself inside of the HA and has formed bonds throughout the substrate.

Discussion

Two force fields of the RGD model were built in steps. The first was attaching the radial and angular springs, which proved to be effective in keeping the molecule together by building bonds between the atoms. Next the model was fine-tuned by the addition of van der Waals forces, which further defined the attraction and repulsion between atoms. This approach was tested and repeated several times until the RGD looked reasonably like the replica of the experimental RGD.

Using the initially developed force field, when RGD was placed in aqueous solution, the molecules quickly disassociated. This could be due to the force at which the RGD contacted the surface of the solution, making the molecules more susceptible to breaking apart. After the RGD force field was improved, the molecules remained intact in the water. In Figure 7, it can be seen that that the negative carboxyl group protrudes vertically above the water film. Future work will seek to find a more gentle approach to placing the RGD on HA in solution so as not to break up the RGD model with the impact.

When the first RGD force field was used, the molecule completely fragmented within the aqueous HA environment. With the improved force field (see Figure 9), RGD retains its structure better but still breaks into two fragments.

The HA-plus-water model performed as expected. Water formed a thin film on top of the HA surface. This is a good starting model for the biological system in which serum, which is mostly water, is in contact with bone. In the HA-plus-water and RGD system, the RGD relatively retains its vertical orientation. The carboxyl group is embedded in HA, while the amine groups point toward the water surface.

Conclusion

The building of a force field is more of an art than a science. The various interactions described were added one at a time so as to make the model more realistic. While there is room for improvement in the model, the primary features were captured. In future research it would be necessary to recalibrate the different force components in order to extend the model to more realistic biological systems. In particular, it would be desirable to begin modeling the attachment of RGD to integrin-binding sites.

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