Molecular Mechanics of Chitin-Protein Interface: Terminus and Side Chain

Zechuan Yu and Denvd Lau*

Department of Civil and Architectural Engineering
City University of Hong Kong

*Corresponding Author
denvid.lau@cityu.edu.hk

Abstract: Chitin and protein are two main building blocks for many natural biomaterials such as crustacean cuticles, sponge skeletons and squid beaks. These biological materials gain growing interests with respect to biomimetic material design, since they possess great mechanical properties due to their evolutionarily refined structures at different hierarchy levels. The interaction between chitin and protein critically determines the properties of the composite biological materials. Moreover, living organisms usually encounter a complex environment, and ambient conditions like water, pH and ions are critical factors towards the structural integrity of biomaterials such as the chitin-protein composites. It is therefore essential to study the chitin-protein interface under different environmental conditions. Here, an atomistic model consisting of a chitin substrate and a protein filament is constructed, which is regarded as a representative of the chitin-protein interface existing in many chitin-based biomaterials. Based on this model, the mechanical properties of chitin-protein interface under different moisture and pH values are investigated through molecular dynamics simulations. The results reveal that there is a weakening effect of water towards the chitin-protein interface, as well as the influence of acidity, i.e. the protonated protein in acidic environment forms a stronger adhesion to chitin than that in the alkaline environment. In addition, the effect from side-chain of protein is studied and it is found that certain kinds of amino acid can form hydrophobic connections to chitin surface, which means that these peptides partly dodge the weakening effect of water. Our observation indicates that terminuses and side-chains in protein are of crucial importance in forming interfacial hydrogen bonds, which connect protein to chitin and contribute to the adhesion. From our full atomistic models, we can observe some molecular mechanisms about how protein interacts with chitin in different conditions, which may spotlight the engineering on biomaterials with similar interfaces.

Key Reference: Jin K, Feng X, Xu Z (2013) Mechanical Properties of Chitin-Protein Interfaces: A Molecular Dynamics Study. BioNanoScience: 1—9
Introduction

Among natural polymers, chitin is a most abundant one, especially in animal kingdom. Chitin fibrils possess good mechanical properties, and they are considered as the loading-bearing scaffolds supporting the fundamental structure of composite material, of which another component is usually protein [1]. The remarkable affinity between chitin and protein makes their combination very common in nature. Lots of biological materials are made of chitin and protein, among which are the exoskeleton of sponges, the cuticles of crustaceans and insects, the beaks of squids and the fangs of spiders, to name a few [2-6]. With respect to biomimetic thinking, these natural materials gain many interests for not only their great mechanical properties (e.g. high stiffness and stability), but also the versatile capabilities. Animals take advantage of the diversity of chitin-based materials during the evolution. Lobster cuticles have different layers, where the outer layer is harder and stiffer than the inner layer. Multi-scale simulations of lobster cuticles elucidate that structural effect as well as mineral content determine the cuticle stiffness [3]. Following this idea, spiders reinforce their fangs via enriching the mineral content. Researchers find that in different regions of fang, different kinds of metal ions may result in different mechanical behaviors [6]. In contrast to spiders, squids tend to cook with no minerals, while they tune the mechanical properties of their beaks via controlling other variables. Experimental studies of squid beaks indicate that the gradient in water content and cross-link degree cause the gradient in the material stiffness [7]. According to these researches, it is noted that the mechanical properties of chitin-protein composite are subject to many factors from both the composition and the environment. With chitin and protein combining together to achieve the versatility, their interaction should have a profound influence on the material properties. Moreover, since the chitin-protein interface is easy to be influenced by the ambient environment, this interface can be considered as the entry where the environmental factors start to take effect.

As mentioned above, the interfacial mechanics between chitin and protein are associated with many environmental factors. Solvent, pH and ions, which usually correlate with biological systems, affect the chitin-protein interface as well. Through performing molecular dynamics simulations, previous study has revealed that water molecules have a weakening effect on the chitin-protein integrity [8]. This phenomenon is reproduced here, from which we have also observed that hydrogen bonds between chitin and protein are diminished by water. Besides, we have proposed a “hydrophobic” conformation of protein, where the sidechain of the histidine, together with the N-terminus, may form a connection to chitin that dodge the effect from water partly. Also, the effect from pH is studied. The solution pH is able to change the protonation state of titratable groups of protein, and thus modulates the interaction between protein and other components [9]. In order to
investigate the acidity effect on the chitin-protein interface, we construct proteins in different environments and compare their interactions with chitin substrate. It is observed that chitin and protein behave differently in acid and alkaline environments.

**Methods**

**Modeling**
A chitin substrate and a protein filament are constructed. The conformation of $\alpha$-chitin has been resolved in the *ab initio* based study [10]. The unit cell of $\alpha$-chitin is replicated ten times in $a$ direction, one in $b$ direction and five in $c$ direction, as shown in Fig. 1a. Protein is in the form of $\beta$-strand, which consists of five glycines. The small protein filament is well placed over the chitin substrate as shown in Fig. 1b. Two terminuses of the protein, i.e., the $-NH_2$ and $-COOH$, are titratable groups, and they are modified to three different states, which are non-ionic, acid and alkaline as shown in Fig. 1c, 1d and 1e, respectively. The protein in Fig. 1f has a different N-terminus, where the glycine is replaced by histidine. In total, four models are built. Three of them represent non-ionized, acid and alkaline environments and an additional one contains the special protein.

**Simulation**
LAMMPS is used to perform all the simulations [11]. CHARMM36 force fields for proteins and carbohydrates are adopted to govern the movement of all the models [12,13]. VMD is used for visualization [14]. The equilibration process for every model is stated as follows. The model is first minimized using the conjugate gradient algorithm, which adjusts the atom coordinates in order to reach a minimum-energy status. It is then heated up from 50 K to 300 K in 20 ps. After equilibrated at 300 K for 50 ps in an NVT ensemble, the model is equilibrated at 300 K and 1 atm for 500 ps in an NPT ensemble, following which the model is equilibrated in NVT ensemble for another 1 ns. After achieving equilibration in dry case, the system is then immersed into water, equilibrated in NVT ensemble for 1 ns and saved for later simulations. Steered molecular dynamics simulations are performed to measure the adhesion strength between chitin substrate and protein filament. The time step is 1 fs during the heating process, and 2 fs in the subsequent equilibrations. Fig. 2 outlines the overall simulation procedures.

**Results and Discussions**

**The “Hydrophobic” Conformation**
As aforementioned, protein models with two sequences (*i.e.* GGGGG and HGGGG) are built and sent for equilibration with chitin substrate. When attached, protein and chitin form hydrogen bonds, the quantity of which can be an indicator of their adhesion strength. We computed the average number of hydrogen bonds formed between two proteins and chitin substrate in dry and wet cases. The criteria to detect hydrogen bonds are 4 Å for the donor-acceptor distance and 35 degree for
donor-hydrogen-acceptor angle [8]. Fig. 3 shows the conformation of proteins in dry and wet cases, from which it is observed that water reduces the number of hydrogen bonds. Comparing these two protein conformations, results are that protein with HGGGGG sequence is capable of forming more hydrogen bonds with chitin in both dry and wet cases.

The Weakening Effect From Water
The non-ionized protein is attached to chitin substrate in both dry and wet circumstances. These equilibrated dry and wet models are tested via steered molecular dynamics approach as shown in Fig. 2d. Free energy is calculated during the peeling process using second-order expansion of Jarzynski’s equality [15]. The change of free energy (referred to as $\Delta F$) from attachment state to the detachment state indicates the adhesion strength between chitin and protein. The $\Delta F$ is 7.1 kcal/mol in wet case, which is around one third of the $\Delta F$ in wet case, implying that water molecules cast a weakening effect towards chitin-protein interface. This result is in line with previous study [8].

The Comparison Between Acid and Alkaline Environments
Models of the acid-state and alkaline-state proteins, together with the chitin substrate, are prepared. The N-terminus and C-terminus of the protein are different. In acid environment, the N-terminus adopts the $-\text{NH}_3^+$ form and the C-terminus is in the form of $-\text{COOH}$, while in the alkaline environment, they are $-\text{NH}_2$ and $-\text{COO}^-$ respectively. Free energy change ($\Delta F$) is measured via the same method as mentioned before. In acid environment, $\Delta F$ is 5.3 kcal/mol, while the $\Delta F$ in alkaline environment is 3.0 kcal/mol. Result shows a stronger adhesion between chitin and acid-state protein, which may have some relationships with the additional hydrogen atom in C-terminus and the nature of hydrogen bond. A hydrogen bond is composed of a donor and an acceptor [16]. In this study, nitrogen (N) and oxygen (O) atoms are regarded as hydrogen bond acceptors. With an attached hydrogen atom (structures like N-H and O-H), they become hydrogen bond donors. Because of the absence of hydrogen atom in $-\text{COO}^-$, it can only act as the hydrogen bond acceptor, while the $-\text{COOH}$ can be both donor and acceptor, providing more opportunities to form hydrogen bonds. This additional hydrogen atom therefore strengthens the adhesion between protein filament and chitin surface. However, it is noted that the N-terminus is rooted into the chitin surface in all the concerning cases. This is probably because the two donors in $-\text{NH}_2$ already permit sufficient hydrogen bonds, so the improvement from the additional hydrogen atom in $-\text{NH}_3^+$ becomes less significant.

Conclusion
Chitin-protein interface plays a profound role in determining the overall properties of the composite material and may act as the entry where the environmental factors cast effect. Following this idea, we investigate the effects of water and acidity on the
interface. Conclusions are that terminuses of protein are important in determining the molecular mechanisms of chitin-protein interface. Through either changing the conformations of terminal part of protein or altering the protonation state of terminuses, water and acidity affect the interfacial strength obviously.

References

1. Dunlop JW, Fratzl P (2013) Multilevel architectures in natural materials. Scripta Materialia 68 (1):8-12
2. Ehrlich H, Simon P, Carrillo-Cabrera W, Bazhenov VV, Botting JP, Ilan M, Ereskovsky AV, Muricy G, Worch H, Mensch A (2010) Insights into chemistry of biological materials: newly discovered silica-aragonite-chitin biocomposites in demosponges. Chemistry of Materials 22 (4):1462-1471
3. Nikolov S, Petrov M, Lymperakis L, Friák M, Sachs C, Fabritius H-O, Raabe D, Neugebauer J (2010) Revealing the Design Principles of High-Performance Biological Composites Using Ab initio and Multiscale Simulations: The Example of Lobster Cuticle. Advanced Materials 22 (4):519--526
4. Vincent JF, Wegst UG (2004) Design and mechanical properties of insect cuticle. Arthropod Structure & Development 33 (3):187--199
5. Miserez A, Schneberk T, Sun C, Zok FW, Waite JH (2008) The transition from stiff to compliant materials in squid beaks. Science 319 (5871):1816-1819
6. Politi Y, Priewasser M, Pippel E, Zaslansky P, Hartmann J, Siegel S, Li C, Barth FG, Fratzl P (2012) A Spider's Fang: How to Design an Injection Needle Using Chitin-Based Composite Material. Advanced Functional Materials 22 (12):2519--2528
7. Miserez A, Rubin D, Waite JH (2010) Cross-linking chemistry of squid beak. Journal of Biological Chemistry 285 (49):38115--38124
8. Jin K, Feng X, Xu Z (2013) Mechanical Properties of Chitin-Protein Interfaces: A Molecular Dynamics Study. BioNanoScience:1--9
9. Wallace JA, Shen JK (2011) Continuous constant pH molecular dynamics in explicit solvent with pH-based replica exchange. Journal of Chemical Theory and Computation 7 (8):2617-2629
10. Petrov M, Lymperakis L, Friák M, Neugebauer J (2013) Ab Initio Based conformational study of the crystalline α-chitin. Biopolymers 99 (1):22--34
11. Plimpton S (1995) Fast parallel algorithms for short-range molecular dynamics. Journal of Computational Physics 117 (1):1-19
12. Huang J, MacKerell AD (2013) CHARMM36 all-atom additive protein force field: Validation based on comparison to NMR data. Journal of computational chemistry 34 (25):2135--2145
13. Guvench O, Mallajosyula SS, Raman EP, Hatcher E, Vanommeslaeghe K, Foster TJ, Jamison FW, MacKerell Jr AD (2011) CHARMM Additive All-Atom Force Field for Carbohydrate Derivatives and Its Utility in Polysaccharide and Carbohydrate-Protein Modeling. Journal of chemical theory and computation 7
14. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. Journal of molecular graphics 14 (1):33--38
15. Park S, Khalili-Araghi F, Tajkhorshid E, Schulten K (2003) Free energy calculation from steered molecular dynamics simulations using Jarzynski’s equality. The Journal of chemical physics 119:3559
16. Reed AE, Curtiss LA, Weinhold F (1988) Intermolecular interactions from a natural bond orbital, donor-acceptor viewpoint. Chemical Reviews 88 (6):899-926

Figure 1 a The chitin substrate. b The protein filament is well placed over the chitin substrate. c d e f Four kinds of protein used in this study.

Figure 2 Flow chart of the simulation process.
Figure 3  a  b  The protein in sequence GGGGG is attached to chitin in dry and wet conditions.  
  c  d  The protein in sequence HGGGG is attached to chitin substrate in dry and wet conditions.