Long-Duration Molecular Dynamics Simulation on Constructed Nacrein Structure

Frank Chang[1]*, Samson Cheung[2], Ming Wong[1], Cathy Bitler[3], Andrew Palma[4]

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1 Introduction

Nacrein (pearl protein) is a water soluble protein and present in the mantle tissue of some mollusc species. Its function is believed to regulate aragonite or calcite polymorphism during biomineralization as in the processes of pearl formation [Miyamoto 1996]. Primary structural analysis of nacrein shows high homology to carbonic anhydrase II (CAII), a protein that regulates intracellular pH homeostasis during osteoclasts bone resorption [Teitelbaum 2000]. A distinct difference between nacrein and CAII is that nacrein has a long glycine-rich repeated sequence between its two carbonic anhydrase domains. The repeated sequence is homologous to that found in proteins of the collagen family.

The role of nacrein in biomineralization has been hypothesized [Miyamoto 1996]; however, its tertiary structure has yet to be determined. We applied long-duration molecular dynamics (MD) simulation to construct the tertiary structure of nacrein protein from *Pinctada fucata* (Japanese pearl oyster). The constructed nacrein structure shows that spatial active sites are similar to CAII (Figure 1 and Figure 2).

We are also interested in identifying the catalytic sites and calcium binding sites (EF-hand like motif) in the nacrein protein. To further investigate this nacrein protein, we will apply MD in an aqueous environment, and this simulated tertiary structure would be the foundation for an *in silico* approach in developing nacrein applications in biomaterial, bone morphogenesis, and pearl formation.

2 Methods

The primary sequence of nacrein was obtained from an earlier report [Miyamoto 1996]. X-ray diffraction structure of carbonic anhydrase II (PDB ID 5CAC) was obtained from the Protein Data Bank. The tertiary structure of nacrein was constructed by a comparative modeling method using ROSETTA [Alm 1999].

Molecular dynamics simulation of both tertiary protein structures were carried out with the NAMD version 2.5b2 and VMD 1.8.1 [Laxmikant 1999 and Humphrey 1999] on Origin 2000 and SGI Altix supercomputers at NAS facility in NASA [NASA-NAS]. Hydrogen atoms were added by using the guesscoord command in NAMD. Force field parameters were adopted from CHARMM22. The van

[1] Changene Inc. NASA Research Park, Moffett Field, CA 94035. * To whom correspondence should be addressed. E-mail: ftchang@changene.com
[2] NASA Advanced Supercomputing Division, Ames Research Center, Moffett Field, CA 94035. E-mail: cheung@nas.arc.nasa.gov
[3] Ecco Biotech, Menlo Park, CA 94025
[4] MDL Information System Inc. San Leandro, CA 94577, E-mail: apalma@mdli.com

der Waals interactions were cut off at 12 Å and 10^7 steps (10 nanoseconds) of energy minimization were performed at 310 K. The nonbonded interaction was recorded every 1000 time steps. The trajectories at given conditions were considered to be the probable structures and were analyzed in details.

3 Results

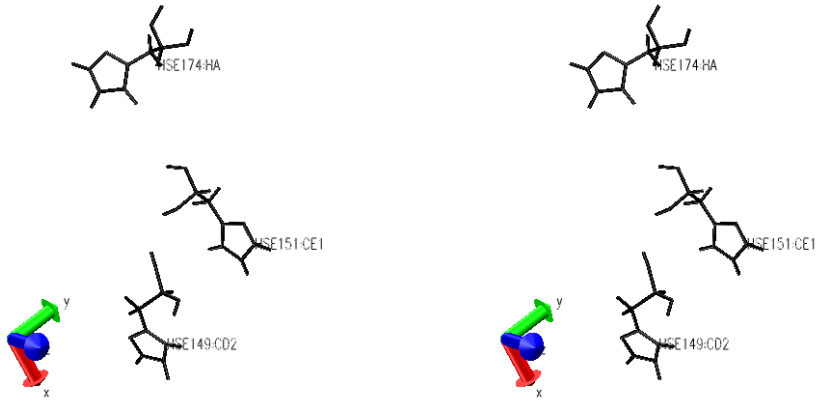

Figure 1. Tertiary structure of nacrein is constructed and applied with MD (entire structure not shown). Nacrein has three domains; Domain 1 (residue 1 – 235) and Domain 3 (residue 314 – 447) are similar to CAII, and Domain 2 (residue 236 – 313) has similarities is similar to collagen family proteins. The figure above is a stereo view of Domain 1 active site in the constructed nacrein structure. The residues shown in the Domain 1 active site are HIS-149, HIS-151, and HIS-174.

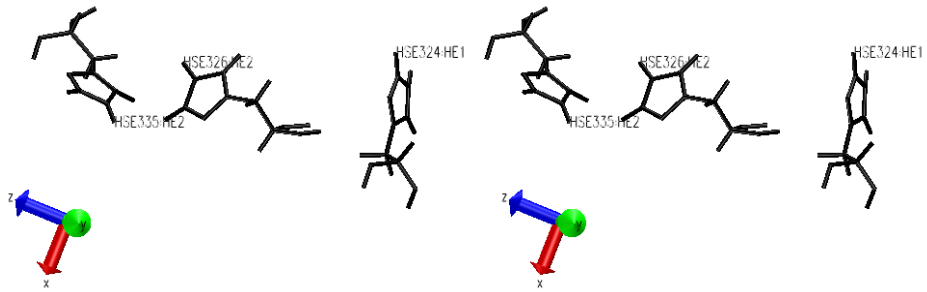

Figure 2. Stereo view of Domain 3 active site in constructed nacrein structure. The residues shown in the Domain 3 active site are HIS-324, HIS-326, and HIS-335. Note: variation of coordinates in Figure 1 and Figure 2 is to enhance visualization of the active sties.

4 References

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