Molecular dynamics simulation of protein effects on interfacial energy between HA surfaces and solutions

Kefeng Wang, Yang Leng, Xiong Lu, Fuzeng Ren

1. Introduction

The formation of hydroxyapatite (HA) in vitro and in vivo plays a key role in biomineralization and osteoconduction of biomaterials. The theoretical studies based on classical theory of crystallization [1–3] indicated that interfacial energy has impacts on nucleation driving force and rate of crystals. Knowledge of HA interfacial energy in physiological solution is required for theoretically understanding the HA formation process in vivo. Several experimental methods such as the crystal growth kinetics and contact angle measurements were adopted to determine the value of interfacial energy between HA and water. However, these data are not quite consistent and exhibit the uncertainty of experimental measurements [4,5]. Note that physiological solution contains inorganic ions and proteins which would make the measurement of the interfacial energy more complicated. The HA interfacial energy in physiological solution should be evaluated because it plays a role in protein effects on HA formation in vivo [3,6].

Molecular dynamics (MD) simulation provides an alternative way to evaluate interfacial energy. It has been used to calculate interfacial energies among water, organic monolayers and calcite crystals based on the Charmm force field [7,8]. In this study, we used the MD simulations to evaluate interfacial energies between HA crystallographic planes and solutions containing proteins as well as inorganic ions of calcium and phosphorous.

2. Computational details

Method: The HA/solutions interfacial energy was calculated from the model as schematically shown in Fig. 1. Interfacial energy is the energy difference between HA/solution block and two separated HA crystal and solution block based on the method of Duffy and de Leeuw [7,8]. The blocks are formed of a slab of HA crystal with the selected crystallographic planes (001), (100) and (110) in H2O molecules box containing Ca2+, HPO42− and/or proteins. The total number of atoms for each simulation model are maintained as the same. The three HA slabs have the same size 48 × 49 × 50 Å and are electrically neutral. The solution blocks also have the same size, including following models: pure water (3020 water molecules for HA(001); 2936 water molecules for HA(100) and 3171 water molecules for HA(110)); water with 0.1 M [Ca2+] and 0.04 M [HPO42−]; 0.5 M [Ca2+] and 0.2 M [HPO42−]; 0.1 M [Ca2+], 0.04 M [HPO42−] and 50 mg/ml Human serum albumin (HSA) and Lysozyme (LSZ); 0.5 M [Ca2+] and 0.2 M [HPO42−] and 50 mg/ml HSA and LSZ. The ratio of [Ca2+] to [HPO42−] is maintained as the same as...
that in physiological environment. Three α-helices in acidic HAS (PDB 1AO6) and basic LSZ (PDB 2NWD) were chosen as the protein models.

**Simulation parameters and procedure:** All the simulations were performed with NAMD [10] using the Charmm27 force field [11] with the addition of HA parameters [12,13]. The long-ranged electrostatic force and the van der Waals interactions were calculated with a cutoff distance of 12 Å. The simulation blocks were periodic in three dimensions (XYZ axes). All the simulations used a time step of 1 fs in the NVT ensemble with a constant temperature of 310.6 K. Then 1 ns MD simulation with all mobile atoms was performed in the slabs.

3. Results and discussion

The MD simulation revealed that the HA interfacial energies changed with increasing simulation time and values of the interfacial energies were calculated at 200 ps to 1 ns. The interfacial energy decreased with increasing adsorption of calcium and phosphate ions on the HA surfaces which occurs during the simulation process. Fig. 2 shows the distances between calcium and phosphate ions and HA surfaces as a function of time. The results indicated that the significant adsorption of inorganic ions occurred after about 230 ps. With severe calcium and phosphate ions absorption on a calcium phosphate crystal, the interfacial energy between the crystal and the solution becomes meaningless. Here, we consider the HA interfacial energy in physiological solution as the one without significant surface absorption of inorganic ions on the surface. By examination of the atom movement during the simulation, we choose the average values of the interfacial energies calculated thrice over the period of 200 to 230 ps as the reference interfacial energies.

The simulation shows that interfacial energies in the aqueous solution exhibit high dependence of HA crystallographic planes as summarized in Table 1. In pure water environment, HA interfacial energies changed with the crystallographic planes within the magnitude of 0.01–0.1 J m⁻². The clear trend of change shows as \( \gamma_{HA(110)} < \gamma_{HA(100)} < \gamma_{HA(001)} \). Rod-like shape of HA with rod-end

![Fig. 1. Schematic representation of the interfacial energy calculation.](image)

\[ \gamma = \frac{(E_{(Solution+HA)} - E_{(Solution)})}{2 - E_{(HA)/2}} \] per surface area.

![Fig. 2. The distances between adsorbed ions and HA surface as a function of time.](image)

(a) HA(100) + 0.1M[Ca]/0.04M[P]. (b) HA (100) + 0.5M[Ca]/0.2M[P].

<table>
<thead>
<tr>
<th>Plane</th>
<th>Pure water</th>
<th>0.1[Ca] 0.04[P]</th>
<th>0.1[Ca] 0.04[P]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HSA1</td>
<td>HSA2</td>
</tr>
<tr>
<td>(001)</td>
<td>0.6</td>
<td>0.856</td>
<td>0.673</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average: 0.628 (–26.6%)</td>
<td>0.143</td>
</tr>
<tr>
<td>(100)</td>
<td>0.13</td>
<td>0.22</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average: 0.153 (–48.6%)</td>
<td>0.073</td>
</tr>
<tr>
<td>(110)</td>
<td>0.022</td>
<td>0.078</td>
<td>0.169</td>
</tr>
<tr>
<td>Plane</td>
<td>0.5[Ca] 0.2[P]</td>
<td>0.5[Ca] 0.2[P]</td>
<td>0.5[Ca] 0.2[P]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSA1</td>
<td>HSA2</td>
</tr>
<tr>
<td>(001)</td>
<td>1.24</td>
<td>0.898</td>
<td>0.845</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average: 0.852 (–31.3%)</td>
<td>0.253</td>
</tr>
<tr>
<td>(100)</td>
<td>0.32</td>
<td>0.232</td>
<td>0.261</td>
</tr>
<tr>
<td>(110)</td>
<td>0.119</td>
<td>0.169 (–42.3%)</td>
<td>0.174</td>
</tr>
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</table>
surface of (001) provides indirect evidence of high interfacial energy of HA (001), as shown in Fig. 3. The good match with experimental study also confirms the validation of our MD simulation for interfacial energy calculations.

The simulation results as shown in Table 1 and Fig. 4 indicate that protein contents lower the interfacial energy between HA and aqueous solutions, except the case of the HSA containing solutions on the HA (110) plane. LSZ lowered the interfacial energy about 45.8–52.7% on HA (001) and (100), but not obviously on HA (110). HSA lowered the interfacial energy about 22.3–34.2% on HA (001) and (100), but increased about 42.3–96.6% on HA (110).

The characteristics of individual HA crystallographic plane leads to the different interactive behavior between proteins and HA surfaces, which had a potential to impact on the interfacial energy between HA surfaces and solutions. The density of atom groups exposed on the HA surfaces shows that HA (110) had the least Ca sites (0.035 atom⁻²; 0.059 for HA (001), 0.046 for HA (100)) and the most PO₄/OH sites (0.070 atom⁻²; 0.051 for HA (001), 0.062 for HA (100)) among three simulated planes. Note that the Ca sites and PO₄/OH sites mainly attract acidic residues and basic residues in protein, respectively [14]. The basic residues of acidic proteins HSA more likely interact with HA (110) than with HA (001) and HA (100). The interaction energy between basic residues of proteins and calcium phosphate is far higher than that between acidic residues and calcium phosphate [15]. Thus HSA has a higher interactive activity on HA (110), which leads to higher

![Fig. 3. TEM images of rod-like HA which was synthesized by a hydrothermal method. (a) Bright-field image, (b and c) HRTEM image with FFT (inset), and (d) SAED pattern with B=|1T0| zone axis.](image)

![Fig. 4. The interfacial energy between HA planes and solution ([Ca], [P] in M).](image)
molecular kinetic energy and potential energy of protein. The increasing HA (110) interfacial energy, which was manifested as the energy change of solution slab, results from the higher molecular kinetic energy and potential energy in HSA. The interfacial energy should be determined by total interaction effects among the ions, proteins and HA surfaces on the energy of solution slab. For instance, the energy of solution slabs was reduced through ions–proteins binding, which could be another reason that different interfacial energies were exhibited when HSA and LSZ were presented.

4. Conclusions

The interfacial energy of HA planes in solutions was evaluated by MD. The simulations provided reference interfacial energies for comparative study of protein effects. The results revealed that generally both acidic HSA and basic LSZ reduce the interfacial energy between HA surfaces and solutions. HSA increases the interfacial energy between HA (110) and solution. These results may help to interpret the phenomena that proteins promote or inhibit nucleation of apatite formation and regulate the morphology of apatite in physiological solutions.

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References