Investigation of the structural conformation and surface interaction of desired chimeric hydrophobin: Interface simulation via molecular dynamics

Hyun Joon Chang[a], Myeongsang Lee[b,c], Sungsoo Na[a,*]

[a] Department of Mechanical Engineering, Korea University, 02841, Seoul, Republic of Korea
[b] Institute of Advanced Machinery Design and Technology, Korea University, 02841, Seoul, Republic of Korea
[c] NanoScience Technology Center, University of Central Florida, Orlando, FL, 32826, United States

A B S T R A C T

Hydrophobins are small amphiphilic fungal proteins that are highly surface-active and are used in various industrial applications such as dispersion, immobilization, and antifouling. At hydrophobic-hydrophilic interfaces, hydrophobins tend to self-assemble as rodlets or monolayers, depending on whether they are class I or II. Several studies have determined the three-dimensional structure and investigated the self-assembly formation mechanism of the class I EAS from *Neurospora crassa* and the class II HFBII from *Trichoderma reesei*. Although some studies have examined the performance of chimeric hydrophobins, they have not been investigated at the atomic scale. Here, we designed chimeric hydrophobins by grafting the L_1 loop of Vmh2 and the L_3 loop of EAS onto the class II hydrophobin HFBII by homology modeling and performed vacuum-water interface molecular simulations to determine their structural behaviors. We found that the chimeric hydrophobin grafted with the L_3 of EAS became unstable under standard conditions, whereas that grafted with the L_1 of Vmh2 became unstable in the presence of calcium ions. Moreover, when both the EAS L_3 and Vmh2 L_1 were grafted together, the structure became disordered and lost its amphiphilic characteristics in standard conditions. In the presence of calcium, however, its structural stability was restored. However, an additional external perturbation is required to trigger the conformational transition. Although our chimeric hydrophobin models were designed through homology modeling, our results provide detailed information regarding hydrophobin self-assembly and their surface-interactive behavior that may serve as a template for designing hydrophobins for future industrial applications.

1. Introduction

Hydrophobins are small fungal proteins found in filamentous fungi and play a key role in penetrating air-water and fungal-host interfaces by self-assembling into amphiphilic monolayers [1,2]. The highly surface-active, amphiphilic hydrophobin monolayers lower the water surface tension to allow hyphae, conidia, and fruiting bodies to grow outside the aqueous media and infect a host [3–5]. These hydrophobins act as coating agents to provide water-resistance to spores for easier dispersion in air and for surface adhesion, as well as to prevent gas transfer channels in fruiting bodies from waterlogged [3,6,7]. Hydrophobins generally have a relatively high content of hydrophobic amino acids. Their structure consists of a β-barrel core composed of four antiparallel β-strands with eight cysteine residues that create four disulfide bonds [8–11]. Because the disulfide bonds stabilize the protein core, the hydrophobic amino acids are exposed on the protein surface, giving hydrophobins a Janus nature [2,12].

Hydrophobins are divided into two classes, class I and class II, which are distinguished by different spacings between the cysteine residues and a different distribution of hydrophobic and hydrophilic residues along the protein sequence [13]. Class II hydrophobins have homologous amino acid sequences with short and regular cysteine spacing, resulting in a closed β-barrel core with a short α-helix. In contrast, class I hydrophobins have long non-homologous amino acid sequences with varying cysteine spacing that result in a relatively open "half-barrel" core. Additionally, prior to amphiphilic monolayer formation, class I hydrophobins form amyloid-like rodlet structures that are difficult to depolymerize, whereas class II hydrophobins form regularly-packed amphiphilic monolayers without rodlets that can be depolymerized easily [14–17]. Class I hydrophobins are also known to undergo conformational changes before aggregating into rodlet structures, while class II hydrophobins have retained their conformation even after self-assembly [18–24]. Because of their distinct amphiphilic characteristics, class I and II hydrophobins have been used in various

industrial applications. The self-assembled layers of class I hydrophobins are suitable for antifouling and immobilization applications, while class II hydrophobin layers are suitable for applications such as dispersion, foaming, and protein expression and purification [2,12,25].

In previous studies, the hybridization of hydrophobin proteins by severing and/or grafting with other molecules has been attempted in order to determine the role of hydrophobin loops in self-assembly [26–28]. Kwan et al. found that the L_1 region of EAS is not necessary for rodlet formation [29], while substituting a specific region within L_3 with glycine had a dramatic effect on self-assembly. The class II hydrophobin NC2 from *Neurospora crassa* was grafted with L_3 segments from EAS; the resulting chimeric hydrophobin NChi2 also exhibited self-assembly with rodlet formation [30]. Furthermore, Lo et al. investigated the effect of grafting the EAS L_3 loop on NChi2 and found that NChi2 could form both rodlets and monolayers depending on the incubation conditions [31]. Similarly, BASF (Ludwigshafen, Germany) produced the new recombinant proteins H*Protein A and H*Protein B using the class I hydrophobins DewA from *Aspergillus nidulans* and a truncated form of yaaD from *Bacillus subtilis* [32]. However, atom-scale studies to determine the effect of grafting class I hydrophobin loops on class II hydrophobin structures have not been conducted. Furthermore, there are no cases in which more than one class I hydrophobin loop has been grafted on a class II hydrophobin. These studies would improve the understanding of overlapping loop effects.

In this study, we investigated the effect of grafting the class I hydrophobin loops L_1 Vmh2 from *Pleurotus ostreatus* and L_3 EAS onto the class II hydrophobin HFBII monomer from *Trichoderma reesei* to evaluate the distinctive characteristics of each loop. To observe the effects on the atomic scale, we implemented molecular dynamics (MD) simulations and compared our results with those of previous studies. In order to observe the role of calcium ions on the aggregation/adsorption of Vmh2 hydrophobin and to further investigate its effect on chimeric hydrophobins, we performed additional simulations with the presence of calcium ions. We focused on the structural stability of the loops and the central β-barrel core of the chimeric hydrophobins to determine its surface-activity and its possible aggregation tendencies, which can provide useful information for designing hydrophobins for industrial applications.

2. Materials and methods

2.1. Materials (modeling)

We obtained the structural information for HFBII and EAS from the Protein Databank (PDB) entries with the IDs 2B97 and 2FMC, respectively. We created the structure of Vmh2 using the *Chimera* program [33]. The modeling methodology for the HFBII, EAS, and Vmh2 structures is explained in the *Materials* section in the Supporting information. As shown in Fig. 1, we classified the hydrophobin sequences into four major parts based on their secondary structure: (1) the β-barrel core consisting of the β-sheets β_1, β_2, β_3, and β_4; (2) loop L_1, which is the sequence located between β_1 and β_2; (3) loop L_2, which is located between β_2 and β_3; and (4) loop L_3, which is located between β_3 and β_4. The sequence classification was based on previous studies of HFBII, EAS, and Vmh2 [12,25,34].

Based on these classifications, we designed three chimeric hydrophobins by grafting Vmh2 L_1 (S15-V45) and/or EAS L_3 (D63-A78) on HFBII: (1) in the first model (H_{HHE}), the sequence V54-L63 of HFBII was replaced with EAS L_3 (Fig. 1(D)); (2) in the second model (H_{VHH}), the sequence L19-L21 was replaced with Vmh2 L_1 (Fig. 1(E)); and (3) in the third model (H_{VHE}), both the L19-L21 and V54-L63 sequences were replaced with Vmh2 L_1 and EAS L_3, respectively (Fig. 1(F)). The chimerization procedure was carried out by comparative homology modeling [33]. The first letter of the names of chimeric hydrophobins indicates the body of its original structure, while the subsequent letters refer to the grafted loop or loops; 'H' indicates 'HFBII', 'V' indicates

'Vmh2', and 'E' indicates 'EAS.' The detailed construction procedure for the chimeric models is provided in the *Materials* section of the Supporting information.

2.2. Simulation and analysis

We performed 300 ns aqueous and 500 ns water-vacuum interface MD simulations of the HFBII, EAS, Vmh2, and chimeric models using the program GROMACS 5.0.7 with the GROMOS 54A7 forcefield [35]. In addition, we considered the effect of the presence of calcium ions on the hydrophobins. Models that were subjected to calcium ion simulation are indicated by a superscript, e.g., $Vmh2^{Ca}$. For the detailed simulation constraints, see the simulation methods and conditions in the Supporting information. After the simulations, we calculated the radius of gyration (R_g), interface-protein distance, and hydrophobic dipole distribution (HDD) to determine the orientation of each hydrophobin model. Additionally, the secondary structure, number of hydrogen bonds (H-bonds), and solvent accessible surface area (SASA) were used to analyze the stability of the hydrophobins. Specifically, we only calculated the Vmh2 L_1, EAS L_3, and HFBII β-barrel core regions to observe the specific structural behavior. Details of the analysis are given in the Supporting information.

3. Results and discussion

For simplicity, we only discuss the interface simulation results of the chimeric hydrophobins in this section. Since the purpose of the aqueous simulation was to equilibrate the hydrophobin in its natural state, the equilibrated states of the hydrophobin models after the aqueous simulation are described in the *Results and discussion* section of the Supporting information, along with the detailed results from the interface simulations of HFBII, EAS, and Vmh2. In this section, the following topics will be discussed: (1) the orientation of the chimeric hydrophobins at the water-vacuum interface, which relates to their global conformational stability and amphiphilicity as well as their interface-interaction behavior; and (2) the structural stability and conformation of the primary segments of the hydrophobins, i.e., the β-barrel core, EAS L_3 region, and Vmh2 L_1 region, to identify the effect of grafting EAS L_3 and Vmh2 L_1 on their possible self-assembly and adsorption behavior.

3.1. Orientation of chimeric hydrophobin models at the water-vacuum interface

Fig. 2 shows the final structures of chimeric hydrophobins after the interface simulations. For H_{HHE} and H_{HHE}^{Ca}, partial regions of L_3, β_1, and β_2 of both models were in contact with the interface, irrespectively of the external condition (Fig. 2(A, D)). Moreover, the L_2 loop region of both models was completely submerged in water throughout the entire simulation. This phenomenon was similar to that observed in HFBII and EAS (Fig. S2(A-B, D-E)), whose L_3, β_1, and β_2 regions were also exposed at the water-vacuum interface. However, the number of interface-contacting residues in H_{HHE} was much greater than for H_{HHE}^{Ca}, resulting H_{HHE} having a larger amount of its area exposed to the vacuum. In Fig. 3(A), the R_g value of H_{HHE} and H_{HHE}^{Ca} were similar; however, as shown in Fig. 3(B), the HDD of H_{HHE} had a broader spectrum than that of H_{HHE}^{Ca}, and its direction was perpendicular to the normal of the water-vacuum interface. Here, we can assume that the L_3 loop of EAS affected the hydrophobic/hydrophilic residue distribution under standard conditions, and that this distribution was altered in the presence of calcium ions.

In the case of H_{VHH}, the L_1 region was exposed to the vacuum for both H_{VHH} and H_{VHH}^{Ca}, but the time of exposure was much smaller for H_{VHH}^{Ca} than H_{VHH} (Fig. 2(B, E)). Specifically, in H_{VHH}^{Ca}, the L_2 region interacted with the vacuum interface, while in H_{VHH} it was the L_3 loop that was exposed to the surface. Comparably, Supplementary Fig. S2(C,