International Journal of Biological Macromolecules 72 (2015) 658–663

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Identification properties of a recombinant class I hydrophobin rHGFI

Wenfeng Li[a,b], Yanbo Gong[c], Haijin Xu[d], Mingqiang Qiao[d,**], Baolong Niu[a,b,*]

[a] Key Laboratory of Interface Science and Engineering in Advanced Materials, Taiyuan University of Technology, Ministry of Education, Taiyuan 030024, PR China
[b] College of Materials Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, PR China
[c] College of Light Textile Engineering & Art, Taiyuan University of Technology, Taiyuan 030024, PR China
[d] State Key Laboratory of Medicinal Chemical Biology, College of Life Sciences, Nankai University, Tianjin 300071, PR China

ARTICLE INFO

Article history:
Received 26 August 2014
Received in revised form
12 September 2014
Accepted 13 September 2014
Available online 19 September 2014

Keywords:
Hydrophobin
Self-assembly
Rodlet structure

ABSTRACT

Hydrophobins fulfill various functions in fungal growth and morphology. These proteins can self-assemble at hydrophilic/hydrophobic interfaces and form amphipathic membranes. Based on their physical properties and hydropathy patterns, hydrophobins are divided into two classes (I and II).

In order to identify the recombinant class I hydrophobin rHGFI, the different properties between rHGFI and the typical class II hydrophobin rHFBI were investigated. In contrast to rHGFI, no rodlet structure was observed on rHFBI coated mica surface, and the membranes formed on siliconized glass surfaces by rHFBI were not robust enough to resist treatment with 60% ethanol and 2% hot SDS. In contrast, the membranes formed by rHGFI on siliconized glass surfaces were so strong that could resist hot detergent and alcohol solution washing. Moreover, self-assembly of rHFBI at the water–air interface was not accompanied by a change in secondary structure. Meanwhile, β-sheet structures dramatically increased after rHGFI self-assembled at water–air interface, which could cause the fluorescence intensity of Thioflavin T increased and Congo Red and CD absorption spectra shift. Water-insoluble erythrosin B dispersion prepared with rHGFI and rHFBI were both stable for more than one month, which indicated that the interaction between erythrosin B and rHGFI/rHFBI was strong. This might promote rHGFI and rHFBI to be considered as potential dispersing agents to stabilize water-insoluble erythrosin B.

1. Introduction

Hydrophobins are a large family of low molecular weight proteins [1,2]. They are uniquely produced by filamentous fungi [3]. The characteristic property of hydrophobins is that they can self-assemble spontaneously at hydrophobic–hydrophilic interfaces into amphipathic membrane and convert the surface from hydrophilic to hydrophobic and vice versa [4–6]. The biological role of these proteins centers upon allowing filamentous structures to break air/water interfaces and so produces aerial spores or fruiting bodies [7–9]. All hydrophobins possess eight conserved cysteine residues that form four intramolecular disulfide bonds, but their amino acid sequences and composition are diverse [10,11].

On the basis of their hydropathy plots and biophysical properties, hydrophobins are discriminated between class I and class II [12,13]. The class I hydrophobins are poorly soluble even under conditions such as in 2% boiling SDS, and can be solubilized only by treatment with solvents such as 100% trifluoroacetic acid (TFA) and formic acid [14]. Meanwhile, rodlets are typically observed by electron microscopy or atomic force microscopy (AFM) when dilute class I hydrophobin solution drip and dry down on a solid support [15–18]. Unlike the class I hydrophobin, the membranes formed by assembled class II proteins can be dissociated under mild conditions (such as 60% ethanol or 2% SDS) [19]. Moreover, no rodlets are observed when dilute class II hydrophobin solutions drip and dry down on a solid support [19]. But the membranes formed by class II hydrophobin imaged by AFM show an organized structure when the dilute solutions drip and dry down onto a mica support [1]. Circular dichroism (CD) shows that the class I hydrophobins contain substantial amount of β-sheet structure in the soluble state and a significant secondary structural changes appear after vortexing the aqueous solution [16,20]. While, except for a slight alteration

* Corresponding author at: Key Laboratory of Interface Science and Engineering in Advanced Materials, College of Materials Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, PR China. Tel.: +86 351 6111197; fax: +86 351 6018740.
** Corresponding author at: State Key Laboratory of Medicinal Chemical Biology, College of Life Sciences, Nankai University, No. 94 Weijin Road, Tianjin 300071, PR China. Tel.: +86 22 23503340; fax: +86 22 23503692.
E-mail addresses: Mingqiangqiao@live.com (M. Qiao), blniunankai@hotmail.com (B. Niu).

http://dx.doi.org/10.1016/j.ijbiomac.2014.09.014
0141-8130/

of intensity, no changes in the CD spectra of class II hydrophobins were observed after vigorous vortexing the solutions [13].

The class I hydrophobin HGFI from *Grifola frondosa* and class II hydrophobins HFBI from *Trichoderma reesei*, respectively, were heterologous expressed in *Pichia pastoris* (*P. pastoris*) [21,22]. Both rHGFI and rHFBI have a good ability of self-assembling into amphipathic membrane to change the wettability of different solid surfaces [21,22]. Compare to rHGFI, rHFBI had the ability to stabilize oil droplets, which was far excess of rHGFI [22].

Here, we studied the properties of the class I hydrophobins rHGFI and compared them to those of the class II hydrophobin rHFBI. Moreover different application possibilities of the two proteins were discussed.

2. Experimental

2.1. Reagents

The fermentation strains (*P. pastoris*) which could overproduce rHGFI and rHFBI were donated by Prof. M.Q. Qiao (State Key Laboratory of Medicinal Chemical Biology, College of Life Sciences, Nankai University, China).

0.1 M phosphate buffer solution (PBS, pH 4.0) consisting of Na_2HPO_4 and KH_2PO_4 were used to dissolve proteins and Congo Red. All other chemical reagents were of analytical grade and purchased from Sigma.

2.2. Fermentation and purification of rHGFI and rHFBI

The fermentation strains (*P. pastoris*) which could overproduce rHGFI or rHFBI were chosen for large-scale production following the procedure reported by Niu et al. [23]. After 96 h of induction time, the *P. pastoris* culture was centrifuged at 8000 × *g* to obtain the supernatant. A two-step process was used to purify the resulting supernatant. The first step was ultrafiltration using a hollow fiber membrane module with 4 kDa molecular weight cut off (Tianjin MOTIMO Membrane Technology Ltd., China) and then lyophilized. RP-HPLC was used to further purify the lyophilized powder with a Vydac C4 reversed-phase column (4.6 mm × 250 mm, GRACE, China). The rHGFI and rHFBI lyophilisates after RP-HPLC purification were identified by 16% Tricine–SDS-PAGE and Western blotting, respectively.

2.3. Proteins self-assembly on hydrophilic or hydrophobic substrates

Wettabilities of bare and hydrophobin-modified solid surfaces were analyzed using water contact angle (WCA) measurements. The cleaned mica and siliconized glass sheets were incubated overnight in rHGFI or rHFBI solutions at 20 °C. Then, the substrates were dried under a stream of nitrogen and gently rinsed with water. Finally, the surfaces were dried once more with a stream of nitrogen gas.

In addition, the proteins coated siliconized glass surfaces were rinsed with 60% (v/v) ethanol solution or 2% boiling SDS solution, respectively.

The stabilities of the membranes formed by the proteins were tested by monitoring the WCA changes after different treatments. WCAs were measured with a 5-μL of water droplet on the modified and unmodified surfaces using an optical contact angle meter (DSA100, Kruss company, German) at room temperature. The average values of WCA were obtained from three water droplet readings at different locations.

2.4. Atomic force microscope measurements

Tapping mode images of dried droplets were obtained using a NanoScope IIIa Multimode atomic force microscope (Veeco Instruments, USA) and silicon nitride cantilevers with a nominal force constant of 50 N/m. Scan rates were approximately 1 Hz. The damping ratio (set-point amplitude/free amplitude) was typically about 0.7–0.8. The scanning probe image processor (SPIP, Image Metrology, Denmark) was used for image analysis. All of the collected images were flattened to rule out any possible tilt.

2.5. Circular dichroism spectropolarimetry

The secondary structure changes of rHGFI and rHFBI were measured by CD after they were self-assembled at an air–water interface. HGFI-AR samples at a concentration of 50 μg/ml in MQW were used in this study. The CD spectra of the assembled hydrophobins were obtained after vigorously shaking on a vortex mixer for 5 min.

The spectra were recorded over the wavelength range from 190 to 250 nm on a Jasco J-715 CD spectrometer (Japan), using a 1-mm quartz cuvette. The temperature was kept at 25 °C, and the sample compartment was continuously flushed with nitrogen gas. The samples' spectra were the result of averaging five scans obtained using a 5-s averaging per point, a bandwidth of 1 nm and a step-width of 0.5 nm. The spectrum of the reference solution without the protein was used to correct for the background signal.

2.6. Thioflavin T (ThT) staining

All the samples used in the rodlet formation assays were tested using fluorescence emission spectroscopy. The samples were excited at 435 nm, and the emission spectra were recorded over the wavelength range of 450–600 nm with emission and excitation slits set at 10 nm. Lyophilized HGFI-AR and rHGFI were dissolved in MQW and added to a stock ThT solution prepared with MQW. The final concentrations in each sample were 5 μM ThT and 40 μg/mL rHFBI or rHGFI, respectively. The spectra were recorded using 5 μM ThT aqueous solution alone as a control. All the samples were measured using a Cary Eclipse Fluorescence Spectrophotometer (Varian Optical Spectroscopy Instruments, Mulgrave, Australia) before and after vortexing for 5 min.

2.7. Congo red (CR) binding assays

CR binding was monitored by a BIO-TEK Microplate Reader model EL312 (BioTek U.S., Winooski, VT, USA). All measurements of the absorption spectra were recorded within 300–700 nm range. The instrument was blanked using phosphate buffer (pH 4.0).

Both CR and the proteins were dissolved in 50 mM phosphate buffer (pH 4.0). Mixtures of CR and rHFBI or rHGFI were incubated at room temperature for 30 min and shaked for 5 min prior to spectral analysis. The final concentrations in each sample were 10 μg/ml CR and 100 μg/mL rHFBI or rHGFI. The absorbance spectra were also measured using 10 μg/ml CR solution alone as a control.

2.8. Dispersion properties of rHGFI and rHFBI

Stock solutions of erythrosin B were prepared in DMSO depending on its solubility characteristic. Erythrosin B was dissolved at 50 mg/ml in DMSO. rHGFI (110 μg/mL) and rHFBI (100 μg/mL) were obtained by adding the required amount of the proteins to distilled water and then gently stirred at room temperature until dissolution was complete. 10 μl above erythrosin B solution was dropped in 5 ml of the prepared protein solutions followed by gentle stirring for 30 min at room temperature. Finally, the resulting