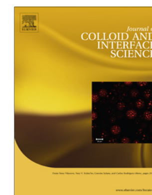
Journal of Colloid and Interface Science 448 (2015) 140–147

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

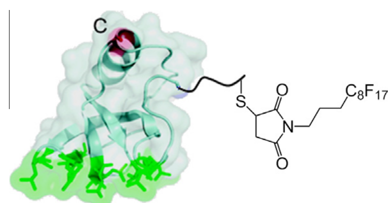


A synthetically modified hydrophobin showing enhanced fluorous affinity

Roberto Milani [a,c,*], Lisa Pirrie [b,d], Lara Gazzera [d], Arja Paananen [b], Michele Baldrighi [d], Evanthia Monogioudi [b], Gabriella Cavallo [d], Markus Linder [b,e,*], Giuseppe Resnati [c,d,*], Pierangelo Metrangolo [b,c,d,*]

[a] VTT Technical Research Centre of Finland Ltd, Biologinkuja 7, Espoo FI-02044 VTT, Finland
[b] VTT Technical Research Centre of Finland Ltd, Tietotie 2, Espoo FI-02044 VTT, Finland
[c] Center for Nano Science and Technology@Polimi, Istituto Italiano di Tecnologia, Via G. Pascoli 70, I-20133 Milano, Italy
[d] NFMLab-DCMIC "Giulio Natta", Politecnico di Milano, Via L. Mancinelli 7, I-20131 Milano, Italy
[e] Aalto University, School of Chemical Technology, Kemistintie 1, P.O. Box 16100, FI-00076 AALTO Espoo, Finland

G R A P H I C A L   A B S T R A C T



A R T I C L E   I N F O

Article history:
Received 27 November 2014
Accepted 2 February 2015
Available online 9 February 2015

Keywords:
Hydrophobin
Surfactant protein
Protein film formation
Fluorinated material
Fluorous tag
Compatibilization

A B S T R A C T

Hydrophobins are natural surfactant proteins endowed with exceptional surface activity and film-forming capabilities and their use as effective "fluorine-free fluorosurfactants" has been recently reported. In order to increase their fluorophilicity further, here we report the preparation of a unique fluorous-modified hydrophobin, named F-HFBI. F-HFBI was found to be more effective than its wild-type parent protein HFBI at reducing interface tension of water at both air/water and oil/water interfaces, being particularly effective at the fluorous/water interface. F-HFBI was also found to largely retain the exceptionally good capability of forming strong and elastic films, typical of the hydrophobin family. Further studies by interface shear rheology and isothermal compression, alongside Quartz Crystal Microbalance and Atomic Force Microscopy, demonstrated the tendency of F-HFBI to form thicker films compared to the wild-type protein. These results suggest that F-HFBI may function as an effective compatibilizer for biphasic systems comprising a fluorous phase.
© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Hydrophobins (HFBs) are small (<100 amino acids) proteins produced by filamentous fungi that are endowed with surface activity and film-forming capabilities unmatched by any other protein [1]. HFBs are exceptionally stable towards denaturation due to the presence of four disulfide bridges in their core structure, and are able to withstand temperatures close to the boiling point of water [2–4]. The high surface activity of HFBs is due to their

* Corresponding authors at: VTT Technical Research Centre of Finland Ltd, Biologinkuja 7, Espoo, FI-02044 VTT, Finland (R. Milani). VTT Technical Research Centre of Finland Ltd, Tietotie 2, Espoo, FI-02044 VTT, Finland (M. Linder and P. Metrangolo). NFMLab-DCMIC "Giulio Natta", Politecnico di Milano, Via L. Mancinelli 7, I-20131 Milano, Italy (G. Resnati). Fax: +39 0223993180 (G. Resnati and P. Metrangolo).

E-mail addresses: roberto.milani@vtt.fi (R. Milani), ext-lisa.pirrie@vtt.fi (L. Pirrie), lara.gazzera@polimi.it (L. Gazzera), arja.paananen@vtt.fi (A. Paananen), michele.baldrighi@iit.it (M. Baldrighi), evanthia.monogioudi@ec.europa.eu (E. Monogioudi), gabriella.cavallo@polimi.it (G. Cavallo), markus.linder@aalto.fi (M. Linder), giuseppe.resnati@polimi.it (G. Resnati), pierangelo.metrangolo@polimi.it (P. Metrangolo).

http://dx.doi.org/10.1016/j.jcis.2015.02.003
0021-9797/© 2015 The Authors. Published by Elsevier Inc.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Janus-type structure where a discrete and well-defined portion of the protein surface is comprised of hydrophobic amino acids and known as the hydrophobic patch, while the remaining surface is hydrophilic in nature [5,6]. The most distinguishing feature of the HFBs is their ability to self-assemble into strong and elastic films at the interface between hydrophilic and hydrophobic phases. Such films have also been reported to be orders of magnitude stronger than those formed by other surface active proteins including β-lactoglobulin and β-casein [7]. Recently, we have demonstrated that HFBs also function as particularly effective "fluorine-free fluorosurfactants" reducing interface energy at the fluorous [8–10]/aqueous interface [11].

The remarkable performance differences between fluorocarbon (FC) and hydrocarbon (HC) surfactants originate from the peculiar features of the fluorine atom such as low polarizability, high electronegativity and ionization potential, as well as a larger size compared to the hydrogen atom. FC chains are thus more hydrophobic than their HC counterparts, and are also substantially lipophobic [12]. One first consequence is that FC-surfactants are more effective in lowering the surface tension of water, being able to attain values as low as 15–20 mN/m, whereas typical HC-based amphiphiles can only reach values of about 30–40 mN/m [13–15]. A second consequence is that FCs tend to segregate into a separate phase in order to avoid unfavorable interactions with other molecules, either hydrophilic or lipophilic, a phenomenon known as *fluorophobic effect* [8–10,16–18].

With the objective of further improving the performance of HFBs at fluorous/aqueous interfaces, and to combine their exceptional film forming capabilities with the superior surface activity of FC-surfactants, we covalently bound a FC chain to the exposed surface of the protein NCysHFBI, which is a genetically engineered variant of the wild-type (WT) hydrophobin HFBI. In this way we obtained a unique fluorous-modified hydrophobin, termed F-HFBI [19].

Protein fluorination strategies have to date mainly relied on the introduction of selectively fluorinated amino acids, typically leucines, in the protein primary structure [20–27]. An alternative approach consists of the site-specific derivatization with fluorous tags by covalently binding FC residues to peptides and proteins, mainly for immobilization, separation, and enrichment purposes [28–31]. Expanding on this strategy, here we report the preparation and subsequent characterization of a HFB derivative covalently functionalized with a single perfluorooctyl chain. This specific FC segment was selected based on the ready availability of reagents, and is intended to act as a first model for the introduction of more environmentally compliant moieties, *e.g.*, small perfluoropolyether chains.

2. Materials and methods

2.1. Materials

NCysHFBI was produced using recombinant strains of *T. reesei*, purified by RP-HPLC as described previously and lyophilized before use.[32] Galden® SV90 (GSV90) is a low molecular weight perfluoropolyether fluid (90 °C boiling point) produced by Solvay Specialty Polymers. Heptane, hexadecane, 1H,1H,2H,2H-perfluorodecane-1-thiol, 11-mercapto-1-undecanol, and hexanethiol were purchased from Sigma Aldrich and used without further purification.

2.2. Reduction of NCysHFBI

The starting protein NCysHFBI was first reduced in order to break the dimers occurring on the formation of disulfide bridges involving the accessible Cys residues close to the N terminus of the protein. Typically, 10 mg of NCysHFBI were dissolved in 1.6 mL of mQ water, then 80 μL of acetate buffer (1 M, pH 5), 32.5 μL of 0.5 M EDTA and 1660 μl of 0.1 M dithiothreitol were sequentially added. The mixture was incubated for 1 h at 37 °C, and the reduced NCysHFBI was purified by reverse phase HPLC on a Vydac C4 column using a gradient of water/acetonitrile (both containing 0.1% TFA). Following overnight freeze-drying, the typical yield of the reaction was around 50–60%. The reduced protein was stored at −80 °C. MALDI-TOF: $m/z = 8677$.

2.3. Preparation of F-HFBI

In a typical synthesis, 5 mg of reduced NCysHFBI were dissolved in a mixture of 2 mL THF and 3.5 mL phosphate buffer (0.1 M, pH 7.2). A solution of 5.8 mg of 3-(perfluorooctyl)-propyl-1-maleimide in 1.5 mL THF was added. The vial containing the mixture was briefly flushed with nitrogen, wrapped in aluminium foil, and gently stirred at room temperature for 5 h. The solution was lyophilized overnight, then the solid was redissolved in 2.5 mL of 50% EtOH for purification by reverse phase HPLC on a Vydac C4 column using a gradient of water/acetonitrile (both containing 0.1% TFA). F-HFBI was subsequently freeze-dried and recovered typically in 65–70% yield. MALDI-TOF: $m/z = 9232$.

2.4. Zeta-potential measurements

The isoelectric point of F-HFBI was measured experimentally with a Nano-ZS zetasizer (Malvern instruments, UK) by performing titrations with 0.01 M and 0.1 M NaOH on 0.10 mg/mL F-HFBI solutions in mQ water. The measurements were highly reproducible, and the experimental *pI* obtained as an average of two different measurements was 4.87 (Fig. S1 in the Supporting information).

2.5. MALDI-TOF Spectrometry

An Autoflex II instrument from Bruker Daltoniks (Bremen, Germany) equipped with a UV/N_2-laser (337 nm/100 μJ) was used to carry out mass spectrometric analyses. Saturated α-cyano-4-hydroxycinnamic acid in a 1:2 (v/v) mixture of acetonitrile and aqueous 0.1% (v/v) trifluoroacetic acid was used as a matrix. The purified NCysHFBI and F-HFBI were dissolved in the same solvent mixture at about 2 mg/mL concentration, mixed with the matrix solutions in 1:1 (v/v) ratio and applied on the stainless steel target plate in 1 μL aliquots. The sample spot was dried in air at room temperature. The mass spectrum (4–20 kDa) was measured in linear positive-ion mode, and Protein standard solution I (Bruker Daltonics) was used for the external molecular mass calibration.

2.6. CD spectroscopy

CD spectra were collected with a Chirascan spectrophotometer (Applied Photophysics) fitted with a Peltier temperature controller, using a rectangular quartz cuvette with an optical path length of 1 mm. Data acquisition was performed in steps of 0.5 nm at a wavelength range from 190 to 280 nm with a spectral bandwidth of 1.0 nm. All spectra were corrected in baseline with mQ water as the blank. Spectra were the average of 10 consecutive scans. The signals were normalized to mean residue ellipticity (MREs) based on the peptide concentration:

$$[\theta]_\lambda = \frac{\theta_{obs}}{(10 \times l \times c \times (n-1))}$$

where $[\theta]_\lambda$ is the MRE at wavelength λ in deg cm^2 dmol^{-1}, l is the path length in cm, c is the molar concentration and $(n-1)$ is the number of peptide bonds.

Download English Version:

https://daneshyari.com/en/article/6996641

Download Persian Version:

https://daneshyari.com/article/6996641

Daneshyari.com