Hydrophobin Enrichment based on the Hydrophobic Interaction

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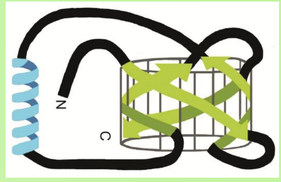
Mohammadreza Khalesi [a]*, Sylvie Deckers [a], James Winterburn [b], Tom Venken [c], Zahra Shokribousjein [a], David Riveros-Galan [a], Kurt Gebruers [a], Hubert Verachtert [a], Jan. A Delcour [a], Marc De Maeyer [c], Frank Delvigne [d], Ivo Vankelecom [e], Peter Martin [b] and Guy Derdelinckx [a]

[a] Department of Microbial and Molecular Systems (M²S), LFoRCe, KU Leuven Kasteelpark Arenberg 33, BE-3001 Heverlee, Belgium.
[b] School of Chemical Engineering and Analytical Science, The Mill, The University of Manchester, Manchester M13 9PL, UK.
[c] Biochemistry, Molecular and Structural Biology Section, KU Leuven, BE-3001 Heverlee, Belgium.
[d] Unite de bio-industries/CWBI, Ulg- Gembloux Agro-Bio Tech, Passage des Deportes 2, BE-5030, Gembloux, Belgium.
[e] Department of Microbial and Molecular Systems (M²S), COK, KU Leuven, Kasteelpark Arenberg 20, BE-3001 Heverlee, Belgium.
*** Corresponding author:** Mohammadreza.Khalesi@biw.kuleuven.be

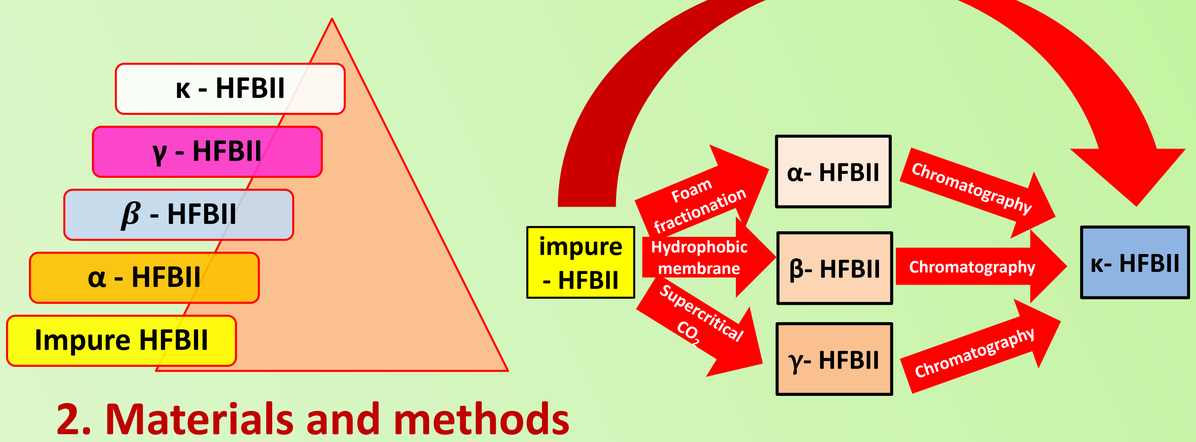
1. Introduction

1.1. Hydrophobins



Hydrophobins HFBII is an exceptional protein produced by *Trichoderma reesei*. Research over the last decade has led to a better understanding of its role in spontaneous self-assembly at hydrophobic/hydrophilic interfaces[1]. This has resulted in many proposals for using hydrophobins in important scientific and technological applications. Hydrophobins may become attractive as **special biosurfactants**, as **foaming agents,** and **in pharmaceutical formulations for stabilization of drugs**[2]. To recognize the positive aspects of hydrophobin, this should be available at large scale.

1.2 Hydrophobin HFBII grades



2. Materials and methods

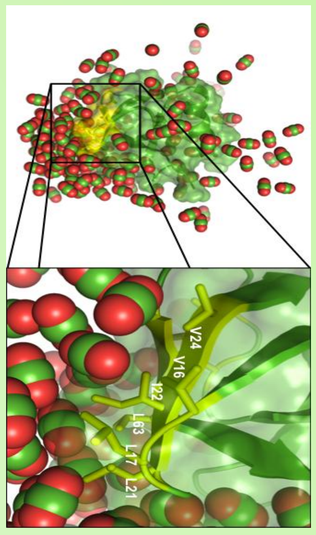
2.1. Production in fermentor

Component	Amount
Carbon source (40g/L)	49.3 g
Peptone	4.8 g
Yeast extract	1.2 g
KH_2PO_4	4.8 g
$(NH_4)_2SO_4$	3.36 g
$MgSO_4.7H_2O$	0.72 g
$CaCl_2$	0.72 g
$CoCl_2.6H_2O$	4.8 mg
$MnSO_4.H_2O$	3.84 mg
$ZnSO_4.7H_2O$	8.28 mg
$FeSO_4.7H_2O$	12 mg

2.2. Extraction

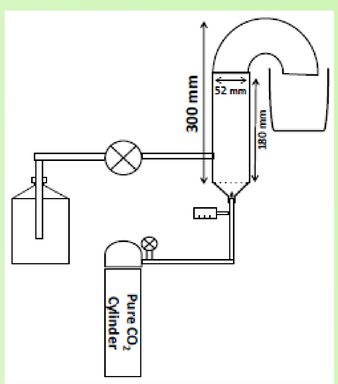
2.2.1. The idea behind the extraction

Using the molecular dynamics (MD) model, we demonstrated the presence of CO_2 aggregates at the hydrophobic patch of HFBII during MD trajectory[3]. As such, the hydrophobic patch has a tendency to cluster the hydrophobic CO_2 molecules close to the protein surface. This effect could be enhanced when multiple hydrophobins are present and polymerize with each other, thereby increasing the hydrophobic patch area and reducing contacts with other parts of the protein.



2.2.2. Foam fractionation system

Foam fractionation of substances is an adsorptive bubble separation method for enriching diluted surface-active substances dissolved in water.
In principle, the strong amphipathic nature of proteins and enzymes, with polar and non-polar groups, causes them to be preferably adsorbed at the gas–liquid interface and foam fractionation can be used to separate and to concentrate such proteins[4]. In this study, CO_2-foam fractionation was used.



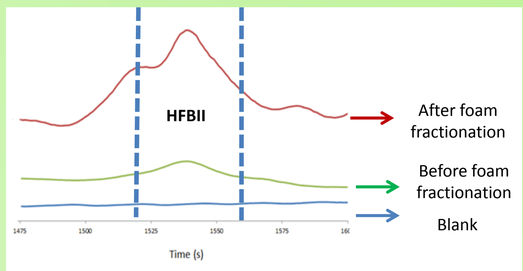
2.2.3. Identification of HFBII

Samples were centrifuged, then directed to **15RPC liquid chromatography**. The fractions of interest were submitted to **MALDI-TOF**. **NanoDrop** ND-1000 at 280 nm wavelength was used for HFBII quantification.

3. Results and discussions

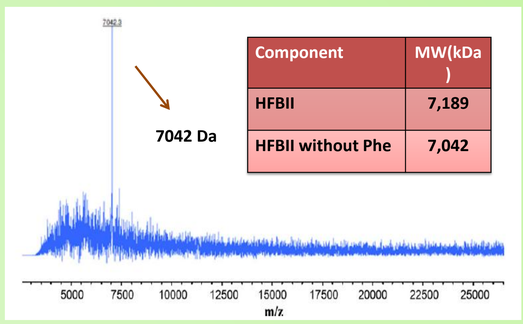
3.1. Chromatography purification

The results before and after CO_2-foam fractionation is observed in the figure. Obviously, the sample after foam fractionation contains more protein than the sample before CO_2 treatment. It demonstrates the fact that CO_2 can enrich the molecule of hydrophobin. The fractions of interest were subjected to the MALDI-TOF for identification.



3.2. MALDI-TOF identification

The fractions of interest seems to contain only one type of protein with a molecular weight (MW) of **7.042 kDa** which was reported previously[5]. This MW is equal to the complete molecule of HFBII minus the last amino acid. HFBII loses ***Phe***, probably due to biodegradation by the fungi at the stationary phase.



Component	MW(kDa)
HFBII	7,189
HFBII without Phe	7,042

3.3. Quantification

Sample	Concentration (mg/mL) before using amicon
Sample before treating	0.10 ± 0.02
Type of frit – the ratio of height of liquid over the height of column–CO_2 flow rate	
G_3–0.13–3 L/min	0.40 ± 0.03
G_3–0.23–1 L/min	0.32 ± 0.02
G_3–0.23–2 L/min	0.41 ± 0.03
G_3–0.23–3 L/min	0.44 ± 0.06
G_3–0.33–3 L/min	0.57 ± 0.04
G_4–0.23–3 L/min	0.33 ± 0.03

4. Conclusion

Our results show that hydrophobin HFBII can be isolated from the growth medium of *T. reesei* by the CO_2-foam fractionation method. This was shown by chromatographic analysis of the extract followed by the MALDI-TOF. The results show that by increasing the flow rate and the initial liquid volume, the obtained protein using liquid chromatography is increased. It was demonstrated that when CO_2 is injected into a sample containing HFBII, it is immediately directed to the hydrophobic amino acids.

Acknowledgement

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