HYDROPHOBINS AS INACTIVE EXCIPIENTS IN THE PHARMACEUTICAL AND FOOD INDUSTRY

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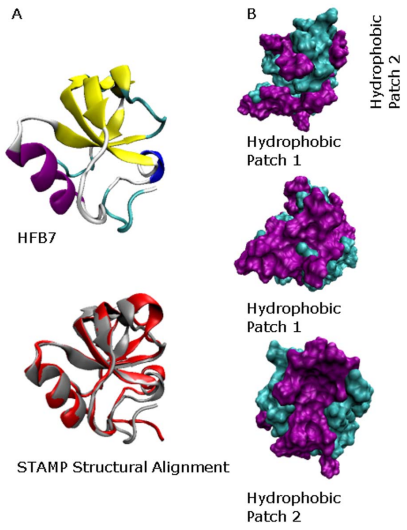
INTRODUCTION

The pharmaceutical and food industries require not only bioactive ingredients but also inactive excipients in formulation development. Pharmaceutical excipients are substances other than the pharmacologically active drug, which are included in the manufacturing process or are contained in a finished pharmaceutical product dosage form. They may be important for keeping the drug from being released too early in the assimilation process or protect the product's stability so that it will be at maximum effectiveness at time of use. Class II hydrophobins (HFBs) from *Trichoderma* spp. can be potentially used as such inactive excipients. In this study, the class II HFB4 proteins from different *Trichoderma* spp. were heterologously produced in *Pichia pastoris*. The extracellular hydrophobins obtained from the fermentation process were purified and their antioxidant properties tested.

EXPERIMENTS / FUNDAMENTAL OF THE PROBLEM / EXAMINATIONS

Filamentous fungi produce a diversity of hydrophobins, a family of low molecular weight amphiphilic surface-active proteins containing four disulfide bridges and a large conserved and exposed hydrophobic patch [Picture 1] [1]. Hydrophobins are traditionally split into class I and class II, by their solubility and hydropathy plots of their amino acid sequences. Class I hydrophobins are not soluble in water whereas the proteins from class II are easily dissolved in the aqueous phase. The large potential of class II hydrophobins in clinical applications has been described in recent literature[1-8]. It was reported that class II HFBs (HFB4 and HFB7) of *Trichoderma* can enhance the rate of enzymatic hydrolysis of aromatic-aliphatic polyesters such as PET [2]. Furthermore, class II hydrophobins have been successfully used for generating stabilized foams in foam-rich products where control of the air phase is especially important [3]. In contrast, the formation of stabilized CO_2 nanobubbles by class II hydrophobins have been reported to induce gushing and are considered to be a negative property of this protein in the carbonated beverages industry [4].



Picture 1: 3D-structure of HFB7. A: structure HFB7 at the end of MD simulations. B:surface representation of HFB7 coloured by hydrophobicity[1].

Przylucka et al. (2017) have proposed the role a novel HFB7 from T. virens in the protection against oxidative stress[1]. The antioxidant activity and ACE-inhibitory of class II HFB2 from *Trichoderma reesei* was reported Khalesi et al. (2016) who

demonstrated the reduction of free radicals of ABTS in the environment [5]. Currently, there are only several antioxidants which are used in industrial applications, such as BHT (Butylated Hydroxy Toluene), BHA (Butylated Hydroxy Anisol), sodium metabisulfite and ascorbic acid. Some of these antioxidants have a negative impact on human health. As hydrophobins have been shown to be immunologically inert [6-7], in this study, we test the antioxidant potential of a collection of class II HFB4 proteins from *Trichoderma* and discuss the possibility of their use as pharmaceutical excipients.

RESULTS AND DISCUSSION

Hydrophobins were originally detected because they enable fungi to grow at the interphase of solids or water and air, which was brought about by their assembly into amphiphilic structures on the outer fungal cell wall. In a previous study, an extended repertoire of class II HFBs was identified in *Trichoderma* species. Among them, HFB4 from 160 different species of *Trichoderma* were studied in detail. In some infrageneric groups of Trichoderma, these HFBs are under positive selection pressure, and some of their residues are positively selected during their evolution. A set of numerous HFB4 genes with different biochemical properties such as pI and hydrophobicity from different *Trichoderma* species were expressed in *P. pastoris* [8]. Subsequently, the antioxidant activity of different HFBs at certain concentrations was determined. The results of this study allowed to detect a few particular HFB4 proteins that significantly reduce the presence of ABTS+ radicals in the solution in comparison with other HFB4s from *Trichoderma* species. The structural analysis of these proteins will be presented and discussed. To test the interaction between HFB4 proteins and industrially, pharmaceutically, and biologically relevant enzymes were assessed using quartz crystal microbalance with dissipation monitoring (QCM-D). For this purpose, quartz crystal sensors, coated with a homogeneous film of either borosilicate or polyethylene terephthalate presenting a hydrophilic and a hydrophobic surfaces, respectively, were used.

CONCLUSION

Class II HFB4 can be potentially used as an inactive excipient in the pharmaceutical or food industry. Its unique amphiphilic properties are promising for the use as antioxidants and stabilizing agents. The interaction between industrially important enzymes and HFBs is the major focus of future investigations as well as their emulsifying properties.

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