Eurasian Journal of Molecular and Biochemical Sciences

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Hydrophobins: The Amphiphilic Proteins Produced in Filamentous Fungi

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Cite:*Turgut B A, Örtücü S. Hydrophobins: The Amphiphilic Proteins Produced in Filamentous Fungi. Eurasian Mol Biochem Sci 2023;2(1): 35-41.*

Received: 10 March 2023, Accepted: 12 April 2023

Abstract

Hydrophobins are proteins of small molecular mass produced by fungi. They are part of various tasks at different stages of the life cycle of fungi, especially made during the formation of aerial structures. Hydrophobins are very stable in their amphiphilic structure and thanks to the four disulfide bonds they contain. They can form a monolayer by self-assembly at the water-air, air-solid interfaces and are important candidates for many industrial applications. For example, in surface modifications, they can make hydrophilic surfaces hydrophobic and hydrophobic ones hydrophilic. In addition, due to their high hydrophobicity, they prevent microorganisms from adhering to the surface or enable the fungi species from which they are produced to adhere to hydrophobic surfaces. The review considers the hydrophobins and their biotechnological applications for future research.

Keywords: Hydrophobins, surfactant, amphiphilic proteins, surface modification, filamentous fungi

Introduction

Hydrophobins are small surface-active proteins of about 100-150 amino acids that are highly stable and found around aerial hyphae, spores and reproductive structures (Figure 1). These proteins mediate the emergence of hydrophobic structures such as aerial hyphae, spores, and conidiophores from hydrophilic environments by reducing surface tension (1, 2). In addition, it is also responsible for detecting and attaching fungal structures to hydrophobic surfaces, thus playing an essential role in pathogenicity (3). These functions arise from hydrophobins amphipathic properties and surface activities (2, 4).

Hydrophobins are natural surfactants and reduce the surface tension by aiding growth. Thus, it allows fungi to form aerial hyphae and sporocarps at the water-air interface. Likewise, spores coated with amphipathic hydrophobin protein have hydrophobic surfaces, can spread quickly in the air, and become water resistance (5, 6). Hydrophobin layers facilitate adhesion to hydrophobic surfaces (8). It is essential to interact with two organisms in a pathogen-host or symbiosis relationship (4, 9). In pathogenic fungi with hydrophobic surfaces, hydrophobins ensure adhesion

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between the fungus and the host such as plants and insects (10, 11). As another function, hydrophobins prevent water absorption but allow gas passage through air channels, increase the durability of cells, and play a role in their survival (5, 6, 7, 9).

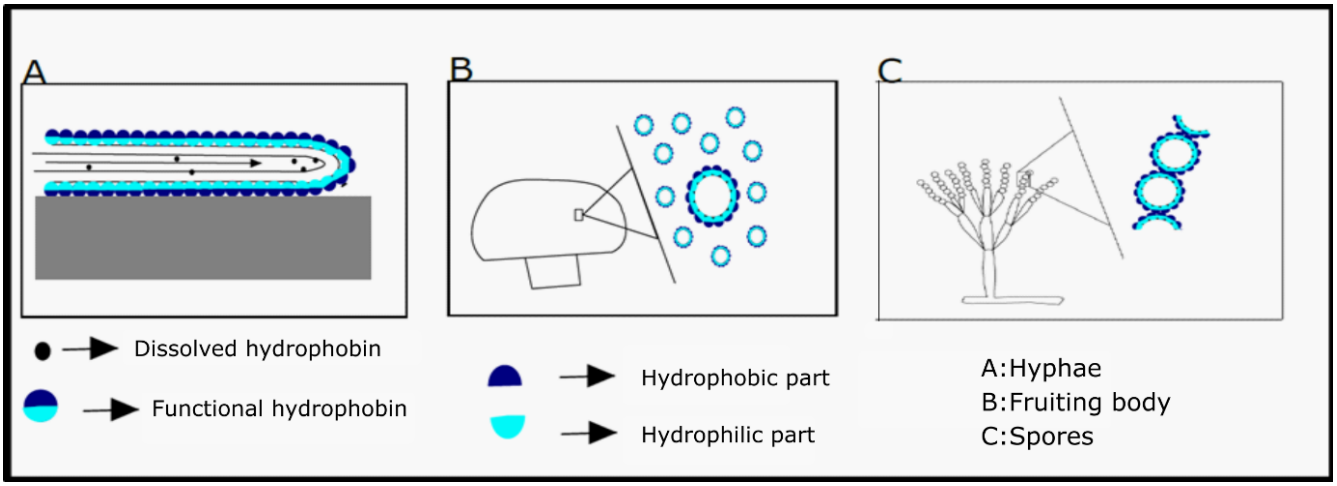

Figure 1. Fungi surfaces containing hydrophobins

Hydrophobins were first isolated from Schizophyllum commune, so the most extensive research on hydrophobins has focused on the Sc1, Sc2, S3, Sc4, Sc5, and Sc6 hydrophobins of this species (12, 13). Other research has focused on investigating the presence and specific roles of hydrophobins in the fungi Agaricus bisporus and Pleurotus ostreatus, which are classified as safe organisms (GRAS) (14). Another widely researched genus is Trichoderma. Hydrophobins of this genus generally have high gene copy numbers (15). In addition, as a result of the screening of Aspergillus species with bioinformatics approaches, it has been shown that there are approximately 74 possible hydrophobins in only eight species (16). Hydrophobins have been successfully isolated and characterized from A. fumigatus, A. oryzae and A. nidulans (14). Isolation and characterization studies in other genera such as Cladosporium, Fusarium and Neurospora have increased recently (13).

Structure of Hydrophobins: Hydrophobins are divided into class I and II according to their stability and differences in the arrangement of the eight highly conserved cysteine residues (6). While both classes of hydrophobins are found in species belonging to ascomycetes, only class I proteins are found in species belonging to basidiomycetes (2, 17). The hydrophobic properties of these two classes and the methods to be followed for purification are different from each other. Class I are more stable hydrophobins than class II, soluble in strong acids such as TFA and formic acid, even in boiling 2% sodium dodecyl sulfate (SDS), and usually found on spore surfaces (18). Class II hydrophobins can be easily dissolved with the help of ethanol and SDS (19). HFBI and HFBII, class II hydrophobins from Trichoderma reesei, have been successfully isolated and characterized for many industrial applications, particularly surface modification (20).

Homology studies of the primary sequence have shown that hydrophobins performing similar roles exhibit similarity among species. The primary sequence of both hydrophobin classes consists of only one polypeptide chain. The folded final conformation consists of α-helix and β-sheets but is rich in β-sheets. The relative amounts of α-helix and β-layers vary according to the hydrophobin class and the protein's location. For example, an increase in the β-layer structure of class I hydrophobins are observed at the water-air interface; there is an increase in the α-helix

structure at the interface between the water-hydrophobic solid. While the α-helix structure occurs within seconds due to the self-organization of proteins, the return to the β-sheet structure takes longer. This structure constitutes the most stable state of self-organization (4).

The primary structure of some Class I hydrophobins, such as EAS and SC3, is known to contain more amino acids than Class II proteins and therefore shows greater diversity (21). Both hydrophobin classes have β-layer structures because they contain the same disulfide bonds, while their amphiphilic structure comes from the aggregation of amino acids with similar properties, as hydrophobic and hydrophilic (22). It is also known that the hydrophobicity between these two classes differs (3). Class I hydrophobins form fibrils similar to proteins of amyloid structure on the conidial surface. These fibrils, called rodlet structures, consist of 4 to 6 precursor filaments and are highly resistant to proteases because they contain mostly β-layer structure (8, 23).

Biotechnological Applications of Hydrophobins: In addition to all these features mentioned above, hydrophobins have become the focus of biotechnological applications due to their non-toxicity. Because it is known that many synthetic molecules used for this purpose have toxicity on the cell. For this reason, researchers have turned to other organic molecules with amphipathic properties, such as biosurfactants. However, due to their thermodynamic properties, biosurfactants interact with cell membranes and disrupt the contents of the membrane. Hydrophobins provide an additional advantage as they do not interact with the plasma membranes of organisms (4, 24) because the surfactant property of hydrophobins is not due to the lipid structure but to certain amino acids in the sequence (25).

In biological processes, various organic surfaces can be covered by proteins in seconds. This is an important factor for maintaining the cell's vital activities. These properties of proteins are widely used in fields such as regenerative medicine and tissue engineering. Studies of coating proteins on surfaces have shown that the adhesion of proteins to a substrate or surface can be controlled by temperature, ionic strength, and buffer composition. In addition, adhesion and surface persistence are closely related to the protein's size, structural stability and composition. Small and rigid proteins are preferred for surface applications as they are not prone to conformational changes after surface adsorption. One such protein, hydrophobins, are very stable small proteins due to the disulfide bonds that stabilize the structure (3, 26, 27).

Hydrophobins have great potential in many practical applications in biotechnology due to the properties they impart to various surfaces. Applications include the bioavailability of drugs, surface coatings, dispersion of hydrophobic materials in aqueous solutions, prevention of foam formation in production processes, biosensors, purification steps of recombinant proteins and production of self-cleaning materials can be listed as the most recent applications of hydrophobins (14, 27). Also, the other applications are self-cleaning surfaces (21, 28) and biomaterial creation (19, 28). In medical biotechnology applications, thanks to the hydrophilic properties imparted to hydrophobic surfaces, cell adhesion to some medically applied surfaces are increased and biocompatible materials are formed (29, 30). On the other hand, the ability of hydrophobins to form highly stable emulsifiers is critical in preparing stable solutions in pharmaceutical applications (3, 19). In addition, hydrophobins have been proposed for various applications, providing unique sites for modifying surface properties (31) and protein immobilization (32). It has been reported that using hydrophobins in nanobiotechnology applications can increase the sensitivity (33), quality and lifetime (34) of biosensors.

Especially since the discovery of water-repellent hydrophobic leaf surfaces of the lotus plant with a contact angle of more than 140° in the 90s, scientists have focused on water and dirt-repellent super-hydrophobic surfaces and have begun to be used in industrial applications in many different sectors. As a result of the hydrophobicity of the surfaces with these high contact angles, the flow of the water droplet that cannot adhere to the surface at an angle of only 3° with the force of gravity causes the removal of dust particles on the surface, and this phenomenon is called self-cleaning (35, 36).

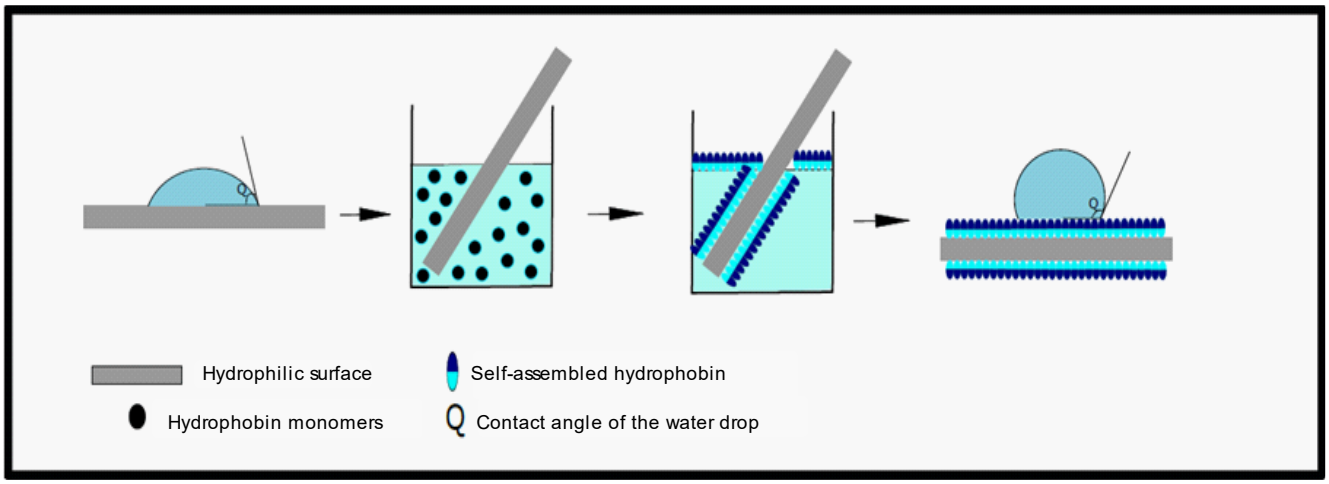

Figure 2. A schematic representation of the ability of hydrophobins to change surface hydrophobicity

Hydrophobins, which have essential roles in the life cycle of fungi, also play a crucial role in many industrial applications due to their ability to form films on hydrophobic and hydrophilic surfaces (Figure 2). Specifically, thanks to the ability of hydrophobins to change the properties of various surfaces, hydrophilic surfaces can be converted to hydrophobic surfaces to obtain super-hydrophobic surfaces, which prevent microorganisms from adhering (21, 28). These applications greatly expand the industrial use of hydrophobins. As is known, the behavior of liquids in solids varies depending on whether the surface is hydrophilic or hydrophobic. The water contact angle (WCA) is a value that can be easily measured when the water comes into contact with the solid and provides valuable information about the hydrophobicity of the surface. Surfaces with a contact angle of less than 10° are called superhydrophilic, and surfaces greater than 150° are called super-hydrophobic (37).

Hidrophobin Production: Researchers emphasize the necessity of further research for successfully producing and purifying these proteins due to the broad application areas of hydrophobins (38). The main strategies used to increase the production and yield of hydrophobins are to increase the expression of hydrophobins using natural producer strains and recombinant DNA technology (39). In particular, the fact that some wild strains do not secrete hydrophobins into the culture medium makes large-scale production difficult (40). Except for some recombinant strains, the hydrophobin production capacity of most fungi is below 150 mg/L. For this reason, researchers emphasized that genetically modified organisms should be used to obtain high production rates (14). It is clear that the production of hydrophobins using recombinant methods is more effective than the production from natural strains using conventional methods (14). However, production studies on genetically modified isolates have not been fully accomplished, especially due to problems in post-translational mechanisms (41). Other disadvantages of using genetically modified organisms; are

environmental hazards, human health risks, product acceptability and economic concerns (33).

For these reasons, many researchers suggest using natural strains to produce hydrophobin (42) and using various production processes to overcome low production yields (43). Although current production methods seem sufficient for small-scale applications such as medical applications, biosensors and drug formulations (40, 41); however, the production efficiency must be increased for larger industrial applications. Another strategy is the optimization of culture. For example, hydrophobin production from Ceratocystsis ulmi increased more than five times using different carbon and nitrogen sources (44). Another example is the production of RodA and RodB, which are hydrophobins extracted from A. fumigatus. These hydrophobins were expressed in Pichia pastoris in a fermentor containing a basal salt medium; then, the expressed RodA and RodB yielded 200-300 mg/L (45). The type of culture is also important; some researchers have emphasized that solid-state fermentation can be an effective alternative to submerged culture in producing hydrophobin (46).

Until recently, many applications of hydrophobins have been patented, but none of them has yet been applied to industry (33). This situation is associated with low production efficiency. Although the production of hydrophobin at the laboratory scale has been achieved, the required yield for industrial production has not been achieved (33). The common solution proposal of many researchers in this regard is the screening, isolation and characterization of hydrophobins in new fungal species. Researchers emphasized that only in this way an effective strategy can be put forward to increase production efficiency (14, 47, 48).

Conclusion

Hydrophobins are highly stable, amphiphilic proteins capable of self-assembly. They are divided into two classes according to their solubility and rodlet structure, but these two classes are similar in that they contain four disulfide bonds. Although not the main factor in determining class differences, both classes have different sequences and amino acid numbers between conserved cysteines. Class I hydrophobins have been studied especially for surface modifications, protein fixation and to produce two-dimensional nanostructures; class II hydrophobins have been studied both for protein purification in two-phase systems and for sending the co-produced protein to a specific region by producing fusion protein recombinantly, and positive results have been obtained. As a result of the studies, it has been said that hydrophobins are good candidates for many biotechnological applications. However, even patented applications could not be transferred to the industry since sufficient production could not be achieved to be used in industrial applications. Screening studies may find the most efficient hydrophobin producer isolates and new effective hydrophobins.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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