真菌疏水蛋白HGFI自组装分子机制和材料表面修饰功能的研究

王波, 徐海津, 乔明强*

南开大学生命科学学院, 分子微生物学与技术教育部重点实验室, 天津 300071

* 联系人, E-mail: qiaomq@nankai.edu.cn

收稿日期: 2019-07-29; 接受日期: 2019-08-12; 网络版发表日期: 2019-09-09

国家自然科学基金(批准号: 31170066)、国家科技部国际合作专项(批准号: 2006DFA32360)和天津市自然科学基金(批准号: 12JCZDJC22600)资助

摘要 真菌疏水蛋白是高等丝状真菌在特定生理时期分泌的一类小分子量、两亲性蛋白质, 其可以在两相界面处通过自我装配形成纳米级蛋白膜, 改变介质表面的亲水性和疏水性. 疏水蛋白独特的自组装性质使其在不同的领域均具有应用潜力, 如材料表面修饰、乳化、蛋白纯化、药物传送和生物传感器制作等. 本文主要介绍了真菌疏水蛋白的国内外研究进展, 并针对本课题组发现的灰树花真菌疏水蛋白, 介绍其自组装分子机制、在材料表面修饰以及药物缓/控释等方面的应用研究.

关键词 真菌疏水蛋白, 自组装, 两亲性, HGFI

真菌疏水蛋白(hydrophobin)是高等丝状真菌在特定时期分泌的^[1-3], 富含8个半胱氨酸的两性蛋白质, 约含有100个氨基酸, 分子量为7~10 kD, 是已知表面活性最强的蛋白质之一^[4,5]. 真菌疏水蛋白能在不同界面自组装, 形成一层约10 nm厚的两性蛋白膜^[6], 改变介质表面的亲疏水性, 是一种新型、天然、纳米级表面活性膜材料.

1991年, 荷兰Wessels研究组^[7]在对裂褶菌中高表达基因的研究中发现了第一个真菌疏水蛋白基因SC3, 随后的研究中发现SC3在裂褶菌菌丝表面形成了高度不溶的聚合物, 最后利用三氟乙酸裂解获得了SC3的单体, 并且提出了“真菌疏水蛋白”这一概念. 真菌疏水蛋白的研究工作自此广泛开展^[8].

早期真菌疏水蛋白的研究集中在蛋白质结构和生理功能两个方面^[9]. 由于真菌疏水蛋白具有优良的两亲性以及两相界面自组装成膜特性、极高的生物相容性和较低的免疫源性, 其应用领域也越来越广泛. 例如, 作为纯化标签提升蛋白纯化效率, 组织工程材料的功能化修饰, 生物传感的器件修饰、探针固定, 多肽类及化学药物的保护、分散和缓释等.

本课题组^[10]首次在食用性真菌灰树花菌丝中发现和分离了功能性疏水蛋白HGFI, 并通过蛋白分子关键部分置换和定点突变技术, 进行分子结构与性质的表征和分析, 阐明关键环对疏水蛋白结构、聚合状态、自组装和固定化方面的作用机理^[11,12]. 还获得了不同等电点的应用范围更广的人工疏水蛋白系列, 研

引用格式: 王波, 徐海津, 乔明强. 真菌疏水蛋白HGFI自组装分子机制和材料表面修饰功能的研究. 中国科学: 生命科学, 2019, 49: 1076–1085
Wang B, Xu H J, Qiao M Q. Self-assembly molecular mechanism and modification function on material surface of hydrophobin HGFI (in Chinese). Sci Sin Vitae, 2019, 49: 1076–1085, doi: [10.1360/SSV-2019-0161](https://doi.org/10.1360/SSV-2019-0161)

究其在不同pH值、离子浓度等条件下在固定化基质表面的吸附能力,最终揭示疏水蛋白自组装机制^[13,14].

本课题组^[15~18]进一步以疏水蛋白独特的自组装性质为基础,研究疏水蛋白在生物材料和多肽药物表面进行吸附和自组装的分子机理,并积极将其应用到生物医学领域,尤其是医学检测、组织工程和药物缓释等^[14,19]领域,为生物医学相关研究奠定理论和实验基础.

1 真菌疏水蛋白的分类与生理功能研究

1.1 真菌疏水蛋白的分类

1994年, Wessels教授^[3]运用Kyte和Doolittle开发的测定蛋白疏水指数的方法对已有的9种疏水蛋白序列进行了分析,以水溶性图谱疏水性和亲水性氨基酸残基的分布及蛋白聚集体的生化稳定性为依据,将疏水蛋白分为两类: I型和II型. 通常I型疏水蛋白形成的聚集体具有高度不溶解性,在高温下,它不能被2% SDS(sodium dodecyl sulfate)或60%的乙醇分解,仅在少数的强酸,如过氧甲酸和三氟乙酸处理后才能够解聚和溶解^[8].

1.2 真菌疏水蛋白的生理功能研究

真菌疏水蛋白在高等丝状真菌特定的生理时期分泌,自组装聚集在特定的部位,并发挥着特定的生理功能^[20,21]. 分布于气生菌丝表面的疏水蛋白SC3,能帮助气生菌丝打破气液界面张力,向空气中生长^[22~25]; 分布于气生孢子表面的疏水蛋白DewA,能协助孢子

的萌发和内容物的保护^[22,26~32]; 分布于稻瘟病菌表面的疏水蛋白MPG1能够提高菌表面的疏水性,有助其在稻叶表面吸附形成附着胞,增强其对宿主的侵染能力^[33~38].

此外,后续的一些研究^[39~42]相继证明了疏水蛋白在孢子表面形成杆状结构的蛋白膜,可以有效地降低动物免疫细胞的识别,从而间接地降低了真菌的免疫原性.

2 真菌疏水蛋白的自组装及分子结构研究

真菌疏水蛋白作为一种两亲性蛋白质,在固液/气液/固气等两相界面具有很强的自组装能力. 通过高分辨的透射电子显微镜(transmission electron microscope, TEM)和原子力显微镜(atomic force microscope, AFM)可以观察到疏水蛋白成膜的微观结构. 如图1所示为AFM观测到的I型和II型疏水蛋白膜结构^[4,43].

研究发现, I型疏水蛋白膜结构都具有相似的棒状结构,而II型疏水蛋白膜结构都具有相似的晶格结构,这可能也是二者膜稳定性不同的原因之一. 膜结构也成为了区分I型和II型疏水蛋白的证据之一.

对已发现的疏水蛋白进行氨基酸序列比对分析后发现,不同真菌来源的疏水蛋白之间的序列同源性很低,通常不同菌种的疏水蛋白序列差异很大^[9],但一般都具有8个保守的半胱氨酸,并且两两组合形成4个二硫键,其中Cys2和Cys3, Cys6和Cys7总是连在一起^[44],如图2所示为真菌疏水蛋白的一级氨基酸结构的通式及二硫键结合方式.

图1 I型(A)和II型(B)疏水蛋白膜结构^[4,43]

Figure 1 Hydrophobin layer structure showing class I (A) and class II (B) types of hydrophobins^[4,43]

图2 真菌疏水蛋白的一级氨基酸结构的通式及二硫键结合方式

Figure 2 General primary structure of a hydrophobin and the exact configuration of four disulfide bonds formed by eight cysteine residues

通过圆二色谱(circular dichroism, CD)、傅里叶红外光谱(Fourier transform infrared spectrometer, FTIR)以及频振动光谱学(vibrational sum frequency generation, VSFG)技术对疏水蛋白自组装过程中二级结构的变化分析发现, 真菌疏水蛋白的自组装是一个 β -sheet结构增多的过程^[45-56]。

目前研究蛋白质三维结构的方法主要有X射线衍射技术和核磁共振(nuclear magnetic resonance, NMR)技术。然而由于疏水蛋白的低水溶性和易聚集的特点, 结晶十分困难, 使得真菌疏水蛋白的晶体结构解析研究比较困难。自1991年第一个疏水蛋白SC3被分离后, 直到2004年芬兰的Linder教授才发表了第一个II型真菌疏水蛋白的晶体结构, 即瑞氏木霉疏水蛋白HFB II高分辨的晶体结构, 从而在分子水平上阐述了

疏水蛋白HFB II的自组装原理^[57-60]。随后2006年, 澳大利亚悉尼大学的Sunde课题组^[61]利用三重核磁共振和X射线衍射技术获得了来自粗糙脉孢菌的I型疏水蛋白EAS单体溶液中的三级结构。在2012年, 该课题组又在*Proc Natl Acad Sci USA*上报道了对EAS自组装分子机制的进一步研究成果^[43]。如图3所示疏水蛋白的三级结构和自组装模型。图3显示, 两种蛋白分子都是以 β -sheet组成的 β -barrel为核心形成蛋白表面的疏水区域, 以 α -helix形成表面的亲水区域。

3 真菌疏水蛋白的应用研究

目前已知的不同真菌疏水蛋白之间存有较大差异, 但由于真菌疏水蛋白优良的两亲性以及在两相界面自组装成膜特性、极高的生物相容性和较低的免疫源性, 使其应用领域越来越广泛。II型疏水蛋白溶解性较高, 其在乳化、发泡和分散方面具有优势; I型疏水蛋白虽然溶解度较低, 但膜稳定性较强, 所以其多应用于材料表面的修饰。目前较为常见的应用领域, 如作为纯化标签提升纯化效率的蛋白纯化, 组织工程材料的功能化修饰, 生物传感的器件修饰、探针固定, 多肽类及化学药物的保护、分散和缓释等^[63]。为了更加高效、大

图3 II型(A)和I型(B)真菌疏水蛋白的三级结构和自组装模型^[62,43]

Figure 3 Tertiary structure and self-assembly model of class II (A) and class I (B) hydrophobins^[62,43]

量地获取疏水蛋白, 其表达系统也越来越多样。

4 真菌疏水蛋白HGFI自组装分子机制及材料表面功能研究

4.1 食用真菌灰树花疏水蛋白的发现及其对材料的表面改性修饰机制

真菌疏水蛋白在生物医学领域具有极大的应用潜力。作为生物医学材料的应用需要具备优良的生物安全性。因此, 本课题组^[10]以食用真菌灰树花作为出发菌株, 发现了新型真菌疏水蛋白资源, 为后期的研究奠定基础。该研究利用cDNA文库技术结合真菌疏水蛋白编码基因特征, 首次鉴定了灰树花真菌疏水蛋白基因, 并将其命名为HGFI, 并且根据cDNA序列确定了其蛋白质序列: QQCTTGQLQCCESTSTANDPATSELLGLIGVVISDVDALVGLTCSPIVIGVSGSAC-TANPVCCDSSPIGGLVSIGCVPVNV。

通过三氟乙酸抽提以及反相高效液相色谱(reversed-phase high performance liquid chromatography, RP-HPLC)进行精纯, 研究人员从灰树花中成功地分离到了单一的HGFI蛋白, 并成功地建立了灰树花真菌疏水蛋白的提取方法和高密度发酵及纯化方法。

经过验证, 利用上述方法分离得到的疏水蛋白HGFI具有良好的自组装活性, 能够有效地降低液体表面张力, 在气液界面进行自组装并且形成具有杆状微观结构的纳米级蛋白膜, 保留了真菌疏水蛋白的基本性质和功能。HGFI是我国首次报道的真菌疏水蛋白, 是当时在国际上少见的具备较高生物安全性的食用真

菌疏水蛋白, 也是迄今为止等电点最低的天然疏水蛋白。

为了扩大疏水蛋白HGFI的发酵产量, 研究人员构建了疏水蛋白HGFI的毕赤酵母异源表达系统, 成功地将HGFI蛋白的表达量提升到了其在水溶液中的溶解饱和值300 mg/L, 同时开发了大体积发酵上清中HGFI蛋白的分离纯化方法, 大大提高了发酵上清中HGFI蛋白的回收率, 相较于传统方法回收率提高了5.5倍, 解决了疏水蛋白HGFI的生产问题, 为其后期广泛的应用研究提供了实验基础。

在国际上, 研究人员利用疏水蛋白HGFI完成了多壁碳纳米管在水溶液中的稳定分散^[6], 相较于报道的其他蛋白BSA(bovine serum albumin)(50 mg/mL), ferritin(70 mg/mL)和lysozyme(10 mg/mL), 疏水蛋白HGFI的有效工作浓度仅为0.1 mg/mL, 具有极强的分散能力。研究人员进一步研究了在碳纳米管表面固定抗体分子以用于制备医疗诊断领域的生物传感器和生物芯片, 如图4所示。

基于碳纳米管修饰研究结果, 本课题组^[64]利用HGFI修饰聚苯乙烯建立了癌胚抗原的时间分辨免疫荧光分析生物传感器。经过HGFI修饰的聚苯乙烯表面, 其时间分辨荧光光谱测定中的灵敏度很高, 且癌胚抗原的标准曲线在4℃保持90天, 以及在37℃保持30天都不会发生明显的改变, 如图5所示。

在疏水蛋白自身结构与自组装机制关系的研究方面, 本课题组^[13,14]通过对I型真菌疏水蛋白HGFI实施定点突变, 构建了6个pI值从3.25~9.08依次递增的系列突变体。上述蛋白经过分离纯化后, 进行了固体表面自

图 4 疏水蛋白HGFI分散的多壁碳纳米管在人源IgG的固定中的应用。A: HGFI分散的多壁碳纳米管; B: 石英晶体微天平测试中, HGFI/碳纳米管复合物及人源IgG的固定; C: 利用纳米金标记的人源IgG抗体检测吸附在HGFI/碳纳米管复合物上的人源IgG活性

Figure 4 Application of hydrophobin HGFI dispersed multiwall carbon nanotubes (MWCNTs) on human IgG immobilization. A: Dispersed MWCNTs obtained using HGFI. B: immobilization of the complex of HGFI/MWCNTs and human IgG in Quartz Crystal Microbalance test. C: activity detection of human IgG adsorbed on HGFI/MWCNTs complex using gold nanoparticles labeled anti-human IgG antibody

图 5 疏水蛋白HGFI修饰的聚苯乙烯表面在癌胚抗原(CEA)固定化中的应用. A: 癌胚抗原的单克隆抗体在HGFI修饰的聚苯乙烯表面的原子力显微镜图; B: 癌胚抗原试剂盒检测标准曲线. (a) 修饰后, (b) 未修饰, (c) 高结合力的聚苯乙烯微板

Figure 5 Application of hydrophobin HGFI modified polystyrene on carcinoembryonic antigen (CEA) immobilization. A: AFM image of uniform CEA antibody layer formed on coated HGFI. B: Standard curves of TR-IFMA assays using modified HGFI. (a) Modified, (b) unmodified, and (c) high-binding polystyrene microplates

组装能力、吸附力、聚合和溶解状态、成膜能力、细胞毒性、体内外细胞增殖、特异性细胞识别黏附能力等的表征和测定分析. 结果表明, 改造的6个pI值不同的疏水蛋白突变体其亲疏水性因点突变而发生了改变. 同时, 研究人员通过蛋白融合技术, 构建了C3-C4环互换的HGFI-AR和HFBI-AR蛋白突变体^[11,12]. 利用接触角测量、X射线光电子能谱、原子力显微镜、圆二色谱、ThT和Congo red荧光标记等技术对疏水蛋白C3-C4环置换突变体进行了表征, 比较了疏水蛋白突变体自组装活性、固体界面上自组装形成膜的稳定性差异, 证明了C3-C4环在I型真菌疏水蛋白杆状结构形成过程中的关键作用. 以上研究成果对疏水蛋白分子结构的认识提供了重要依据.

4.2 真菌疏水蛋白在组织工程领域的应用研究

组织工程的核心就是建立细胞与生物材料的三维空间复合体. 在组织工程血管构建的过程中发现, 真菌疏水蛋白能够在人造血管材料聚己内酯(polycaprolactone, PCL)表面进行良好的吸附形成两亲性的蛋白膜, 并且能够大大地提高生物材料PCL表面的生物相容性, 使其表面的高疏水性变为相对亲水的表面^[15]. 相较于传统的修饰方法, 该技术不会对PCL材料的机械性能造成影响, 而且操作中不会引入其他试剂成分,

具有操作简便和安全等特性.

在人造血管形成的过程中, 支架材料表面内皮层细胞的吸附和生长是重要的先决条件. 为了特异性地捕获体内的内皮化细胞(endothelial cell, ECs)进而加速PCL表面内皮化细胞层的形成, 本课题组^[15]利用疏水蛋白修饰的PCL进行CD31单克隆抗体的固定化, 依靠抗原抗体特异性反应促进ECs在PCL表面的聚集. 研究结果表明, 实验组PCL表面ECs的吸附数量是空白PCL表面的14.9倍, 而且ECs细胞保持了较强的增殖能力.

本课题组^[17,18]还尝试将血管内皮化生长因子VEGF(vascular endothelial growth factor)与疏水蛋白HGFI进行融合, 并且进行PCL材料的功能性修饰. 体内外实验均表明, 融合蛋白VEGF-HGFI能在PCL人造血管表面形成稳定的蛋白膜, 并显著提高人脐静脉内皮细胞HUVECs(human umbilical vein endothelial cells)在PCL表面的吸附、迁移和增殖. 相较于空白PCL材料, 在动物体内VEGF-HGFI修饰的PCL人造血管表面的细胞化程度、内皮组织的形成、平滑肌的再生、毛细血管的形成能力都得到了显著提升.

本课题组^[65]还将抗菌肽片球菌素PA-1与疏水蛋白HGFI进行融合表达. 利用融合蛋白PA-1-HGFI (PH), 成功地将细菌素PA-1固定在生物材料PCL表面, 使修饰后的PCL表面具有了抗菌能力. 这部分工作为医用

材料抗菌界面的设计提供了新的方法, 如图6所示。

4.3 真菌疏水蛋白在药物缓释领域的应用研究

由于真菌疏水蛋白具有良好的生物相容性、高温/酸碱/蛋白酶耐受性、自组装和低免疫原性等特性, 因此其在药物缓释应用方面具有潜在的科学研究价值。目前对真菌疏水蛋白在药物缓释的研究大多局限于小分子的化学类药物, 对真菌疏水蛋白作为多肽类药物载体的应用研究却鲜有报道。

胰高血糖素类似物GLP-1(glucagon-like peptide-1)在体内能够迅速地被二肽基肽酶4(dipeptidyl peptidase 4, DPP4)所降解而失去活性, 体内半衰期仅为2 min。基于灰树花真菌疏水蛋白HGFI在溶液中的自我聚集性质, 以其作为GLP-1的包被载体, 增强GLP-1在体内的

稳定性进而提高其降血糖效果^[19]。实验设计思路如图7所示。结果表明, 真菌疏水蛋白HGFI与GLP-1多肽能够在体外通过简易操作形成稳定的药物复合物, 较之GLP-1单一的注射其半衰期延长了近700倍。该项研究为药物缓释载体的设计提供了新的思路和理论基础, 并且拓展了真菌疏水蛋白的应用范畴。

在生物医学应用中, 真菌疏水蛋白在疏水药物的制备和传递方式方面具有重要的应用价值。疏水药物通常使用表面活性剂, 但在免疫功能低下的患者(如癌症患者)中被证明具有免疫原性^[66]。本课题组^[14]利用真菌疏水蛋白HGFI的突变体MGF6分散消炎药吲哚美辛(indomethacin, IND), 相较于野生型HGFI, MGF6的分散作用更明显, 纳米颗粒在100 nm左右, 并且在酸性和中性pH下(模拟胃肠环境)表现出了良好的缓释效果,

图6 PH修饰PCL的抑制金黄色葡萄球菌生物被膜活性检测。(A1, A2) 未修饰PCL; (B1, B2) PH修饰PCL; (C1, C2) PA-1修饰PCL; (D1, D2) HGFI修饰PCL^[65]

Figure 6 SEM images of the biofilm formed using *Staphylococcus aureus* ATCC 6538 on the surface of PCL films (small magnifications, A1–D1; large magnification, A2–D2); bare PCL film, (A1, A2); PCL modified by pH, (B1, B2); PCL modified by PA-1, (C1, C2); and PCL modified by HGFI, (D1, D2)^[65]

图7 真菌疏水蛋白作为GLP-1药物包被载体示意图^[19]

Figure 7 Scheme illustrating the transport of GLP-1 by hydrophobin and drug stability determination^[19]

如图8所示. 这为后期疏水蛋白制剂口服给药方式的研究提供了理论基础.

5 总结

真菌疏水蛋白具有两亲性、自组装性、表面结合与修饰活性, 且具有良好的酸碱耐受性, 其在药物传输系统的应用亦成为近几年研究的热点之一. 深入解析 I 型真菌疏水蛋白的分子结构, 以期阐明HGFI蛋白分子结构与自组装机制的关系及其与界面间相互作用机理, 明确疏水蛋白对药物分子包裹机制和颗粒纳米化机制, 增加对疏水蛋白与药物分子互作机理的认识, 研制更加稳定缓慢释放的策略及新型纳米颗粒药物制备的技术, 将为生物医药的基础研究和临床应用提供非常重要的参考价值.

图8 疏水蛋白包裹的IND在动态pH条件下的释放曲线^[14]
Figure 8 Release curve of IND coated with hydrophobins in dynamic pH conditions^[14]

致谢 感谢中国农业农村部畜禽产品质量监督检验检测中心于雷、天津大学王泽方、山西太原理工大学牛宝龙、河北师范大学赵立强、诺维信(中国)生物技术有限公司宋东民、西北工业大学王翔翔和天津市第一中心医院杨玖霞等博士对本研究做出重要贡献. 感谢南开大学白艳玲教授、张秀明高工、孔德领教授、陈强教授、王恺副教授、天津医科大学龚珉教授、广东科伦药业有限公司丁靖志高工和天津科技大学梁俊教授等对本研究的贡献.

参考文献

- Rosenberg M, Jellebergs K. Hydrophobic interactions: Role in bacterial adhesion. In: Marshall K C, ed. *Advances in Microbial Ecology*. Boston: Springer, 1986
- Wessels J G H, De Vries O M H, Asgeirsdottir S A, et al. The *thn* mutation of *Schizophyllum commune*, which suppresses formation of aerial hyphae, affects expression of the *Sc3* hydrophobin gene. *J General Microbiol*, 1991, 137: 2439–2445
- Wessels J G H. Developmental regulation of fungal cell wall formation. *Annu Rev Phytopathol*, 1994, 32: 413–437
- Wessels J G H. Fungal hydrophobins: Proteins that function at an interface. *Trends Plant Sci*, 1996, 1: 9–15
- Wösten H A B, de Vocht M L. Hydrophobins, the fungal coat unravelled. *BBA-Rev Biomembranes*, 2000, 1469: 79–86
- Dang X, Wang Y, Hu C, et al. Preparation and application of a novel electrochemical sensing material based on surface chemistry of polyhydroquinone. *Mater Sci Eng-C*, 2014, 40: 9–15
- Wessels J G H, de Vries O M H, Asgeirsdottir S A, et al. Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in *Schizophyllum*. *Plant Cell*, 1991, 3: 793
- Wessels J G H. Hydrophobins, unique fungal proteins. *Mycologist*, 2000, 14: 153–159
- Linder M B, Szilvay G R, Nakari-Setälä T, et al. Hydrophobins: The protein-amphiphiles of filamentous fungi. *FEMS Microbiol Rev*, 2005, 29: 877–896
- Yu L, Zhang B, Szilvay G R, et al. Protein HGFI from the edible mushroom *Grifola frondosa* is a novel 8 kD class I hydrophobin that forms rodlets in compressed monolayers. *Microbiology*, 2008, 154: 1677–1685
- Niu B, Gong Y, Gao X, et al. The functional role of Cys3-Cys4 loop in hydrophobin HGFI. *Amino Acids*, 2014, 46: 2615–2625
- Niu B, Li B, Wang H, et al. Investigation of the relationship between the rodlet formation and Cys3-Cys4 loop of the HGFI hydrophobin. *Colloid Surface B*, 2017, 150: 344–351

- 13 Wang X, Song D, Wang B, et al. A mutant of hydrophobin HGFI tuning the self-assembly behaviour and biosurfactant activity. *Appl Microbiol Biotechnol*, 2017, 101: 8419–8430
- 14 Yang J, Wang B, Ge L, et al. The enhancement of surface activity and nanoparticle stability through the alteration of charged amino acids of HGFI. *Colloid Surface B*, 2018, 175: 703–712
- 15 Zhang M, Wang Z, Wang Z, et al. Immobilization of anti-CD31 antibody on electrospun poly(ϵ -caprolactone) scaffolds through hydrophobins for specific adhesion of endothelial cells. *Colloid Surface B*, 2011, 85: 32–39
- 16 Huang Y, Zhang S, Niu B, et al. Poly(ϵ -caprolactone) modified with fusion protein containing self-assembled hydrophobin and functional peptide for selective capture of human blood outgrowth endothelial cells. *Colloid Surface B*, 2013, 101: 361–369
- 17 Zhao L, Ma S, Pan Y, et al. Functional modification of fibrous PCL scaffolds with fusion protein VEGF-HGFI enhanced cellularization and vascularization. *Adv Healthc Mater*, 2016, 5: 2376–2385
- 18 Wang K, Zhang Q, Zhao L, et al. Functional modification of electrospun poly(ϵ -caprolactone) vascular grafts with the fusion protein VEGF-HGFI enhanced vascular regeneration. *ACS Appl Mater Interfaces*, 2017, 9: 11415–11427
- 19 Zhao L, Xu H, Li Y, et al. Novel application of hydrophobin in medical science: A drug carrier for improving serum stability. *Sci Rep*, 2016, 6: 26461
- 20 Wösten H A B. Hydrophobins: Multipurpose proteins. *Annu Rev Microbiol*, 2001, 55: 625–646
- 21 Gierasch L, Lynn D, Schneider J. Two forms and two faces, multiple states and multiple uses: Properties and applications of the self-assembling fungal hydrophobins. *Biopolymers*, 2013, 100: 601–612
- 22 Wosten H A B, de Vries O M H, Wessels J G H. Interfacial self-assembly of a fungal hydrophobin into a hydrophobic rodlet layer. *Plant Cell*, 1993, 5: 1567–1574
- 23 Wösten H A, Asgeirsdóttir S A, Krook J H, et al. The fungal hydrophobin Sc3p self assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. *Eur J Cell Biol*, 1994, 63: 122–129
- 24 van der Vegt W, van der Mei H C, Wösten H A B, et al. A comparison of the surface activity of the fungal hydrophobin SC3p with those of other proteins. *Biophys Chem*, 1996, 57: 253–260
- 25 Wösten H A B, van Wetter M A, Lugones L G, et al. How a fungus escapes the water to grow into the air. *Curr Biol*, 1999, 9: 85–88
- 26 Stringer M A, Timberlake W E. *dewA* encodes a fungal hydrophobin component of the *Aspergillus* spore wall. *Mol Microbiol*, 1995, 16: 33–44
- 27 Muñoz G, Nakari-Setälä T, Agosin E, et al. Hydrophobin gene *srh1*, expressed during sporulation of the biocontrol agent *Trichoderma harzianum*. *Curr Genets*, 1997, 32: 225–230
- 28 Whiteford J R, Spanu P D. The hydrophobin HCF-1 of *Cladosporium fulvum* is required for efficient water-mediated dispersal of conidia. *Fungal Genets Biol*, 2001, 32: 159–168
- 29 Klimes A, Dobinson K F. A hydrophobin gene, *VDH1*, is involved in microsclerotial development and spore viability in the plant pathogen *Verticillium dahliae*. *Fungal Genets Biol*, 2006, 43: 283–294
- 30 Zhang S, Xia Y X, Kim B, et al. Two hydrophobins are involved in fungal spore coat rodlet layer assembly and each play distinct roles in surface interactions, development and pathogenesis in the entomopathogenic fungus, *Beauveria bassiana*. *Mol Microbiol*, 2011, 80: 811–826
- 31 Dubey M K, Jensen D F, Karlsson M. Hydrophobins are required for conidial hydrophobicity and plant root colonization in the fungal biocontrol agent *Clonostachys rosea*. *BMC Microbiol*, 2014, 14: 18
- 32 Grünbacher A, Throm T, Seidel C, et al. Six hydrophobins are involved in hydrophobin rodlet formation in *Aspergillus nidulans* and contribute to hydrophobicity of the spore surface. *PLoS ONE*, 2014, 9: e94546
- 33 Beckerman J L. *MPG1*, a gene encoding a fungal hydrophobin of *Magnaporthe grisea*, is involved in surface recognition. *Mol Plant Microbe In*, 1996, 9: 450–456
- 34 Talbot N J, Ebbole D J, Hamer J E. Identification and characterization of *MPG1*, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. *Plant Cell*, 1993, 5: 1575–1590
- 35 Lau G, Hamer J E. Regulatory genes controlling *MPG1* expression and pathogenicity in the rice blast fungus *Magnaporthe grisea*. *Plant Cell*, 1996, 8: 771–781
- 36 Talbot N J, Kershaw M J, Wakley G E, et al. *MPG1* encodes a fungal hydrophobin involved in surface interactions during infection-related development of *Magnaporthe grisea*. *Plant Cell*, 1996, 8: 985–999
- 37 Kershaw M J, Wakley G, Talbot N J. Complementation of the *MPG1* mutant phenotype in *Magnaporthe grisea* reveals functional relationships

- between fungal hydrophobins. *EMBO J*, 1998, 17: 3838–3849
- 38 Soanes D M, Kershaw M J, Cooley R N, et al. Regulation of the *MPG1* hydrophobin gene in the rice blast fungus *Magnaporthe grisea*. *Mol Plant Microbe In*, 2002, 15: 1253–1267
- 39 Heddergott C, Bruns S, Nietzsche S, et al. The *Arthroderma benhamiae* hydrophobin *HypA* mediates hydrophobicity and influences recognition by human immune effector cells. *Eukaryot Cell*, 2012, 11: 673–682
- 40 Carrion S J, Leal Jr S M, Ghannoum M A, et al. The RodA hydrophobin on *Aspergillus fumigatus* spores masks Dectin-1- and Dectin-2-dependent responses and enhances fungal survival *in vivo*. *J Immunol*, 2013, 191: 2581–2588
- 41 Dagenais T R T, Giles S S, Aimaniananda V, et al. *Aspergillus fumigatus* LaeA-mediated phagocytosis is associated with a decreased hydrophobin layer. *Infect Immun*, 2010, 78: 823–829
- 42 Rambach G, Blum G, Latgé J P, et al. Identification of *Aspergillus fumigatus* surface components that mediate interaction of conidia and hyphae with human platelets. *J Infect Dis*, 2015, 212: 1140–1149
- 43 Macindoe I, Kwan A H, Ren Q, et al. Self-assembly of functional, amphipathic amyloid monolayers by the fungal hydrophobin EAS. *Proc Natl Acad Sci USA*, 2012, 109: E804–E811
- 44 Khalesi M, Gebruers K, Derdelinckx G. Recent advances in fungal hydrophobin towards using in industry. *Protein J*, 2015, 34: 243–255
- 45 de Vocht M L, Reviakine I, Wösten H A B, et al. Structural and functional role of the disulfide bridges in the hydrophobin SC3. *J Biol Chem*, 2000, 275: 28428–28432
- 46 de Vocht M L, Scholtmeijer K, van der Vegte E W, et al. Structural characterization of the hydrophobin SC3, as a monomer and after self-assembly at hydrophobic/hydrophilic interfaces. *Biophys J*, 1998, 74: 2059–2068
- 47 Butko P, Buford J P, Goodwin J S, et al. Spectroscopic evidence for amyloid-like interfacial self-assembly of hydrophobin SC3. *Biochem Biophys Res Commun*, 2001, 280: 212–215
- 48 de Vocht M L, Reviakine I, Ulrich W P, et al. Self-assembly of the hydrophobin SC3 proceeds via two structural intermediates. *Protein Sci*, 2002, 11: 1199–1205
- 49 Wang X, Graveland-Bikker J F, de Kruif C G, et al. Oligomerization of hydrophobin SC3 in solution: From soluble state to self-assembly. *Protein Sci*, 2004, 13: 810–821
- 50 Wang X, Permentier H P, Rink R, et al. Probing the self-assembly and the accompanying structural changes of hydrophobin SC3 on a hydrophobic surface by mass spectrometry. *Biophys J*, 2004, 87: 1919–1928
- 51 Wang X, de Vocht M L, de Jonge J, et al. Structural changes and molecular interactions of hydrophobin SC3 in solution and on a hydrophobic surface. *Protein Sci*, 2002, 11: 1172–1181
- 52 Lugones L G, Wös H A B, Wessels J G H. A hydrophobin (ABH3) specifically secreted by vegetatively growing hyphae of *Agaricus bisporus* (common white button mushroom). *Microbiology*, 1998, 144: 2345–2353
- 53 Mackay J P, Matthews J M, Winefield R D, et al. The hydrophobin EAS is largely unstructured in solution and functions by forming amyloid-like structures. *Structure*, 2001, 9: 83–91
- 54 Banerjee G, Robertson D L, Leonard T J. Hydrophobins *Sc3* and *Sc4* gene expression in mounds, fruiting bodies and vegetative hyphae of *Schizophyllum commune*. *Fungal Genets Biol*, 2008, 45: 171–179
- 55 Askolin S, Linder M, Scholtmeijer K, et al. Interaction and comparison of a class I hydrophobin from *Schizophyllum commune* and class II hydrophobins from *Trichoderma reesei*. *Biomacromolecules*, 2006, 7: 1295–1301
- 56 Askolin S, Nakari-Setälä T, Tenkanen M. Overproduction, purification, and characterization of the *Trichoderma reesei* hydrophobin HFB I. *Appl Microbiol Biotech*, 2001, 57: 124–130
- 57 Hakanpää J, Parkkinen T, Hakulinen N, et al. Crystallization and preliminary X-ray characterization of *Trichoderma reesei* hydrophobin HFBII. *Acta Crystallogr D*, 2004, 60: 163–165
- 58 Hakanpää J, Linder M, Popov A, et al. Hydrophobin HFBII in detail: Ultrahigh-resolution structure at 0.75 Å. *Acta Crystallogr D*, 2006, 62: 356–367
- 59 Kallio J M, Linder M B, Rouvinen J. Crystal structures of hydrophobin HFBII in the presence of detergent implicate the formation of fibrils and monolayer films. *J Biol Chem*, 2007, 282: 28733–28739
- 60 Nakari-Setälä T, Aro N, Ilmén M, et al. Differential expression of the vegetative and spore-bound hydrophobins of *Trichoderma reesei* cloning and characterization of the *Hfb2* gene. *Eur J Biochem*, 1997, 248: 415–423

- 61 Kwan A H Y, Winefield R D, Sunde M, et al. Structural basis for rodlet assembly in fungal hydrophobins. *Proc Natl Acad Sci USA*, 2006, 103: 3621–3626
- 62 Hakanpää J, Paananen A, Askolin S, et al. Atomic resolution structure of the HFBII hydrophobin, a self-assembling amphiphile. *J Biol Chem*, 2004, 279: 534–539
- 63 Berger B W, Sallada N D. Hydrophobins: Multifunctional biosurfactants for interface engineering. *J Biol Eng*, 2019, 13: 10
- 64 Wang Z, Huang Y, Li S, et al. Hydrophilic modification of polystyrene with hydrophobin for time-resolved immunofluorometric assay. *Biosens Bioelectron*, 2010, 26: 1074–1079
- 65 Wang X, Mao J, Chen Y, et al. Design of antibacterial biointerfaces by surface modification of poly(ϵ -caprolactone) with fusion protein containing hydrophobin and PA-1. *Colloid Surface B*, 2017, 151: 255–263
- 66 Weiszhar Z, Czucz J, Révész C, et al. Complement activation by polyethoxylated pharmaceutical surfactants: Cremophor-EL, Tween-80 and Tween-20. *Eur J Pharmaceut Sci*, 2012, 45: 492–498

Self-assembly molecular mechanism and modification function on material surface of hydrophobin HGFI

WANG Bo, XU HaiJin & QIAO MingQiang

The Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China

Hydrophobins are typically small and amphiphilic protein molecules produced by filamentous fungi in specific physiology conditions. These proteins can be self-assembled at the nanoscale forming hydrophobic and hydrophilic layer interfaces, which changes the properties of the material surface. This special ability of hydrophobins makes them widely applicable in various areas, such as surface modification, formation emulsions, protein purification, drug delivery and usage in biosensors. Herein, an overview of the research progress on hydrophobin is discussed. In particular, insights into the self-assembly molecular mechanism of hydrophobin HGFI, as specifically isolated from *Grifola frondosa*, and its applications in surface modification and drug sustain control release are elucidated.

hydrophobin, self-assembly, amphiphilic, HGFI

doi: [10.1360/SSV-2019-0161](https://doi.org/10.1360/SSV-2019-0161)