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Review

Yeast cell surface display for lipase whole cell catalyst and its applications

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ABSTRACT

The cell surface display technique allows for the expression of target proteins or peptides on the microbial cell surface by fusing an appropriate protein as an anchoring motif. Yeast display systems, such as *Pichia pastoris*, *Yarowia lipolytic*a and *Saccharomyces cerevisiae*, are ideal, alternative and extensive display systems with the advantage of simple genetic manipulation and post-translational modification of expressed heterologous proteins. Engineered yeasts show high performance characteristics and variant utilizations. Herein, we comprehensively summarize the variant factors affecting lipase whole cell catalyst activity and display efficiency, including the structure and size of target proteins, screening anchor proteins, type and chain length of linkers, and the appropriate matching rules among the above-mentioned display units. Furthermore, we also address novel approaches to enhance stability and activity of recombinant lipases, such as VHb gene co-expression, multi-enzyme co-display technique, and the micro-environmental interference and self-assembly techniques. Finally, we represent the variety of applications of whole cell surface displayed lipases on yeast cells in non-aqueous phases, including synthesis of esters, PUFA enrichment, resolution of chiral drugs, organic synthesis and biofuels. We demonstrate that the lipase surface display technique is a powerful tool for functionalizing yeasts to serve as whole cell catalysts, and increasing interest is providing an impetus for broad application of this technique.

Contents

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1. Introduction

(Triacylglycerol hydrolase, EC 3.1.1.3) are a class of versatile hydrolases with specific three-dimensional conformations. Many researches have demonstrated that lipases can catalyze the hydrolysis of triglycerides yielding glycerol and free fatty acids [1–3]. Lipases also can catalyze the transesterification and synthesis of esters as well as exhibit high chemo-, regio- and/or enantioselective properties [4]. Lipases displayed on the yeast cell as whole cell catalysts omit the tedious and time-consuming traditional purification and immobilization steps of lipases. Therefore, lipase whole cell catalysts have been widely used for a variety of applications such as biofuels, food, pharmaceuticals, and chemical synthesis [5].

The cell surface display technique allows for the expression of a target peptide or protein on the cell surface through linkage with a genetically fused anchor protein. Thereby, it combines gene expression, protein purification and enzyme immobilization. Fig. 1 shows the structural units of the surface display system, including the target protein, anchor protein and host cell, as well as N-terminal (target protein–anchor protein fusion) and C-terminal fusion (anchor protein–target protein fusion) methods. Occasionally, the histidine acid (HA) tag was added to the C terminal of the target protein for purification. This technique has been extensively used to develop whole cell catalysts, vaccines and antibodies, library screens, bioconversions, synthesis of valuable chemicals and bio-sorbents, etc. [6–8]. Yeast display systems including *Pichia pastoris*, *Yarowia lipolytic*, and *Saccharomyces cerevisiae*, are ideal, alternative and wide utilization display systems with many advantages including simple genetic manipulation and post-translational modification of the expressed target protein [9,10]. Engineered yeast expressing lipases show improved enzyme characteristics and considerable potential applications in organic synthesis [11,12], drug resolution [13] and bioenergy [14,15]. Table 1 summarizes numerous useful lipases successfully displayed on yeast cell surfaces for whole cell catalysis. It was demonstrated that the shifts of the displayed lipase conformation and activity depend on the features of the target protein, anchor protein, promoter and linker, which affect the exogenous protein display efficiency and post-translation modification and secretion [43].

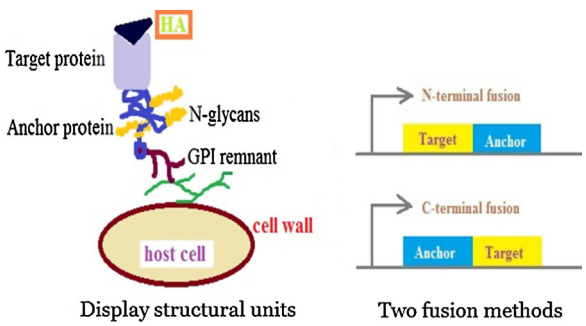

Fig. 1. The displaying structural units of the surface display system, including the target protein, anchor protein and the host cell, as well as two ways for the fusion between target and anchor proteins. A histidine acid (HA) tag can also be added with the gene C terminal of the target protein for protein purification.

From Table 1 it can be seen that more and more lipases have been successfully displayed on the yeast cell as whole cell catalysts with good stability and high activity [11,14]. Rapid advances in the life sciences, such as recombinant DNA techniques, have greatly increased the availability of whole cell catalysts. However, the matching of anchor protein, target enzyme, promoters and linkers is an important consideration in cell surface display development. In this review, we describe the recent progress on yeast cell surface display lipases as whole cell catalysts, focusing on the crucial factors affecting whole cell catalyst activity and stability. Then, a basic principle of lipase surface display on yeast cells is described. We also summarize some useful approaches for manipulating the stability and activity of recombinant lipases, including VHb gene co-expression, multi-lipase co-display methods, microenvironment regulation and self-assembly techniques. In the end, we elucidate the versatile applications of whole cell catalysts of lipase surface-displayed on yeast cells, such as the synthesis of esters, polyunsaturated fatty acid (PUFA) enrichment, resolution of chiral drugs, organic synthesis and biofuels production.

2. Crucial factors of lipase whole cell catalyst displayed on yeast cells

2.1. Target protein conformation and molecular size

Lipase activity is closely related to its conformation. In the lipase structure a lid of an α-helix, catalytic triad shelters the lipase active site, which commonly consists of three amino acids: Ser, His and Asp. The structure of lipase from *Y. lipolytica* (YlLip2) is given as an example in Fig. 2.

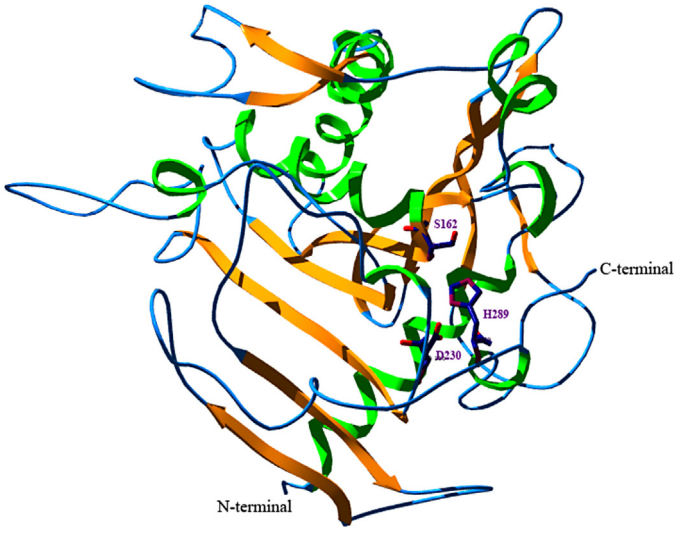

Fig. 2. The conformation and structure of *Y. lipolytica* lipase 2 (YlLip2). It represents the typical α/β fold in lipases with nine α-helices and eleven β-strands and the central β-sheet; it is made up of nine β-strands and five α-helices being packed on both sides of the sheet. The YlLip2 catalytic triad is composed of S162, H289 and D230 and an oxyanion hole and forms a stereo structure, and its C-terminal domain is closer to the catalytic triad than the N-terminal domain.