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Module recombination and functional integration of oligosaccharide-producing multifunctional amylase

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ABSTRACT

The oligosaccharide-producing multifunctional amylase (OPMA-N) has both hydrolytic and transglycosyl activities. Our previous reports demonstrated that the function and catalytic versatility of OPMA-N is closely related to its oligomerization, and its oligomeric state is affected by several conserved residues, such as Trp358, and by the cooperation of its small, noncatalytic N-terminal module and the catalytic module. We have demonstrated that the residue Trp358 exposed on the surface of OPMA-N molecule and has an obvious impact on OPMA-N oligomerization mainly by the charge effects. In this study, we investigated the effects of module recombination on the functional integration of OPMA-N. A series of module recombinants of the N-terminal and catalytic modules revealed that the intramolecular, semi-intramolecular and semi-intermolecular interactions of the N-terminal module with two or more catalytic modules enhanced the substrate affinity of the catalytic modules and facilitated the transglycosyl activity and functional integration of the enzyme by mediating positive cooperativity between the catalytic modules. Free N-terminal module alone did not contribute to these effects. Based on the results of this study, we speculated that the substrate affinity, but not the maximal catalytic activity, was the primary driving force in the natural evolution of enzymes, and that molecules of natural or modular enzymes may have occupied some evolutionary spaces that could be expanded or exhumed through module recombination to increase their overall catalytic efficacies or eventual catalytic efficiencies. All of the results in this study could be applied to integration of enzyme function and for creation of novel enzymes. Additionally, our results may provide important insights into the evolution of enzymes or organisms.

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1. Introduction

The overlapping of genes is a prevalent phenomenon in lower organisms such as viruses. Overlapping genes provide more information or meaning using fewer nucleotides or words. However, the effects of overlapping in functional protein molecules are unclear. In nature, many functional proteins, such as immunoglobulins, monomeric enzymes (e.g., multifunctional amylases [MFAs] [1] or laccases [2], and oligomeric enzymes (e.g., phosphorylase [3], influenza virus neuraminidase [4] or influenza virus RNA polymerase [5])), generally have a modular structure, and a module spatially composed of one or more domains is usually a functional unit for its host protein, contributing directly to the function of the protein. Because of this, the concept of the single-chain variable fragment (scFv) antibody has been proposed by recombining two variable modules of the heavy (VH) and light chains (VL) of an immunoglobulin molecule.

The activity of oligomeric enzymes is generally regulated by the association/dissociation of its subunits. In contrast, the activity of some monomeric enzymes may be regulated by oligomerization. However, most enzymes have certain modular characteristics at different levels, including protein sequence, conformation, function, and interaction networks. Combination or separation of enzyme modules is an important mechanism for the natural evolu-

Abbreviations: OPMA-N, of oligosaccharide-producing multifunctional amylase, N isoform; ΔOPMA-N, OPMA-N lacking the N-terminal domain; MFAs, multifunctional amylases; scFv, single-chain variable fragment; VH, heavy light; VL, light chains; OPMA-MR, recombinants of OPMA-N modules; OPMA-MM, equimolecular mixtures of different OPMA-MR; G2, maltose; G3, maltotriose; IG3, isomaltotriose; IG4, isomaltotetraose; DNS, 3,5-dinitrosalicylic acid; K_m, Michaelis constant; k_{cat}, kinetic constants; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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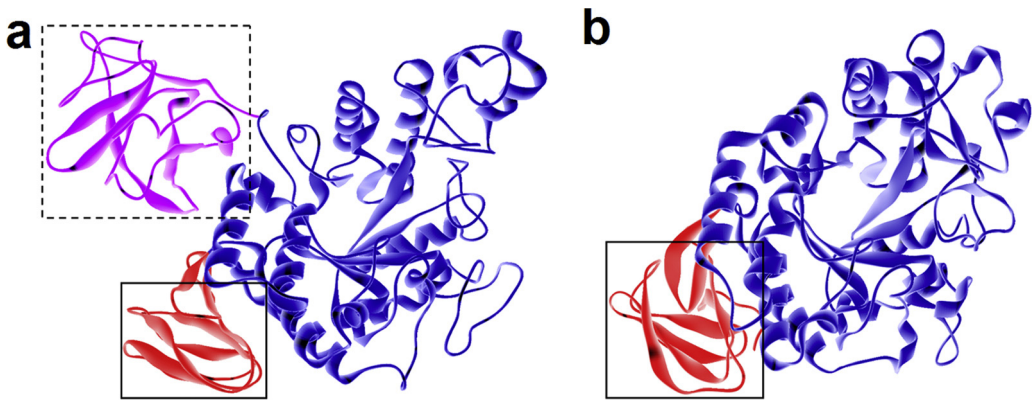

Fig. 1. Ribbon diagrams of three dimensional structures of OPMA-N monomer and TAA. Helices are shown as spiral ribbons and beta-strands are drawn as arrows from the amino end to the carboxy end of the beta-strand. The catalytic modules (core modules) composed of the TIM-barrel (corresponding to the A-domain) and B domain are shown in blue. The N-terminal and C-terminal modules are indicated by the larger and smaller black boxes, respectively.

tion of enzymes. Moreover, this also provides a more effective way for exploring protein sequence spacing than point mutations [6–9]. However, it is unclear whether functional integration is achieved by the incorporation or sharing of a particular module in an enzyme molecule. If this occurs, it may be possible to utilize the limited modules of enzymes to catalyze more reactions, resulting in expansion of the protein sequence space, thereby having implications in the field of synthetic biology.

We have reported the catalytic versatility of the oligosaccharide-producing multifunctional amylase (OPMA-N) secreted by *Bacillus* sp. ZW2531-1 [10,11]. OPMA-N is a novel type of amylolytic enzyme with the carbohydrate-binding module family 34 (CBM34) within the Carbohydrate-Active enzymes (CAZy) database according to its unique substrate and product specificities [12]. OPMA-N exclusively digests starch, but not other glucan or polysaccharides such as β-cyclomaltodextrin and pullulan, to produce maltose (G2), maltotriose (G3), isomaltotriose (IG3) and isomaltotetraose (IG4), but not glucose (G1). Therefore, OPMA is not only distinguished from other amylolytic enzyme such as alpha-amylase(EC 3.2.1.1), but alos from other multifunctional amylases such as maltogenic amylase (MAase), neopullulanase (NPase) and cyclodextrin-hydrolyzing enzymes (CDase) by the substrate and product specificities. So, OPMA-N is also considered a novel member of the neopullulanase subfamily of the alpha-amylase family GH13 based on its same catalytic triad (Asp. . .Glu. . .Asp) and four highly conserved regions (I–IV) as the alpha-amylase family, and its fifth conserved region as the neopullulanase subfamily [13] though OPMA-N has a high degree of sequence homology with some other members of the neopullulanase subfamily [14,15].

We have also examined the effect of the conserved residue Trp358 of OPMA-N through site-directed mutageneses and have revealed that the size and charge at this site directly affect the orientation of the crucial catalytic residues, the catalytic specificity, the oligomerization and the thermal stability of the enzyme [1]. Many other crucial studies have demonstrated based on the detailed bioinformation obtained from the present in silico and experimental structure/function analyses that some conserved residues such as tryptophan residues in TIM-barrel or at the substrate-binding site [16,17], or those at the interface between the N-terminal and the catalytic modules [18] play a significant role in modulate the function of GH13 family or determine the specificity of GH13 subfamily such as GH13_20 [19] or the intermediary group such as GH13_36 [20].

However, almost multifunctional amylases with CBM34 such as OPMA-N have a typical modular structure consisting of two small modules (N- and C-terminal) and a core catalytic module (Fig. 1a) which is corresponding to the total of domain A and domain B of a tipycal alpha-amylase [19]. Relative to the typical alpha-amylase (e.g., TAA from *Aspergillus oryzae*, 478 amino acids) (Fig. 1b), OPMA-N has an extra N-terminal module (amino acid residues 1–124) which is the so-called starch-binding domain of the family CBM34 as the N-terminal module in cyclodextrin-hydrolyzing enzymes (CDase), Thermoactinomyces vulgaris R-47 alpha-amylase and other multifunctional amylases [12,21,22]. The N-terminal module in these multifunctional amylases is relatively separated in three-dimensional space from all other modules (the core catalytic module along with the small C-terminal module) [18,20,23,24] which are considered the "generalized" catalytic module. Therefore, for these multifunctional amylases, the catalytic module is equivalent to the entire alpha-amylase molecule which includes the core catalytic module and the small C-terminal module (amino acid residues 125–588).

We have proposed that the hydrolytic activity of OPMA-N may be ascribed to its monomeric form, whereas the transglycosyl activity of OPMA-N may be dependent on its oligomeric form (particularly the dimeric form) [1]. Our previous study has also demonstrated that the extra N-terminal module modulates the catalytic activity of OPMA-N or the functional (active) balance between the hydrolytic and transglycosyl activities, despite the fact that the oligomerization and catalytic versatility of OPMA-N does not depend on this module [14]. Moreover, the intact transglycosylation center of OPMA-N is located at the dimeric interspace, and the individual monomer has its own hydrolysis center [1,25]. These results indicate that there may be a functional cooperativity among the N-terminal module, the hydrolysis-catalyzing module, and the transglycosylation-catalyzing half-module in the OPMA-N molecule.

To support this cooperativity hypothesis, in this study, we designed several module recombinants based on the N-terminal and the catalytic modules of OPMA-N. We aimed to develop a feasible approach for the functional integration of enzyme molecules by examining the effects of the recombination or rearrangement of the N-terminal module (amino acid residues 1–124) and the catalytic module (amino acid residues 125–588) (Fig. 2) on the catalytic features or functional integration of OPMA-N.

2. Materials and methods

2.1. Materials

pET28a-OPMA-N (GenBank: EU368579) and pET28a-ΔOPMA-N was constructed as described previously [14]. Module recombinants were obtained by PCR using the designed primer pairs

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