

COMMENTARY

Transglycosylation, a new role for multifunctional cellulase in overcoming product inhibition during the cellulose hydrolysis

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ABSTRACT

Cellulase mainly consisting of exo-glucanase, endo-glucanase and β -glucosidase, was considered as the most important biocatalyst for bioconversion of ethanol and other biofuels, feedstuffs and pharmaceuticals. Hydrolysis product inhibition, especially of glucose inhibition, is one of the critical difficulty awaiting to be overcome during cellulose bioconversion. Recently, several studies showed that some multifunctional cellulases (e.g., Umcel9y-1, Td2F2 and CoGH1A) could eliminate or relieve the product inhibition through transglycosylation actions during the cellulose hydrolysis. Transglycosylation confers multifunctional cellulases insensitive character to the end products (glucose and/or cellobiose), and provides a potential access in overcoming the inhibition of biofuels conversion. Moreover, transglycosylation harboring cellulases are also attracted as substitute of glycosyltransferase in synthesis of functional foods, nutraceuticals, or pharmaceuticals. Here, several interested transglycosylation harboring cellulases were summarized and assessed for the potential values in bioengineering application.

ARTICLE HISTORY

Received 20 June 2016
Revised 8 July 2016
Accepted 13 July 2016

KEYWORDS



β -glucanase; bioconversion;
multifunctional cellulase;
product inhibition;
transglycosylation

Introduction

Energy crisis and environmental pollution are the 2 noticeable topics in world, and have raised the importance of renewable energy development and bioresource utilization. As we known, cellulose is the most abundant and renewable polysaccharide, and cellulose constitutes almost one-third of all existing plant cell-wall material which is the largest potential bioresource for bioconversion of biofuels, feedstock chemicals, and pharmaceuticals.^{1–3} In China, millions of tonnes of straws are produced each year. Straw returning seems a good choice, but takes too long and is relatively limited in economic benefit. Other than returning field, burning will lead to serious air pollution. Degradation of cellulose and hemicellulose to short chains of oligosaccharides by cellulase is the key step for biomass conversation and has become a research hotspot in the areas of biocatalyst.

Cellulase as the basal bioenzyme for cellulose degradation mainly consisting of exo-glucanase (or cellobiohydrolase, $_{EC}3.2.1.91$), endo-glucanases ($_{EC}3.2.1.4$) and

β -glucosidases ($_{EC}3.2.1.21$). Generally, endo-glucanases randomly hydrolyze internal β -1,4-glycosidic bond to decrease the length of cellulose chains, exo-glucanases split off cellobiose from the shortened oligosaccharides, and β -glucosidases degrade cellobiose to glucose.⁴ However, the product inhibition especially glucose inhibition (the end hydrolysis product) is the most important restriction factor (or bottleneck problem) when the cellulase selected as the key biocatalyst for industrial application. Lately, several studies focused on multifunctional cellulase which exhibited transglycosylation activity, and considered the cellulase as the candidate of hydrolysis product insensitive biocatalyst. The investigations of hydrolysis product insensitive cellulase might provide an effective choice in overcoming the product inhibition.^{5,6} For years, glycosyltransferase was the primary considered biocatalyst for glycosidic synthesis (the core chemical group of functional materials, nutraceuticals, or pharmaceuticals), but the industrial utilization was restricted by the expensive nucleotide sugar precursors, narrow

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substrate specificity and low enzyme availability.^{5,6} By contrast, multifunctional cellulase is easily available and have a broad acceptor-substrate specificity with simple substrates.^{6,7} Therefore, transglycosylation harboring cellulase is also attracted as a potential substitute of glycosyltransferase for the synthesis of stereo- and regio-specific glycosides (or oligosaccharides).

The significance of transglycosylation harboring cellulase

As a valued multifunctional cellulase (including exo-glucanase, endo-glucanase), CelA showed efficient degradation ability on natural celluloses (i.e., Avicel, corn stover, switchgrass), and displayed considerable bioconversion ratios at high temperature (~50% bioconversion was observed on alkaline peroxide pretreated corn stover at 75°C). The outstanding merit of the CelA suggests that the cellulase has a good potential for native cellulose bioconversion.⁸ Similar as many other cellulases, the hydrolysis efficiency of CelA was inhibited by means of the end product cellobiose. For example, the performance of CelA acting on Avicel was markedly improved (producing 50% increased) within 7 d by the addition of β -D-glucosidase to the conversion system. The result suggested that β -glucosidase relieved the product inhibition (cellobiose) of CelA and improved natural cellulose

bioconversion.⁸ As indicated by CelA, if the downstream products were transferred and/or converted in time, the products inhibition could be relieved and the bioconversion efficiency was improved.

Other than the method of downstream product transfer, search for multifunctional cellulase that insensitive to downstream products of cellobiose and/or glucose has been considered as the newly reinvigorated program.^{5,6,9-10,15} Several studies indicated that the downstream hydrolysis products (i.e., cellobiose, glucose and oligosaccharides) could be further consumed, and other disaccharides and/or oligosaccharides may synthesized by transglycosylation reaction of multifunctional cellulase. More specifically, during cellulose degradation process, the hydrolysis products of cellobiose and/or glucose are quickly consumed as substrates by transglycosylation to produce gentiobiose, sophorose, laminaribiose, and other disaccharides and oligosaccharides. As the result, the products cellobiose and/or glucose are transferred, and the transglycosylation products of other disaccharides (except for cellobiose) and oligosaccharides are not the intensive inhibitors of cellulase, and the product inhibition was relieved (Fig. 1). And therefore, transglycosylation activity not only capacitates the multifunctional cellulase insensitive to downstream products of cellobiose and/or glucose during cellulose hydrolysis, but also confers the possibility of

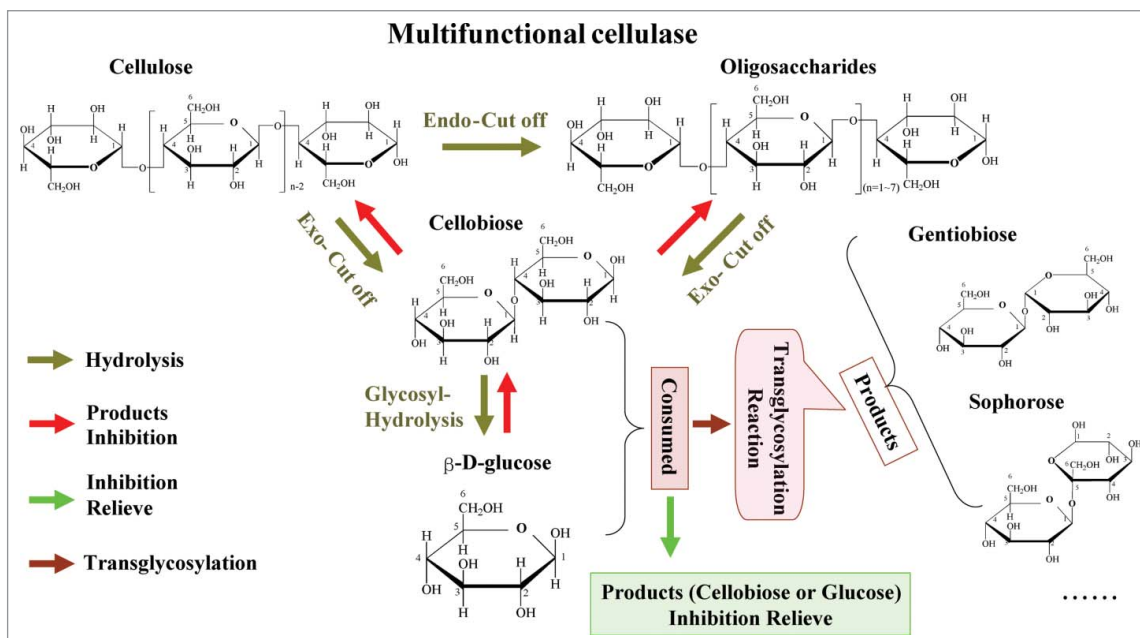


Figure 1. Schematic description of multifunctional cellulase in overcoming the end products inhibition.

stereo- and regiospecific glycosides (or oligosaccharides) synthesis which implies the potential as biocatalysts for functional materials.^{5,6}

Several transglycosylation harboring multifunctional cellulases

A amount of multifunctional cellulases with transglycosylation activity have been isolated and identified as glucose and/or cellobiose insensitive bioenzymes, and these cellulases were obtained from various sources (Table 1). The Umcel9y-1 (β -glucanase subfamily) which isolated from the paddy soil microbial metagenome, manifests efficient endo-glucanase activity to barley glucan, high activities of transglycosylation and exo-glucanase (identified as p-nitrophenol-D-cellobioside hydrolysis). During the polysaccharide (barley glucan) hydrolysis, Umcel9y-1 consumed the end products glucose and cellobiose as the substrates, and then trisaccharose (G3) was produced by transglycosylation reactions.⁵ Through transglycosylation, the end product inhibition was relieved, the multifunctional β -glucanase achieved the glucose and cellobiose tolerant characteristic during the barley glucan degradation. Other than the end products tolerance, the efficient hydrolysis activity and halotolerance were the 2 prominent advantages of Umcel9y-1. The k_{cat}/K_m value for barley glucan was higher than that of most reported cellulases from bacteria, fungi and metagenomes.⁵ As indicated by the original study, the Umcel9y-1 showed more than 70% relative activities after 10 d of pre-incubation with 4 M NaCl (or 4 M KCl), which was higher than that of the well-known halotolerant cellulase Cel5G.^{5,7}

A similar result was also obtained from the multifunctional cellulase Td2F2 that allocated to β -glucosidase subfamily. The cellulase Td2F2 was isolated from

a compost microbial metagenome and characterized as an efficient β -glucosidase with strong transglycosylation activity.⁶ Unlike the most other cellulases, some monosaccharides even improved the hydrolysis efficiency of Td2F2 toward cellobiose. The mechanisms of transglycosylation and glucose tolerance for Td2F2 (glycoside hydrolase family 1, GH1 family) were further disclosed by the high-resolution crystal structure and random mutagenesis test. The mutagenesis test showed that the Asn²²³ residue between subsites +1 and +2 was the critical site for transglycosylation activity and glucose tolerance.¹¹ That is to say, transglycosylation activity conferred the Td2F2 tolerance to the end hydrolysis product of glucose, and the β -glucosidase activity was even improved by the addition of some monosaccharides.^{6,11}

Another multifunctional cellulase CoGH1A belonging to GH1 family manifests activities of β -glucosidase, exo-glucanase, β -xylosidase, β -galactosidase and transgalactosylation, and the CoGH1A was obtained from an extremely thermophilic bacterium *Caldicellulosiruptor owensensis*.¹² Compare to the above multifunctional cellulases (Umcel9y-1 and Td2F2), the prominent advantage of CoGH1A is the excellent thermostability by retaining almost 100% relative activity after 12 hours incubation at 75°C. The multifunctional cellulase CoGH1A has high capabilities in saccharification of lignocellulosic biomass, decomposition of lactose, and synthesis of galactooligosaccharides. It was a promising bioenzyme to be used for bioconversion on the industrial scale. Although the end product tolerance was not evaluated directly for CoGH1A in the original study, the time course of 40 g·L⁻¹ lactose hydrolysis result suggested that high concentration of D-glucose was accumulated, but the product inhibition was not observed. The glucose insensitive character of

Table 1. Several important multifunctional glycoside-hydrolases with transglycosylation activityzyme.

| Name | GH Family | Activities | | | | Glucose tolerance | Transglycosylation product | Ref. |
|----------------------------|-----------|-----------------|----------------|----------------------|------------------------|-------------------|----------------------------|------|
| | | Endo -glucanase | Exo -glucanase | β -glucosidase | β -galactosidase | | | |
| Umcel9y-1 | GH9 | + | + | — | — | + | CeLOS, Gen, Lam, Sop | 5 |
| Td2f2 | GH1 | — | — | + | — | + | Sop, Lam, Gen, Cel | 6 |
| CoGH1A | GH1 | — | + | + | + | + | GalOS | 12 |
| β -glucosidase | NA | — | — | + | — | NA | CeLOS | 13 |
| Bga5 | GH1 | — | — | + | + | NA | GalOS | 14 |
| Cel1A, Cel3A, Cel3B, Cel3E | GH1, GH3 | — | + | + | — | + | Sop, Lam, Cell | 15 |

Abbreviation: GH, glycoside hydrolase; Gen, Gentiobiose; Lam, Laminaribiose; Sop, Sophorose; Cel, Cellobiose; Cell, cellotriose; GalOS, Galactooligosaccharides; CeLOS, celooligosaccharides; NA, unavailable.

CoGH1A might also attributed to transglycosylation activity which is similar as the Td2F2.^{11,12}

Perspective

End product inhibition has become a critical restriction for cellulose bioconversion and biofuels industrial production. Various studies indicated that multifunctional cellulase with transglycosylation activity was the promising candidate in overcoming the technological difficulty during the bioconversion process. As the mechanisms of transglycosylation and end product tolerance disclosed gradually, the development of biofuels are optimistically in future. On the other hand, find a proximal perfect or superfunctional cellulase (or as cellulosome) is always a difficult work to research scientists and technological engineers, much efforts are essential for the improvement of cellulase application.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The study is supported by Natural Science Foundation of Anhui Province for Outstanding Youth (Grant No. 1608085J08), Natural Science Foundation of Anhui Province (Grant No. 1608085QC57) and the Startup Foundation for Advanced Talents of Anhui Agricultural University.

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