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# Activation of enzymatic chitin degradation by a lytic polysaccharide monooxygenase

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ABSTRACT

For decades, the enzymatic conversion of recalcitrant polysaccharides such as cellulose and chitin was thought to solely rely on the synergistic action of hydrolytic enzymes, but recent work has shown that lytic polysaccharide monooxygenases (LPMOs) are important contributors to this process. Here, we have examined the initial rate enhancement an LPMO (CBP21) has on the hydrolytic enzymes (ChiA, ChiB, and ChiC) of the chitinolytic machinery of *Serratia marcescens* through determinations of apparent $k_{cat}$ ($k_{cat}^{app}$) values on a β-chitin substrate. $k_{cat}^{app}$ values were determined to be 1.7±0.1 $s^{-1}$ and 1.7±0.1 $s^{-1}$ for the *exo*-active ChiA and ChiB, respectively and 1.2±0.1 $s^{-1}$ for the *endo*-active ChiC. The addition of CBP21 boosted the $k_{cat}^{app}$ values of ChiA and ChiB giving values of 11.1±1.5 $s^{-1}$ and 13.9±1.4 $s^{-1}$, while there was no effect on ChiC (0.9±0.1 $s^{-1}$).



Cellulose and chitin are the two most abundant biopolymers in nature having an annual production around hundred billion and one trillion tons making the enzymatic degradation of them very important, both biologically and economically.[1,2] They are insoluble polymers composed of β-(1,4)-linked units of D-glucopyranose and N-acetyl-D-glucosamine (GlcNAc) respectively.

Depolymerization of chitin and cellulose is efficiently achieved by synergistic enzyme cocktails composed of glycoside hydrolases (GHs) and lytic polysaccharide monooxygenases (LPMOs).[3–7] The glycoside hydrolases comprising these cocktails can have different modes of action including *endo*- and *exo*-activity as well as varying degrees of processivity.[8] Another feature is that they can have different preferences for which chain end they choose to attack (reducing or non-reducing end).

LPMOs have lately been reorganized into the Auxiliary Activity Family (AA) in the CAZy database.[9] They are a recently discovered group of oxidative copper-enzymes capable of cleaving chitin and cellulose chains using an unprecedented oxidative mechanism.[4,10,11] Their catalytic activity is dependent on the presence of molecular oxygen and an external electron donor, and the reaction leads to the generation of new chain ends.[4] It has been shown that LPMOs can enhance both the rate and extent of polysaccharide degradation.[4,12,13]

In this work, we have looked at the initial rate enhancement an LPMO has on GH catalyzed hydrolysis of a recalcitrant polysaccharide using the well-characterized chitinolytic machinery of *Serratia marcescens*. This machinery consists of one processive chitinase working from the reducing end (chitinase A (ChiA)), one processive chitinase working from the non-reducing end (chitinase B (ChiB)), a nonprocessive *endo*-acting chitinase (chitinase C (ChiC)), and an LPMO (CBP21).[5,14–16] CBP21 by itself has a relatively low activity (~1 $min^{-1}$), which is ~100 fold slower compared to the hydrolytic activity of the individual chitinases (albeit determined under non-saturating substrate conditions).[4,17] The product of CBP21 catalysis are new chain ends on the polymer as well as soluble oxidized chitooligosaccharides in small amounts.[4] When CBP21 and an individual chitinase (i.e., ChiA) are co-incubated with chitin, the only detectable product is $(GlcNAc)_2$ (see below).

To assess the rate enhancing effect, apparent catalytic rate constants for two forms of β-chitin degradation by ChiA, ChiB, and ChiC, alone or together with CBP21 were determined. Firstly, all GHs were incubated with different concentrations of β-chitin of 3 μm size (10, 20, and 30 mg/ml), and initial rates were determined by monitoring the concentration of product formation with respect to time (0, 4, 8, 12, and 16 min) from three independent measurements using HPLC (Fig. 1 and Fig. 2). Fig. 1 shows that initial rates

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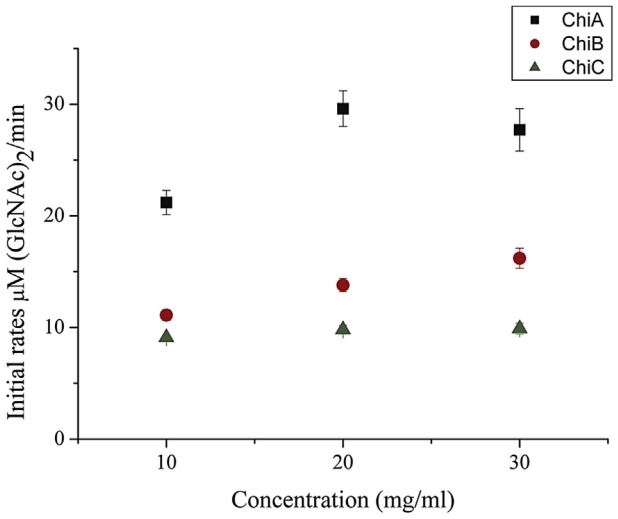


**Fig. 1.** Rate saturation plot from the degradation of 10, 20, and 30 mg/ml 3 μm β-chitin in 20 mM in Tris–HCl pH 8.0 by ChiA (black squares), ChiB (red circles), and ChiC (green triangles). Initial rates (μM $(GlcNAc)_2$/min) is plotted versus the concentration of β-chitin (mg/ml). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were constant from 20 mg/ml and higher for all three chitinases, hence being a measure of apparent $V_{max}$. The reaction conditions were thereafter changed; first the pH was changed to 6.1 before the substrate was changed to that of 180 μm size. Both conditions showed also substrate saturation at 20 mg/ml.

When the 3 μm β-chitin was hydrolyzed by ChiA and ChiB at pH 8.0, $(GlcNAc)_2$ was the only product while minor amounts of $(GlcNAc)_3$ and $(GlcNAc)_4$ were produced as well by ChiC. From initial rates, apparent rate constants ($k_{cat}^{app}$) were determined to be 1.7±0.1 $s^{-1}$, 1.7±0.1 $s^{-1}$, and 1.2±0.1 $s^{-1}$ (Table 1, Fig. 2) for ChiA, ChiB, and ChiC respectively. By adding the LPMO CBP21 a 6- and 9-fold increase in $k_{cat}^{app}$ was seen for the two *exo*-active chitinases ChiA and ChiB, respectively; equaling $k_{cat}^{app}$ values of 11.1±1.5 $s^{-1}$ and 13.9±1.4 $s^{-1}$ (Table 1, Fig. 2). Interestingly, the *endo*-active chitinase ChiC showed that addition of CBP21 has no or little effect on $k_{cat}^{app}$ having a value of 0.9±0.1 $s^{-1}$ (Table 1, Fig. 2).

The effect was also tested on another β-chitin variant (180 μm microparticulate) at pH 6.1 (Table 2). For ChiA, the kinetic values increased from 2.7±0.4 $s^{-1}$ to 13.7±3.5 $s^{-1}$ when CBP21 was added. Finally, pH was varied to 6.1 for the 3 μm β-chitin substrate (Table 2). Again, a significant increase in $k_{cat}^{app}$ was observed for ChiA from 2.2±0.3 $s^{-1}$ to 9.2±2.7 $s^{-1}$ in the presence of CBP21. Over the pH-range investigated in this study and by looking at two different substrates there are no significant effects on $k_{cat}^{app}$.

It has been postulated that oxidative cleavage of glycosidic bonds by a lytic polysaccharide monooxygenase creates new chain ends generating new points of attack on the recalcitrant polysaccharide.[4] Our results clearly show that the presence of an LPMO greatly enhances the initial rate of chitin degradation for the two *exo*-acting chitinases ChiA and ChiB being in line with this postulation. Since ChiA and ChiB have different directionalities and both experience a rate enhancement, this suggests that the presence of CBP21 generates new ends over the whole substrate. Interestingly, the presence of CBP21 did not affect the initial rate for the *endo*-acting ChiC on the substrate under the conditions used in our study. This contrasts the findings in the initial study by Vaaje-Kolstad et al. from 2010 where such a rate enhancement was observed for ChiC.[4] A major difference is that we operate with a~120 times higher substrate to enzyme ratio (20 mg/ml and 170 nM vs 0.45 mg/ml chitin and 500 nM respectively) to have substrate saturating

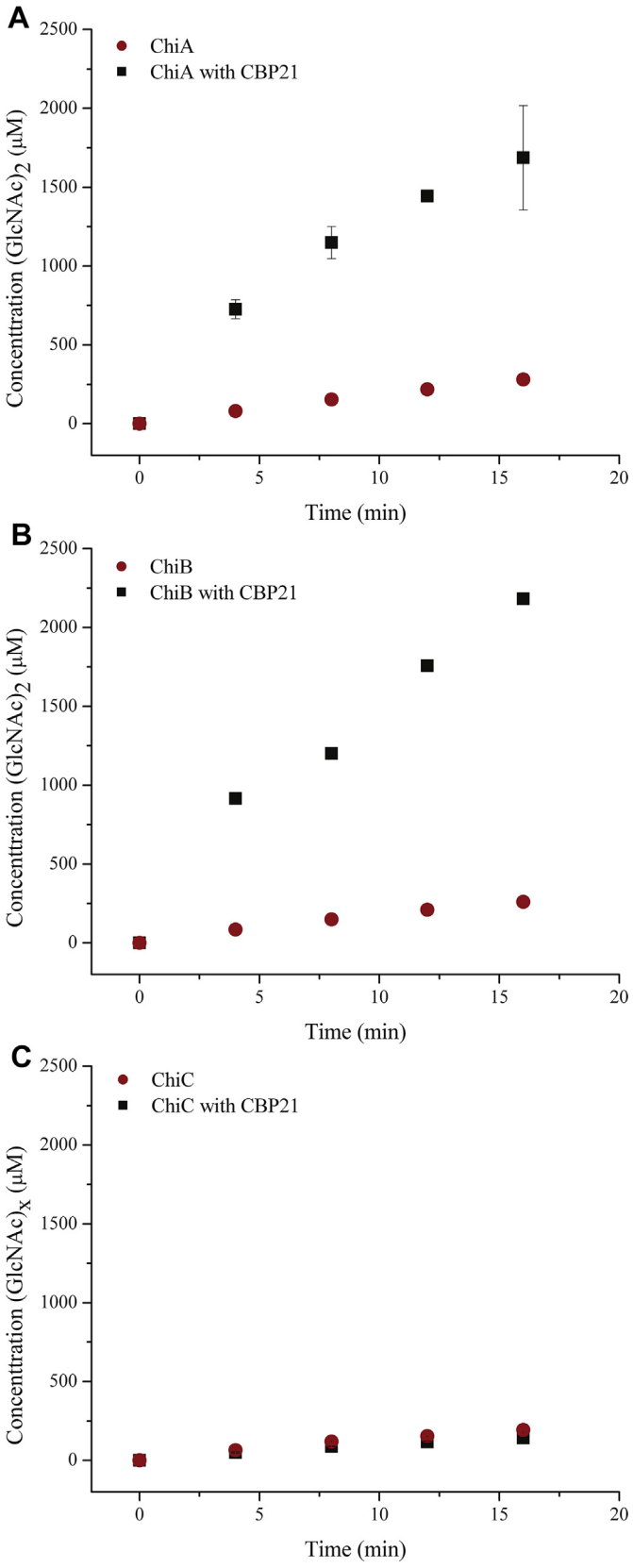


**Fig. 2.** Initial rates of $(GlcNAc)_2$ or $(GlcNAc)_x$ formation after the degradation of 20 mg/ml 3 μm β-chitin in 20 mM in Tris–HCl pH 8.0 by 170 nM ChiA (left), 156 nM ChiB (middle), and 170 nM ChiC (right) alone (red circles) and with 1 μM CBP21 present (black squares). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)