Stereochemical Course of the Action of the Cellobioside Hydrolases I and II of *Trichoderma reesei*

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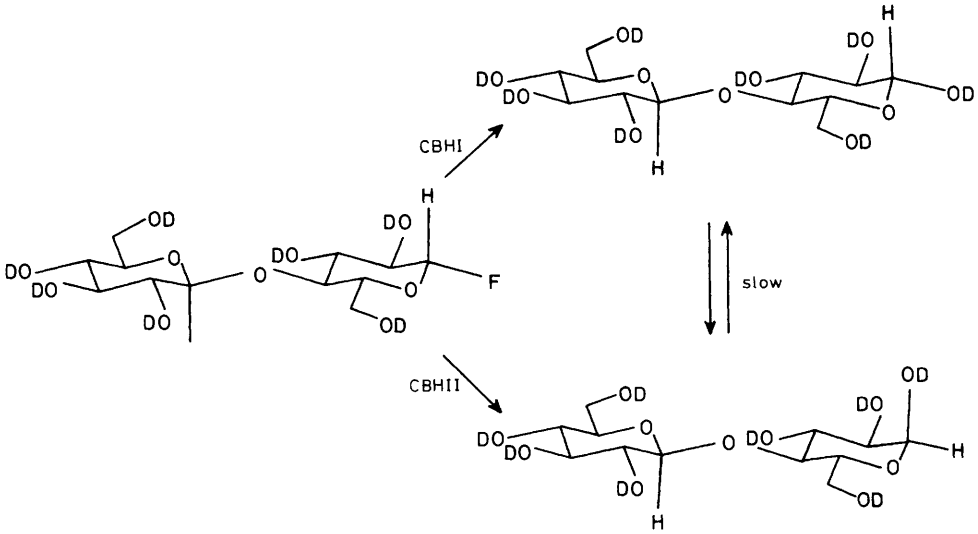
With β-cellobiosyl fluoride as substrate, CBHI gives β-cellobiopyranose as the first product, whereas CBHII gives α-cellobiopyranose (CBH = cellobioside hydrolase).

The degradation of cellulose by micro-organisms is the subject of wide-ranging current investigations.[1] The genetics of the cellulase machinery of white-rot fungi is now well-understood,[2] although the synergistic roles of the gene-products in solubilising crystalline cellulose remains unclear.[3] In the case of the extracellular enzymes of the most studied fungus, *Trichoderma reesei*, it appears that the two cellobioside hydrolases, CBHI and CBHII, initiate the attack on crystalline cellulose and can by themselves degrade it.[4,5] The gene-coding sequences for these two enzymes are known,[6,7]



Scheme 1. Reactions catalysed by the cellobiohydrolases I and II of *T. reesei*, showing substrate anomeric protons.

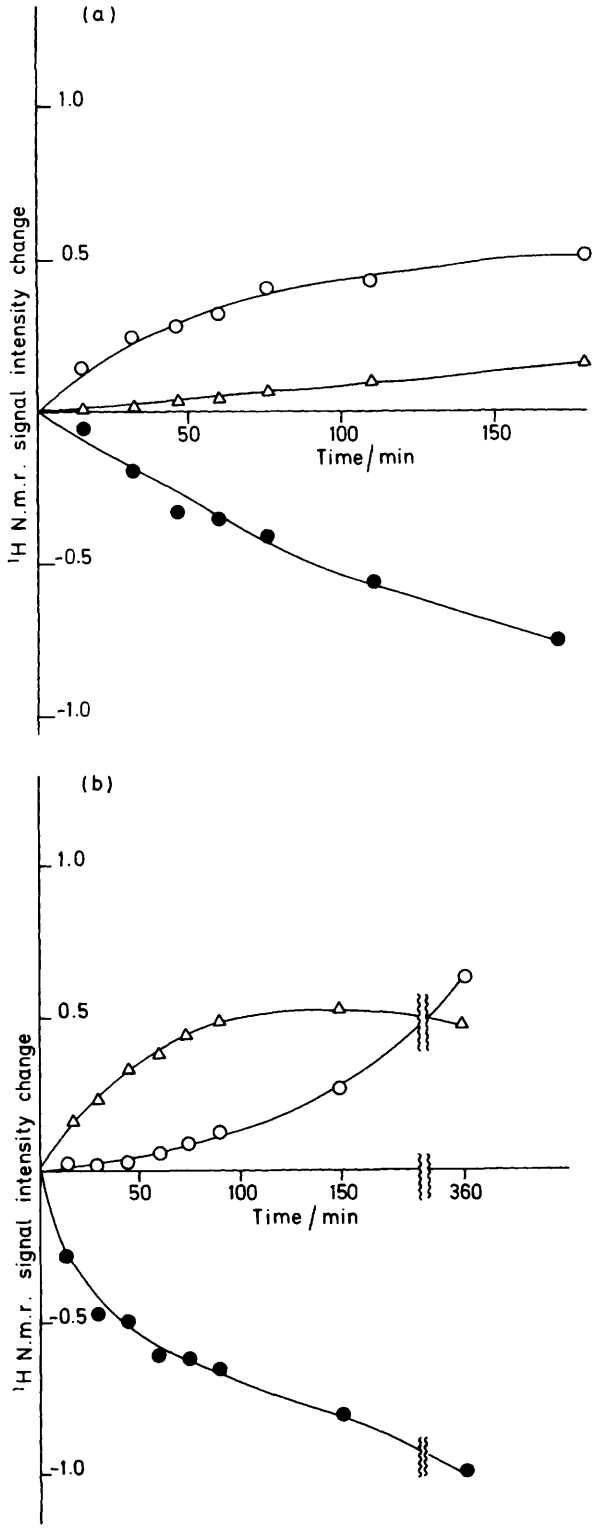


Figure 1. Changes in intensities of anomeric proton resonances during hydrolysis of β-D-cellobiosylfluoride by (a) CBHI and (b) CBHII (for conditions, see text); ○ β-OH, Δ α-OH, ● β-F.

but the fundamental mechanistic data[8] of their stereochemical courses are not, probably because k_{cat} values for substrates of low molecular weight are very low (< 1 s^{-1}).[9]

We now report that by using β-cellobiosyl fluoride[10] as a substrate,[11] we have been able to achieve rates of hydrolysis faster than mutarotation. Solutions containing the fluoride (28 mM) and CBHI[5] (14 μM), or the fluoride (23 mM) and CBHII[5] (39 μM), in D_2O buffer (0.1 M sodium–acetic acid, pD 5.64) were monitored by ^{1}H n.m.r. spectroscopy at 400 MHz (probe temperature 19 °C), in the region of the anomeric protons. The fluoride anomeric hydrogen signal (dd, δ 5.185, $J_{1—2}$ 7.3, $J_{1—F}$ 53.0 Hz) was replaced by signals due to the anomeric H of α-[δ 5.139 (d, $J_{1—2}$ 3.8 Hz)] or β-cellobiose [δ 4.578 (d, $J_{1—2}$ 7.9 Hz)]; changes in the signal of the internal anomeric hydrogen [δ 4.429 (d, $J_{1—2}$ 7.8 Hz)] were also just detectable.

Figure 1(a) and (b) show changes in signal intensities with time; because of the closeness of the large HOD peak, these intensities were estimated from peak heights, using the signals due to 1,6-anhydrocellobiose impurity [δ 5.379 (bs) and 4.531; (d, $J_{1—2}$ 7.8 Hz)] as internal standards.

It is clear that CBHI is acting with overall retention of configuration, and CBHII with overall inversion (Scheme 1). The catalytic apparatus of the two enzymes must therefore be radically different, with CBHI acting through a glycosyl-enzyme intermediate, and CBHII probably using a single displacement by a nucleophilic water molecule.[8,12] Hypotheses about the role of these two enzymes in cellulose hydrolysis should recognise this difference. It may be that both inverting and retaining glucanases are required for cellulose hydrolysis: they have been detected with the (fungal) *Phanerochaete chrysosporium*[13] and (bacterial) *Cellulomonas fimi*[14] systems.

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