Efficient chemoenzymatic oligosaccharide synthesis by reverse phosphorolysis using cellobiose phosphorylase and cellodextrin phosphorylase from Clostridium thermocellum

Inverting cellobiose phosphorylase (CiCBP) and cellodextrin phosphorylase (CiCDP) from Clostridium thermocellum ATCC27405 of glycoside hydrolase family 94 catalysed reverse phosphorolysis to produce cellobiose and cellodextrins in 57% and 48% yield from α-d-glucose 1-phosphate as donor with glucose and cellobiose as acceptor, respectively. Use of α-d-glucosyl 1-fluoride as donor increased product yields to 98% for CiCBP and 68% for CiCDP. CiCBP showed broad acceptor specificity forming β-glucosyl disaccharides with β-(1→4)-regioselectivity from five monosaccharides as well as branched β-glucosyl trisaccharides with β-(1→4)-regioselectivity from three (1→6)-linked disaccharides. CiCDP showed strict β-(1→4)-regioselectivity and catalysed linear chain extension of the three β-linked glucosyl disaccharides, cellobiose, sophorose, and laminaribiose, whereas 12 tested monosaccharides were not acceptors. Structure analysis by NMR and ESI-MS confirmed two β-glucosyl oligosaccharide product series to represent novel compounds, i.e., β-d-glucopyranosyl-[(1→4)-β-d-glucopyranosyl]n-(1→2)-d-glucopyranose, and β-d-glucopyranosyl-[(1→4)-β-d-glucopyranosyl]n-(1→3)-d-glucopyranose (n = 1–7). Multiple sequence alignment together with a modelled CiCBP structure, obtained using the crystal structure of Cellvibrio gilvus CBP in complex with glucose as a template, indicated differences in the subsite +1 region that elicit the distinct acceptor specificities of CiCBP and CiCDP. Thus Glu636 of CiCBP recognized the C1 hydroxyl of β-glucose at subsite +1, while in CiCDP the presence of Ala800 conferred more space, which allowed accommodation of C1 substituted disaccharide acceptors at the corresponding subsites +1 and +2. Furthermore, CiCBP has a short Glu496-Thr500 loop that permitted the C6 hydroxyl of glucose at subsite +1 to be exposed to solvent, whereas the corresponding longer loop Thr637–Lys648 in CiCDP blocks binding of C6-linked disaccharides as acceptors at subsite +1. High yields in chemoenzymatic synthesis, a novel regioselectivity, and novel oligosaccharides including products of CiCDP catalysed oligosaccharide oligomerisation using α-d-glucosyl 1-fluoride, all together contribute to the formation of an excellent basis for rational engineering of CBP and CDP to produce desired oligosaccharides.

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