Abstract

The N-terminal cellulose-binding domain (CBDN1) from Cellulomonas fimi beta-1,4-glucanase CenC binds amorphous but not crystalline cellulose. To investigate the structural and thermodynamic bases of cellulose binding, NMR and difference ultraviolet absorbance spectroscopy were used in parallel with calorimetry (Tomme, P., Creagh, A. L., Kilburn, D. G., & Haynes, C. A., (1996) Biochemistry 35, 13885-13894) to characterize the interaction of soluble cellooligosaccharides with CBDN1. Association constants, determined from the dependence of the amide 1H and 15N chemical shifts of CBDN1 upon added sugar, increase from 180 +/- 60 M-1 for cellotriose to 4,200 +/- 720 M-1 for cellotetraose, 34,000 +/- 7,600 M-1 for cellopentaose, and an estimate of 50,000 M-1 for cellohexaose. This implies that the CBDN1 cellulose-binding site spans approximately five glucosyl units. On the basis of the observed patterns of amide chemical shift changes, the cellooligosaccharides bind along a five-stranded beta-sheet that forms a concave face of the jelly-roll beta-sandwich structure of CBDN1. This beta-sheet contains a strip of hydrophobic side chains flanked on both sides by polar residues. NMR and difference ultraviolet absorbance measurements also demonstrate that tyrosine, but not tryptophan, side chains may be involved in oligosaccharide binding. These results lead to a model in which CBDN1 interacts with soluble cellooligosaccharides and, by inference, with single polysaccharide chains in regions of amorphous cellulose, primarily through hydrogen bonding to the equatorial hydroxyl groups of the pyranose rings. Van der Waals stacking of the sugar rings against the apolar side chains may augment binding. CBDN1 stands in marked contrast to previously characterized CBDs that absorb to crystalline cellulose via a flat binding surface dominated by exposed aromatic rings.