INVESTIGATION OF STRUCTURAL CHANGES IN Cel7A CELLULASE WHEN BOUND TO CELLULOSE SUBSTRATES

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Cellulose is the major component of plant cell walls, accounting for almost half of their net weight, and so has the potential to be a plentiful feedstock for the production of ethanol for biofuels. It is converted to glucose by the enzyme cocktails secreted by fungi and bacteria. A deeper mechanistic understanding of this saccharification of cellulosic biomass, which is recognized as a bottleneck in biorefining applications, could enhance the efficiency of biofuel development. Cellobiohydrolase I (Cel7A) is a major component of the cellulytic enzyme cocktail secreted by the fungus *Trichoderma reesei*. We investigated the solution structure of *T. reesei* Cel7A using small-angle neutron scattering (SANS) at pH values between pH 4.2 and 7.0, corresponding to a pH range from maximum enzymatic activity to minimal activity, respectively. SANS showed that the protein is ~100 Å long, and its structure varies subtly with changes in the pH value of the buffer solution. At the enzyme's optimal pH of 4.2, the tight packing of the polypeptide chain in the catalytic core observed at the higher pH values is disrupted without changing the secondary structure. This suggests that the increased flexibility afforded by such a state is important for function. We have extended this work to investigate of the structure of Cel7A when bound to cellulose substrates to gain new insight into the mechanism of action of this protein. SANS is ideally suited for this investigation because it is possible to distinguish the scattering contributions of different components in a complex mixture using the contrast variation technique. Approaches for producing deuterated crystalline cellulose substrates were developed to enable these studies. The techniques employed in this research are broadly applicable to investigating solution structures of proteins under a wide range of environmental conditions and can provide structural information on complex systems that is not attainable by other means.