Biochemical and Structural Characterizations of Two Dictyostelium Cellobiohydrolases from the Amoebozoa Kingdom Reveal a High Level of Conservation between Distant Phylogenetic Trees of Life

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Glycoside hydrolase family 7 (GH7) cellobiohydrolases (CBHs) are enzymes commonly employed in plant cell wall degradation across eukaryotic kingdoms of life, as they provide significant hydrolytic potential in cellulose turnover. To date, many fungal GH7 CBHs have been examined, yet many questions regarding structure-activity relationships in these important natural and commercial enzymes remain. Here, we present the crystal structures and a biochemical analysis of two GH7 CBHs from social amoeba: Dictyostelium discoideum Cel7A (DdiCel7A) and Dictyostelium purpureum Cel7A (DpuCel7A). DdiCel7A and DpuCel7A natively consist of a catalytic domain and do not exhibit a carbohydrate-binding module (CBM). The structures of DdiCel7A and DpuCel7A, resolved to 2.1 angstrom and 2.7 angstrom respectively, are homologous to those of other GH7 CBHs with an enclosed active-site tunnel. Two primary differences between the Dictyostelium CBHs and the archetypal model GH7 CBH, Trichoderma reesei Cel7A (TreCel7A), occur near the hydrolytic active site and the product-binding sites. To compare the activities of these enzymes with the activity of TreCel7A, the family 1 TreCel7A CBM and linker were added to the C terminus of each of the Dictyostelium enzymes, creating DdiCel7ACBM and DpuCel7ACBM, which were recombinantly expressed in T. reesei. DdiCel7ACBM and DpuCel7ACBM hydrolyzed Avicel, pretreated corn stover, and phosphoric acid-swollen cellulose as efficiently as TreCel7A when hydrolysis was compared at their temperature optima. The K_i of cellobiose was significantly higher for DdiCel7ACBM and DpuCel7ACBM than for TreCel7A: 205, 130, and 29 μM, respectively. Taken together, the present study highlights the remarkable degree of conservation of the activity of these key natural and industrial enzymes across quite distant phylogenetic trees of life.

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