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Nat Struct Biol. 1998 Sep;5(9):812-8.

Insights into transition state stabilization of the beta-1,4-glycosidase Cex by covalent intermediate accumulation in active site mutants.

Nolenboom V, Bisan C, Nitik M, Rose DB, Warren RA, Withers SG.

Protein Engineering Network of Centres of Excellence, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Canada.

Abstract

The catalytic mechanism of 'retaining' beta-glycosidases has been the subject of considerable interest and debate for many years. The visualization of a covalent glycosyl enzyme intermediate by X-ray crystallography was first accomplished with a saccharide substrate substituted with fluorine at its 2-position. The structure implicated major roles for residue His 205 and for the 2-hydroxyl position of the proximal saccharide in binding and catalysis. Here we have studied the kinetic behavior of various His 205 mutants. One of these mutants, a double mutant H205N/E127A, has been used to stabilize a covalent glycosyl-enzyme intermediate involving an unsubstituted sugar, permitting crystallographic analysis of the interactions between its 2-hydroxyl group and the enzyme.

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