

Abstract

The 20 kDa xylanase from *Bacillus circulans* carries out hydrolysis of xylan via a two-step mechanism involving a covalent glycosyl-enzyme intermediate. In this double-displacement reaction, Glu78 functions as a nucleophile to form the intermediate, while Glu172 acts as a general acid catalyst during glycosylation, protonating the departing aglycone, and then as a general base during deglycosylation, deprotonating the attacking water. The dual role of Glu172 places specific demands upon its ionization states and hence pKa values. ¹³C-NMR titrations of xylanase, labeled with [δ -¹³C]glutamic acid, have revealed pKa values of 4.6 and 6.7 for Glu78 and Glu172, respectively. These agree well with the apparent pKa values obtained from a study of the pH dependence of k_{cat}/K_m and demonstrate that, at the enzyme's pH optimum of 5.7, the nucleophile Glu78 is deprotonated and the general acid Glu172 initially protonated. Remarkably, the pKa for Glu172 drops to 4.2 in a trapped covalent glycosyl-enzyme intermediate, formed by reaction with 2', 4'-dinitrophenyl 2-deoxy-2-fluoro-beta-xylobioside [Miao et al. (1994) *Biochemistry* 33, 7027-7032]. A similar pKa is measured for Glu172 when a glutamine is present at position 78. This large decrease in pKa of approximately 2.5 units is consistent with the role of Glu172 as a general base catalyst in the deglycosylation step and appears to be a consequence of both reduced electrostatic repulsion due to neutralization of Glu78 and a conformational change in the protein. Such "pKa cycling" during catalysis is likely to be a common phenomenon in glycosidases.