Abstract

The pH optima of family 11 xylanases are well correlated with the nature of the residue adjacent to the acid/base catalyst. In xylanases that function optimally under acidic conditions, this residue is aspartic acid, whereas it is asparagine in those that function under more alkaline conditions. Previous studies of wild-type (WT) Bacillus circulans xylanase (BCX), with an asparagine residue at position 35, demonstrated that its pH-dependent activity follows the ionization states of the nucleophile Glu78 (pKa 4.6) and the acid/base catalyst Glu172 (pKa 6.7). As predicted from sequence comparisons, substitution of this asparagine residue with an aspartic acid residue (N35D BCX) shifts its pH optimum from 5.7 to 4.6, with an approximately 20% increase in activity. The bell-shaped pH-activity profile of this mutant enzyme follows apparent pKa values of 3.5 and 5.8. Based on 13C-NMR titrations, the predominant pKa values of its active-site carboxyl groups are 3.7 (Asp35), 5.7 (Glu78) and 8.4 (Glu172). Thus, in contrast to the WT enzyme, the pH-activity profile of N35D BCX appears to be set by Asp35 and Glu78. Mutational, kinetic, and structural studies of N35D BCX, both in its native and covalently modified 2-fluoro-xylobiosyl glycosyl-enzyme intermediate states, reveal that the xylanase still follows a double-displacement mechanism with Glu78 serving as the nucleophile. We therefore propose that Asp35 and Glu172 function together as the general acid/base catalyst, and that N35D BCX exhibits a "reverse protonation" mechanism in which it is catalytically active when Asp35, with the lower pKa, is protonated, while Glu78, with the higher pKa, is deprotonated. This implies that the mutant enzyme must have an inherent catalytic efficiency at least 100-fold higher than that of the parental WT, because only approximately 1% of its population is in the correct ionization state for catalysis at its pH optimum. The increased efficiency of N35D BCX, and by inference all "acidic" family 11 xylanases, is attributed to the formation of a short (2.7 A) hydrogen bond between Asp35 and Glu172, observed in the crystal structure of the glycosyl-enzyme intermediate of this enzyme, that will substantially stabilize the transition state for glycosyl transfer. Such a mechanism may be much more commonly employed than is generally realized, necessitating careful analysis of the pH-dependence of enzymatic catalysis.