Abstract

NMR-monitored pH titration curves of proteins provide a rich source of structural and electrostatic information. Although relatively straightforward to measure, interpreting pH-dependent chemical shift changes to obtain site-specific acid dissociation constants (pK (A) values) is challenging. In order to analyze the biphasic titrations exhibited by the side chain (13)C(γ) nuclei of the nucleophilic Glu78 and general acid/base Glu172 in Bacillus circulans xylanase, we have revisited the formalism for the ionization equilibria of two coupled acidic residues. In general, fitting NMR-monitored pH titration curves for such a system will only yield the two macroscopic pK (A) values that reflect the combined effects of both deprotonation reactions. However, through the use of mutations complemented with ionic strength-dependent measurements, we are able to extract the four microscopic pK (A) values governing the branched acid/base equilibria of Glu78 and Glu172 in BcX. These data, confirmed through theoretical calculations, help explain the pH-dependent mechanism of this model GH11 xylanase by demonstrating that the kinetically determined pK (A) values and hence catalytic roles of these two residues result from their electrostatic coupling.