

## 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  $(^{13}C(\gamma))$  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  $pK(A_i)$  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.