

Salt bridge interaction and optimum pH shift in
Bacillus circulans xylanase

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Electrostatic interactions play important role in determining structure and function of proteins. Likewise the protein folding, binding, flexibility and stability, pK_a s of the charged residues are modulated by electrostatic interactions. Long-range interactions, like salt bridge interactions and electrostatic repulsion are the major interactions that modulate the pK_a s of the charged residues involved. Here, we study the possibility of long range interactions to modulate the pK_a s of catalytic residues of *Bacillus circulans* xylanase, to shift optimum pH of the enzyme towards alkaline or acidic side. 11 mutation sites, far from the catalytic glutamates, were selected randomly, and substituted by positively charged residues. The change in pK_a of the catalytic residue was predicted by using Delphi module in Insight II. Upon site directed mutagenesis and characterization of the mutants, seven mutants showed shift in optimum pH towards alkaline side. Two mutants showed shift in optimum pH towards acidic side, while the other two did not show any change in optimum pH. Analyses of interacting residues and electrostatics showed that these interactions possess enormous potential in engineering pH dependence of enzyme catalysis.