

Analysis of the mechanism of the Serratia nuclease using site-directed mutagenesis

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Abstract

Based on crystal structure analysis of the Serratia nuclease and a sequence alignment of six related nucleases, conserved amino acid residues that are located in proximity to the previously identified catalytic site residue His89 were selected for a mutagenesis study. Five out of 12 amino acid residues analyzed turned out to be of particular importance for the catalytic activity of the enzyme: Arg57, Arg87, His89, Asn119 and Glu127. Their replacement by alanine, for example, resulted in mutant proteins of very low activity, <1% of the activity of the wild-type enzyme. Steady-state kinetic analysis of the mutant proteins demonstrates that some of these mutants are predominantly affected in their $k(\text{cat})$, others in their $K(\text{m})$. These results and the determination of the pH and metal ion dependence of selected mutant proteins were used for a tentative assignment for the function of these amino acid residues in the mechanism of phosphodiester bond cleavage by the Serratia nuclease.

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