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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(cat), others in their K(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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