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Mechanism of DNA cleavage by the DNA/RNA-no--specific Anabaena sp. PCC 7120 endonuclease NucA and its inhibition by NuiA

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Abstract

A structural model of the DNA/RNA non-specific endonuclease NucA from Anabaena sp. PCC7120 that has been obtained on the basis of the three-dimensional structure of the related Serratia nuclease, suggests that the overall architecture of the active site including amino acid residues H124, N155 and E163 (corresponding to H89, N119 and E127 in Serratia nuclease) is similar in both nucleases. Substitution of these residues by alanine leads to a large reduction in activity (<0.1%), similarly as observed for Serratia nuclease demonstrating that both enzymes share a similar mechanism of catalysis with differences only in detail. NucA is inhibited by its specific polypeptide inhibitor with a K1 value in the subpicomolar range, while the related Serratia nuclease at nanomolar concentrations is only inhibited at an approximately 1000-fold molar excess of NuiA. The artificial chromophoric substrate deoxythymidine 3',5'-bis--p-nitrophenyl phosphate) is cleaved by NucA as well as by Serratia nuclease. Cleavage of this analogue by NucA, however, is not inhibited by NuiA, suggesting that small molecules gain access to the active site of NucA in the enzyme-inhibitor complex under conditions where cleavage of DNA substrates is completely inhibited. The active site residue E163 seems to be the main target amino acid for inhibition of NucA by NuiA, but R93, R122 and R167 (corresponding to K55, R87, R131 in Serratia nuclease) are also involved in the NucA/NuiA interaction. NuiA deletion mutants show that the structural integrity of the N and C-terminal region of the inhibitor is important for complex formation with NucA and inhibition of nuclease activity. Based on these results a mechanism of DNA cleavage by NucA and its inhibition by NuiA is proposed. (C) 2000 Academic Press.

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Keywords

Enzyme mechanism, Inhibitor, Nuclease, Structural model