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Kinetic analysis of the cleavage of natural and synthetic substrates by the Serratia nuclease

Friedhoff P., Meiss G., Kolmes B., Pieper U., Gimadutdinow O., Urbanke C., Pingoud A.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

The extracellular nuclease from Serratia marcescens is a non-specific endonuclease that hydrolyzes double-stranded and single-stranded DNA and RNA with high specific activity. Steady-state and pre-steady-state kinetic cleavage experiments were performed with natural and synthetic DNA and RNA substrates to understand the mechanism of action of the Serratia nuclease. Most of the natural substrates are cleaved with similar k(cat) and K(m) values, the k(cat)/K(m) ratios being comparable to that of staphylococcal nuclease. Substrates with extreme structural features, like poly(dA) · poly(dT) or poly(dG) · poly(dC), are cleaved by the Serratia nuclease with a 50 times higher or 10 times lower K(m), respectively, as salmon testis DNA. Neither with natural DNA or RNA nor synthetic oligodeoxynucleotide substrates did we observe substrate inhibition for the Serratia nuclease as reported recently. Experiments with short oligodeoxynucleotides confirmed previous results that for moderately good cleavage activity the substrate should contain at least five phosphate residues. Shorter substrates are still cleaved by the Serratia nuclease, albeit at a rate reduced by a factor of more than 100. Cleavage experiments with oligodeoxynucleotides substituted by a single phosphorothioate group showed that the negative charge of the proR(p)-oxygen of the phosphate group 3' adjacent to the scissile phosphodiester bond is essential for cleavage, as only the R(p)-phosphorothioate supports cleavage at the 5' adjacent phosphodiester bond. Furthermore, the modified bond itself is only cleaved in the R(p)-diastereomer, albeit 1000 times more slowly than the corresponding unmodified phosphodiester bond, which offers the possibility to determine the stereochemical outcome of cleavage. Pre-steady-state cleavage experiments demonstrate that it is not dissociation of products but association of enzyme and substrate or the cleavage of the phosphodiester bond that is the rate-limiting step of the reaction. Finally, it is shown that Serratia nuclease accepts thymidine 3',5'-bis(p-nitrophenyl)phosphate as a substrate and cleaves it at its 5'-end to produce nitrophenol and thymidine 3'-(p-nitrophenylphosphate) 5-phosphate. The rate of cleavage of this artificial substrate, however, is 6-7 orders of magnitude smaller than the rate of cleavage of macromolecular DNA or RNA.

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Keywords

Deoxyribonuclease, Modified oligonucleotide, Pre-steady-state kinetics, Ribonuclease, Steady-state kinetics