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Chemical Rescue of Active Site Mutants of S. pneumoniae Surface Endonuclease EndA and Other Nucleases of the HNH Family by Imidazole

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Abstract

The His-Asn-His (HNH) motif characterizes the active sites of a large number of different nucleases such as homing endonucleases, restriction endonucleases, structure-specific nucleases and, in particular, nonspecific nucleases. Several biochemical studies have revealed an essential catalytic function for the first amino acid of this motif in HNH nucleases. This histidine residue was identified as the general base that activates a water molecule for a nucleophilic attack on the sugar phosphate backbone of nucleic acids. Replacement of histidine by an amino acid such as glycine or alanine, which lack the catalytically active imidazole side chain, leads to decreases of several orders of magnitude in the nucleolytic activities of members of this nuclease family. We were able, however, to restore the activity of HNH nuclease variants (i.e., EndA (Streptococcus pneumoniae), SmaNuc (Serratia marcescens) and NucA (Anabaena sp.)) that had been inactivated by His→Gly or His→Ala substitution by adding excess imidazole to the inactive enzymes in vitro. Imidazole clearly replaces the missing histidine side chain and thereby restores nucleolytic activity. Significantly, this chemical rescue could also be observed in vivo (Escherichia coli). The in vivo assay might be a promising starting point for the development of a high-throughput screening system for functional EndA inhibitors because, unlike the wild-type enzyme, the H160G and H160A variants of EndA can easily be produced in E. coli. A simple viability assay would allow inhibitors of EndA to be identified because these would counteract the toxicities of the chemically rescued EndA variants. Such inhibitors could be used to block the nucleolytic activity of EndA, which as a surface-exposed enzyme in its natural host destroys the DNA scaffolds of neutrophil extracellular traps (NETs) and thereby allows S. pneumoniae to escape the innate immune response.

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Keywords

Chemical rescue in vivo, DNA cleavage, Enzyme catalysis, Nonspecific nucleases, Reaction mechanisms