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Identification of functionally relevant histidine residues in the apoptotic nuclease CAD

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Abstract

The caspase-activated DNase CAD (DFF40/CPAN) degrades chromosomal DNA during apoptosis. Chemical modification with DEPC inactivates the enzyme, suggesting that histidine residues play a decisive role in the catalytic mechanism of this nuclease. Sequence alignment of murine CAD with four homologous apoptotic nucleases reveals four completely (His242, His263, His304 and His308) and two partially (His127 and His313) conserved histidine residues in the catalytic domain of the enzyme. We have changed these residues to asparagine and characterised the variant enzymes with respect to their DNA cleavage activity, structural integrity and oligomeric state. All variants show a decrease in activity compared to the wild-type nuclease as measured by a plasmid DNA cleavage assay. H242N, H263N and H313N exhibit DNA cleavage activities below 5% and H308N displays a drastically altered DNA cleavage pattern compared to wild-type CAD. Whereas all variants but one have the same secondary structure composition and oligomeric state, H242N does not, suggesting that His242 has an important structural role. On the basis of these results, possible roles for His127, His263, His304, His308 and His313 in DNA binding and cleavage are discussed for murine CAD.