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Involvement of conserved histidine, lysine and tyrosine residues in the mechanism of DNA cleavage by the caspase-3 activated DNase CAD

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Abstract

The caspase-activated DNase (CAD) is involved in DNA degradation during apoptosis. Chemical modification of murine CAD with the lysine-specific reagent 2,4,6-trinitrobenzenesulphonic acid and the tyrosine-specific reagent N-acetylimidazole leads to inactivation of the nuclease, indicating that lysine and tyrosine residues are important for DNA cleavage by this enzyme. The presence of DNA or the inhibitor ICAD-L protects the enzyme from modification. Amino acid substitution in murine CAD of lysines and tyrosines conserved in CADs from five different species leads to variants with little if any catalytic activity, but unaltered DNA binding (K155Q, K301Q, K310Q, Y247F), with the exception of Y170F, which retains wild-type activity. Similarly, as observed for the previously characterised H242N, H263N, H308N and H313N variants, the newly introduced His→Asp/Glu or Arg exchanges lead to variants with <1% of wild-type activity, with two exceptions: H313R shows wild-type activity, and H308D at pH 5.0 exhibits ~5% of wild-type activity at this pH. Y170F and H313R produce a specific pattern of fragments, different from wild-type CAD, which degrades DNA non-specifically. The recombinant nuclease variants produced in Escherichia coli were tested for their ability to form nucleolytically active oligomers. They did not show any significant deviation from the wild-type enzyme. Based on these and published data possible roles of the amino acid residues under investigation are discussed.