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Biochemical characterization of Anabaena sp. strain PCC 7120 non- specific nuclease NucA and its inhibitor NuiA

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Abstract

We have established overexpression systems and purification protocols for NucA and NuiA, a sugar non-specific nuclease and its protein inhibitor from Anabaena sp. strain PCC 7120, in order to characterize these proteins in detail. CD spectroscopy revealed that NucA has a similar secondary-structure composition, 13% a helix and 20% β sheet, to the related Serratia nuclease, while NuiA represents a protein with a higher α-helical (29%) and β-sheet (24%) content than NucA. Denaturation experiments showed that the stabilities of NucA and NuiA are in the typical range for proteins of mesophilic organisms, NuiA with ΔG(o)(H2O)= 63.4 J* mol-1, (residue), being slightly more stable than its target NucA with ΔΔ(o)(H2O)= 46.3 J * mol- 1(residue). The nuclease requires divalent metal ions as cofactors, the optimum concentration being around 5 mM for Mn2+ or Mg2+. The order of effectiveness of various divalent cations to function as cofactors for the hydrolytic activity of NucA is Mn2+ = Co2+ > Mg2+≤ Ni2+> Ca2+ = Cd2+ at a concentration of 5 mM. Nuclease activity decreases with increasing concentration of monovalent salt. The activity of NucA shows a pH optimum at pH 5.5-7.5. The temperature optimum is around 35°C, the activation energy was calculated to be 53 kJ mol-1. The specific activity of the nuclease towards high molecular-mass DNA is 8.4X 106 Kunitz-units * mg-1, which means that NucA is one of the most active nucleases known. Kinetic constants for the cleavage of various DNA and RNA substrates by NucA are all in the range K(m) ≤ 0.1 mg * ml- and k(cm) 1000 s-1. As other non-specific nucleases, NucA exhibits sequence preferences, similar to the related Serratia nuclease, NucA avoids cleavage of d(A) d(T) tracts. The nucleolytic activity of NucA is completely inhibited at equimolar concentrations of nuclease and inhibitor. An ultracentrifugation analysis showed that NucA and NuiA form a 1:1 complex. The interaction of NucA with NuiA was also investigated by CD spectroscopy and revealed no major conformational changes upon complex formation of the two proteins.

Keywords

Nuclease, Nuclease inhibitor, Protein-protein interaction, Steady-state kinetics