Appendix

Mung Bean Nuclease

Sung and Laskowski (1962) originally reported the presence of an endonuclease activity in mung bean sprouts. The purified enzyme specifically hydrolyzed heat-denatured DNA and RNA, and was designated mung bean nuclease I, single-strands-specific (Johnson and Laskowski 1968). The digestion products with the nuclease range from mononucleotides to at least heptanucleotides that are terminated by a 5' - phosphate and 3' - hydroxyl. The nuclease shows a pH optimum at 5.0 and is quite sensitive to ionic strength, 0.025-0.05 M being optimal. The preference for single-stranded DNA, as assayed by the formation of acid-soluble material, depends on the type of DNA as assayed by the formation of acid-soluble material, depends on the type of DNA and the reaction conditions. At 37°C under the optimal conditions it is 30,000-fold for T7 DNA. 65-fold for crab poly (dA-dT), and less than 2-fold for synthetic poly (dA-dT). No hydrolysis of poly (dG):poly (dC) occurs under these conditions, even with a 100-fold excess of enzyme. Mung bean nuclease I does not recognize synthetic poly (dA-dT) as a typically double-stranded structure. The enzyme exhibits a preference for Ap↓Nd and T(U)↓pN in single-stranded nucleic acids (Johnson and Laskowski 1970).

The purified enzyme can dephosphorylate 3' -mononucleotides. Also, dinucleotides NpNp are first dephosphorylated to NpN and then the internucleotide linkage is cleaved to form N + pN. This dephosphorylation activity is a 3' - w - monophosphatase rather than a 3' -nucleotidase (Mikulski and Laskowski 1970). The w-monophosphatase hydrolyzes ribose mononucleotides 50 - to 100-fold faster than the corresponding deoxyribose compounds. It also shows preference for bases in the order A>T(U)> C>G. A similar preference is shown by the nuclease activity, except that poly (dT) or poly (U) were bydrolyzed faster than the corresponding adenine polymers presumably because the former lack an ordered structure.

The nuclease is a glycoprotein consisting of 29% carbohydrate by weight and has a molecular weight of 39,000 as determined by polyacrylamide gel electrophoresis in the presence of SDS (Kowalski et al. 1976). In the absence of the latter, it gives several bands, presumably caused by aggregation. The enzyme contains one sulfhydryl group and three disulfide bonds per molecule. It has a high content (12.6 mole %) of aromatic residues. A portion of the enzyme molecules contains a peptide bond cleavage at a single region in the protein. The two polypeptides, 25K and 15K. are covalently linked by a disulfide bond(s). Both the cleaved and intact forms of the enzyme are equally active in the hydrolysis of phosphate ester linkages in either DNA, RNA, or adenosine 3' -monophosphate.

Like Pl and S1 nucleases, mung bean nuclease I is a zinc metalloenzyme with multiple metal ion-binding sites (Kowalski et al. 1976), The enzymatic activity can be stabilized at pH 5.0 in the presence of 0.1 mM zinc acetate, 1.0 mM cysteine, and 0.001% Triton X-100. After removal of Zn^{2+} and cysteine by dialysis for 24 hours against 0.05 M sodium acetate (pH 5.0) containing 0.001% Triton X-100, the enzyme loses 70-80% of its original activity against denatured DNA. The dialyzed enzyme can be reactivated by addition of Zn^{2+} (0.1 mM) and cysteine (1mM) and 20-30 minutes incubation at 23°C. Zinc acetate without a sulfhydryl compound is also capable of reactivating the dialzyed enzyme. The rate is very slow, however, since only one-half of the maximal reactivation is achieved in 24 hours with 0.1 mM or 1.0 mM Zn^{2+}, Co^{2-}, Mg^{2+}, Mn^{2+}, Ca^{2+}, Fe^{2+}, and Cu^{2+} under the same conditions were incapable of reactivating the enzyme.

Dialysis of the enzyme against 0.05 M sodium acetate (pH 5.0) containing 1 mM EDTA, followed by further dialysis to remove the EDTA, results in complete loss of the activity against single-stranded DNA. Incubation with 1 mM Zn^{2+} and other divalent cations failed to produce reactivation after 18 hours at 23°C in the presence of 1 mM cysteine and 0.001% Triton X-100. Apparently, zinc ions are totally removed by EDTA dialysis, resulting in irreversible inactivation. This essential metal ion is presumably not totally removed by dialysis in the absence of EDTA, Since, under these conditions, both sulfhydryl

compounds and Zn^{2+} reactivate the enzyme (compare S1 nuclease above). Also, 0.01% SDS (pH 5.0) completely inactivates the enzyme. Mung bean nuclease 1 is stable to heat treatment (60-70°C) at pH 5 in the presence of both Zn^{2+} and sulfhydryl compounds.

With a high concentration of the enzyme, mung bean nuclease I introduces limited number of endonucleolytic, double-strand cleavages in linear duple DNA. Phage DNA is cleaved most rapidly in the AT-rich region in the center of the molecule (Johnson and Laskowski 1970) and preferentially at total of six loci, all of which fall within the three AT-rich blocks revealed by partial denaturation mapping (Kroeker and Kowalski 1978). In addition to these sites, there are other sites that are hydrolyzed more slowly. For phag DNAs of T7, gh-1, and PM2, the digestion products by the nuclease are genesized (300-2100 bp), suggesting that cleavage occurs at specific site (Kroeker and Kowalski 1978).

Under conditions that do not favor a tight helical structure and with high concentrations of the enzyme, large duplex polymers such as T7 DNA, fragments (300-2100 bp) are completely degraded from their termini with continuous accumulation of mono-,di-, and trinucleotides (Kroeker et al 1976). The terminally directed activity is an intrinsic property of the enzyme molecule because:(1) it is inactivated and reactivated in parallel with the single-strand activity and (2) the two activities coelectrophorese on analytical gels. The pH optimum

for both the hydrolysis of denatured DNA and terminally directed hydrolysis of native DNA becomes more acidic with increasing salt concentration. The relative preference for single-stranded structures increases as the pH becomes more basic.

At near neutral pH, supercoiled DNA is the preferred substrate over the relaxed topoisomer by 30,000-fold. The enzyme converts supercoilded PM2 DNA to the nicked circular form but not to the linear form (Wang 1974; D Kowalski, J.P. Sanford, and T.J. Foels, pers, comm.). With a high concentration of the enzyme at more acidic pH, the nicked circular DNA is converted to small-sized DNA fragments.