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## Abstract

DNA-dependent DNA-polymerases

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DNA-dependent DNA-polymerases from Neurospora crassa.

Until now DNA-dependent DNA-polymerases have been isolated from the cytoplasm (Wintersberger et al. 1974 Eur. J. Biochem. 80:41) and the mitochondria (Wintersberger et al. 1976 Eur. J. Biochem. 68:199) of only two fungi, Ustilago maydis and Saccharomyces cerevisiae. In lower eukaryotes generally two different "cytoplasmic" i.e. nonmitochondrial DNA-polymerases have been found (named A and B according to their order of elution from a DEAE-cellulose column). However, Banks (G. Banks et al. 1976 Eur. J. Biochem. 62:131) reported finding only a single enzyme in Ustilago maydis. The cytoplasmic polymerases of Saccharomyces cerevisiae showed little difference from each other although the mitochondrial enzyme can easily be distinguished from them.

Table 1

Some preliminary results with the DNA-polymerase of Neurospora crassa.

| | cytoplasmic | | mitochondrial |
|---|---|---|---|
| | A | B | |
| $Mg^{2+}$ optimal concentration (mM) | 5 | 5 | 30 |
| $K^+$ optimal concentration | 40 | 40 | 60 |
| pH-optimum | 8.2 | 8.2 | 8.5 |
| temperature optimum (°C) | 37 | 37 | 37 |
| inhibition by 30 μM p-chloromercuribenzoate | + | + | - |
| thermal inactivation at 50°C (under standard assay conditions) HMT | 2' | 1' | nt |
| molecular weight (aldolase used as marker protein) | · 150000 | ·150000 | 70000 |

Preliminary results with the DNA-polymerases from Neurospora are reported here. DNA-polymerase activity was assayed by measuring the incorporation of $^3$H-dTTP into acid-insoluble material. The standard enzyme assay contained 50 mM Tris-HCl Buffer (pH 8.2), 1 mM mercaptoethanol, 4 μg bovine serum albumin, 80 μM of each of the four deoxynucleosidetriphosphates, 6 μg (20 μg) activated calf thymus DNA (Prepared according to the method of Aposhian et al. 1962 J. Biol. Chem. 237:519), 5 mM (30 mM) $Mg^{2+}$, 40 mM (60 mM) $K^+$ and 2 μCi dTTP in a total volume of 250 μl (conditions for the mitochondrial enzyme in brackets). The mixture was incubated for 30 minutes at 37° C and the reaction was stopped by the addition of carrier and TCA. After 30 minutes on ice the precipitate was collected by filtration and the radioactivity was determined. One unit is the amount of enzyme that catalyzes the incorporation of one nanomole dTTP in 30 minutes under standard incubation conditions. The protein content was determined according to the method of Schaffner (Schaffner et al. 1973 Anal. Biochem. 56:502).

The polymerases were purified from Neurospora crassa wt 74 A as follows. Hyphae were homogenized and disrupted in a "Weiss-mill" (Weiss et al 1970 Eur. J. Biochem. 14:75) and suspended in Tris-HCl buffer (pH 8.0) containing 0.44 M sucrose. After low-speed centrifugation the mitochondria were collected from the supernatant in a Sorvall continuous flow rotor. The mitochondria were purified by several centrifugation steps at low and high speed and an additional sucrose gradient. After lysis they were extracted with 0.5 M ammonium chloride. The cytoplasmic polymerase activity was obtained in the supernatant fraction following high speed centrifugation (100,000 g).

Further purification procedures for the cytoplasmic and for the mitochondrial enzymes included chromatography on Sephadex G-25, DEAE-cellulose, phosphocellulose, DNA-cellulose, Sepharose 6B and centrifugation on glycerol gradients. DEAE-cellulose chromatography of the supernatant fraction resolved two activities, termed A and B. Specific activities of 50 U/mg for polymerase A, 70 U/mg for B and 130 U/mg for the mitochondrial enzyme were obtained with the purified polymerase species.

All three polymerases worked 3 to 4 times better with an activated primer template than with native DNA and were unable to use synthetic ribonucleic primer-template complexes (e.g. poly rCdG). Some properties of the polymerases are listed in Table 1. Activities A and B isolated from the cytoplasm appear to be different with respect to molecular weight and thermal lability and therefore may represent different enzymes. The mitochondrial polymerase can be distinguished clearly from the cytoplasmic enzymes by molecular weight, salt concentration optima and inhibition by the sulfhydryl reagent (pCMB). Our preparations were free from terminal deoxynucleotidyl transferase and only very low amounts of endonuclease contamination were present as measured with covalently closed circular DNA (· 10 ng equivalent pancreatic endonuclease/mg protein). Furthermore, no single or double-strand-specific exonuclease could be detected. This demonstrates that the polymerases are not associated with an exonucleolytic activity. - - - Max-Planck-Institut fur Biochemie, D-8033 Martinsried bei Munchen.