SHORT COMMUNICATION

MITOCHONDRIAL DNA POLYMERASE FROM CAULIFLOWER INFLORESCENCE

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Received July 24, 1980

DNA polymerase was isolated from the mitochondrial fraction of cauliflower inflorescence and characterized. The enzyme is clearly separable from the nuclear DNA polymerases by high salt elution on DEAE-cellulose column chromatography. Template-primer utilization, response to KCl and sensitivity to N-ethylmaleimide of the enzyme are similar to those of DNA polymerase of animal mitochondria.

It has been described that mitochondrial DNA polymerases from several different sources of animal tissues or yeast are different in several characteristics from the nuclear fraction (Myer and Simpson 1968, 1970; Kalf and Ch'ih 1968; Tibbetts and Vinograd 1973; Fry and Weissbach 1973; Hecht 1975; Wintersberger and Blutsh 1976; Bertazzoni *et al.* 1977). Mitochondria of higher plant tissues are known to possess different properties from those of animal sources (Bonner 1965). There has been, however, no precise information about the nature of DNA polymerase in mitochondria from higher plants, presumably because of its low activity. Cauliflower head mitochondria can be isolated well and are excellent experimental material (Macrae and Moorhouse 1970). In this communication we described some properties of a DNA polymerase isolated from the mitochondrial fraction of cauliflower inflorescence. This enzyme differs from other three DNA polymerases found in the whole tissue extract of apical portion of the inflorescence.

Preparation of mitochondrial fraction: The mitochondrial fraction was prepared from cauliflower head according to the method described by Sarkissian and Srivastava (1968) and Macrae and Moorhouse (1970) with a slight modification. The frozen cauliflower tissues were homogenized in five volumes of a grinding medium (50 mM Tris-HCl pH 8.0, 1 mM dithiothreitol, 0.5 M sucrose, 1 mM EDTA) by a polytron (Kinematica Co.), filtered with two layers of nylon mesh (40 μ m), and the filtrate was centrifuged at $8,000\times g$ for 20 min. The sediment was suspended in the grinding medium by a teflon homogenizer, centrifuged at $500\times g$ for 10 min, and the sediment was discarded. The supernatant was centrifuged at $7,000\times g$ for 20 min, and thus precipitated mitochondrial

fraction was used immediately or stored in liquid nitrogen until use.

Preparation of DNA polymerase extract: Frozen or fresh mitochondrial fraction was suspended in TGED-buffer (50 mM Tris-HCl pH 7.7, 1 mM dithiothreitol, 1 mM EDTA, 25% glycerol) containing 1 M KCl, homogenized by a teflon homogenizer, followed by repeated thawing and freezing (three times), and incubated at 4°C for one hour. The homogenate was centrifuged at $105,000\times g$ for 60 min. The supernatant was dialyzed overnight against TGED-buffer.

DNA polymerase assay: Two standard reaction mixtures, system I and system III, were used as described in the previous paper (Yamaguchi *et al.* 1979) with slight modifications. System I contained 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, 60 mM KCl, 0.1 mM each of dATP, dCTP, dGTP and 0.5 μCi of [3H]dTTP (20 cpm/p mol), 80 $\mu l/ml$ activated-DNA (isolated from cauliflower, Fukasawa *et al.* 1980), 10 μl of the enzyme, and 400 $\mu g/ml$ bovine serum albumin in 30 μl of assay mixture. Incubation was conducted at 37°C for 60 min. System III contained 50 mM Tris-HCl (pH 8.0), 0.5 mM $MnCl_2$, 1 mM dithiothreitol, 0.1 mM [3H]dTTP (0.5 μCi , 20 cpm/p mol), 2.5 μg poly(rA)(dT)₁₀, 120 mM KCl, 10 μl of the enzyme, and 400 $\mu g/ml$ bovine serum albumin in 30 μl of assay mixture. Incubation was conducted at 35°C for 30 min. After incubating, 25 μl of the medium was placed on a DEAE-cellulose paper (Whatman DE 81, 23 mm) and the radioactivity was counted in a LKB liquid scintillation counter.

Separation of DNA polymerase from mitochondria fraction: The dialyzed enzyme extract containing 18–30 mg of protein was adsorbed onto a DEAE-cellulose column (1.6 $\times$ 22 cm). The column was washed with two bed volumes of TGED-buffer and eluted with a linear gradient from 0 to 0.8 M KCl containing TGED-buffer. 3.5 ml-fractions were collected and assayed for DNA polymerase activity (Fig. 1). The first activity peak assayed with system I was eluted at 0.15 M KCl in TGED-buffer. The elution position corresponded to that of the polymerase-A from whole tissue homogenate (Yamaguchi *et al.* 1979). The second activity peak assayed with system I was eluted at 0.44 M KCl-concentration; this peak was not recognized in the DEAE-cellulose column chromatography of the extract from whole tissue homogenate (Yamaguchi *et al.* 1979; Fukasawa *et al.* 1980). Accordingly, the second activity peak must be attributed to the cauliflower mitochondria. The third activity peak assayed with system III was eluted at higher concentration of KCl (0.49 M) and the existence of two DNA polymerases in cauliflower mitochondria was suggested.

The second and third peak fractions (Nos. 15–20) from the DEAE-cellulose column were combined, diluted with two volumes of TGED-buffer and loaded to a CM-Sephadex column (1.0 $\times$ 18 cm), and eluted with a linear gradient from 0 to 0.8 M KCl containing TGED-buffer. 1.25 ml-fractions were collected and assayed for DNA polymerase activity (Fig. 2). Both the activity peaks assayed with system I and system III coincided well in elution position, though the former peak was low and broad with shoulders. By this reason it would be reasonable to consider that the mitochondria contain one DNA polymerase and two activity peaks in DEAE-cellulose column chromatography might be attributed to a contaminant which interfered to the two assay systems in a

Fig. 1. Separation of mitochondrial DNA polymerase by DEAE cellulose column chromatography. Procedural details are described in the text. ●—●, DNA polymerase activity assayed with system I. ○---○, assayed with system III.

Fig. 2. CM-Sephadex column chromatography. Procedural details are described in the text. ●—●, DNA polymerase activity assayed with system I. ○---○, assayed with system III.

Table 1. Template specificity of mitochondrial DNA polymerase

Template	DNA polymerase activity of CM-fractions			
	No. 12		No. 14	
	System I	System III	System I	System III
Native DNA	40	—	41	—
Denatured DNA	172	—	110	—
Activated DNA	122	195	99	60
Poly(rA)(dT) ₁₀	81	904	87	671
Poly(dA)(dT) ₁₀	741	93	608	111
Poly(rA)	—	86	—	48

Activity was expressed as cpm of [³H]dTTP-incorporation in assay system I and III.

different way.

Properties of mitochondrial DNA polymerase: Several templates were examined to see the template specificity of mitochondrial DNA polymerase. Two CM-fractions (Nos. 12 and 14) were assayed with system I and system III, and found that both of them utilize some extent any of the templates used (Table 1). It is noticeable that in both fractions poly(dA)(dT)₁₀ was utilized well with system I, while poly(rA)(dT)₁₀ was utilized well with system III like found in rat liver mitochondrial DNA polymerase (Tanaka and Koike 1977).

The effect of varying concentration of divalent cations on the activity of our mitochondrial DNA polymerase (CM-fraction) were examined. No prominent effect was observed in the test of Mg²⁺ with assay system I, but the effect of Mn²⁺ was markedly dependent on the concentration with assay system III. A remarkable stimulation to polymerase activity was observed in the reaction mixture containing 0.6 mM Mn²⁺. These results are somewhat different from the data in mitochondrial DNA polymerases from other sources (Myer and Simpson 1970; Wintersberger and Blutsh 1976; Fujisawa *et al.* 1977). This difference appears to be due to the difference in the reaction mixtures used.

The activity of mitochondrial DNA polymerase was markedly influenced by the ionic strength of the reaction mixture. The enzyme has shown its optimum activity at 150 mM KCl with the assay system III. The same high salt requirement has been reported in rat liver mitochondrial DNA polymerase (Myer and Simpson 1968; Fujisawa *et al.* 1977; Bolden *et al.* 1977).

The effect of sulfhydryl-blocking reagent, N-ethylmaleimide (NEM), on the activity of our mitochondrial DNA polymerase was examined, and marked inhibition was observed; a 75% inhibition of the reagent at 1 mM. NEM-sensitivity of mammalian mitochondrial DNA polymerases has likewise been reported (Hecht 1975; Fujisawa *et al.* 1977).

From these results it would be said that plant mitochondrial DNA polymerase is basically not different from mammalian DNA polymerase of mitochondria and play a role in replication of mitochondria DNA.

ACKNOWLEDGMENT

This work was partly supported by a grant from the Ministry of Education, Science and Culture of Japan.

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