SUBCELLULAR LOCALIZATION OF HIGH AND LOW MOLECULAR WEIGHT DNA POLYMERASES OF RAT LIVER

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SUMMARY:

DNA polymerases from isolated rat liver organelles have been characterized by sucrose gradient centrifugation and gel filtration. Mitochondrial DNA polymerase has a molecular weight of about 150,000. The nuclear DNA polymerase has a molecular weight of about 35,000.

INTRODUCTION:

Basic to an understanding of the mechanisms and regulation of gene replication in eukaryotes, is the necessity to determine the number, subcellular location and metabolic function of each of the DNA polymerases found in the cell. In rat liver there are at least three and possibly more distinct enzymes now identified (1-5). Many laboratories have used crude cell extracts as a source of enzyme and usually report two enzyme activities separable on Sephadex columns (6-8). In such preparations it is difficult to draw any conclusions regarding their origin. A second approach has been to isolate and purify specific organelles from which the enzyme is subsequently extracted. This can provide more definitive evidence for subcellular localization. In order to sort out the DNA polymerases of whole cell extracts, to identify their functions and changes

during the cell cycle, development, hyperplasia or neoplasia, it would be advantageous to have criteria by which some of these enzymes can be identified. During further characterization of the tightly bound DNA polymerases of rat liver mitochondria and nuclei, we have found they differ markedly in molecular weight, and provide a useful criterion for distinguishing between them.

MATERIALS AND METHODS:

Mitochondria and nuclei were isolated from 175-225 g rats. Mitochondrial DNA polymerase was purified as previously described (2). Chromatin-bound nuclear DNA polymerase was purified by a new procedure, involving chromatography on DNA-cellulose, hydroxylapatite and DEAE-Sephadex (9). DNA polymerase and alkaline phosphatase assays were performed as described (1,2).

For sedimentation velocity studies, 5.0 ml 6-21% sucrose gradients in TME buffer (0.025 M Tris-HCl pH 8.0, 0.005 M 2-mercaptoethanol, 0.001 M EDTA) were overlaid with 0.2 ml of enzyme to which 1 μ l of *E. coli* alkaline phosphatase (Worthington Biochemicals, Freehold, N.J.) was added as a marker. After centrifuging 18 hr at 35,000 rpm in the Spinco 50L rotor, 8-drop fractions were collected and assayed for DNA polymerase and alkaline phosphatase. Sedimentation coefficient and approximate molecular weights were calculated according to Martin and Ames (10).

Sephadex G-100 and G-200 columns, 2.5 x 32 cm, were equilibrated with TME containing 0.15 M NaCl, and standardized with blue dextran, human gamma globulin II, aldolase, *E. coli* alkaline phosphatase, bovine serum albumin (BSA), ovalbumin, chymotrypsinogen, ribonuclease A and horse heart cytochrome c according to the method of Andrews (11). When DNA polymerase

samples were run, the column was equilibrated with buffer containing 1.0 mg/ml BSA to stabilize the purified enzymes.

RESULTS:

Sucrose gradient profiles for the two enzymes are shown in Fig. 1. Rat liver mitochondrial DNA polymerase has an S value of 9.2 corresponding to a molecular weight of 141,000, assuming an S value of 6.3 and molecular weight of 80,000 for E. coli alkaline phosphatase (12). The nuclear polymerase, on the other hand , is much smaller with an S value of 3.3, corresponding to a molecular weight of about 32,000.

Elution profiles for the two enzymes on Sephadex G-100 are shown in Fig. 2. This method gave slightly higher values of 159,000 for the mitochondrial DNA polymerase and 38,000 for the nuclear DNA polymerase.

DISCUSSION:

The results indicate that the tightly bound DNA polymerases of rat liver mitochondria and nuclei have averaged molecular weight of 150,000 and 35,000 respectively. These molecular weights are only approximate since the two methods used to characterize them rely upon molecular conformation as well as molecular weight. Neither the mitochondrial nor the nuclear enzyme has been purified to homegeneity or in sufficient quantities to use other methods for physical characterization of the proteins.

The rat liver mitochondrial DNA polymerase is one of the largest DNA-dependent DNA polymerase reported so far, and is approximately the same size as the mitochondrial DNA polymerases of calf liver (13) and yeast (14), and nuclear polymerase from sea urchin eggs (15).

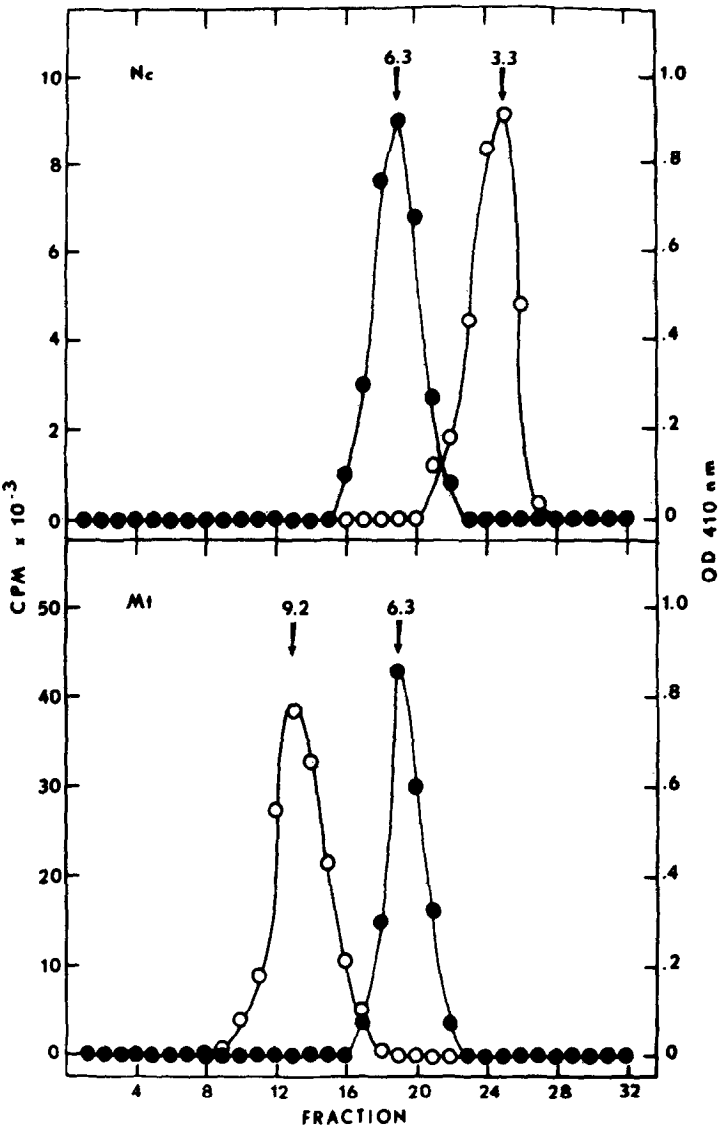

Fig. 1. Sucrose gradient profiles for rat liver nuclear and mitochondrial DNA polymerases. Mitochondrial reaction mixes contained 25mM Tris-HCl pH 8.0, 5mM 2-merceptoethanol, 0.5mM EDTA, 150mM NaCl, 0.015mM each of dATP, dCTP, dGTP and 10μC/ml ^{3}H-dTTP (specific activity 11 C/m mole), 7.5mM Mg-acetate, 100 μg/ml of denatured calf thymus DNA, and 25μl of the fraction tested in a final volume of 125μl. Incubation was for one hr. at 37° and radioactivity was determined by the filter paper disc method (20). Nuclear reaction mixes contained the same components except 50mM KCl was substituted for NaCl and native DNA was used in place of denatured DNA. Alkaline phosphatase assays contained 1mM p-nitrophenyl phosphate, 100mM Tris-HCl pH 8.0 and 25 μl of the fraction in a final volume of 0.5ml. After 30 min. incubation at 37°, 0.5ml of 1N NaOH was added and absorbance was read at 410nm.

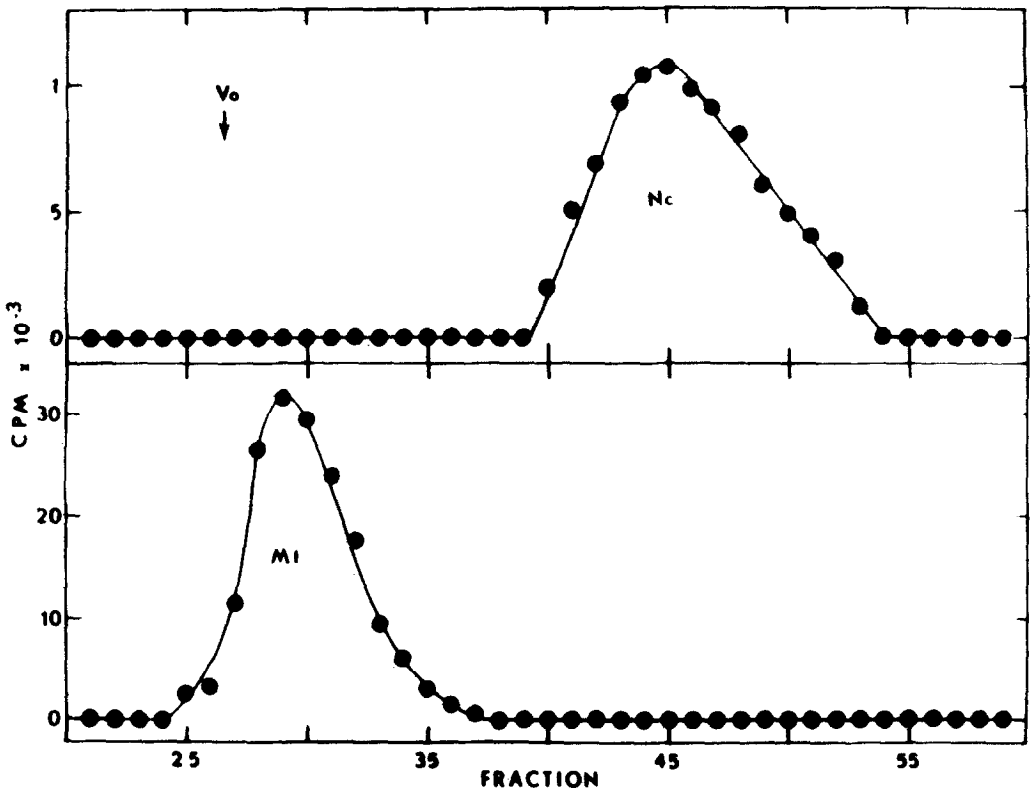

Fig. 2 Nuclear and mitochondrial DNA polymerase elution profiles on Sephadex G-100. Assays were performed as described in the legend to Fig. 1.

The chromatin-bound nuclear DNA polymerase is similar in size to the recently purified enzyme from rabbit bone marrow (16-17). It is much smaller than the highly purified calf thymus DNA polymerase (18).

At present we have found only one species of DNA polymerase in isolated mitochondria. Nuclei prepared in sucrose buffers contain only the low molecular weight enzyme detectable on sucrose gradients in agreement with the findings of Chang and Bollum (16). However, we do observe three peaks of activity on DEAE-cellulose columns (1), suggesting the possibility of multiple species of DNA polymerase in mammalian nuclei.

The relation of the 3.3S nuclear enzyme to those purified in other laboratories remains to be elucidated. The major DNA polymerase activity of crude mammalian cell extracts is found in the cytoplasm. Chang and Bollum, in a survey of several mammalian cell extracts, (16) report the presence of a 6-8S (about 100,000 daltons) soluble enzyme. On the other hand, Greene and Korn (19) found the soluble DNA polymerase of KB cells to be 2.4S (21,000 daltons), Roychoudhury and Bloch (20) report 4.8S for the Ehrlich ascites tumor enzyme, and Berger and Huang found a 3.5S (40,000 daltons) enzyme in regenerating rat liver cytoplasm (4). These large variations in size of the soluble mammalian DNA polymerase suggest the possibility of active subunits or aggregates of the cytoplasmic enzyme. It is unlikely that the cytoplasmic enzyme is identical to the mitochondrial enzyme (2), since the mitochondrial DNA polymerase is not easily extracted with low ionic strength buffer even from the broken organelle, and it has a much higher molecular weight than the soluble DNA polymerase reported by others. The relationship and possible interactions of the small nuclear DNA polymerase and the soluble DNA polymerase of rat liver in nuclear DNA replication is currently under investigation.

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