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BOUND FORMS OF NUCLEAR DNA POLYMERASE IN REGENERATING AND NEOPLASTIC RAT LIVERS

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1. Introduction

Although the DNA polymerase activity in whole cell extract from rat liver is very low [1, 2], isolated nuclei contain much more active enzyme which is present in a tightly bound form [2]. This enzyme can be solubilized only at relatively high ionic strength. According to recent reports the activity of this bound form of DNA polymerase increases considerably in rat liver nuclei after partial hepatectomy [3, 4]. This is in contrast to some previous findings according to which the nuclear DNA polymerase activity remains relatively constant during liver regeneration [5, 6].

To obtain a better characterization of the nuclear DNA polymerase enzymes, we have solubilized and partially purified the bound form of DNA polymerase. We report here that the activity of the bound enzyme (3–4 S) increased considerably after partial hepatectomy and in the livers of rats fed with a diet containing the hepatocarcinogen *N,N*-dimethyl-*p*-(*m*-tolylazo)-aniline (3′-MDAB). A new DNA polymerase similar to reverse transcriptase also appears in the livers of 3′-MDAB rats during the process of neoplastic transformation. By using this method, this DNA polymerase can not be detected in the livers of partially hepatectomized rats.

2. Materials and methods

Male albino rats (Sprague-Dawley) fed ad libitum and weighing between 150–200 g were subjected to partial hepatectomy under ether anesthesia [7]. No liver was removed in sham-operated animals. All operations were performed between 9–11 a.m. Male Fisher rats (120–150 g initial weight) were maintained on Wayne Laboratory Meal (Allied Mills, Inc.) containing 10% (w/w) corn oil (Mazola). This basal control diet was supplemented with 0.06% 3′-MDAB which was dissolved in corn oil and allowed to soak into the Wayne Laboratory Meal pellets replacing pure 10% corn oil given to the control rats. Every experimental point represents an average of 2–3 experiments, each consisting of 4–5 rats.

The liver nuclei were isolated by the method of Chauveau et al. [8]. The tightly bound DNA polymerase was solubilized with 0.2 M phosphate buffer, pH 7.4 [2] and its activity was assayed according to the method of Chiu and Sung [9]. For sucrose density gradient centrifugation, the sample was carefully layered over a sucrose gradient (5–20% w/v) in cellulose nitrate tubes [10] and centrifuged at 37 000 rpm at 0°C for 16 hr. After centrifugation the bottom of each tube was punctured and 0.2 ml fractions were collected. Each fraction was assayed for DNA polymerase activity. Partial purification of the DNA polymerase was achieved by ammonium sulfate fractionation and DEAE-cellulose column chromatography [11].

3. Results and discussion

As shown in table 1, the activity of solubilized DNA polymerase increases considerably in the liver nuclei of rats treated with carcinogen 3′-MDAB as well as after partial hepatectomy. In contrast to the controls,

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Table 1
Solubilized DNA polymerase activities in 3′-MDAB and regenerating rat liver nuclei.

Sample		DNA polymerase activity (pmoles/mg protein/min)
3′-MDAB treated rats	Days on the diet	
Control	12	11.60
Control	28	11.17
Control	112	9.57
DAB	8	9.39
DAB	18	12.03
DAB	28	25.10
DAB	40	25.92
DAB	72	15.29
Partially hepatectomized rats Time after operation		
0		11.34
6		9.62
12		10.59
18		13.50
24		20.78
48		35.88
72		20.68

Complete system for measuring the activity of DNA polymerase contained in a total volume of 0.4 ml: 20 μmoles Tris–HCl buffer, pH 7.4; 2 μmoles dithiothreitol; 20 nmoles each dATP, dGTP and dCTP with 1 μCi [^{3}H]dTTP (52 Ci/mmole), and 20 μg calf thymus native DNA. After incubation at 37°C for 30 min, the reaction was terminated and the radioactivity was determined by the method of Chiu et al. [9].

the activities of solubilized DNA polymerases begin to increase about 18 days on 3′-MDAB or 18 hr after partial hepatectomy. They reach a prominent peak between 30–40 days of the exposure to carcinogens or 48 hr of regeneration. This indicates that the tightly bound DNA polymerase activity rises parallel with the DNA synthesis in both systems [12, 13].

Results of the sucrose density gradient centrifugation are presented in fig. 1a and fig. 1b. All enzymatic activities were correlated to the protein contents of the individual fractions. The protein assays were based on the Lowry et al. [14] procedure. As can be seen, only the 3–4 S DNA polymerase activity can be detected in the control and hepatectomized rats or during the first 8 days of carcinogenic diet. The high molecular 6–8 S enzyme appears prominently only in rats exposed to 3′-MDAB diet for 24 days, peaks around 32 days, decreases rapidly and disappears after 104 days of the exposure to this carcinogen (fig. 1a).

The 6–8 S bound form of DNA polymerase which could be detected only in the livers of rats exposed to 3′-MDAB was further purified and characterized. The phosphate buffer extract of the enzyme solution (DABp) from 3′-MDAB rats fed for 32 days was first subjected to ammonium sulfate concentration between 0.25–0.45 saturation and then to 0.45–0.7 saturation. Both ammonium sulfate fractions were dissolved in 0.01 M phosphate buffer (pH 7.4) containing 2 mM 2-mercaptoethanol and 5% glycerol. The dissolved enzymes were applied to a DEAE-cellulose column (1.3 × 20 cm) equilibrated with the same buffer. The enzymes were eluted with a linear KCl gradient from 0 to 0.5 M dissolved in the same buffer. The DABpl enzyme was obtained from ammonium sulfate saturation between 0.25–0.45 and eluted from the DEAE-cellulose column with KCl concentrations between 0.2–0.3 M. The DABp2 enzyme was obtained from ammonium sulfate saturation between 0.45–0.7 and eluted from the column with 0.1 M KCl. The sucrose density gradient centrifugation profiles of these two enzymes were almost identical to the positions of corresponding enzymes in the original sedimentation patterns of the purified nuclear extracts (i.e. the DABpl enzyme sediments around 6–8 S and the DABp2 enzyme sediments around 3–4 S).

The properties of the partially purified DNA polymerases DABpl and DABp2 using natural and synthetic templates are shown in table 2. It is shown here that the DABp2 enzyme has virtually no activity with the poly AU and poly A·poly U templates. On the other hand, the DABpl enzyme highly catalyzed the incorporation of [^{3}H]TTp into the acid insoluble polymer using poly AU and poly A·poly U as template. The incorporation of [^{3}H]TTP increases slightly when denatured DNA was used as a template for the DABpl enzyme. The partially purified enzyme DABp2 strongly preferred the native DNA as template over denatured DNA.

The DABpl and DABp2 enzymes differed in their Mg^{2+} ion requirements, the former having a maximum activity in about 10 mM $MgCl_2$ as compared with the 15–25 mM $MgCl_2$ necessary for the maximum activity of the latter. These two enzymatic activities also differed in their responses to monovalent ion concentrations. While the DABp2 activity increased about

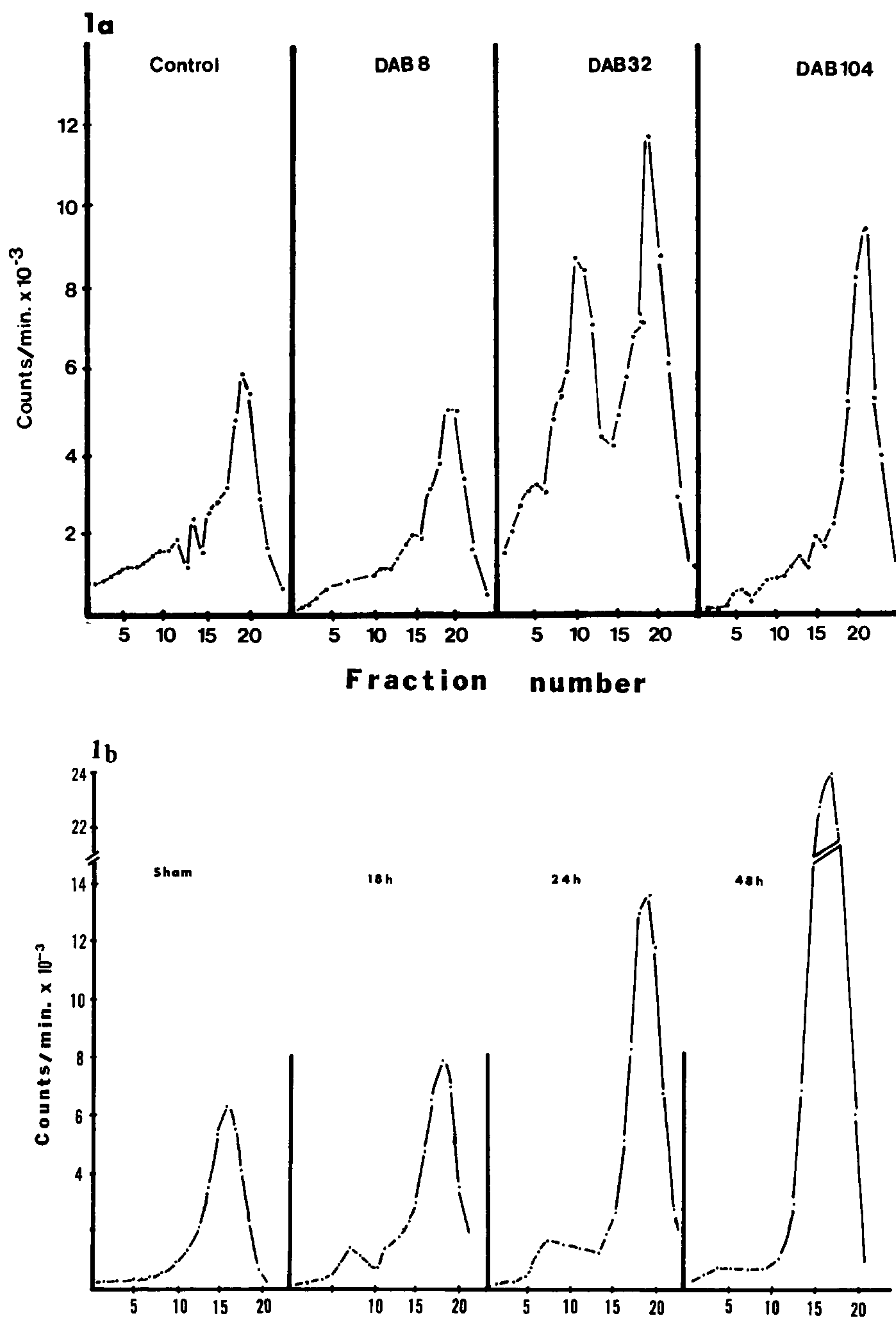

Fig. 1. Sucrose density gradient centrifugation of rat liver nuclear DNA polymerases in: 1a) control and 3'-MDAB treated rats; 1b) sham-operated and hepatectomized rats. Nuclear extract (0.4 ml containing approximately 1 mg of protein) was layered over 4.6 ml of a 5–20% (w/v) linear sucrose gradient. The sucrose was prepared in 0.1 M phosphate buffer pH 7.4 containing 2 mM 2-mercaptoethanol. DAB 8 (etc) days on 3'-MDAB diet. Sham: 24 hr after sham-operation; 6 hr (etc) time after hepatectomy. The bottom of the gradient is to the left.

Table 2
Template specificity of partially purified DNA polymerases in azo dye-treated liver nuclei.

Sample	Enzyme activity (cpm)				
	Poly dAT	Poly AU	Poly A·poly U	Native DNA	Heated DNA
Enzyme DABp1	3513	3102	3069	3839	4571
Enzyme DABp2	3602	242	252	6409	1618

Enzymatic activities were compared using natural and synthetic templates. The conditions of incubation were the same as described in table 1 except that dCTP and dGTP were omitted.

200% in the presence of 30 mM KCl, the DABp1 enzyme was stimulated only about 50%.

It can be concluded that the exposure of rats to a carcinogenic diet elicits the appearance of a bound, high molecular weight DNA polymerase with properties similar to those reported for the reverse transcriptase enzymes.

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