MOLECULAR PHARMACOLOGY, 24:329–335

The Fidelity of Mouse Liver Mitochondrial DNA Polymerase following Long-Term Administration of Carbon Tetrachloride, Diethylnitrosamine, or Phenobarbital

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Received January 10, 1983; Accepted May 12, 1983

SUMMARY

The fidelity of liver mtDNA polymerase was compared in control mice, in mice treated chronically with diethylnitrosamine (DEN), carbon tetrachloride (CCl_4), or phenobarbital sodium (PBNa). Liver mitochondria were isolated, and the mtDNA polymerase activity was obtained by high-salt extraction and subjected to chromatography first on DEAE-cellulose (DE 52) and then on heparin-Sepharose. Fidelity of the mtDNA polymerase was determined, after heparin-Sepharose chromatography, by measuring the relative incorporation of a complementary nucleotide, [^{32}P]dTMP, and a noncomplementary nucleotide, [^{3}H]dGMP, into acid-precipitable material with enzyme activity directed by the template-primer poly(A)·oligo(dT)$_{12-18}$. A decrease in fidelity (increase in relative incorporation [^{3}H]dGMP) was observed after 12 or 13 weeks of treatment with DEN. The fidelity of mouse liver mtDNA polymerase also was decreased after 12 weeks of treatment with PBNa. By contrast, treatment for 12 weeks with CCl_4 was accompanied either by increased fidelity (decrease in the relative incorporation of [^{3}H]dGMP) or no change in fidelity.

INTRODUCTION

Chemicals such as the dialkylnitrosamines as well as others which initiate liver cancer in experimental animals produce genotoxic effects by alkylating liver nDNA and mtDNA (1–3). Alkylation damage to DNA (4, 5), the persistence of DNA damage (6), and its incorporation into newly synthesized DNA following cell division (7) amplify the damage to DNA into successive generations of cells. Adaptation to alkylation damage to DNA may result in significant reorganization of chromosomal DNA.

Many enzymes contribute to the process of DNA synthesis, but the DNA polymerases probably represent the final common pathway in the assembly of the deoxyribonucleotide polymer. Alterations in DNA polymerase activity and fidelity may influence the structure of subsequently synthesized DNA and the expression of the genome (8–10). DNA damaged by alkylation may code for an error-prone DNA polymerase which in turn could lead to the synthesis of DNA with an increased number of noncomplementary bases and possibly with altered sequences. Thus the process would become self-perpetuating, leading to a reorganized genome.

This work was supported by United States Public Health Service Grant CA 21863. It was abstracted from a thesis presented by L. S. M. to the Graduate College of the University of Vermont in partial fulfillment of the requirements for the Ph.D. degree in pharmacology.

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The liver tumor promoter PB[2] (11, 12), in contrast to chemical initiators of liver tumor formation, has no genotoxic potential but appears to sustain the cellular changes initiated by a chemical carcinogen. CCl_4 also appears to be devoid of any genotoxic potential (6, 13) and may be considered a promoter of liver tumor formation. The regeneration of liver cells following CCl_4-induced liver cell necrosis, similar to the regeneration which follows partial hepatectomy, enhances liver tumor formation in animals treated with an initiating regimen of a chemical carcinogen (14). The molecular events which direct liver tumor promotion by chemicals such as PB and CCl_4 are unknown.

We have studied the effects of chronic administration of the chemical carcinogen DEN on the fidelity of mouse liver mtDNA polymerase and compared these effects with changes in liver mtDNA polymerase fidelity in mice treated chronically with CCl_4 or with PB. Mitochondria contain a single DNA polymerase considered to be identical with DNA polymerase γ and which probably directs mtDNA synthesis (15, 16). Changes in mtDNA polymerase fidelity may be representative of the alterations in the fidelity of the DNA polymerase(s) which is (are) responsible for nuclear DNA synthesis.

[2] The abbreviations used are: PB, phenobarbital; CCl_4, carbon tetrachloride; DEN, diethylnitrosamine; PBNa, phenobarbital sodium; 2-ME, 2-mercaptoethanol; PMSF, phenylmethylsulfonylfluoride; SDS-PGE, sodium dodecyl sulfate-polyacrylamide electrophoresis.

0026-895X/83/050329-07$02.00/0

EXPERIMENTAL PROCEDURES

Materials

DEAE-cellulose (DE-52) was obtained from Whatman, Inc. (Clifton, N. J.). Heparin-Sepharose CL-6B was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Calf thymus DNA (Type II), bovine serum albumin (Fraction V), and the deoxyribonucleotide triphosphates were obtained from Sigma Chemical Company (St. Louis, Mo.). Polyadenylate and oligodeoxythymidylate were from Miles Laboratories, Inc. (Elkhart, Ind.). New England Nuclear Corporation (Boston, Mass.) was the source for the radioactive chemicals [*methyl*-^{3}H]thymidine-5′-triphosphate, [8-^{3}H]deoxyguanosine-5′-triphosphate, and [α-^{32}P]thymidine-5′-triphosphate, as well as for Omnifluor premixed LSC fluor.

Adult male Swiss mice (COBS CD-1; Charles River Canada, St. Constant, Que., Canada) weighing 22–25 g were randomly divided into treatment groups of 25–35 mice. A reverse 12-hr light/dark cycle was established, and the animals were allowed a 1-week acclimation period before the treatment regimen was begun.

Methods

DEN was added to the drinking water at a concentration of 4 mg/100 ml; the solution was prepared fresh twice weekly. Control animals for this treatment group received plain tap water. PBNa also was administered in the drinking water, at a concentration of 50 mg/100 ml, and this solution was prepared fresh twice weekly. Plain tap water was supplied to the control animals. Mice were treated with CCl_4 according to the following schedule: A CCL_4/corn oil (Mazola) mixture in a total volume of 0.1 ml was administered by gavage twice weekly on a Tuesday-Friday sequence. The dosage was increased gradually by starting with 0.07 μl of CCl_4/g of body weight for the 1st week, 0.1 μl of CCl_4/g of body weight for the 2nd week, 0.15 μl of CCl_4/g of body weight during the next 2 weeks, and 0.2 μl of CCl_4/g of body weight for the remainder of the treatment period. Control animals received 0.1 ml of corn oil by gavage on the same schedule.

The PBNa-treated mice, CCl_4-treated mice, and their appropriate control groups were maintained for a period of 12 weeks. DEN was administered to groups of mice for periods of 12 or 13 weeks. Mice treated with CCl_4 and their controls were killed 48 hr after the last dose.

Isolation of mitochondria and mtDNA polymerase. The isolation of liver mitochondria was performed as follows: Mice were killed by decapitation and were exsanguinated, and livers were removed and trimmed of extraneous tissue. Livers from two or three animals were combined and minced in 20 ml of a sucrose buffer, Buffer A [0.3 M sucrose, 0.01 M Tris-HCl (pH 7.9), 3 mM $CaCl_2$, 0.5% polyoxyethylene-20-cetyl ether (Brij 58), 2 mM 2-ME, and 1 mM PMSF]. This and all subsequent procedures were performed on ice. The tissue was homogenized in a glass Dounce homogenizer fitted with a loose (B) pestle, and the mitochondrial pellet was obtained by standard differential centrifugation.

Enzymes were solubilized from the mitochondria by extraction with 0.5 M potassium phosphate (pH 7.4) containing 1.0 mM dithiothreitol and 1.0 mM PMSF. Usually two to three mitochondrial extracts were combined at this time, and the combined extracts were centrifuged at 105,000 × *g* for 60 min. The supernatant fraction, containing the crude enzyme preparation, was decanted and dialyzed against a total of 2 liters of Buffer B [0.02 M potassium phosphate (pH 7.5), 10% (v/v) glycerol, 5 mM 2-ME, and 0.1 mM PMSF].

Chromatography. The crude enzyme preparation was subjected to ion-exchange chromatography on DEAE-cellulose (Whatman DE 52), as described by Bolden *et al.* (15). Elution of the enzyme was carried out with a linear gradient of 0.02–0.5 M potassium phosphate (pH 7.5) containing 10% (v/v) glycerol, 5 mM 2-ME, and 0.1 mM PMSF; 25–30 fractions were recovered. Immediately following collection of the column fractions, 25-μl aliquots of each fraction were assayed for mtDNA polymerase activity.

Fractions from the ion-exchange chromatography containing peak enzyme activity were pooled and dialyzed against a total of 2 liters of Buffer C [0.02 M potassium phosphate (pH 7.5), 30% (v/v) glycerol, 5 mM 2-ME, and 0.1 mM PMSF]. Affinity chromatography on heparin-Sepharose was performed as described by Brennessel *et al.* (17). Elution of the enzyme was carried out with a linear gradient of 0.0–1.0 M KCl in Buffer C. Assays for DNA polymerase activity were performed on 25-μl aliquots of each fraction immediately after heparin-Sepharose chromatography. Fractions containing the peak enzyme activity were pooled and dialyzed against Buffer C to remove the KCl. The samples were concentrated by dialysis, either in standard dialysis tubing or in collodion bags, against Buffer C containing 30% (w/v) polyethylene glycol. Enzyme preparations were stored at −70°.

Polymerase assay. DNA polymerase activity was measured by determining the rate of conversion of ^{3}H-labeled deoxyribonucleoside triphosphates into acid-insoluble material following incubation with enzyme. The DNA polymerase assay described by Linn *et al.* (18) was used with a minor modification: either activated calf thymus DNA or the synthetic polynucleotide poly(A)·$(dT)_{12-18}$ served as template-primer. The reaction mixtures, in a total volume of 0.1 ml, contained the following: 50 mM Tris-HCl (pH 7.5 or 8.6); 0.5 mM dithiothreitol; 7.5 mM $MgCl_2$ or 0.5 mM $MnCl_2$; 0.1 M KCl; bovine serum albumin (0.5 mg/ml); 50 μM each of dATP, dCTP, dGTP, and dTTP (dTTP labeled with ^{3}H at 50–100 cmp/pmole); 20 μg of template; and the DNA polymerase preparation. When poly(A)·$dT)_{12-18}$ was used as the template, the only deoxyribonucleoside triphosphate included in the reaction mixture was dTTP. The reaction was initiated by the addition of the enzyme preparation, and the mixture was incubated at 37° for 30 min.

The reaction mixtures were then placed on ice and mixed with cold 10% trichloroacetic acid containing 10 mM sodium pyrophosphate; they were kept on ice for at least 45 min to ensure complete precipitation. Glass microfiber filters (Whatman GF/C) were soaked in 10 mM sodium pyrophosphate, and acid-insoluble product was collected on the filters. The filters were washed in sequence with cold 5% trichloroacetic acid, iced 95% ethanol, and ether and allowed to air dry. The dried filters were placed into plastic vials, 3 ml of fluor was added, and radioactivity was assayed by liquid scintillation spectrometry in a Packard PRIAS PLD liquid scintillation spectrometer. The amount of [^{3}H]dTMP incorporated in the presence of enzyme was corrected for the amount of incorporation in the absence of enzyme. One unit of enzyme incorporates 1 nmole of dTMP into acid-insoluble product per milligram of protein per hour.

Fidelity assay. The fidelity assays were performed as described by Battula and Loeb (19), with modifications appropriate to analysis of mitochondrial enzyme. The fidelity assay is designed to measure the accuracy with which the DNA polymerase is able to copy a synthetic polynucleotide template when supplied with both complementary and noncomplementary deoxyribonucleoside triphosphates. The noncomplementary nucleotide is labeled with ^{3}H and the complementary nucleotide is labeled with ^{32}P so that the error rate of the enzyme can be calculated as the ratio of noncomplementary to complementary nucleotide incorporation in the polynucleotide product.

The reaction mixture, in a total volume of 50 μl, contained 50 mM Tris-HCl (pH 8.6), 0.5 mM dithiothreitol, 7.5 mM $MgCl_2$, bovine serum albumin (0.5 mg/ml), 0.1 M KCl, 20 μM [α-^{32}P]dTTP (25–50 cpm/pmole), 20 μM [8-^{3}H]dGTP (10,000 cpm/pmole), 4 μg of poly(A)·$(dT)_{12-18}$, and the DNA polymerase preparation. Following incubation at 37° for 30 min, the reaction was terminated with the addition of 100 μg of heat-denatured calf thymus DNA followed by cold 1.0 M perchloric acid containing 0.05 M sodium pyrophosphate. The percipitate was mixed with 0.2 N NaOH containing 0.05 M sodium pyrophosphate and was precipitated again with perchloric acid. This procedure was repeated two more times in order to minimize soluble nucleotide contamination. The acid-insoluble product was collected on Whatman GF/C filters, washed extensively with cold 1.0 M perchloric acid containing 0.05 M sodium pyrophosphate, and rinsed in cold 95% ethanol and then in ether. The filters were allowed to air dry, and radioactivity was determined using a dual label program for ^{3}H and ^{32}P in a Beckman LS

7,000 liquid scintillation spectrometer. All reactions were carried out in triplicate, and the experimental values were corrected for the amount of radioactivity incorporated in the absence of enzyme protein.

Mixing experiments. Mixing experiments were performed to determine any possible contributions to enzyme activity from soluble inhibitory or stimulatory factors. The assay conditions and procedures were similar to those described for the mtDNA polymerase assay. Aliquots from two enzyme preparations were present in one incubation mixture, and the amount of [^{3}H]dTMP incorporated into acid-insoluble product was compared with the amount of incorporation by twice the concentration of each separate enzyme. The amount of [^{3}H]dTMP incorporated by the mixture of enzyme preparations should be additive if no inhibitory or stimulatory factors were present.

Lineweaver-Burk analysis was performed to determine the Michaelis constant (K_m) of the mtDNA polymerase, using dTTP as the substrate and poly(A)·oligo(dT)$_{12-18}$ as the template-primer. Only preparations obtained after heparin-Sepharose chromatography were used, and the concentrations of dTTP varied from 5 to 100 μM.

RESULTS

The DNA polymerase extracted from mouse liver mitochondria effectively utilized poly(A)·oligo(dT)$_{12-18}$ as a template-primer (Table 1). Mg^{2+} was the preferred divalent cation at either pH 7.5 or 8.6, and pH 7.5 appeared to be the optimal pH when mtDNA polymerases copied an activated DNA template. When directed by poly(A)·oligo(dT)$_{12-18}$, Mn^{2+} was the preferred divalent cation at pH 7.5. The highest enzyme activities, when directed by the synthetic template-primer, were observed at an incubation medium pH of 8.6, and at this higher pH there was little difference in enzyme activity whether the divalent cation was Mn^{2+} or Mg^{2+} (Table 1). The addition of KCl to 0.1 M in the incubation mixture further increased the incorporation of [^{3}H]TMP by 60% (data not shown).

The DNA polymerase activity extracted from mouse liver mitochondria eluted from a DEAE-cellulose column as a single peak at 0.13–0.15 M potassium phosphate (Fig. 1). Much of the protein in the sample preceded the peak of enzyme activity. A peak of poly(A)·oligo(dT)$_{12-18}$ transcribing activity eluted from a heparin-Sepharose column between 0.48 and 0.54 M KCl (Fig. 2).

Mouse liver mtDNA polymerase activity. Table 2 summarizes the results of the assays of mtDNA polymerase preparations obtained from mice treated with DEN, CCl_4, or PB and their controls after DEAE-cellulose (DE 52) chromatography and after heparin-Sepharose chromatography. Statistically significant differences in mtDNA polymerase activity were apparent in a few instances. However, the variations in enzyme activity were large, and no major trends could be observed.

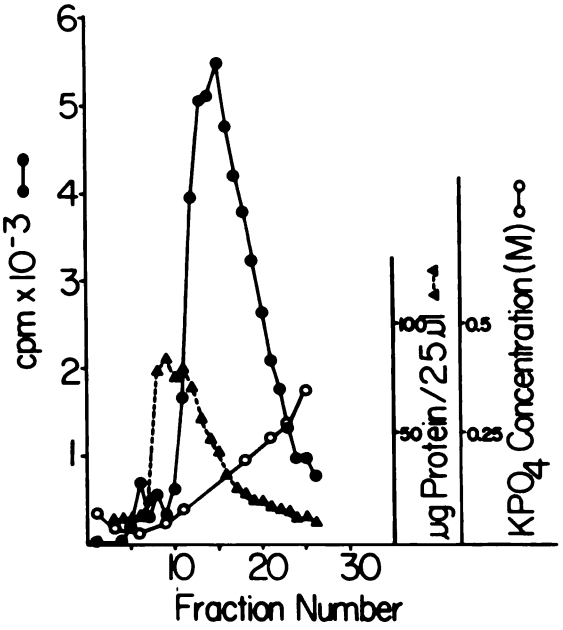

FIG. 1. *Elution of mtDNA polymerase from a DEAE-cellulose column*

The preparation is from a control sample, and mtDNA polymerase activity was directed by 5 μg of poly(A)·oligo(dT)$_{12-18}$.

Fidelity of mtDNA polymerase. Preparations following heparin-Sepharose chromatography served as the source of enzyme, and the results are summarized in Table 3.

The misincorporation frequency of the enzyme preparation obtained from mice treated with DEN for 12 or 13 weeks was 1/570 to 1/1,421, whereas values of the corresponding control enzyme preparations were 1/4,596 and 1/5,482. The mtDNA polymerase from treated animals incorporated a noncomplementary nucleotide into polynucleotide product approximately 5 times more often than did the control enzyme.

The average misincorporation frequency of the enzyme preparations from mice treated with CCl_4 was found to be 1/36,058, whereas that of the control enzyme preparation was 1/7,478. MtDNA polymerase obtained from mice treated with CCl_4 may copy the poly(A)·(dT)$_{12-18}$ template with a 4- to 5-fold increase in accuracy as compared with control enzyme preparations.

The mtDNA polymerase preparations from mice

TABLE 1

Effect of template, pH, and divalent cations on mouse liver mtDNA polymerase activities[a]

Enzyme source	N[b]	Template[c]	pH	Divalent cation		Mn^{2+}/Mg^{2+}
				Mn^{2+}	Mg^{2+}	
DE 52 preparation	4	DNA	7.5	0.32 ± 0.03	0.57 ± 0.04	0.56
			8.6	0.18 ± 0.01	0.32 ± 0.03	0.56
		poly(A)·oligo(dT)$_{12-18}$	7.5	1.60 ± 0.16	1.25 ± 0.06	1.30
			8.6	3.19 ± 0.27	3.02 ± 0.25	1.06

[a] Activity in units (1 unit = 1 nmole of dTMP incorporated per milligram of protein per hour).

[b] N = number of enzyme preparations assayed. Each preparation was derived from the combined mitochondria from two or three mouse livers. All data in this and subsequent tables are expressed as the mean ± 1 SEM when N = 3 or more samples.

[c] 20 μg of DNA; 5 μg of poly(A)·oligo(dT)$_{12-18}$

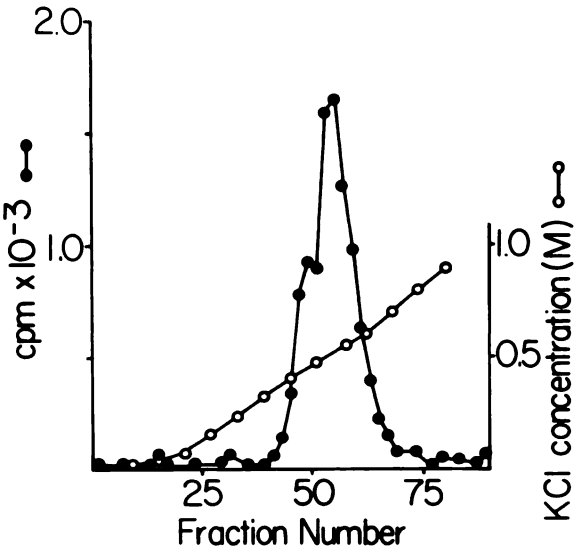

FIG. 2. *Elution of mtDNA polymerase from a heparin-Sepharose column*

A sample from mice treated with DEN was subjected first to chromatography on DEAE-cellulose; the peak fractions were pooled, dialyzed against Buffer C, and then applied to the heparin-Sepharose column. mtDNA polymerase activity was directed by 5 μg of poly(A)·oligo(dT)$_{12-18}$.

TABLE 2

Mouse liver mitochondrial DNA polymerase activity[a]

Enzyme source	*N*	DE 52 preparations	*N*	Heparin-Separose preparations
Control	1	2.30	1	48.1
13-Week DEN	2	1.01, 1.11	2	64.9, 76.7
Control	3	2.09 ± 0.35[b]	3	22.3 ± 3.6
12-Week CCl_4	3	3.11 ± 0.43[b]	3	37.9 ± 3.5[e]
Control	4	1.84 ± 0.20[b]	4	55.6 ± 5.6
12-Week PB	4	2.05 ± 0.26[b]	4	37.8 ± 7.3

[a] Activity in units (1 unit = 1 nmole of dTMP incorporated per milligram of protein per hour) directed by poly(A)·oligo(dT)$_{12-18}$.
[b] Assays performed with 5 μg of template.
[c] Value significantly different from control ($p < 0.01$).
[d] Value significantly different from control ($p < 0.02$).
[e] Value significantly different from control ($p < 0.05$).

treated with PB copied the poly(A)·(dT)$_{12-18}$ template with an average misincorporation frequency of 1/1,028, whereas the average error rate of the control enzyme was 1/8,956. The difference represents an 8- to 9-fold increase in the frequency of misincorporation in enzyme preparations obtained from mice treated with PB.

The results of the mixing experiments are summarized in Table 4. dTMP incorporation was additive when two enzyme preparations were combined, indicating that there were no soluble inhibitory or excitatory factors in the enzyme preparations.

K_m values for mouse liver mtDNA polymerase were between 15.2 and 21.8 μM (Table 5). No major differences were observed between the K_m values for the mtDNA polymerases of control or treated mice.

A limited degree of purification of the mtDNA polym-

TABLE 3

Incorporation of complementary and noncomplementary nucleotides by mtDNA polyerases copying poly(A)·(dT)$_{12-18}$

The enzyme preparations were obtained following heparin-Sepharose chromatography, and fidelity assays were performed as described under Methods, using 4 μg of poly(A)·(dT)$_{12-18}$. *N* is the number of enzyme preparations assayed. The misincorporation frequency is calculated as the ratio of noncomplementary to complementary nucleotide incorporation as measured in polynucleotide product.

Enzyme source	*N*	[^{3}H]dGMP incorporated	[α-^{32}P]dTMP incorporated	Misincorporation frequency (dGMP/dTMP)
		fmoles	*pmoles*	
Control	1	7.1	32.6	1/4,596
12-Week DEN	1	15.3	15.8	1/1,030
Control	1	4.4	24.3	1/5,482
13-Week DEN	2	8.6	12.2	1/1,421
		23.6	12.0	1/510
Control	3	4.6	17.1	1/3,711
		4.4	18.5	1/4,204
		1.5	21.8	1/14,520
				1/7,478 ± 3,523
12-Week CCl_4	3	1.0	38.4	1/38,040
		0.3	21.5	1/65,121
		2.3	11.4	1/5,013
				1/36,058 ± 17,380
Control	2	1.3	9.9	1/7,857
		1.9	18.6	1/10,054
				1/8,956
12-Week PB	2	7.7	11.6	1/1,512
		41.7	22.7	1/545
				1/1,028

TABLE 4

Effect of mixing enzyme preparations from control and treated mice on incorporation of dTMP

Mixing experiments were performed as described under Methods, using 5 μg of poly(A)·$(dT)_{12-18}$. The enzyme preparations were assayed following DEAE-cellulose chromatography. The total amount of protein in each assay (control, treated, and mixture of control and treated) was approximately 40 μg. *N* is the number of enzyme preparations assayed.

Enzyme source	*N*	Enzyme specific activity		Treated control
		Experimental	Predicted[a]	
Control	2	2.21		
12-Week DEN	2	1.16		0.53
Control + 12-week DEN	2	1.46	1.68	
Control	3	1.78 ± 0.15		1.86
12-Week CCl_4	3	3.31 ± 0.59		
Control + 12-week CCL_4	3	2.65 ± 0.27	2.54	
Control	4	1.51 ± 0.14		0.79
12-Week PB	4	1.19 ± 0.16		
Control + 12-week PB	4	1.31 ± 0.07	1.35	

[a] Value calculated assuming no soluble stimulatory of inhibitory factors present in enzyme preparations.

erase was achieved as indicated by SDS-PGE. Among the 8 or 10 protein bands which were present in the DE 52 preparations, a sharp band was observed consistently corresponding to 52,000 M_r (Fig. 3). A protein of 52,000 M_r was the most prominent band remaining after heparin-Sepharose chromatography (Fig. 3). The SDS-PGE profiles from treated mice did not differ from those of controls (Fig. 4).

DISCUSSION

The DNA polymerase activity which was isolated from mouse liver mitochondria was, by several criteria, that of mtDNA polymerase or DNA polymerase γ. The effective utilization of the synthetic ribohomopolymer template at pH 8.6 in the presence of 0.1 M KCl particularly characterizes this enzyme (15, 16). Divalent cation requirements, either Mn^{2+} or Mg^{2+}, correspond to other experimental results when the interaction with the pH of the incubation medium is taken into account (16, 20). These enzyme preparations were only partially purified, but the consistent band obtained on SDS-PGE at 52,000 M_r is close to the molecular weight of 47,000 assigned to the subunit of a highly purified preparation of DNA polymerase (21).

MtDNA codes for only a few of the mitochondrial proteins, and mtDNA polymerase appears not to be among them (22). MtDNA polymerase must be one of

TABLE 5

Michaelis constant of mtDNA polymerase from mouse liver

Assays were performed as described under Methods, except for the use of the varying concentrations of dTTP and of 5 μg of poly(A)·$(dT)_{12-18}$ as template. The enzyme preparations were assayed following heparin-Sepharose chromatography. *N* is the number of enzyme preparations assayed. Values of K_m were determined by Lineweaver-Burk type analysis of kinetic experimental data.

Enzyme source	*N*	K_m
		μM
Control	1	21.0
12-Week DEN	1	15.9
Control	1	15.8
12-Week CCl_4	2	15.2, 18.2
Control	1	18.7
12-Week PB	1	21.8

FIG. 3. *SDS-PGE of liver mtDNA polymerase from control mice: 7.5% polyacrylamide tube gels stained with Coomassie blue*

Tubes *A*, *B*, *C*, and *D* are from preparations concentrated after DE 52 chromatography, Tube *E* following heparin-Sepharose chromatography. The *arrows* on the *left* indicate, from *top* to *bottom*, molecular weights of 165,000, 135,000, 102,000, and 52,000.

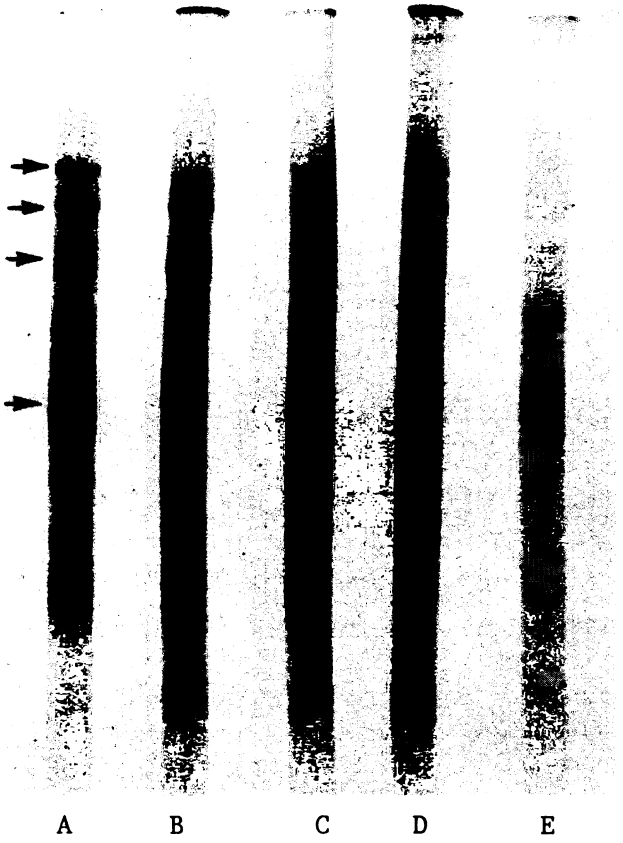

FIG. 4. *SDS-PGE of liver mtDNA polymerase from mice treated for 12 weeks with DEN*

Tubes *A*, *B*, *C*, and *D* are from preparations concentrated after DE 52 chromatography, Tube *E* following heparin-Sepharose chromatography. Other conditions were similar to those described in Fig. 3.

the many mitochondrial proteins which are synthesized by cytoplasmic organelles and then transported and incorporated into the mitochondria. The synthesis of mtDNA polymerase, therefore, would be directed by nuclear genes and chemicals which alter genetic expression and could lead to the synthesis of mtDNA polymerase with altered fidelity. Thus, nuclear DNA damaged by DEN-induced alkylation could code for an increasingly error-prone mtDNA polymerase. It is also possible that metabolic activation of DEN may lead to direct alkylation of newly synthesized mtDNA polymerase before the polymerase is transported into the mitochondira.

The decrease in fidelity of liver mtDNA polymerase from mice treated with PB is more difficult to explain. That liver drug-metabolizing enzyme-system inducers bind with specificity to nuclear receptors has been demonstrated directly (23) and may be inferred from the patterns of cytochrome P-450 which are induced (24, 25). Chemically induced changes in genetic expression when maintained over long time periods may result in maladaptative changes, one of which may be the synthesis of an error-prone mtDNA polymerase. Degenerative changes in the liver that are reflections of maladaptation have been described in rats treated chronically with liver drug-metabolizing enzyme-system inducers, including PB (26). Whatever the cause of this decrease in enzyme fidelity may be, the tumor-promoting potential of PB, and possibly of complex chlorinated hydrocarbons as well (11, 12), may be a consequence of their induction of an mtDNA polymerase with decreased fidelity.

A number of nuclear enzymes in addition to the DNA polymerases play a significant role in both semiconservative and repair DNA synthesis (10, 27). Both DEN and PB, through their effects on nuclear transcription, may induce the formation of a modified accessory protein(s) which could alter DNA polymerase fidelity. The possible presence of such modified accessory proteins should be considered, since the K_m values of the liver mtDNA polymerases were the same whether or not they were derived from control or treated mice. The small quantities of enzyme protein remaining after heparin-Sepharose chromatography did not permit us to carry out further characterization and purification of the enzyme, which would be essential to working out the molecular basis for these alterations in the fidelity of mtDNA polymerases.

Liver regeneration following a necrotizing dose of CCl_4 appears to be comparable to regeneration following partial hepatectomy (14). During chronic CCl_4 administration, as performed in our experiments, each dose of CCl_4 induces a sequence of necrosis and regeneration (28). Mitochondrial DNA replication is dramatically enhanced in response to the regenerative stimulus of partial hepatectomy, being several-fold greater than nuclear DNA replication (29); a similar relationship probably occurs in regeneration following CCl_4. An mtDNA polymerase with increased fidelity (a decrease in error-prone DNA synthesis) would provide a means for ensuring the accuracy of mitochondrial DNA synthesis during regeneration, particularly over a period in which regeneration is stimulated repetitively by a hepatotoxic chemical.

The distortions in liver produced by CCl_4 particularly and by DEN result in marked alterations in the populations of liver cells (30). The heterogeneity of these populations becomes exaggerated and, under these circumstances, enzyme activity and fidelity must represent the mean derived from these varying populations. For example, in CCl_4-treated mice, hepatotoxicity is accompanied by degeneration, necrosis, subsequent regeneration of liver parenchymal cells, and proliferation of stromal elements (28, 30). mtDNA polymerase fidelity may be markedly increased in regenerating cells, but lysosomal alteration in degenerating cells results in marked decreases in fidelity; the net result would be the sum of these two fidelities. A similar situation would occur in the livers of mice treated with diethylnitrosamine over relatively long time periods. Even PB treatment produces at least two different populations of hepatocytes, the hypertrophied centrilobular parenchymal cells in which fidelity of the enzyme may be depressed and the portal hepatocytes which remain normal in appearance and may have mtDNA polymerase whose fidelity matches that of control hepatocytes. It is possible, therefore, that lysosomal enzymes may alter the mtDNA polymerase, particularly in mice treated with CCL_4 or DEN, although this is much less likely in PD-treated mice.

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