An inhibitor of DNA polymerases α and δ in calf thymus DNA

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ABSTRACT

Most, although not all, samples of commercial calf thymus DNA were strongly inhibitory to DNA polymerase α ; the inhibition made the DNA useless as a template for this enzyme. In a pre-assembled DNA polymerase assay mixture (minus enzyme but including activated DNA) the inhibition tended to diminish with time but at a rate that was not predictable, and some inhibition usually persisted. It was concluded that the inhibition was the result of contamination of the DNA by a heparin-like material on the basis of the following: 1) the inhibition could be reversed by treatment of the DNA with heparinase; 2) both the endogenous inhibitory effect of calf thymus DNA as well as the inhibitory effect of heparin on DNA polymerase α are reversed by protamine (which is known to prevent the antithrombin activity of heparin); 3) both the endogenous inhibition and inhibition by heparin are also reversed by ampholyte (which also prevents the antithrombin activity of heparin); and 4) both the endogenous and the heparin-induced inhibitory effects display the same spectrum of activity against mammalian DNA polymerases, i.e. both DNA polymerases α and δ are extremely sensitive whereas, DNA polymerases β and γ are resistant. The last result also suggests the use of heparin as a specific inhibitor of purified mammalian DNA polymerases α and δ , similar to the use of aphidicolin.

INTRODUCTION

Calf thymus DNA has been used widely as a reagent in nucleic acid biochemistry, including its role as a template for DNA and RNA polymerases. In particular, many assays for DNA polymerase activity use 'activated' calf thymus DNA (i.e. partially digested with pancreatic DNase) as template since its use with bacteriophage T2 DNA polymerase 28 years ago by Aposhian and Kornberg (1). We have found that most samples of calf thymus DNA are initially inactive and, in fact, are strongly inhibitory in assays with DNA polymerases α and δ . We report here how to prevent the inhibition and provide evidence on the probable nature of the contaminant responsible for the inhibition. The results suggest that heparin, which reproduces the effects of the endogenous inhibitor in calf thymus DNA, may be useful as a specific inhibitor of purified DNA polymerases α and δ .

MATERIALS AND METHODS

Enzymes

The procedures for purification of DNA polymerases α /primase (2), β (3), γ (mitochondrial and cytoplasmic) (3) and δ (4) from cultured mouse lymphoid (L1210) cells have been described. The DNA polymerase δ was very similar to the calf thymus enzyme of Lee *et al.* (5), including sensitivity to PCNA (6–8). Crude extract was $27,000\times$ g supernatant from Dounce lysate of cultured mouse L1210 cells. Heparinase, from *Flavobacterium heparinum*, was purchased from Sigma (H7505; 3120 units/mg).

DNA

Calf thymus DNA: Source A was Sigma (cat. no. D1501); A0, lot no. unknown, A1, lot no. 45F9515; A2, lot no. 66F9645; A3, lot no. 67F9730; A4, lot no. 92F9600. Source B was Worthington (cat. no. LS-02106, code DNA); B1, lot no. 35H7687; B2, lot no. 36J8373. Source C was Pharmacia (cat. no. 27-4562-02); C1, lot no. 00544562. Source D was Calbiochem (cat. no. 2618); D1, lot no. 704275. Salmon testis DNA was from Sigma (cat. no. D1626); E1, lot no. 82F7085; E2, lot no. 96F7160. *E. coli* DNA was prepared from a lysozyme/EDTA lysate of *E. coli* by the procedure described below for repurification of DNA carried out twice. Calf thymus DNA purchased in activated form was from Pharmacia (cat. no. 27-4575-01); F1, lot no. QE97439; F2, lot no. OG93149. Salmon testis DNA purchased in activated form was from Pharmacia (cat. no. 27-4576-01); lot no. 0080124.

Other reagents

Ampholyte, when not specified, was LKB Ampholine, 3–10, at a final concentration of 0.2%. Ampholyte A was Pharmacia Pharmalyte, 3–10; B, Serva Servalyte, 3–10; C, BDH Resolyte, 3.5–10; D, Schwarz-Mann/ICN Isolyte, 3–10; E, Bio-Rad Biolyte, 3–10.

Heparin (sodium), from porcine intestinal mucosa, was from Sigma (H3125; 170 USP K units/mg). Protamine sulfate, from salmon, was from Sigma (P4380; grade II). Thrombin, USP, bovine, was from Parke-Davis (N-0071-4173-35; Thrombostat).

Reaction conditions

DNA polymerase reactions were carried out in 20 μ l of either mixture A or B, both of which contained 1 mM dithiothreitol; 100 μ M each of dATP, dCTP, dGTP and 20 μ M [3 H]dTTP (0.25 μ Ci/nmol); 100 μ g/ml of bovine plasma albumin, and 100

$\mu\text{g/ml}$ activated DNA. Mixture A had, in addition, 20 mM Tris(Cl⁻) pH 7.6, 2 mM Tricine (K⁺) pH 7.6, 5 mM potassium acetate, 5 mM magnesium acetate, 10% glycerol and 0.01% Nonidet P-40; whereas mixture B had, in addition, 50 mM Hepes(K⁺) pH 7.6 and 7 mM MgCl₂. All reactions used mixture A unless stated otherwise. The complete mixture A was usually made up 5 $\times$ and stored at -20°C ; mixture B was usually made up 2 $\times$, either without the [³H]dTTP and stored (for extended periods) at -20° ; or including the [³H]dTTP and stored only briefly at -20°C (e.g. 2–7 days.) For the purpose of this study, unless stated otherwise, the DNA was added to the reaction mixture shortly before the reactions were carried out. For 'stored mixtures' DNA was included in the 5 $\times$ mixture A (or 2 $\times$ mixture B) and stored at -20°C for periods that ranged from days to months (this is also the way that the mixtures are used in normal practice, and can be stored for up to 1 year).

The reactions received DNA polymerase (0.05–0.5 units) and were incubated at 37°C for 30 min following which acid-insoluble radioactivity was determined (2). One unit of DNA polymerase activity was 1 nmol of total nucleotide in 1 h at 37°C .

Activation of DNA was carried out in a mixture containing 20 mM Tris(Cl⁻) pH 8.1, 20 mM KCl, 5 mM MgCl₂, 0.1 mM CaCl₂, 2.5 mg/ml DNA and 1–2 ng pancreatic DNase (Worthington DSV). It was incubated at 37°C until 15–20% of the DNA was acid soluble; this took from 30 min to several hours and occasionally required additional DNase. The reaction was stopped by adding 10 μl 1 M EDTA per ml, and the mixture was extracted with phenol $\times 3$, and with CHCl₃ $\times 5$; precipitated twice with ethanol; dissolved in 10 mM Tris pH 7.6, 1 mM EDTA; and stored at -20°C .

Treatment of DNA with heparinase was carried out in a mixture containing 50 mM Hepes(K⁺) pH 7.0, 2 mM CaCl₂, 1 mg/ml activated DNA, plus heparinase in amounts indicated. Incubation was at 30°C (9); portions were removed at 1 and 4 h, made 10 mM in EGTA, and used in the DNA polymerase reaction without further treatment.

Blood coagulation reactions were carried out in a final volume of 0.3 ml; this consisted of 0.2 ml human plasma (containing 0.01 M sodium citrate) + 0.1 ml of 20 mM Hepes(K⁺) pH 7.6, 0.14 M NaCl, containing 1 unit thrombin plus additional components as specified. The mixture was assembled in a 10 $\times$ 75 mm glass tube in a 37°C water bath. The thrombin was added last, beginning the reaction; the tube was tilted $\sim 1\times/\text{s}$ and the time (s) required for formation of a visible clot was measured.

Other methods

Repurification of DNA (before or after activation) consisted of treatment with 50 $\mu\text{g/ml}$ RNase A plus 10 $\mu\text{g/ml}$ RNase T₁ at 37°C for 5 h, and 50°C for 16 h; followed by 1% SDS plus 200 $\mu\text{g/ml}$ Proteinase K at 37°C for 5 h and 50°C for 16 h; then extraction with phenol $\times 3$ and CHCl₃ $\times 5$; and precipitation with ethanol $\times 2$.

RESULTS

The initial observation on the effect to be described was that if the standard assay mixture for DNA polymerase α was assembled from its components and used immediately, it was inactive. The same mixture completely assembled including DNA (but not enzyme) and stored for weeks or months was active. When the problem was examined it was found that 'activation' of the assay mixture through 'aging' could sometimes be speeded up at higher temperatures (e.g. 37°C , 1h), or by freezing and thawing several times (using mixture B, Materials and Methods) but this could not be relied upon. The time required for aging varied from hours to months but in some instances the mixture remained inactive (<5% expected activity) even after aging for two years.

The freshly assembled assay mixture was not only inactive, but was also inhibitory; i.e. it would prevent the activity of an already aged and active mixture; and the inhibitory effect was a property of the activated DNA and not the other components of the mixture (Table 1). Inhibition by the activated DNA was also indicated by the fact that there was *more* DNA synthesis with *less* activated DNA in the reaction mixture (Table 1).

It was found that addition of a carrier ampholyte would result immediately in full activity of the assay mixture (Table 2), and that the optimal concentration of ampholyte was $\sim 0.2\%$ (Fig. 1). Even mixtures that were active after aging were usually stimulated by ampholyte although to a much lesser extent than a freshly assembled mixture (Table 2).

Aging of the activated DNA alone did not eliminate the inhibitory effect; the DNA had to be aged as a component of the assay mixture. The particular mixture used for most of the DNA polymerase assays (mixture A, Materials and Methods) was not essential to demonstrate the inhibitory effect of activated calf thymus DNA in its renewal with aging or ampholyte; the same results were seen with a different assay mixture (mixture B, Materials and Methods).

Different DNAs were activated and tested for evidence of an inhibitory effect that could be reversed with ampholyte (Table

TABLE 1. Inhibition of DNA polymerase α by activated calf thymus DNA^a

Aged Mixture (5 $\times$), Complete ^b (μl)	Fresh Mixture (5 $\times$), Minus DNA (μl)	Activated DNA ^c (μg)	DNA Polymerase α Activity (cpm)
4	0	0	15,610
0	4	2	81
2	2	1	310
2	0	0	5,330
2	2	0	7,930
2	0	1	27
0	4	0.2	1,430

^a The DNA was calf thymus A0; final reaction volume, 20 μl ; reaction conditions are given in Materials and Methods.

^b The aged mixture (which included the DNA) had been stored 3 mos (-20°C).

^c The final reaction mixture normally contained 2 μg DNA (Materials and Methods).

TABLE 2. Effect of ampholyte on the reaction of DNA polymerase α with activated DNA^a

Activated DNA	DNA Polymerase α Activity (cpm)	
	Ampholyte –	+
Calf thymus, A0	230	49,300
Calf thymus, A0 (aged) ^b	30,500	57,100
Calf thymus, A1	899	42,500
Calf thymus, A1 (repurified) ^c	459	44,500
Calf thymus, B1	23,000	42,400
Calf thymus, C1	1,248	40,100
Calf thymus, D1	876	41,600
Salmon testis, E1	31,300	26,400
<i>E. coli</i>	26,400	26,700

^a Sources of DNA and reaction conditions are given in Materials and Methods.^b The complete 5 $\times$ mixture (containing the DNA) had been stored for 2 mos, –20°C (Materials and Methods).^c DNA was repurified prior to activation (Materials and Methods). **Figure 1.** Titration of ampholyte effect in assay for DNA polymerase α . The reactions were carried out with activated calf thymus DNA (A0) and varying amounts of ampholyte ('%' refers to the final concentration in the reaction mixture), as described in Materials and Methods.**TABLE 3.** Effect of ampholyte on mammalian DNA polymerases

Enzyme ^a	DNA Polymerase Activity ^b (cpm)	
	Ampholyte –	+
DNA polymerase α /primase	371	75,700
DNA polymerase β	23,500	27,600
Mitochondrial DNA polymerase γ	10,780	13,040
Cytoplasmic DNA polymerase γ	6,710	8,670
DNA polymerase δ	890	43,000

^a The enzymes were purified from cultured mouse cells (Materials and Methods).^b DNA was calf thymus A2.**TABLE 4.** Effect of heparinase on inhibitory calf thymus DNA in DNA polymerase α assay^a

Heparinase (units/mg DNA)	DNA Polymerase α Activity (cpm)			
	1 h ^b Ampholyte –	+	4 h Ampholyte –	+
0	263	26,300	316	27,300
0.2	219	27,700	337	25,700
1	457	27,600	1,651	27,100
5	5,870	28,100	13,240	27,900
25	19,860	27,000	22,600	26,000

^a Activated calf thymus DNA A2 was treated with heparinase in the amounts indicated, following which the DNA was used in assays of DNA polymerase α /primase (Materials and Methods).^b Period of incubation of DNA with heparinase.**TABLE 5.** Effects of heparin and ampholyte on DNA polymerase α assay^a

Heparin ^b	DNA Polymerase α Activity (cpm)	
	Ampholyte –	+
0	17,840	29,800
2 pg	19,450	29,400
20 pg	12,070	32,200
0.2 ng	346	32,900
2 ng	150	32,100
20 ng	196	31,400
0.2 μ g	91	28,800
2 μ g	151	10,120

^a The DNA was calf thymus DNA already activated by the supplier (F1) (Materials and Methods).^b Amount per 20- μ l reaction.

2). All 5 of the samples of calf thymus DNA from one source (A0–4, Materials and Methods) showed the effect (activity without ampholyte was <1–10% activity with ampholyte), as did two samples from a second source (C1,2) (<5%) and one from a third (D1) (Table 2). The only calf thymus DNAs that were active (20–50%) immediately after addition to the assay mixture, without ampholyte, were two samples from a fourth source (B1,2) (Table 2). Two samples of salmon testis DNA (E1,2) and one of *E. coli* (Materials and Methods) were not stimulated by ampholyte but were somewhat less active than calf thymus DNA with ampholyte (Table 2).

Of three samples of commercially activated DNA, a calf thymus (F1) was active and was stimulated ~2-fold by ampholyte; a second calf thymus (F2) and a salmon testis worked very poorly (5–10%) and were unaffected by ampholyte (Materials and Methods).

The ampholyte did not stimulate other reactions of DNA polymerase α /primase. The effect of ampholyte in the self-primed reactions of DNA polymerase α /primase on unprimed poly(dT) and single-stranded circular DNA, as well as the reactions with primed circular DNA and poly(dA)·oligo(dT), was slight to moderate inhibition (10–30%) rather than stimulation (not shown).

Repurification of inhibitory calf thymus DNA (Materials and Methods), either before (Table 2) or after (not shown) activation, had no discernible effect on the inhibition. Passage of the calf thymus DNA over a Sephadex G-50 or Sephacryl S-300 spin column prior to activation, or fractionation of activated DNA

TABLE 6. Effect of protamine on the reaction of DNA polymerase α with activated DNA

Activated DNA ^a	Heparin ^b (2 ng)	Ampholyte	Protamine ^b (ng)	DNA Polymerase α Activity (cpm)
Calf thymus B2	—	—	0	2,800
	—	+	0	10,110
	+	—	0	86
	+	+	0	10,200
	+	—	2	110
	+	—	4	7,020
	+	—	8	15,130
	+	—	16	13,380
Calf thymus A4	—	—	0	143
	—	+	0	13,990
	—	—	0.8	95
	—	—	2	1,759
	—	—	4	5,520
	—	—	8	9,070
	—	—	16	8,470

^a Calf thymus DNA B2 was selected for its activity without ampholyte; A4, for the absence of activity without ampholyte.

^b The amounts of heparin and protamine are per reaction (20 μ l).

over an S-200 column, also did not reduce the inhibitory effect.

With the notable exception of DNA polymerase δ , the other well-characterized mammalian DNA polymerases, DNA polymerase β and both mitochondrial and cytoplasmic forms of DNA polymerase γ , were insensitive to the inhibitory effects of activated calf thymus DNA (Table 3). Two other mammalian cytoplasmic DNA polymerases that utilize poly(rA)·oligo(dT), and are not identified with any of the well-recognized mammalian DNA polymerases (3), were also not affected by inhibitory activated calf thymus DNA (not shown). The sensitivity to inhibition by calf thymus DNA was also not seen with crude mammalian cell extracts, in which most of the DNA polymerase activity was polymerase α . Instead, the effect of ampholyte on the activity of crude extracts was a small, but fairly consistent *inhibition* with different activated DNAs, regardless of source. Both the *E. coli* DNA polymerase I large fragment (Klenow) and bacteriophage T4 DNA polymerase were active with the inhibitory calf thymus DNAs and showed only slight stimulation by ampholyte, usually not greater than 2-fold (data not shown).

The standard ampholyte used in the results presented here was for the pH range 3–10; the current form of the standard ampholyte, 3.5–10, as well as the 5–7 and 7–9 ranges, gave results that were indistinguishable from the 3–10. However, the 2.5–4 and 9–11 produced only 20–30% of the stimulation obtained with the 3–10 standard ampholyte (all tested at 0.2%). Of the pH 3(3.5)–10 ampholytes from five other sources (Materials and Methods), two, ampholyte B at 0.2% and C at 0.04%, gave results equivalent to the standard ampholyte at 0.2%; the other three, ampholytes A, D and E, gave a maximum of 20–70% of the stimulation of the standard ampholyte at the concentrations tested (0.04, 0.2 and 1%).

A possible clue to the explanation for the inhibitory effect of calf thymus DNA appeared in a paper by Wahl *et al.* (10), which referred to an inhibitory effect of calf thymus DNA on DNA polymerase δ and described the use of histone and spermidine, and also treatment with heparinase, in attempts to overcome it. In an earlier paper from the same laboratory treatment of calf thymus DNA with RNase A (2 mg/ml) was reported to reduce inhibition of DNA polymerase α that occurred at 'high concentrations' of activated DNA (100 μ g/ml, or greater) (11). We were unable to reproduce an effect with histone or spermidine

TABLE 7. Effect of ampholyte on antithrombin (anticoagulant) activity of heparin^a

Heparin ^b (240 ng)	Protamine ^b (240 ng)	Ampholyte ^c (%)	Coagulation Time (s)
—	—	0	10
+	—	0	25
+	+	0	8
+	—	0.1	18
+	—	0.5	16
+	—	1	9
—	+	0	9
—	—	0.1	8
—	—	1	10

^a The mixtures contained plasma and thrombin plus the additions, as indicated (Materials and Methods).

^b Amount per coagulation reaction (300 μ l).

^c Final concentration in the reaction mixture.

(although several levels were tested it is possible that we did not achieve optimal conditions), nor with RNase treatment (see repurification of DNA, above); however, heparinase treatment clearly reversed the inhibition (Table 4). The DNA could also be pretreated with heparinase, i.e. before activation with DNase, with the same result (not shown). Further, the addition of very small amounts of heparin to an activated DNA that was relatively free of inhibition, caused severe inhibition; and this was reversed by ampholyte (Table 5).

Protamine, known for its activity in reversing the anticoagulant effect of heparin, was similarly effective in reversing the inhibition of DNA polymerase α by heparin (Table 6). Protamine was also active against the endogenous inhibitor in calf thymus DNA (Table 6). Conversely, ampholyte was able to inhibit the anticoagulant (antithrombin) activity of heparin, similar to the effect of protamine (Table 7).

The mammalian DNA polymerases (Table 3) were tested for their sensitivity to inhibition by heparin, as in Table 5. The amounts of heparin required for 50% inhibition (per 20 μ l reaction with 0.1 unit enzyme) were: DNA polymerase α /primase, 16 pg; DNA polymerase β , 48 ng; mitochondrial DNA polymerase γ , 3.0 ng; DNA polymerase δ , 46 pg.

DISCUSSION

The results indicate that the inhibitor in commercial calf thymus DNA is a heparin-like material; this conclusion is based on: 1) the elimination of inhibition by treatment with heparinase; 2) ability to reproduce a similar inhibitory effect with heparin, including reversal with ampholyte; 3) reversal of both the endogenous and heparin-induced inhibition by protamine; and 4) both the endogenous and heparin-induced inhibitory effect had the same pattern of activity against mammalian DNA polymerases, i.e. DNA polymerases α and δ were highly sensitive, whereas β and γ were resistant.

We assume that a portion of the heparin-like glycosaminoglycans from calf thymus tissue are frequently retained with the DNA by the methods used for its preparation by most commercial sources, which were described as a 'modified Schmidt-Thanhauser' procedure by one (source A). In the case of another (source B), from whom two samples were less inhibitory, it was said to be based on the procedure of Zamenhof (12). We were unable to get any information concerning the procedures used by the other two sources for calf thymus DNAs tested here (sources C and D). It is hard to understand why gel filtration did not free the DNA of the heparin-like contaminant unless there is an association via basic groups on the glycosaminoglycan, or covalent attachment, possibly produced as a chemical artifact of the procedure used to purify the DNA.

In using activated calf thymus DNA with DNA polymerase δ the concentration of DNA is ordinarily kept low, which minimizes the problem of inhibition; and the activities found with activated DNA are relatively poor (5), although this is not the case when higher concentrations of DNA are used together with ampholyte (4). It is less clear to us how (or whether) the problem of inhibition is circumvented by laboratories that use activated calf thymus DNA with DNA polymerase α . Wahl *et al.* recognized the inhibitory effect of calf thymus DNA and described its correction with heparinase (10), providing for us the essential link of the similarity of the inhibitor to heparin. Although Wahl *et al.* referred to the enzyme with which they observed the inhibition as DNA polymerase δ (10) their enzyme has recently been renamed DNA polymerase ϵ (13). It, therefore, seems very likely that what has been described here for DNA polymerases α and δ also applies to DNA polymerase ϵ , an enzyme that resembles DNA polymerase δ in some of its properties but is larger in size and insensitive to PCNA (13).

Others had already made the observation that heparin inhibits mammalian DNA polymerases (14–18), and that DNA polymerase α is more sensitive than DNA polymerases β (14,15) or γ (16). Heparin has also been used as an inhibitor of DNA polymerases but at concentrations that did not even distinguish between DNA polymerases α and β (17); and both the sensitivities and the differences between polymerases in previous reports were generally 10- to 100-fold less than reported here. It has been found that the inhibitory effect of the heparin family of glycosaminoglycans is determined by particular structural features and does not result simply from charge density, although the latter plays a part (18).

Carrier ampholytes were not previously known to have heparin-inactivating properties, whereas the basic protein, protamine, is commonly used for this purpose in blood coagulation. The effect of ampholyte here may be related to an ability of the amphoteric molecules to shield the strongly negative groups in heparin. This might explain why the inactivating effect requires much larger amounts of ampholyte compared to protamine, which may act

stoichiometrically in titrating the heparin. It was reported by Wahl *et al.* (10) that histone or spermidine also had an effect on the activity of DNA polymerase δ with activated calf thymus DNA. An earlier reference to the use of RNase treatment for a similar effect with DNA polymerase α (11) may be rationalized if some of the RNase, which also is basic, remained after treatment of the DNA. The possibility must be considered that some of the other reports of basic proteins or polyamines stimulatory to DNA polymerase α (19–21) may have their basis in this effect.

In practical terms, in carrying out assays for DNA polymerases α and δ , inclusion of ampholyte (or, possibly, protamine) in the assay mixture provides a simple solution to the inhibition problem. There is also the alternative of using salmon testis DNA, especially if conditions can be found that increase its activity to levels ordinarily found with calf thymus (+ ampholyte). (The amounts of heparinase required would make that approach an expensive solution to the problem).

Ampholyte has been used in other laboratories to improve recovery and/or stability of DNA polymerase α (22,23). We have attempted to use ampholyte in the past to stabilize DNA polymerase α /primase, without evident success, but had also noticed that it stimulated enzyme activity in the standard assay with activated calf thymus DNA. It was for this reason that ampholyte was tried when the problem with inactivity/inhibition of the DNA polymerase assay using activated calf thymus DNA was encountered. Our results raise a question about whether some of the observations that were thought to represent improved stability or recovery (due to ampholyte) might instead be explained by the phenomenon described here; if the more purified enzyme that resulted (e.g. from glycerol gradient) had become more susceptible to inhibition by the activated calf thymus DNA and dependent on the ampholyte for its detection in the assay.

The sensitivity of DNA polymerases α and δ to heparin, and insensitivity of DNA polymerases β and γ , mirrors the sensitivity of the same enzymes to the widely used inhibitor, aphidicolin (24,25). DNA polymerases α and δ are now regarded presumptively as the principal replicative DNA polymerases in mammalian cells, DNA polymerase δ on the leading strand of the replication fork, and DNA polymerase α /primase on the lagging strand (26–28). It is possible that the unusual sensitivity of the two enzymes to heparin, or to aphidicolin, or both, reflects a structural/functional feature that the two enzymes share as a requirement for their unique but closely coordinated roles in the cell. The results indicate that heparin may be useful as a reagent for the specific inhibition of purified DNA polymerases α and δ (3,4), similar to the use of aphidicolin for this purpose (24,25). No tests were carried out here with less pure fractions of the enzymes but the insensitivity of DNA polymerase α in crude extracts to the endogenous heparin-like inhibitor in commercial calf thymus DNA cautions against assuming that heparin can be generally substituted for aphidicolin, which appears able to inhibit DNA polymerase α and/or δ even in the intact cell (24,25).

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