Comparison of DNA Polymerases α and δ from Bone Marrow†

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ABSTRACT: DNA polymerases α and δ from rabbit bone marrow were purified to specific activities greater than 30 000 nM TMP incorporated (mg of protein)$^{-1}$ h^{-1}. α is quantitatively predominant. α and δ have the same reaction requirements and are both similarly sensitive to *N*-ethylmaleimide. The primary functional distinction is the association of 3′ to 5′ exonuclease activity with δ. Sedimentation coefficients obtained from zone sedimentation in glycerol gradients and Stokes radii values from gel filtration allow the calculation of true molecular weight and frictional ratios. α exhibits a bimodal pattern, sedimenting at 6 and 8 S on glycerol gradients and demonstrating components corresponding to 40.5- and 65-Å Stokes radii upon gel filtration. The calculated molecular weights of the two forms of α are 100 000 and 215 000; the frictional ratios are 1.34 and 1.65. This and other data suggest a possible monomer–dimer relation. In contrast, δ sediments uniformly at 6.5 S and also behaves uniformly upon gel filtration at 45.5 Å. The molecular weight of δ is distinct at 122 000; its frictional ratio is 1.39. Because of similarities of the DNA polymerizing activities of both forms of α and of δ, it is postulated that α is derived from δ by structural modification, resulting in a decrease in molecular weight, the tendency to aggregate as dimers, and a concomitant loss of 3′ to 5′ exonuclease activity.

The focus of the search for the eukaryotic DNA polymerase responsible for genetic replication has been DNA polymerase α, for reasons recently reviewed (Weissbach, 1977). Although this enzymatic activity was first described more than 18 years ago (Bollum, 1960), it has been resistant to purification and precise definition; evidence for molecular heterogeneity is present in most reports. Consequently, the function of DNA polymerase α in the cell and the nature of the replicative DNA polymerase in eukaryotes remain undetermined.

Recently DNA polymerase δ was discovered in rabbit bone marrow cells (Byrnes et al., 1976). Many characteristics of this enzyme are similar to DNA polymerase α obtained from the same source, and a possible common origin or interconversion is suggested. However, δ is distinct in its association with a 3′ to 5′ exonuclease activity.

A 3′ to 5′ exonuclease is generally found in association with prokaryotic DNA polymerases (Gefter, 1975) and functions as proofreader by removing erroneously incorporated nucleotide bases, thus helping to maintain DNA replication fidelity (Brutlag & Kornberg, 1972). Because DNA polymerase α has no associated exonuclease activity, it has been postulated that replication fidelity in eukaryotes must be augmented by other means (Loeb, 1974; Sedwick et al., 1975; Bollum, 1975). The 3′ to 5′ exonuclease associated with DNA polymerase δ also removes misincorporated nucleotides and augments the fidelity of DNA synthesis (Byrnes et al., 1977). Thus, the discovery of DNA polymerase δ indicates that this vital function may be served by the same mechanism in eukaryotes as in prokaryotes. Therefore, in order to better define both eukaryotic DNA polymerases, comparative physical, functional, and quantitative studies of DNA polymerases α and δ were undertaken.

Materials and Methods

^{3}H-labeled deoxyribonucleoside triphosphates were purchased from Amersham/Searle; unlabeled deoxyribonucleoside triphosphates were obtained from P-L Biochemicals. Oligo($dT_{\overline{16}}$) and poly(dA) were obtained from P-L Biochemicals and annealed in the ratio of 1:40 A_{260}U to form poly(dA)/oligo($dT_{\overline{16}}$). Calf thymus DNA, obtained from P-L Biochemicals, and poly(dA-dT), obtained from General Biochemicals, were activated as described by Aposhian & Kornberg (1962). All template primers were dialysed against 0.01 M Tris[1]-HCl buffer, pH 7.4, containing 0.06 M KCl before use. Dithiothreitol (DTT), *N*-ethylmaleimide (NEM), and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes) were purchased from Calbiochem. Amido black 10B was purchased from Baker. Diethylaminoethyl (DEAE)-Sephadex A-25 was obtained from Pharmacia. Phosphocellulose powder (P11) and GF/C filters were purchased from Whatman. Hydroxylapatite was obtained from Bio-Rad. Ultrogel AcA 34 was purchased from LKB Instruments. Beef liver catalase, bovine serum albumin, horse spleen ferritin, and rabbit muscle aldolase were obtained from Boehringer Mannheim. Ovalbumin was purchased from Worthington. Purified monoclonal IgGκ and human fibrinogen were a gift from Dr. Duane Schultz and rat liver tRNA was a gift from Dr. Karl Muench. All other chemicals were reagent grade.

DNA Polymerase Assays. The assay for DNA polymerases α and δ contained in a final volume of 0.25 mL: 80 mM Hepes buffer, pH 7.0; 90 mM KCl; 0.4 mM $MnCl_2$; 0.75 A_{260} unit/mL of poly(dA)/oligo(dT); 25% (v/v) glycerol; 2 μM [^{3}H]-TTP 400 Ci/mol; and DNA polymerase. The reaction mixture was incubated at 37 °C for 15 min and stopped by the addition of 2 mL of cold 5% trichloroacetic acid containing 10 mM sodium pyrophosphate. The precipitate was collected on a glass fiber filter (Whatman GF/C) and washed with 15 mL of 5% trichloroacetic acid followed by 5 mL of ethanol. The filter was dried and counted in 10 mL of toluene Omnifluor (New England Nuclear) solution in a liquid scintillation spectrometer. When either activated calf thymus DNA or poly(dA-dT) was used as template/primer, the assay was as previously described

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[1] Abbreviations used: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; NEM, *N*-ethylmaleimide; DTT, dithiothreitol.

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TABLE I: Representative Purification of DNA Polymerases α and δ from Rabbit Bone Marrow.[a]

step	procedure	units obtained/units applied sp act. resulting (U/mg)						
IV	phosphocellulose column chromatography	5100/7600 51						
V	DEAE-Sephadex column chromatography	δ rich 2125/5100 163			+	α rich 2579/5100 175		
VI	hydroxylapatite column chromatography	δ = a 438/2125 875	+	unresolved = b 400/2125 not done	+	α = c 965/2125 1137		
VII	second phosphocellulose column chromatography	262/438 4943				683/965 3553		
VIII	Ultrogel AcA 34 column chromatography	δ 127/173 25 546			α_1 192/450 >33 000	+	α_2 208/420 11 071	

[a] Experimental details were as described under Materials and Methods. Poly(dA)/oligo(dT) was used as template/primer to determine DNA polymerase activity. The starting material was the 30 000*g* supernate of the bone marrow cell lysate (step I) which generally contained about 8000 U of DNA polymerase with a specific activity of 0.2–0.4 U/mg of protein.

(Byrnes et al., 1976). One unit of DNA polymerase catalyzes the incorporation of 1 nmol of TMP/h at 37 °C.

3′ to 5′ Exonuclease Assay. The 3′ to 5′ exonuclease activity was assayed by measuring the release of [^{3}H]TMP from acid precipitable poly(dA-dT)·[^{3}H]TMP as previously described (Byrnes et al., 1977).

Other Methods. Protein concentrations were measured using the method of Schaffner & Weissman (1973); bovine serum albumin was the standard. Sample aliquots were adjusted to contain 0.5–5.0 μg of protein. The pH and conductivity of buffer solutions were measured at room temperature with a Radiometer digital pH meter and a Yellowspring conductivity bridge.

Purification of DNA Polymerases α and δ. The starting material, approximately 120 g of rabbit bone marrow, was obtained and processed through step V as previously described (Byrnes et al., 1976). All procedures were carried out between 0 and 4 °C. Steps IV to VII were assayed with poly(dA)/oligo(dT) (Table I). The phosphocellulose elution of DNA polymerase activity (step IV) is very broad; therefore, no attempt was made to separate δ from α. The fractions containing DNA polymerase were pooled, dialyzed, and subsequently chromatographed upon DEAE-Sephadex (step V), whereupon DNA polymerases α and δ begin to separate but overlapped considerably. The fractions containing 3′ and 5′ exonuclease activity were pooled for hydroxylapatite chromatography. Fractions not containing exonuclease provided an additional source of DNA polymerase α. In step VI, approximately 50 mL of DEAE-Sephadex eluate was applied directly onto a hydroxylapatite column (2.0 cm^2 × 15 cm) which had been equilibrated with 0.02 M potassium phosphate, pH 7.5, 20% (v/v) glycerol, and 0.5 mM DTT. The column was washed with the same buffer solution and a 200-mL linear gradient, 0.02–0.5 M potassium phosphate, pH 7.5, was applied. Assay of the eluate for DNA polymerase activity and 3′ to 5′ exonuclease activity revealed two peaks of DNA polymerase: one, DNA polymerase δ was coincident with exonuclease activity; and another, larger DNA polymerase peak, α, which had no associated exonuclease activity (Figure 1). For further studies and purification, the eludate was separated and then pooled into the following fractions: (a) the region of the δ profile outside the projected base of the DNA polymerase α peak; (c) the region of α uncontaminated by δ as indicated by the absence of exonuclease activity, and (b) the intervening area of unresolved α and δ. At this stage of purification, DNA polymerases α and δ were 10 000-fold more purified than the starting material and had specific activities in excess of 2000 U/mg of protein.

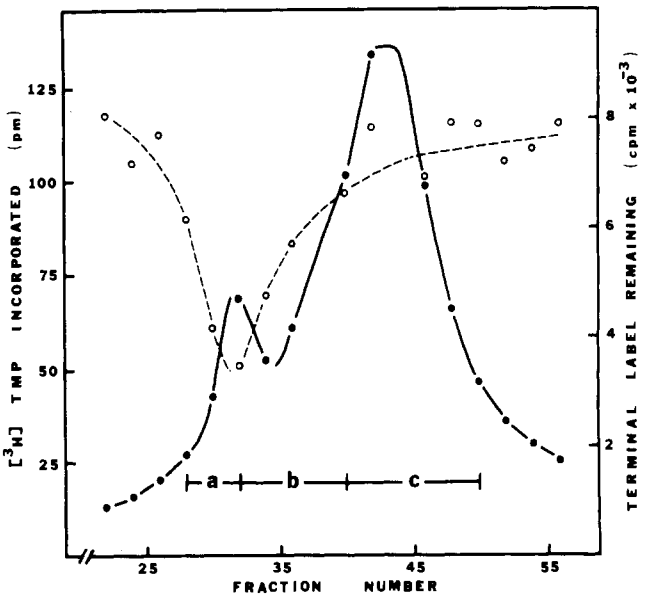

FIGURE 1: Resolution of DNA polymerase δ from DNA polymerase α upon hydroxylapatite chromatography. In Figures 1, 2, 3, 5, and 6, experimental details were as described in the text except 50-μL aliquots were included in the DNA polymerase and 3′ to 5′ exonuclease assays. DNA polymerase activity was assayed using poly(dA)/oligo(dT) as template to direct TMP incorporation (●—●), expressed as picomoles (pm) incorporated. Exonuclease activity (O- - -O) was measured by the release of 3′-terminal label from poly(dA-dT)·[^{3}H]TMP.

Step VII was designed to concentrate the enzymes and transfer them into the appropriate buffer solution for the subsequent procedure, either gel filtration chromatography or glycerol gradient centrifugation. Further purification was also obtained, about three- to fivefold. Ten to 14 mL of hydroxylapatite eluate, containing either DNA polymerase δ (fraction a) or DNA polymerase α (fraction c), was applied directly to a small phosphocellulose column (0.64 cm^2 × 1 cm). The column was equilibrated with a buffer solution containing:

50 mM Tris (pH 7.8); 15% (v/v) glycerol; 0.1 mM EDTA; 1 mM DTT; and 0.1 M KCl. The enzyme was eluted with a stepwise application of buffer solution containing 0.3 M KCl. At this point, DNA polymerase δ generally had a specific activity of about 5000 U/mg and a protein content of about 50 μg in a volume of 2–4 mL. DNA polymerase α had a specific activity of about 4000 U/mg and a protein content of about 200 μg in a similar volume.

In step VII, Ultrogel AcA 34, equilibrated in a buffer solution containing 50 mM Tris, pH 7.8, 15% (v/v) glycerol, 0.1 mM EDTA, 1 mM DTT, and 0.3 M KCl, was poured into a column of 2 $cm^2 \times 107$ cm. A constant hydrostatic pressure of 105 cm of buffer solution was maintained using a Marriotte flask; the flow rate was 5.4 mL/h. Two milliliter aliquots of step VII enzyme, containing either 150–200 U of DNA polymerase δ or 300–500 U of DNA polymerase α, were chromatographed and 1.8-mL fractions were collected. Fifty-microliter aliquots were assayed for DNA polymerase and for 3′ to 5′ exonuclease activity.

Determination of Stokes Radius and Apparent Molecular Weight. Siegel & Monty (1966) demonstrated that the elution position of a protein, from molecular sieving gels, correlated with its effective molecular radius (Stokes radius) but not necessarily with its molecular weight. Therefore, the Ultrogel AcA 34 column was calibrated for both apparent molecular weight and Stokes radius by chromatography of proteins of known behavior (Andrews, 1965, 1970). Two-milliliter samples containing 2 to 8 mg of pure protein standard were applied and 1.8-mL fractions were collected. Protein elution was followed by absorption, at the appropriate wavelengths, using a Gilford spectrophotometer. The void volume of the column was determined by the exclusion of Blue Dextran 2000. The mean elution volumes were determined for six runs of α and five of δ. V_e/V_0 of the standards and DNA polymerases were plotted against the values for both log apparent molecular weight (data not shown) and Stokes radii (Figure 4). The values for apparent molecular weights and Stokes radii of DNA polymerases α and δ were then determined by inspection.

Glycerol Gradient Centrifugation. The sedimentation coefficients of DNA polymerases α and δ were determined by the ratio of distance traveled, the method of Martin & Ames (1961). Linear, 15–30% (v/v) glycerol gradients were formed with a Buchler universal density gradient mixer in 4.8 mL of buffer solution containing: 50 mM Tris, pH 7.8; 0.1 mM EDTA; 1 mM DTT; and 0.3 M KCl. Before use, the gradients were allowed to stand at 4 °C for 6 h. Samples of 0.2 mL, from step VII, were layered upon the gradients and centrifuged at 55 000 rpm for 14 h at 2 °C in a SW 65 rotor (Beckman Instruments Co., Palo Alto, Calif.). The experimental gradient samples contained either 6 U of DNA polymerase α or δ with 200 μg of monoclonal purified IgGκ as an internal protein standard. Identical control gradients were run simultaneously in the same rotor and contained 100 μg of rat liver tRNA and 300 μg of beef liver catalase. After centrifugation, the bottoms of the tubes were punctured in a Buchler piercing unit and 8-drop fractions were collected by hand. Fifty-microliter aliquots of each fraction were used for the DNA polymerase and for the 3′ to 5′ exonuclease assays. IgG was quantified by the method of Mancini et al. (1965), using anti-IgG impregnated radial immunodiffusion plates obtained from Meloy Laboratories (Springfield, Va.). tRNA was detected by its absorption at 260 nm and catalase at 406 nm. All three markers had constant ratios of the distance traveled to their $s_{20,w}^{0.725}$ values.

Molecular Weight and Frictional Ratio Determination. The molecular weight and frictional ratio (f/f_0) values for DNA polymerases δ and the two forms of δ were calculated from classical eq 1 and 2:

$$M = 6\pi\eta Nas/(1 - \bar{v}\rho) \quad (1)$$

$$f/f_0 = a \Big/ \left(\frac{3\bar{v}M}{4\pi N}\right)^{1/3} \quad (2)$$

where M = molecular weight, a = Stokes radius, s = sedimentation coefficient, $\bar{v}$ = partial specific volume, f/f_0 = frictional ratio, η = viscosity of medium, ρ = density of medium, and N = Avogadro's number. A value of 0.725 was assumed for $\bar{v}$ (Martin & Ames, 1961).

TABLE II: Comparative Template Utilization.

template	% act.[a] α	% act.[a] δ
oligo(dT)$_{16}$/poly(dA)	100	100
poly(dA-dT)	5	46
activated calf thymus DNA	73	44

[a] Relative nucleotide incorporation when different templates are used to direct DNA synthesis with DNA polymerases α and δ. Twenty microliters of α or δ, purified through step VII, was used in the assays. DNA polymerase assays were performed as described in Materials and Methods. One hundred percent activity of α was 1.2 nmol of nucleotide incorporated/h; for δ, it was 0.85 nmol.

Resolution and Quantitation of DNA Polymerases α and δ. The purification of DNA polymerase through step VI was essentially as described previously, the primary difference being that poly(dA)/oligo(dT) was used as template/primer in the DNA polymerase assay. Previously, the purification procedure included two DNA polymerase assays, one with poly(dA-dT) to identify δ and another with activated calf thymus DNA to find α. As shown in Table II, poly(dA-dT) gives good activity with DNA polymerase δ but not with DNA polymerase α. On the other hand, the DNA polymerase assay with activated calf thymus DNA as template/primer is demonstrative of DNA polymerase α. We have found that poly(dA)/oligo(dT) is an excellent template/primer for both polymerases. It directs synthesis of an equivalent amount or more of DNA by DNA polymerase α as does activated calf thymus DNA and, likewise, it is as good or better template for DNA polymerase δ as is poly(dA-dT).

Assay of the eluate of the hydroxylapatite column with poly(dA)/oligo(dT) generated the profile shown in Figure 1. As when assayed with poly(dA-dT) (Byrnes et al., 1976), DNA polymerase δ eluted at 0.04 M potassium phosphate, coincident with 3′ to 5′ exonuclease activity. At 0.075 M potassium phosphate DNA polymerase α eluted and had no associated 3′ to 5′ exonuclease activity.

At this point, quantitational comparison of the resolved DNA polymerase α and δ indicates that δ is about 20% of the DNA polymerase activity. Taking into account the elimination of α in the previous step, the relative amount of δ present is small compared with that of α and is in the order of 10% of the DNA polymerase activity.

Gel Filtration Studies. Our previous attempts to purify and characterize these enzymes by gel filtration were limited by severe loss of enzymatic activity during the course of the procedure. The high percentage of glycerol required to preserve enzyme activity and the column dimensions required for adequate resolution gave very slow flow rates and large elution volumes. Consequently, the enzymes were in a dilute state at 4 °C for long periods of time; this resulted in substantial loss of activity. Fortunately, Ultragel AcA 34 provided a matrix

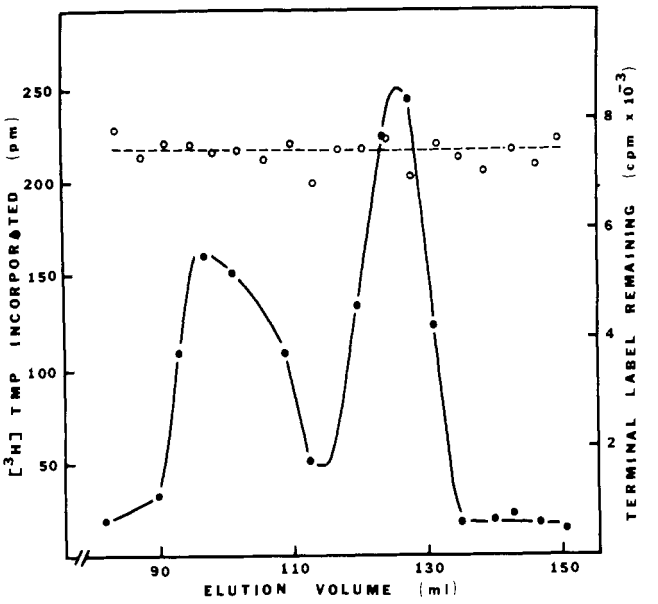

FIGURE 2: Ultrogel column chromatography of DNA polymerase α.

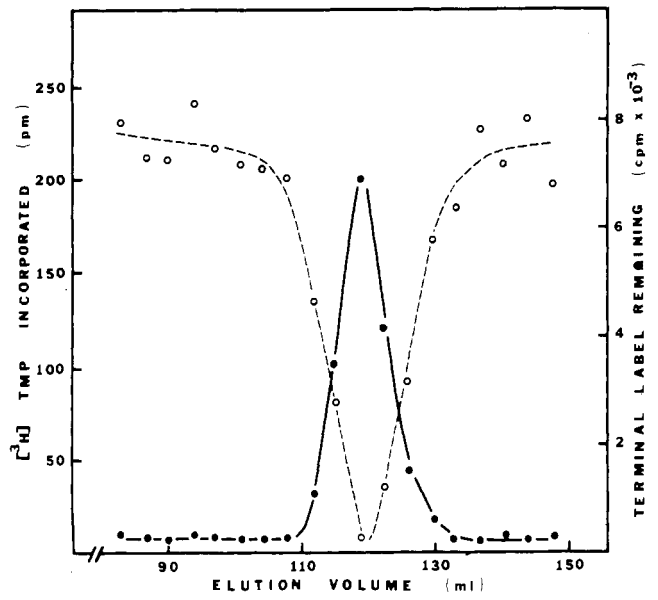

FIGURE 3: Ultrogel column chromatography of DNA polymerase δ.

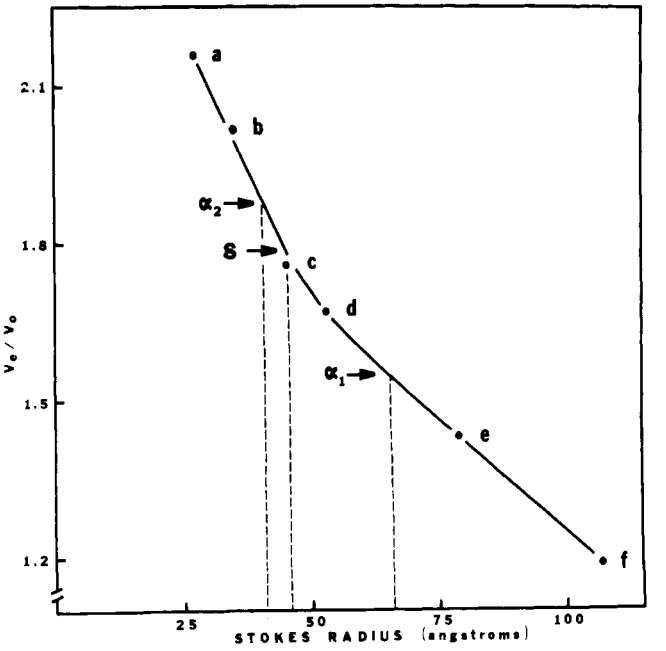

FIGURE 4: Effective Stokes radii of α_1, α_2, and δ. Values were determined on a calibrated Ultrogel AcA 34 column utilizing known values for (a) ovalbumin, (b) bovine serum albumin, (c) aldolase, (d) human IgG, (e) horse spleen ferritin, and (f) fibrinogen, which are plotted against V_e/V_0 of standards and unknowns. Values for a, b, c, and d are from Andrews (1965); e and f are from Siegal & Monty (1966).

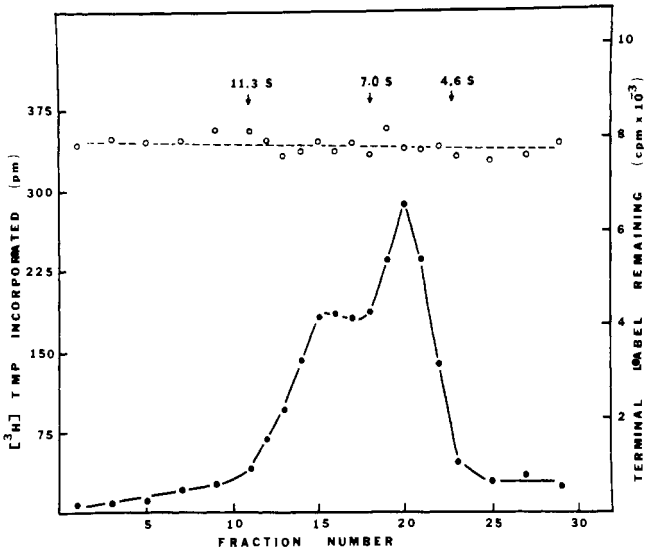

FIGURE 5: Glycerol density gradient centrifugation of DNA polymerase α.

that was able to withstand the hydrostatic pressure required to obtain a more rapid flow rate in 15% glycerol and thus allowed chromatography on a column of sufficient dimensions to give the necessary resolution. Recovery of applied enzyme activity was generally better than 70%.

Chromatography of DNA polymerase α upon Ultragel AcA 34 was performed six times and a consistent pattern was obtained (Figure 2). A broad based, asymmetrical peak of DNA polymerase activity, α_1, eluted at 103.7 mL. This trailed into a narrower based, taller and sharper peak, α_2, at 126.2 mL. No exonuclease activity was associated with either DNA polymerase form. The elution of α_1 corresponds to an apparent molecular weight of 300 000, whereas the behavior of α_2 is that of a globular protein of 110 000. α_1 has an effective Stokes radius of 65 Å and α_2, 40.5 Å (Figure 4).

Substantial purification was accomplished by gel filtration. Protein in the region of α_1 was below the lower limit of the sensitive protein quantitation technique used; therefore, the specific activity of this form was in excess of 33 000 U/mg. α_2 eluted at the front of a small protein peak and its specific activity was generally about 12 000 U/mg.

In contrast to the elution profile of DNA polymerase α, DNA polymerase δ consistently eluted as a uniform entity (Figure 3). Five preparations of DNA polymerase δ demonstrated a narrow based, sharp peak eluting at 119.8 mL. This corresponds to an apparent molecular weight of 140 000 and an effective Stokes radius of 45.5 Å (Figure 4). The 3′ to 5′ exonuclease activity eluted in an identical manner with the DNA polymerase activity. The specific activity of DNA polymerase δ was generally about 30 000 U/mg after this procedure.

Glycerol Gradient Sedimentation Studies. Glycerol gradient sedimentation was carried out in a solution of high ionic strength and conditions identical with the buffer solution used

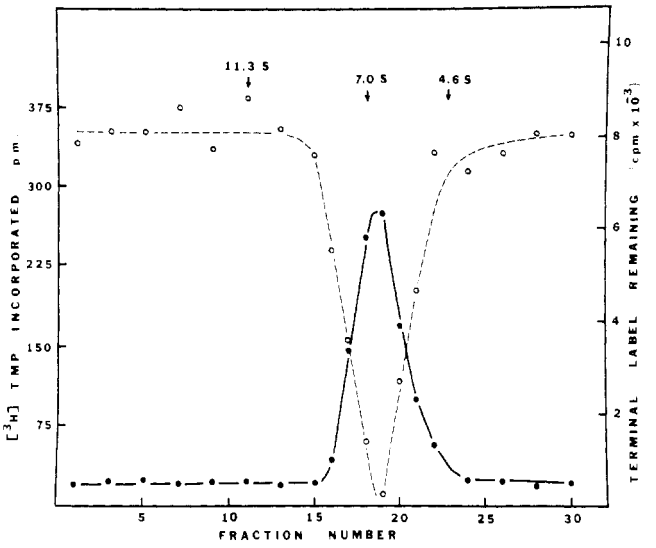

FIGURE 6: Glycerol density gradient of DNA polymerase δ.

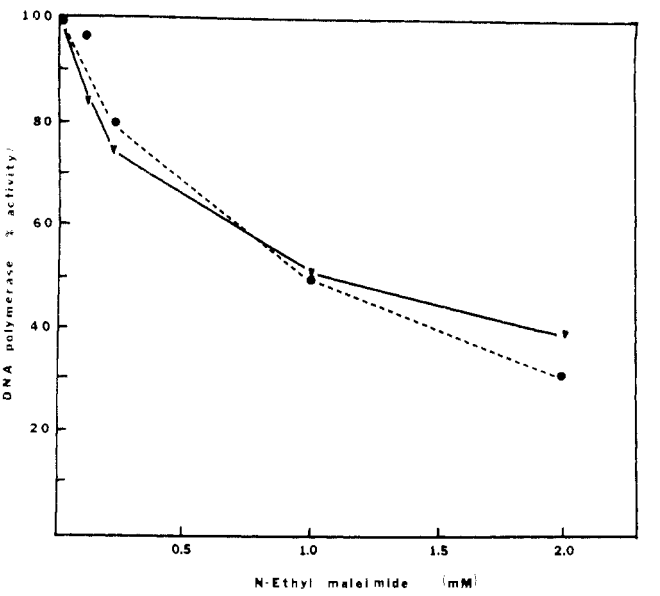

FIGURE 7: Comparative sensitivity of DNA polymerases α and δ to the sulfhydryl inhibitor *N*-ethylmaleimide. The DNA polymerase activity was assayed as described using 20μL of step VII enzyme. *N*-Ethylmaleimide was added to the assay at the start of incubation at 37 °C. One hundred percent activity of α was 390 pmol of TMP incorporated and 100% δ was 500 pmol.

TABLE III: Differentiating Physical Parameters of DNA Polymerases α and δ.

enzyme	app mol wt (× 10^{-3})	Stokes radius (Å)	$s_{20,w_{13}}^{0.725}$ (× 10^{13} s)	true mol wt (× 10^{-3})	f/f_0
α_1	300	65.0	8.0	215	1.65
α_2	110	40.5	6.0	100	1.34
δ	140	45.5	6.5	122	1.39

in the gel filtration studies. The behavior of DNA polymerases α and δ were distinct. α, similar to its behavior upon gel filtration, sedimented in a bimodal manner at 6 and 8 S (Figure 5). There was no exonuclease detectable in association with either form of α. In contrast, DNA polymerase δ sedimented in a uniform fashion at 6.5 S with cosedimenting 3′ to 5′ exonuclease activity (Figure 6).

Determination of True Molecular Weights and Frictional Ratios. The values independently obtained for $s_{20,w}^{0.725}$ and Stokes radius for the three forms of DNA polymerase were used to calculate molecular weight values of 215 000 for α_1, 100 000 for α_2, and 122 000 for δ. The apparent molecular weight obtained for α_1 (300 000) on gel filtration, anomalous when compared with the calculated value, 215 000, is characteristic of an elongate rather than a globular molecular form. The high frictional ratio of 1.69 for α_1 is likewise indicative of an asymmetric molecular configuration. The skewed, trailing pattern of α_1 upon gel filtration is suggestive of aggregation phenomena. α_2 and δ have frictional ratios more typical of globular proteins (Table III).

Functional Comparison of DNA Polymerases α and δ. The primary functional distinction between the two forms of α and δ is the association of 3′ to 5′ exonuclease activity with delta. As noted previously, there is also a difference in template specificity. DNA polymerase δ uses poly(dA-dT) well, whereas α does not (Table II).

The two forms of α are identical in terms of their activation requirements: pH optima for each are betwen 6.5 and 7.0; ionic strength conditions are best satisfied at 90 mM KCl; and Mn^{2+} concentrations of 0.1 mM or Mg^{2+} of 1.2 mM are required for optimal activity (data not shown). We have previously shown that the DNA polymerase requirements are interdependent and influenced by the other components of the reaction mix (Byrnes et al., 1973, 1974); thus, these values pertain specifically to the conditions described in Materials and Methods.

The matter is more complex for DNA polymerase δ as simultaneous activation of the exonuclease reaction influences the net DNA synthesis that is measured. If the exonuclease is selectively inhibited by 5′-AMP, then activation requirements are obtained similar to those of DNA polymerase α (data not shown).

DNA polymerases α and δ exhibit identical sensitivity to increasing concentrations of the sulfhydryl inhibitor *N*-ethylmaleimide. Both are 50% inhibited at 1 mM (Figure 7) under the conditions described.

Discussion

This comparative physical, functional, and quantitational study of the high molecular weight, cytoplasmic DNA polymerases from rabbit bone marrow clearly resolves three distinct forms: α_1, α_2, and δ. Previous studies from this laboratory depended, in part, upon differential poly(dA-dT) utilization to facilitate the isolation of DNA polymerase δ from α (Byrnes et al., 1976). However, it is conceivable that the action of an exonuclease would allow α to better use poly(dA-dT) as template and that demonstration of association of exonuclease activity with the poly(dA-dT) polymerizing activity might be a functional association consequent to overlapping α and exonuclease activities. In this case, δ would not be a unique entity. In order to eliminate this objection, template and assay conditions used in the current study are very sensitive for the detection of both DNA polymerases α and δ. Thus, any ambiguity of DNA polymerase identity or exonuclease association would be apparent.

Under these stringent conditions, DNA polymerase δ is clearly physically distinct and separable from α. δ has molecular values different from α when studied by zone sedimentation and gel filtration procedures (Table III). Furthermore, δ behaves in a uniform manner rather than in the bimodal pattern of DNA polymerase α.

α is the quantitatively predominant form of DNA polymerase obtained from the microsomal extract. Relative

quantitation of δ and α, after resolution, suggests that δ constitutes about 10–20% of the DNA polymerase activity present.

The gel filtration studies, in addition to their usefulness in determining the molecular parameters of the enzymes, contribute substantially to the purification procedure. Although the enzyme preparations began with the bone marrow from 48 rabbits, made hyperplastic by phenylhydrazine injections, the resultant quantities of highly purified enzymes were extremely small and dilute. Consequently, the standard methodologies used as criteria of purity, such as disc gel electrophoresis, were not applicable and the exact degree of purity of DNA polymerases α and δ obtained after step VII could not be determined. However, the specific activities obtained are comparable to, or greater than, most previous reports for mammalian-derived cytoplasmic DNA polymerases and represent a greater than 70 000-fold increase in specific activity over the starting material. (Studies now in progress with a more sensitive method for the determination of protein suggest that the ultimately purified enzymes will be of comparable specific activity to that of purified prokaryotic DNA polymerases.)

The major functional distinction between α and δ is the association of 3′ to 5′ exonuclease activity with DNA polymerase δ. Examination of both the glycerol gradients and the gel filtrate with the sensitive exonuclease assay confirms the absence of 3′ to 5′ exonuclease activity associated with DNA polymerase α and reaffirms its association with δ. The better use of poly(dA-dT) as template/primer by DNA polymerase δ may be due to the association of exonuclease activity. However, selective inhibition of the 3′ to 5′ exonuclease activity by 5′ adenosine monophosphate does not impair the ability of δ to use this template; rather, it stimulates net DNA synthesis by eliminating the turnover reaction (Byrnes et al., 1977). The role of the exonuclease in template specificity and utilization is the subject of continuing investigation in this laboratory.

The primary purpose of this study was to clearly distinguish DNA polymerases α and δ. However, the characteristics they share in common and the nature of the differences between them make it appropriate to speculate about a common derivation. The two forms of α are functionally identical in the studies we have performed (pH, ionic strength, divalent cation optima). α_1 has double the molecular weight and thus may be a dimer of α_2. This interpretation is given further support by the high frictional coefficient of α_1, indicative of molecular assymetry, and the trailing elution profile upon gel filtration suggestive of an aggregation phenomena. Molecular asymmetry has also been described of DNA polymerases from rat liver (Holmes & Johnson, 1973), and from BHK cells (Craig & Keir, 1975).

Inhibition of the polymerase activity by *N*-ethylmaleimide is a characteristic of DNA polymerase α. DNA polymerase δ has similar sensitivity. This, along with similarities of pH, ionic strength, and divalent cation optima, when the exonuclease reaction is inhibited, suggests the DNA polymerizing component of both α and δ is the same.

δ has a molecular weight of 122 000 in comparison with 100 000 for the putative α monomer. The primary functional difference between α and δ is the association of 3′ to 5′ exonuclease with δ. Hence, we are considering the possibility that α is derived from δ by the loss of a 22 000 molecular weight component, accompanied by a concomitant loss of exonuclease activity and the acquisition of the tendency to associate as dimers. Explicit demonstration of possible structural relations between DNA polymerases α and δ awaits further purification, allowing subunit studies of the enzymes.

DNA polymerase α has been considered the probable replicative DNA polymerase in eukaryotes; however, introduction of DNA polymerase δ makes reassessment of the question of the replicative DNA polymerase necessary. The prevailing notion, that eukaryotic DNA replication fidelity is not assisted by the same mechanism as in the well-elucidated prokaryotic enzymes, also must be challenged.

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