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Purification of the α Subunit of Avian Myeloblastosis Virus DNA Polymerase by Polyuridylic Acid-Sepharose

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The α subunit of the avian myeloblastosis virus DNA polymerase could be readily purified to near homogeneity using a polyuridylic acid-Sepharose column chromatography step.

The major RNA-directed DNA polymerase isolated from avian oncornaviruses, termed $\alpha\beta$, has been purified by a variety of techniques including nucleic acid affinity chromatography (2, 3, 5-7). The minor DNA polymerase species found in these avian viruses, termed α, can be separated from $\alpha\beta$ holoenzyme by phosphocellulose chromatography (2). The α subunit, like $\alpha\beta$, both of which possess RNase H activity, can be purified by using a combination of ion-exchange column chromatography, glycerol gradient centrifugation, and nondissociating gel electrophoresis (2, 8). But these steps are time consuming and the final quantity of purified α is limited. This report demonstrates that the phosphocellulose-purified α enzyme can be readily prepared to a high purity using a polyuridylic acid [poly(U)]-Sepharose 4B column chromatography step.

As previously stated, the α subunit of avian myeloblastosis virus (AMV) DNA polymerase can be separated from $\alpha\beta$- or β-enriched enzyme complex by phosphocellulose chromatography (Fig. 1A) (2, 4). As shown in Fig. 1B, the α subunit represents approximately 10% of the protein in the phosphocellulose-purified enzyme. At this stage of purification, α AMV DNA polymerase preparations purified from virus that was obtained from plasma of virus-infected chickens has more lower-molecular-weight contaminants than similar enzyme preparations prepared from virus grown in tissue culture (2).

α AMV DNA polymerase can be purified by adsorption to and elution from poly(U)-Sepharose 4B by a linear salt gradient. As shown in Fig. 2A, α eluted from poly(U)-Sepharose as a single sharp peak of polymerase-RNase H activity. Although α AMV DNA polymerase also bound to polycytidylic acid-agarose, the enzyme was eluted from the column over a wider range of salt concentrations (data not shown). The peak of polymerase activity eluted from poly(U)-Sepharose at 0.26 M KCl. The α polypeptide was purified to a high degree, as demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, in a single step using this nucleic acid affinity chromatography system (Fig. 2B). Recovery of DNA polymerase activity was between 65 and 85% when the enzyme was assayed immediately after elution from the column. Storage of the enzyme on ice at this step resulted in a rapid loss of polymerase activity (~60% overnight) unless the enzyme was immediately concentrated by cyclic dialysis against 50% glycerol (2) or against 30% polyethylene glycol-20% glycerol. The concentrated enzyme was stable for at least several weeks at $-20°C$. Addition of bovine serum albumin (500 μg/ml) to the unconcentrated enzyme stabilized the enzyme at 0°C for several days. Phosphocellulose-purified α AMV DNA polymerase is very stable because of the higher protein concentration in these preparations (1 to 2 mg/ml).

The DNA endo- and exonuclease activity in poly(U)-Sepharose-purified α AMV DNA polymerase preparations was determined. There was no detectable exonuclease activity as measured by release of acid-soluble counts when 17 U of DNA polymerase activity was incubated for 30 min at 37°C with [^{3}H]poly(deoxyadenylic acid-deoxythymidylic acid) (1,570 cpm/μmol). A unit of polymerase activity was defined as the amount of enzyme needed to catalyze the incorporation of 100 pmol of TMP in DNA polymer in 15 min at 37°C using $(A)_n \cdot (dT)_{12-18}$ as template-primer. To measure endonuclease activity, the circular, superhelical colicinogenic factor, ColE1 of *Escherichia coli*, which was grown in the presence of chloramphenical (G. F. Gerard, manuscript in preparation), was utilized. When this plasmid replicates in the presence of chloramphenical, a ribonucleotide segment is covalently inserted into one of the DNA strands (1). Using this factor, the α DNA polymerase (7 U) did not convert [^{3}H]thymidine-labeled 23*S* ColE1 DNA to 17*S* after 30 min of

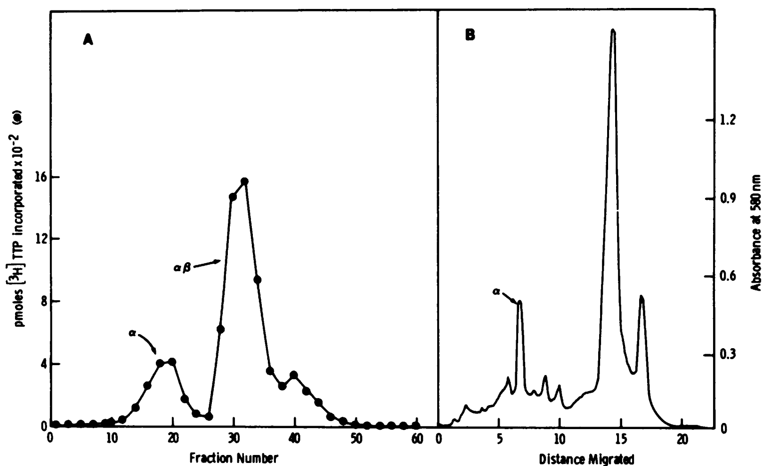

FIG. 1. *Phosphocellulose chromatography of DEAE-cellulose-purified AMV DNA polymerase. (A) DEAE-cellulose-purified AMV DNA polymerase was diluted, adsorbed onto a phosphocellulose column (1.5 by 20 cm) and eluted as previously described (2). The fractions (1.8 ml) were assayed (3 μl) for DNA polymerase activity with $(A)_n \cdot (dT)_{12-18}$ (4). α DNA polymerase was pooled (fractions 16 through 22), concentrated, and stored in buffer A with 0.15 M KCl and 50% glycerol at −20°C (see Fig. 2). (B) A portion of the pooled enzyme was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were stained with Coomassie blue, and a densitometric tracing at 580 nm was made with a Gilford spectrophotometer. Electrophoresis was from left to right.*

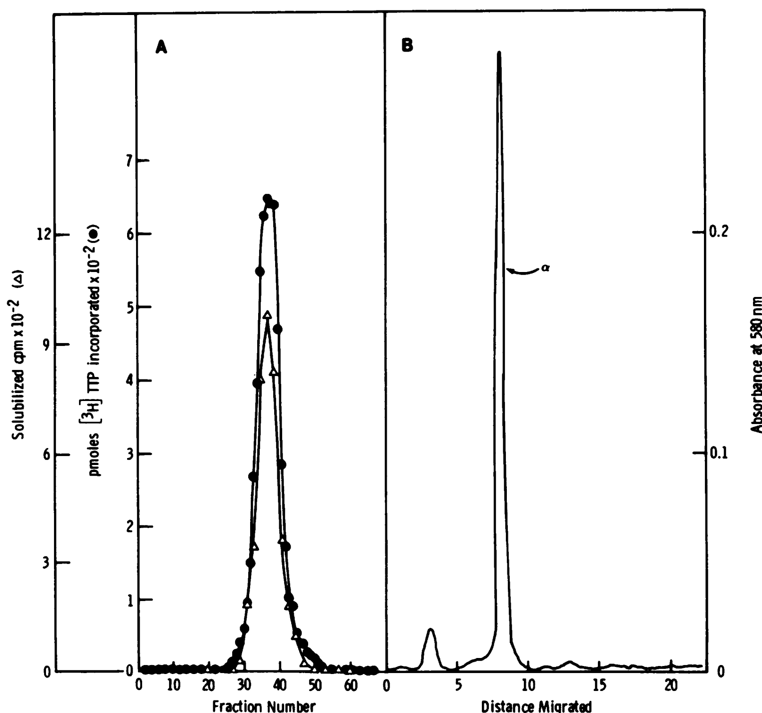

FIG. 2. *Purification of α AMV DNA polymerase by poly(U)-Sepharose 4B. (A) Phosphocellulose-purified α AMV DNA polymerase (diluted 1:6 with buffer A, see below) was adsorbed onto a poly(U)-Sepharose 4B (Pharmacia) column (0.6 by 8 cm) at 4°C. The column had been previously washed with a buffer containing 0.8 M KCl, 50 mM Tris-hydrochloride (pH 7.5 at 20°C), 0.1 mM EDTA, 3 mM dithiothreitol, 0.02% Nonidet P-40, and 10% glycerol (vol/vol). After this high-salt wash, the column was equilibrated with the above buffer (buffer A) minus KCl before adsorption of the enzyme. The adsorbed enzyme was washed with 6 ml of buffer A and then 10 ml of buffer A with 0.1 M KCl. The enzyme was eluted with a 100-ml linear gradient of 0 to 0.6 M KCl in buffer A at a flow rate of 8 ml/h. Aliquots (20 μl) were taken from each fraction (1.5 ml) and assayed for DNA polymerase activity using $(A)_n \cdot (dT)_{12-18}$ (●) and 10 μl for RNase H activity with $[^3H](A)_n \cdot (dT)_n$ (△) as substrate (4). (B) Fractions 36 to 39 were pooled and dialyzed in water (48 h) and then in 0.1% sodium dodecyl sulfate, followed by lyophilization and analysis on sodium dodecyl sulfate-polyacrylamide gels as described in Fig. 1. The minor high-molecular-weight contaminant had a molecular weight of approximately 140,000. Electrophoresis was from left to right.*

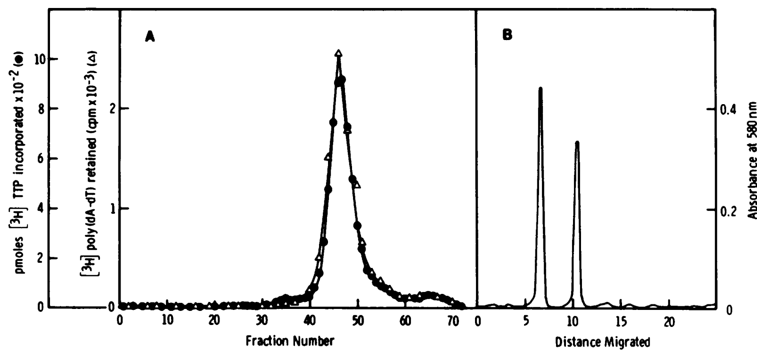

FIG. 3. *Purification of αβ AMV DNA polymerase by poly(U)-Sepharose 4B. (A) Phosphocellulose-purified αβ AMV DNA polymerase was adsorbed and eluted from poly(U)-Sepharose as described in Fig. 2. Aliquots (20 μl) were taken from each fraction and assayed for DNA polymerase activity using* $(A)_n \cdot (dT)_{12-18}$ *(●) and 10 μl for nucleic acid binding activity using [³H]poly-(deoxyadenylic acid-deoxythymidylic acid) (△) as substrate. The conditions for the binding assay using the nitrocellulose filter assay technique were previously described (4). (B) Fractions 44 through 48 were pooled and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in Fig. 2.*

incubation at 37°C. *E. coli* RNase H (0.5 μg), an endoribonuclease, readily converted this 23*S* DNA to 17*S*. Using the same assay conditions described for DNA exonuclease activity, the purified enzyme did not solubilize ^{3}H-labeled AMV 4*S* or 70*S* RNA (10^4 cpm/μg). Several preparations contained trace amounts of RNase activity by measuring acid-soluble counts using $[^3H](U)_n$ (80 cpm/pmol) as substrate. As described above, the purified enzyme preparations were not able to nick the DNA or RNA segment of ColE1 DNA, suggesting little RNase contamination.

αβ AMV DNA polymerase can also be extensively purified by using poly(U)-Sepharose chromatography (Fig. 3). The enzyme eluted from poly(U)-Sepharose at 0.36 M KCl with a 90% recovery of polymerase activity. In contrast to α, the αβ enzyme was quite stable upon purification.

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