# BINDING OF HeLa DNA-POLYMERASE α TO BLUE DEXTRAN: A POSSIBLE ARTEFACT IN MOLECULAR WEIGHT DETERMINATION BY GEL FILTRATION

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## 1. Introduction

We have previously described the isolation of a major soluble cytoplasmic protein fraction from HeLa cells by chromatography on poly(A)-Sepharose [1]. This protein fraction is eluted from the poly(A) column by 0.2 M NaCl and appears to contain a major species of 38 000 daltons by SDS-acrylamide gel electrophoresis along with several minor bands. In the course of a systematic search for enzymes in this fraction, we have looked for a DNA-dependent DNA-polymerase activity. The present paper reports on the presence of such an activity. Its physico-chemical and enzymological characteristics, which are those of the α DNA-polymerase [2,3], clearly distinguish it from the major polypeptide of 38 000 daltons present in the above protein fraction.

During the course of this study, it was observed that the polymerase could bind to the Blue Dextran used to measure the void volume of Sephadex columns. It is the purpose of this paper to bring attention to this artefact which may be misleading in the interpretation of gel filtration data in terms of molecular shape [4].

## 2. Materials and methods

Conditions for growth and labeling of HeLa cells, cellular fractionation and chromatography on poly(A)-Sepharose column of the cytosol were as described previously [1].

Reaction mixtures for DNA-polymerase assay contained in a final volume of 200 μl: 10 μmol Tris–HCl, pH 9 (25°C), 2 μmol $MgCl_2$, 0.2 μmol β-mercaptoethanol, 40 μg bovine serum albumin, 25 μg calf thymus DNA (Sigma type I) 'activated' according to Aposhian and Kornberg [5] 20 nmol each of dGTP, dCTP and dATP (Boehringer, France), 0.14 nmol of [$^3$H]dTTP (28 Ci/mmol, Amersham, England) and up to 70 μl of enzyme fraction. After 30 min of incubation at 37°C, the reactions were stopped by addition of 0.2 ml of 0.1 M Na-pyrophosphate and 3 ml 10% trichloracetic acid. The precipitates were collected on Whatman GF/C glass filters and counted in an Intertechnique SL 30 scintillation counter. Protein concentrations were estimated either by absorbance at 280 nm or according to Kuno and Kihara [6] using bovine serum albumin as a standard.

All other experimental details are given in the legends to figures.

## 3. Results

Fig.1 shows that the protein fraction from HeLa cytosol which is eluted from poly(A)-Sepharose by 0.2 M NaCl (referred to as F 0.2 M) [1] does contain a DNA-dependent DNA-polymerase activity. This activity appears to be quite sensitive to ionic strength (65% inhibition at 0.2 M KCl) and to the thiol reagent *p*-chloromercuribenzoate (80% inhibition at 0.5 mM), both of these features being known characteristics of the cytoplasmic DNA-polymerase-α [2,3]. In order to decide whether this activity is carried by the major constituent of F 0.2 M of which we had previously reported the physico-chemical properties [1], we compared its behavior on a glycerol



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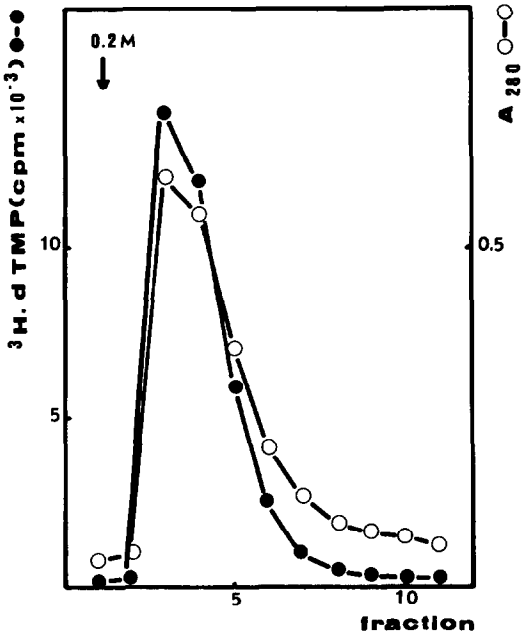


Fig.1. Poly(A) Sepharose chromatography of HeLa cytosol. 60 mg of cytosol in buffer A (10 mM Tris–HCl pH 7.4 25°C, 10 mM NaCl, 1.5 mM $MgCl_2$, 1 mM β-mercaptoethanol) containing 5% glycerol was loaded onto a 2 ml poly(A) Sepharose column and eluted by 0.2 M NaCl in buffer A as previously described [1]. Each fraction was assayed for protein concentration ($A_{280}$, o———o) and DNA polymerase activity (•———•) as detailed in Materials and methods. The flow through of the column which contains most of the protein and around 70% of the total DNA-polymerase activity has not been represented.

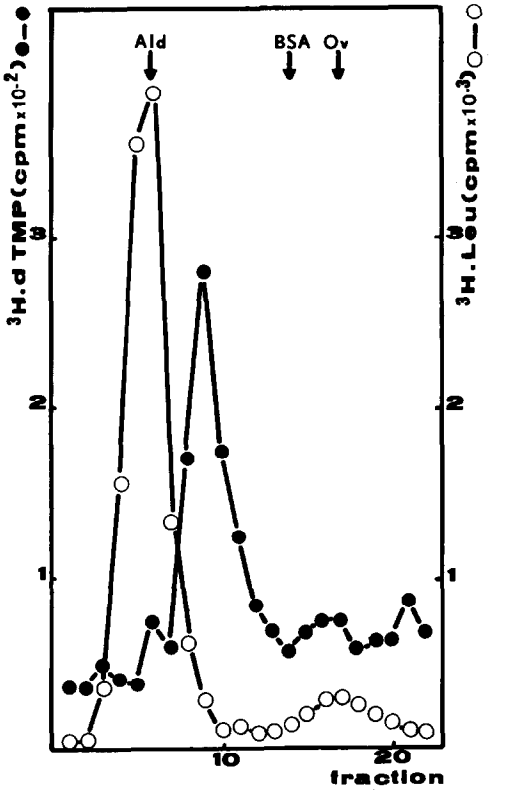


Fig.2. Glycerol gradient centrifugation. Composite diagram of two separate gradients (14 ml of 15–30% glycerol w/v in buffer A) containing either 30 μg of [$^3$H]leucine labeled F 0.2 M ($10^4$ cpm) or 1 mg of cold F 0.2 M. In the latter case, the gradient contained 0.5 mg/ml bovine serum albumin. These two gradients as well as three others containing respectively aldolase, bovine serum albumin and ovalbumine as markers were run in parallel. Centrifugation was at 4°C for 37 h at 38 000 rev/min in the SB 283 rotor of the IEC B60 ultracentrifuge. 0.5 ml fractions were collected and assayed either for acid precipitable $^3$H-F 0.2 M (o———o) or for DNA polymerase activity (•———•).

gradient and a Sephadex column with that of the major polypeptide. For pratical reasons, a radioactive preparation of F 0.2 M labeled with [$^3$H]leucine was used and run in parallel.

Fig.2 shows that the peak of activity sediments at 6.5 S while that of the marker F 0.2 M is at 7.5 S. The same sedimentation profile was observed when the gradient was run at 0.5 M NaCl. This result already demonstrates that the polymerase is distinct from the major constituent of F 0.2 M.

The composite diagram of fig.3 shows that the activity profile is exactly superimposed to that of the Blue Dextran and therefore that the polymerase appears to be excluded from Sephadex G-200. However, when the same preparation is run under strictly identical conditions but in the absence of Blue Dextran, the activity appears as a broad peak eluting between the F 0.2 M and ovalbumin markers. In addition to confirming the non-identity of the polymerase and the major protein of F 0.2 M, this experiment clearly demonstrates that the enzyme can quantitatively bind to Blue Dextran in our conditions.

In order to confirm this binding and as a possible means of purification, we have prepared a Blue Dextran–Sepharose column. Fig.4 shows that the polymerase from crude cytosol 4B does indeed bind

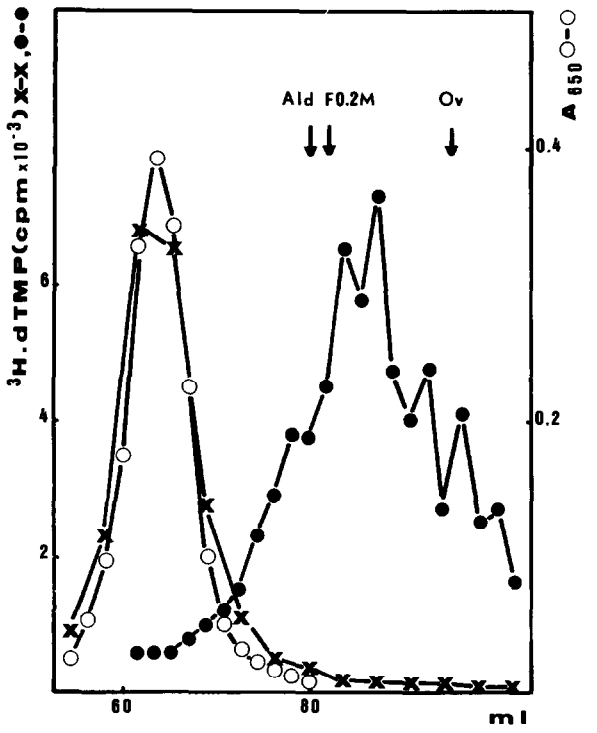


Fig.3. Sephadex G-200 column chromatography. Composite diagram of the elution profile of F 0.2 M DNA-polymerase activity chromatographed with (x———x) or without (•———•) 2 mg Blue Dextran 2000 in the loaded sample ( 1mg protein in 1 ml). A 1.5 X 90 cm column was equilibrated and developped at 12–15 ml/h under 20 cm hydrostatic pressure by buffer A containing 0.1 M NaCl. 2 ml fractions were collected in tubes containing 0.1 ml of 10 mg/ml bovine serum albumin solution and aliquots assayed for enzyme activity. Blue Dextran was monitored by its absorbance at 650 nm. $^{3}$H-F 0.2 M, aldolase and ovalbumin were run successively on the same column.

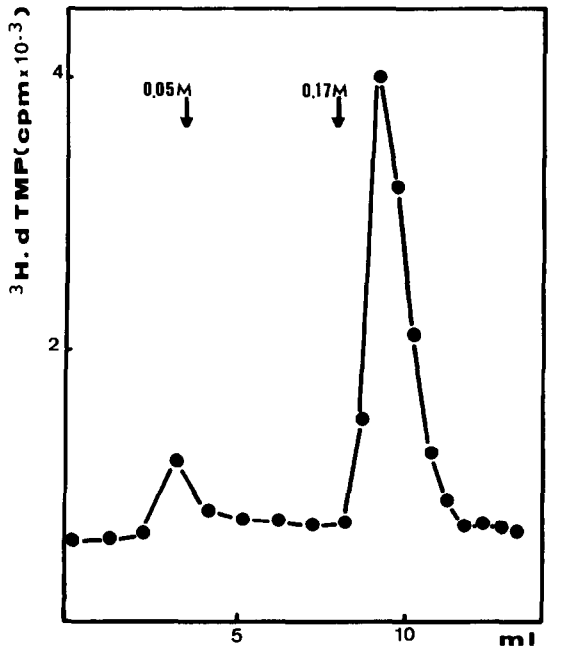


Fig.4. Blue Dextran–Sepharose chromatography of crude cytosol. Blue Dextran–Sepharose containing 10 mg dye per ml of packed Sepharose 4-B was prepared according to Ryan and Vestling [7]. A 1 ml column was packed in a pasteur pipet and equilibrated with buffer A in which 50 mM KCl had been substituted for 10 mM NaCl. 3.5 ml of cytosol containing 14 mg protein were loaded at 6 ml/h followed by a 5 ml wash with the same buffer. Elution was performed by a 0.17 M KCl step in the same buffer. Enzyme activity was assayed as usual.

to such a column from which it can be eluted by 0.17 M KCl.

## 4. Discussion

The data presented in this paper show that the previously described [1] protein fraction from HeLa cytosol which binds to poly(A)-Sepharose and is eluted by 0.2 M NaCl (F 0.2 M) contains as a contaminant a DNA-dependent DNA-polymerase which by all criteria can be identified to the cytoplasmic α polymerase.

In the course of this study, we found that this enzyme could bind to the Blue Dextran used to measure the void volume of the column. A striking discrepancy between the sedimentation coefficient and the gel filtration behavior had already been observed by several authors [4,8,9] and had led Holmes and Johnston [4] to postulate a highly asymmetric shape for the cytoplasmic enzyme from rat liver. Running a Sephadex G-200 column at low ionic strength, Sedwick et al. [8] found that the α polymerase from KB cells elutes with the Blue, exactly as does in our hands the HeLa enzyme in 0.1 M NaCl. At 0.45 M NaCl, the KB enzyme does no longer travel with the Blue [8]. This agrees well with our result of fig.4 which shows that the enzyme can be eluted from a Blue Dextran–Sepharose column by

0.17 M KCl. Also working with the HeLa enzyme, Weissbach et al. [9] found that it eluted close to but not coincident with the dye. As they were using a 0.2 M phosphate buffer, they may have encountered an intermediate situation in which the enzyme is only weakly bound to the Blue Dextran and is therefore progressively displaced from it as they travel through the column. As long as the above data have been obtained with the dye incorporated into the sample, we think they should be reconsidered in view of the observations reported here.

Several enzymes using nucleotides as substrates including *E. Coli* DNA-polymerase I have recently been shown to bind to Blue Dextran–Sepharose columns [10]. We are currently investigating the use of such columns for the purification of HeLa $\alpha$ DNA polymerase.

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