A SIMPLE AND EFFICIENT METHOD FOR THE ISOLATION OF DNA POLYMERASE I FROM BACTERIA

Ulla WÄHNERT

Department of Molecular Biochemistry, Central Institute for Microbiology and Experimental Therapy of the GDR Academy of Sciences, DDR-69 Jena, GDR

Received 1 April 1981

1. Introduction

The widespread use of DNA polymerase I from microorganisms for the enzymatic preparation of DNA model polymers and for labeling DNA in vitro by nick translation requires an ample supply of the enzyme. Despite the improvement of the isolation procedure [1] the removal of cellular nucleic acids by autolysis remained the crucial event in the purification of DNA polymerase. Without this removal the cellular DNA accompanying preparations of DNA polymerase would prevent the adsorption of the enzyme on phosphocellulose columns necessary for further purification. The use of polymin P at low concentration for the isolation of DNA polymerase I from *Escherichia coli* has been shown to be a remarkable improvement in the removal of nucleic acids from the enzyme [2].

This paper alternatively describes a rapid and safe method for the isolation of DNA polymerase I from various bacterial strains. The introduction of gel filtration on Bio-Gel A-0.5 m at high ionic strength in the first step of purification circumvents the autolysis as well as precipitation and high speed centrifugation steps previously used. The stability and ready availability of the DNA polymerase peak fractions from gel filtration is a great advantage of our method for isolating DNA polymerase I.

2. Materials and methods

2.1. *Assays*

DNA polymerase was assayed as in [3], except that 50 mM Tris–HCl (pH 7.8), 5 mM $MgCl_2$ and calf thymus DNA as template were used. A unit of enzyme is defined as 10 nmol total nucleotide incorporated in 30 min at 37°C. Protein was determined following [4].

2.2. *Bio-Gel A-0.5 m filtration*

Bio-Gel A-0.5 m (200–400 mesh) was obtained from BioRad. *Escherichia coli* K-12 cells (30 g) suspended in 60 ml buffer A (0.02 M Tris–HCl (pH 7.5), 10 mM 2-mercaptoethanol) containing 0.4 M NaCl were sonicated (Schoeller Schall) 10 × 30 s with cooling. After centrifugation at 100 000 × *g* the supernatant (fraction 1) was adjusted to 1 M NaCl and applied to the agarose column (5 × 77 cm gel bed) pre-equilibrated with buffer A containing 1 M NaCl. The column was washed with the same buffer system (flow rate 1 ml/min) and fractions active in DNA polymerase were pooled (fig.1).

3. Results and discussion

A typical elution pattern of an *E. coli* cell extract from gel filtration on a Bio-Gel A-0.5 m column is shown in fig.1. This gel filtration technique was applied directly to the bacterial cell extract after centrifugation (fraction 1). The appearance of nucleic acids in the effluent could be easily followed by the turbidity of the fractions starting with an elution volume equal to the void volume of the Bio-Gel A-0.5 m column. Immediately after the nucleic acids have passed through the column, the DNA polymerase peak eluted as shown by the elution profile in fig.1. The peak fractions of DNA polymerase activity obtained from Bio-Gel A-0.5 m column at 1 M NaCl in buffer A were pooled and could be stored at −5°C for 3 weeks without loss of activity (fraction 2). Fraction 2 was dialyzed against

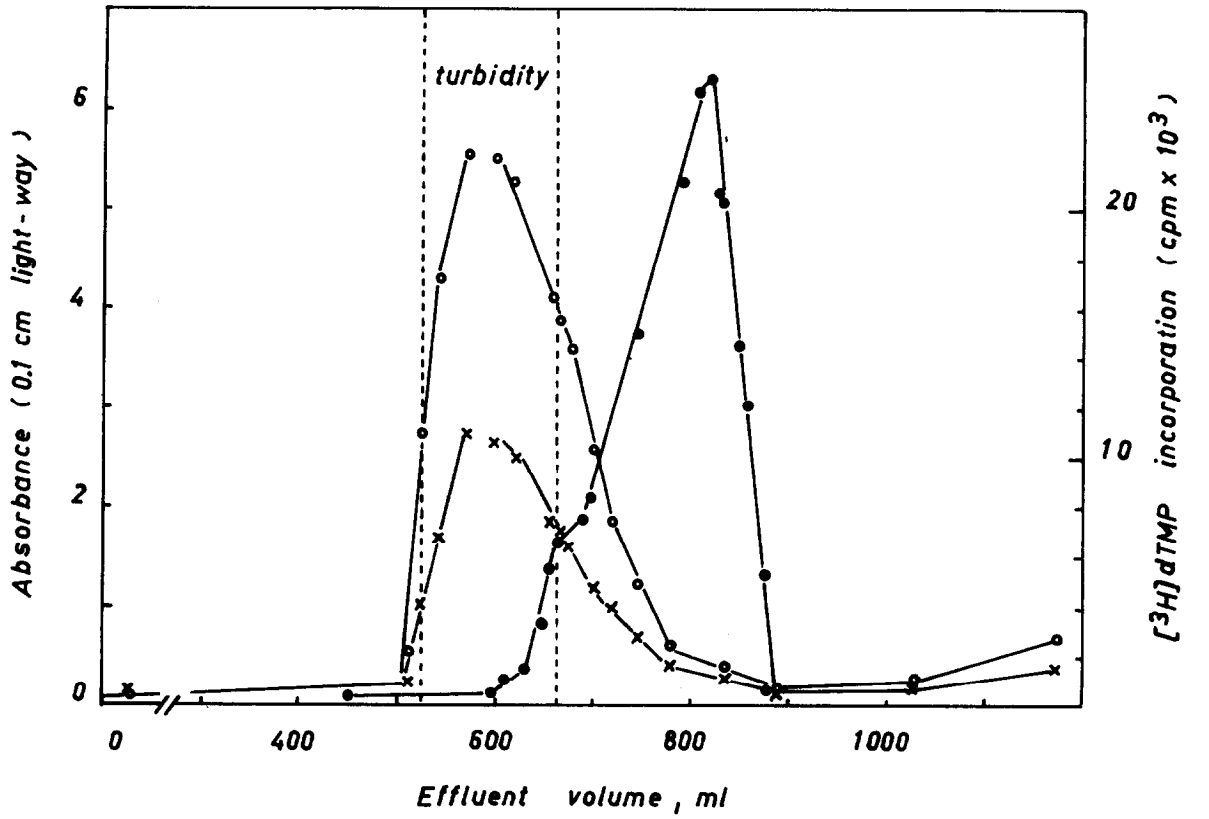

Fig.1. Elution profile of *E. coli* K-12 cell extract from a Bio-Gel A-0.5 m column (5 × 77 cm) at 1 M NaCl. (•) Activity of DNA polymerase; (○) absorbance at 260 nm; (×) absorbance at 280 nm.

0.2 M potassium phosphate (pH 6.7), 10 mM 2-mercaptoethanol overnight and loaded onto a DEAE-cellulose column (Whatman, DE 52) (1.6 × 28 cm) pre-equilibrated with the dialysis buffer. For large-scale preparation the DNA polymerase of the Bio-Gel fractionation can be concentrated by precipitation with ammonium sulfate (66% saturation) until sufficient quantities are collected for the following procedures. Subsequent steps in the polymerase purification included fractionation on DEAE-cellulose and phosphocellulose as in [1].

Results of a typical preparation of DNA polymerase I from *E. coli* K-12 are summarized in table 1. The electrophoretic pattern of fraction 4 (phosphocellulose) in 9% acrylamide in the presence of sodium dodecyl sulfate was compared with that of commercial preparations of DNA polymerase I (Boehringer, Mannheim) and standard proteins. The main protein band corresponds to $M_r \sim 110\,000$ daltons, a second protein to $M_r \sim 67\,000$ moving faster than the Setlow enzyme.

The present isolation method for DNA polymer-

Table 1
Purification of DNA polymerase I from 30 g *Escherichia coli* K-12

Fraction	Total protein (mg)	Protein (mg/ml)	Specific activity (units/mg)	Yield (%)
1. Extract	1800	22	26.7	100
2. Bio-Gel A-0.5 m	408	1.7	88	74
3. DEAE-cellulose	312	1.3	93	60
4. Phosphocellulose	22	0.13	674	30

ase I from *E. coli* provides a facile and rapid purification of the enzyme within a few days. The initial removal of the bulk amount of nucleic acids from the proteins by filtration on Bio-Gel A-0.5 m proved to be as useful for the isolation of DNA polymerase from *E. coli* as from *Streptomyces* strains demonstrating the general applicability of the method. Furthermore the gel filtration enables the simultaneous preparation not only of DNA polymerase I but also of other DNA-dependent enzymes as methylases [5] and restrictive endonucleases [6,7] as they have been found to elute in separate fractions.

Acknowledgements

The author is grateful to Drs A. Schmidt and M. Hartmann for helpful comments and making available their results on gel filtration.

References

[1] Jovin, T. M., Englund, P. T. and Bertsch, L. L. (1969) J. Biol. Chem. 244, 2996–3008.
[2] Rhodes, G., Jentsch, K. D. and Jovin, T. M. (1979) J. Biol. Chem. 254, 7465–7467.
[3] Wähnert, U., Zimmer, Ch., Luck, G. and Pitra, Ch. (1975) Nucleic Acids Res. 2, 391–404.
[4] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[5] Schmidt, A., Kahl, H., Reinert, H. and Venner, H. (1980) in: DNA-Recombination, Interactions and Repair (Zadrazil, S. and Sponar, J. eds) pp. 171–180, Pergamon, Oxford.
[6] Walter, F., Hartmann, M. and Roth, M. (1978) 12th FEBS Meet., Dresden, abst. 648.
[7] Roberts, R. J. (1976) CRC Crit. Rev. Biochem. 4, 123–164.