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Polyacrylamide gel electrophoresis of DNA polymerase from Ehrlich ascites tumor cells and recovery of active enzyme

Polyacrylamide gel electrophoresis should be useful for monitoring DNA polymerase (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) preparations at different stages of purification and for identification of the band or bands representing active protein. CAVALIERI AND CARROLL[1] reported studies on the *Escherichia coli* DNA polymerase; however, considerable activity was found to be lost after recovery of the protein from the gel. NEUHOFF AND LEZIUS[2] have described two assay methods for detecting the *E. coli* enzyme in microgels. To date there has been no report of electrophoresis of mammalian DNA polymerase or of success in recovering enzyme with a high yield of activity from gels.

The maintenance of the ascites cells and the preparations of the whole cell hypotonic extract and its pH-5 precipitate was as given by ROYCHOUDHURY AND BLOCH[3]. Samples of the pH-5 precipitate, subjected to chromatography on a sequence of DEAE-cellulose, phosphocellulose, hydroxylapatite and Sephadex G-100 columns were provided by Dr. R. Roychoudhury. All enzyme samples were dialyzed overnight against 2 l of cold Buffer A (50 mM potassium phosphate, pH 7, 10 mM 2-mercaptoethanol, 1 mM EDTA, 20% ethylene glycol).

The Tris–HCl–glycine electrophoresis system[4] showed a clear protein banding pattern with hypotonic extract protein, however, DNA polymerase activity was lost in protein extracted from the gel. Three buffer systems running at pH 7–7.5 did not produce banding patterns (lysine–HCl–glycine, imidazole–asparagine, and imidazole–glycine). The following procedure was found to permit resolution of protein bands and at the same time maintain the enzyme in an active form. Stock solutions were: A (80 ml of 1 M potassium phosphate, pH 7.5, 0.23 ml *N,N,N,N'*-tetramethylethylenediamine

TABLE I

RECOVERY OF DNA POLYMERASE ACTIVITY FROM 7% POLYACRYLAMIDE GELS LOADED WITH HYPOTONIC EXTRACT PROTEIN

Five gels were prerun, loaded with 180 μg of sample each, and run as described in the text. The gels were pooled and the protein extracted, concentrated, and assayed for polymerase activity.

	DNA polymerase activity (nmoles dATP per mg protein)
After electrophoresis	1.68
Control enzyme	1.55

TABLE II

LOCALIZATION OF DNA POLYMERASE ACTIVITY IN 5% POLYACRYLAMIDE GEL SEGMENTS AFTER ELECTROPHORESIS OF THE PHOSPHOCELLULOSE FRACTION

Two gels were prerun, loaded with 75 μg of sample each, and run as described in the text. The gels were cut into 3 segments (see Fig. 2A for divisions). Corresponding segments were pooled and the protein extracted, concentrated, and assayed for polymerase activity.

	DNA polymerase activity (nmoles dATP per mg protein)
Top gel segment	41.1
Middle gel segment	0.4
Bottom gel segment	—
Control enzyme	9.2

TABLE III

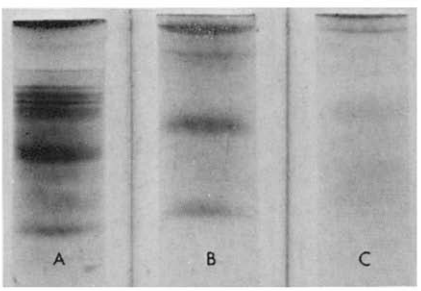
LOCALIZATION OF DNA POLYMERASE ACTIVITY IN 5% POLYACRYLAMIDE GEL SEGMENTS AFTER ELECTROPHORESIS OF THE SEPHADEX G-100 FRACTION

A gel prepared with DNA substrate was prerun, loaded with 14 μg of sample and run as described for the Sephadex fraction modification. The gel was cut into 4 segments (see Fig. 2C for divisions). Segments were individually homogenized and used directly for polymerase assay. Representative data from a band cutting experiment and from a whole gel homogenate are given.

	DNA polymerase activity (pmoles dATP per assay)
Segment 1 (slowest bands)	60
Segment 2	95
Segment 3	555
Segment 4	60
Total in 4 segments	770
Total gel homogenate	814
Control enzyme (14 μg)	977

and water to make 100 ml), B-5% (20 g acrylamide recrystallized from chloroform, 0.526 g *N,N'*-methylene bis-acrylamide and water to make 100 ml), B'-7% (28 g acrylamide and 0.735 g *N,N'*-methylene bis-acrylamide and water to make 100 ml), C (ethylene glycol), D (0.1 M dithiothreitol), E (0.28 g ammonium persulfate and water to make 100 ml). The acrylamide and methylene bis-acrylamide were products of Eastman Organic Chemicals (Rochester, N.Y.). The working gel solution was 1 part A, 2 parts B, 1 part C, 0.08 part D and 4 parts catalyst E. Gels of 1-ml volume were formed in glass tubes of 0.5-cm internal diameter. The electrophoresis buffer was 0.1 M potassium phosphate (pH 7.5), 50 mM glycine and 1 mM EDTA. The following operations were carried out at 4°. The gels were prerun at 10 mA per tube (Spinco, Duostat) for 1 h to remove impurities. After sample loading, the gels were run at 10 mA per tube for 4 h. The reservoir buffers were changed after the prerun and after 2 h of the run. Figs. 1 and 2 show the protein banding patterns of enzyme samples at different stages of purification.

Whole gels or gel segments (cut according to a reference gel stained with 1% Amido black) were homogenized in twice their volume of Buffer B (0.01 M potassium phosphate, pH 7.5, 0.15 M KCl, 1 mM EDTA, 1 mM dithiothreitol and 20% ethylene glycol). The homogenates were centrifuged at 88 000 $\times g$ for 20 min in a Beckman L-2 ultracentrifuge in a rotor 30 at 4°, and the resulting clear supernatants were poured off



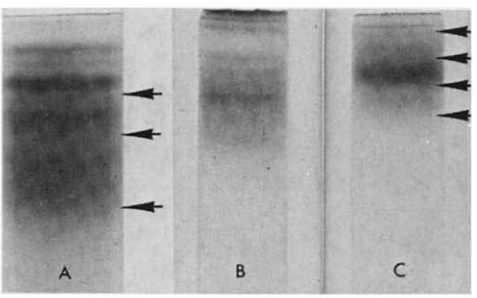


Fig. 1. 7% polyacrylamide gel electrophoresis of DNA polymerase fractions with the potassium phosphate (pH 7.5)–glycine buffer system. A. Initial hypotonic extract (75 μg). B. pH-5 precipitate (75 μg). C. DEAE-cellulose fraction (60 μg). The μg quantities of protein loaded on the gels are given in parentheses.

Fig. 2. 5% polyacrylamide gel electrophoresis of DNA polymerase fractions with the potassium phosphate (pH 7.5)–glycine buffer system. A. Phosphocellulose fraction (150 μg). B. Hydroxylapatite fraction (30 μg). C. Sephadex G-100 fraction (14 μg). The arrows indicate the location of the gel divisions referred to in Tables II and III.

and adjusted to 70% satd. $(NH_4)_2SO_4$ solution. A control enzyme sample was prepared by adding Buffer B to a sample of the loading protein and subjecting it to the 70% satd. $(NH_4)_2SO_4$ precipitation. The precipitates were collected by centrifugation at 13 000 $\times g$ for 10 min at 4°, dissolved in cold Buffer A and dialyzed overnight against the same buffer. Protein concentration was determined by the method of LOWRY *et al.*[5]. DNA polymerase activity was assayed by the method of ROYCHOUDHURY AND BLOCH[3] with the exception that glycylglycine buffer was used in place of potassium phosphate and 100 μg heat-denatured DNA rather than "activated" DNA. A fully active hypotonic extract enzyme was recovered from whole gel homogenates (Table I). The possibility that this activity was influenced by the removal of inhibitors (*e.g.* nucleases)

during electrophoresis cannot be ruled out. Band-cutting experiments on gels loaded with phosphocellulose samples located activity in the top segment of the pattern (Table II).

The technique of placing DNA substrate in the gel[6] was used in the assay for DNA polymerase activity of the Sephadex fraction[3]. "Activated" heat-denatured salmon-sperm DNA was prepared according to OLESON AND KOERNER[7] and polymerized with the gels at a concentration of 30 μg/ml. After electrophoresis the stained reference gel showed one dense and at least four faint protein bands (Fig. 2C). Four segments were cut from the unstained gel and individually homogenized in Buffer A bringing the volume to 0.2 ml. The whole segment samples were taken for the polymerase assay. Activated DNA was added to give a final concentration of 30 μg/ml (including the amount already present in the gel segment). DNA was thus available as primer for polymerase that was eluted from the gel or that was still in the gel fragments. The segment containing the dense protein band was found to contain over 70% of the total activity recovered in the four segments (Table III). Recovery of activity from the whole pattern was 83% of the activity initially loaded.

The present polyacrylamide gel system incorporates several factors which stabilize this enzyme and permit both protein banding and recovery of polymerase activity. The method applied to samples at early stages of purification[3] showed DNA polymerase activity to be located in the slow-moving zones of the pattern. The major protein band in the preparation with the highest specific enzyme activity appeared to indicate the primary position of the polymerase.

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