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SHORT COMMUNICATION

DNA POLYMERASE ACTIVITY OF MUNG BEAN SEEDLINGS

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Abstract—DNA polymerase activity has been demonstrated in extracts and protein fractions of the seedlings of a higher plant, *Phaseolus aureus*, the mung bean. The bulk of the activity appears to be concentrated in the roots. The level of activity is consistent with the assumption that polymerase activity reflects the DNA content of an organism.

INTRODUCTION

THE presence of DNA polymerase in microorganisms, viruses, and in animal tissue is well documented.[1] The major mode of *in vivo* replication of DNA in higher plants does not apparently differ from that of other organisms.[2,3] The present communication presents evidence for the presence of DNA polymerase in extracts and in a protein fraction of the tissues of a higher plant, *Phaseolus aureus*, the mung bean.

RESULTS

The polymerase was extracted from the mung bean tissue at pH 7·0, partially purified by ammonium sulfate, and assayed in the presence of added DNA and the four deoxynucleoside triphosphates by measuring the incorporation of ^{3}H-dATP into the $HClO_4$-insoluble fraction.[4–6] Table 1 shows that the incorporation of labeled dATP into acid-insoluble material retained by glass filter paper is entirely dependent upon the presence of the four deoxynucleoside triphosphates (dNTP) and magnesium chloride, and is partially dependent upon added DNA. DNase completely abolishes incorporation. The data thus demonstrate the presence of DNA polymerase in mung bean sprout extracts. Neither EDTA (5 μmoles) nor mercaptoethanol profoundly influences the incorporation rate. The amount of DNA synthesized is time-dependent. It reaches a maximum at about 60 min (Fig. 1), after which time the newly synthesized DNA is again degraded. Upon further addition of dNTP at

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TABLE 1. REQUIREMENTS FOR MUNG BEAN DNA POLYMERASE

System	Experimental (cpm)	Control (cpm)	Δ cpm
Complete system	133±3	40±8	93±11
Omit dCTP, dGTP, dTTP	58±7	50±10	8±4
Omit Mg^{2+}	13±7	41±5	−28±3
Omit DNA	56±9	38±7	18±4
Add DNase, 1·7 μg	16±5	43±3	−27±3
Omit 2-mercaptoethanol	146±15	39±4	102±8

Complete system (standard incubation mixture A, see Experimental) contained 0·09 ml of mung bean extract. The DNase was bovine pancreas deoxyribonuclease I (Sigma). "Enzyme" control was used. Δ cpm is average Δ cpm of two separate experiments.

145 min DNA synthesis is resumed. The rate of DNA synthesis increases with increasing enzyme (Fig. 1) and increasing dNTP (Table 2).

Eighty per cent of the polymerase activity was destroyed after heating the extract for 5 min at 60° and all activity completely abolished after 10 min at 60°. The presence of 1 M $(NH_4)_2SO_4$ also destroyed the enzyme activity.

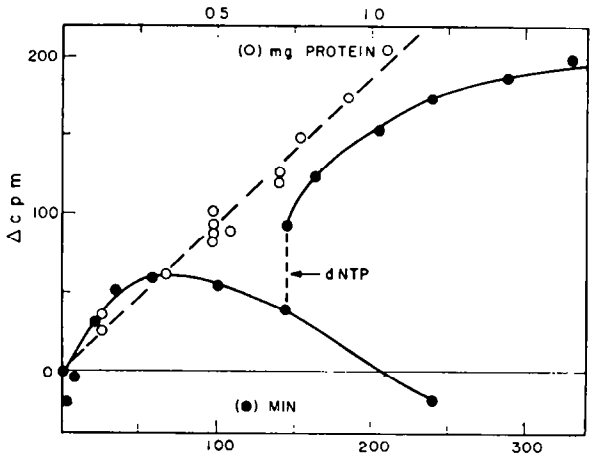

FIG. 1. TIME COURSE OF DNA SYNTHESIS BY 0·05 ml OF MUNG BEAN EXTRACT STANDARD INCUBATION MIXTURE I.

At 140 min 70 mμmoles of dNTP were added and aliquot volume corresponding to 0·3 ml before addition of dNTP removed (●). Effect of concentration of extract on DNA polymerase (○).

DNA polymerase activity was not detected in the low salt fraction. About 12·2 units of activity[7] were recovered in the high salt fraction starting with 10·6 units in 50 ml of extract. The increase in specific activity from 0·3 to 1·2 units of polymerase (24 mμmoles of deoxynucleoside incorporated per hr/mg of protein) represents a 4-fold purification.

Activity was consistently demonstrated in fresh batches of seedlings purchased and extracted the same day received from the supplier. Seedlings which had obviously deteriorated

[7] C. C. RICHARDSON, C. L. SCHILDKRAUT, H. V. APOSHIAN and A. KORNBERG, *J. Biol. Chem.* **239**, 222 (1964).

TABLE 2. EFFECT OF dNTP CONCENTRATION ON MUNG BEAN DNA POLYMERASE

dNTP, mμmoles/assay	Δ cpm—I	Δ cpm—II
25	87	104
50	178	201
90	202	227

Incubation mixture B (see Experimental), standard except for variation in dNTP substrate concentration, contained 0·13 ml mung bean extract. Δ cpm—I are values with "enzyme" control and Δ cpm—II are values in which control consisted of complete reaction system without Mg^{2+}.

as evidenced by wilting and brown discoloration did not consistently yield enzymatically active extracts. The highest activity was observed with a fresh batch of seedlings washed with 0·007 M sodium hypochlorite prior to preparation. These observations appear to rule out contaminating microorganisms as the source of DNA polymerase activity.

Each of 100 seedlings was separated into roots, hypocotyl, cotyledons and epicotyl. One part by weight of tissue was ground for 5 min by hand via chilled mortar and pestle with two parts of glass beads and two parts of grinding medium. After allowing the suspension to settle for 1 hr, the supernatant liquid was decanted and assayed for DNA polymerase activity. The roots contained about three-quarters of the polymerase activity and one-quarter of the protein per seedling but constituted less than one-fifth of the fresh weight. The hypocotyl was practically devoid of activity.

DISCUSSION

The data presented demonstrated the presence of DNA polymerase in a higher plant, the mung bean. The requirement for DNA (Table 1), in contrast to that for bacterial extracts, is probably owing to the low DNA content of plant tissue. Negative values of Δ cpm in the absence of Mg^{2+} or in the presence of DNase can best be explained by postulating the instantaneous combination of dATP with a substance in the enzyme preparation, to form a complex that is retained by the glass filter paper. During incubation this complex is presumably destroyed by enzyme action.

The specific activity of the extract is less than 0·1 of that in bacterial extracts.[7] Bacteria are about 1000 times as active as mung bean on per-gram-of-tissue basis. But since a plant cell is about 10^6 larger than a bacterial cell, there is about 1000 times more enzyme activity per plant cell than per bacterial cell. However since the plant cell contains about 1000 times as much DNA as does the bacterial cell, the DNA-polymerase activity of both plant and bacterial cells per unit DNA are of the same order of magnitude.

EXPERIMENTAL

Preparation of Extract

Fresh mung bean sprouts were purchased from a local grocery and 200 g were frozen and then ground at half speed for 2·5 min at 0–10° in a Waring Blendor with 400 g of glass beads (Superbrite, average diameter 200 μ) and 150 ml of 0·02 M glycylglycine buffer, pH 7·0,

containing 0·002 M EDTA and 0·002 M glutathione.[4] The mixing was continued for another 10 min at a lower speed after the addition of an additional 130 ml of buffer. After 30 min at 0°, the upper layer was decanted and centrifuged for 30 min at 11,000 *g* to yield 180 ml of supernatant. The supernatant was filtered through Celite to yield a clear yellow mung bean extract. The extract was frozen, distributed among ten tubes and stored at −13°. After thawing and freezing, a new precipitate formed which was devoid of polymerase activity. It was removed by centrifugation at 6000 *g*. The resulting clear supernatant was used for most of the experiments.

Salt Fractionation

To 50 ml of the mung bean extract at 0° were added, with stirring, 15 g of ammonium sulfate (0·48 saturation). After 30 min the precipitate was centrifuged off to yield the "low salt" fraction. To the supernatant were added an additional 15 g of ammonium sulfate (0·84 saturation). This precipitate was centrifuged off to yield the "high salt" fraction. Each fraction was dissolved in 5 ml of 0·02 M potassium phosphate buffer, pH 7·4.

DNA Polymerase Assay

Standard incubation mixture A (0·3 ml) contained 20 μmoles of potassium phosphate buffer, pH 7·2, 2 μmoles of $MgCl_2$, 0·3 μmole of 2-mercaptoethanol, 25 μg of calf thymus DNA (Sigma) in 0·016 M NaCl (or 0·05 ml, 12·5 μg of "activated" DNA;[5] this DNA yields results similar to those with thymus DNA), 10 mμmoles each of TTP, dCTP, dGTP and ^{3}H-dATP (Schwarz, $1{\cdot}5 \times 10^6$ cpm (counts/min) per μmole), (the mixture of equivalent amounts of the four deoxynucleoside triphosphates is hereafter referred to as dNTP) and enzyme diluted with enzyme diluent of Okasaki and Kornberg.[4] Standard incubation mixture B was similar to A except that it contained no 2-mercaptoethanol and 50 mμmoles of dNTP. After 60 min at 37°, the reaction mixture was chilled and treated with 0·5 ml of M $HClO_4$. The acid-insoluble radioactivity was determined routinely by the glass filter paper assay of Okasaki and Kornberg[4] or by the centrifuge assay described by Lehman *et al.*[6] with the modification that the final precipitate was collected on glass filter paper. In a separate experiment, with standard reaction mixture A containing 0·07 ml of mung bean extract incubated at 37° for 1 hr, the glass filter and centrifuge methods[6] yielded Δ cpm values of 89 and 84 respectively with "enzyme" controls. When $MgCl_2$ was omitted, the corresponding values were −25 and −27.

Two types of controls were used. In the "enzyme" control, the enzyme was added to the reaction mixture after the incubation period. In the "requirements" control, $MgCl_2$ and DNA were added to the reaction mixture after the incubation and the reaction mixture contained 30 mμmoles of cold dATP and 10 mμmoles of ^{3}H-dATP instead of 40 mμmoles of dNTP.

Protein was determined by the method of Lowry *et al.*[8] on cold trichloroacetic acid-precipitated samples.

[8] O. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).