A MODIFIED NUCLEAR DNA POLYMERASE IN A CASE OF ACUTE LYMPHOBLASTIC LEUKEMIA

José Mordoh[§] and Bertold R. Fridlender

Instituto de Investigaciones Bioquímicas "Fundación Campomar"
Obligado 2490, Buenos Aires (28), Argentina

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SUMMARY: Cells from a patient with acute lymphoblastic leukemia contained an altered nuclear DNA polymerase. This enzymatic activity differs from the normal enzyme in its KCl sensitivity, heat stability and reactivity with antibody against HeLa cytoplasmic DNA polymerase-α . The results also show that in these leukemic cells the cytoplasmic DNA polymerase-α is different to the nuclear DNA polymerase.

The presence of at least three different DNA polymerases has been reported in animal cells (1-9). DNA polymerase-α is a large MW enzyme (6-8S) found mainly in the cytoplasmic extract, although a similar activity has also been detected in nuclei isolated from actively dividing cells; a second class of enzyme generally recovered from the nuclear fraction is a low MW protein (2.5-3.5S) denominated DNA polymerase-β ; a third type, named DNA polymerase-γ , is the enzyme isolated from either cytoplasm or nuclei that catalyzes the synthesis of poly (dT) on a poly (rA) template. The physiological role of these enzymes has not yet been clearly established, although in a number of animal systems an increased rate of proliferation and DNA synthesis is accompanied by an increased activity of DNA polymerase-α (6, 7, 10-13).

It has also been observed in vitro that only DNA polymerase-α can initiate DNA synthesis on an RNA primer (5, 14). Based on its general properties, the DNA polymerase-α isolated from either cytoplasm or nuclei has been assumed to be the same enzyme.

We have previously reported the content and subcellular distribution of DNA polymerases-α and β in normal human lymphocytes (15, 16). We observed that a change occurred when resting cells were stimulated by phytohemagglutinin (PHA). In resting nuclei only DNA polymerase-β was detected; when the enzymes were purified from nuclei isolated from stimulated cells DNA polymerase-α as well as β were found (15). These results are in agreement with the idea of DNA poly-

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§ Scholar of the Leukemia Society of America

Abbreviation: PHA: phytohemagglutinin

merase-α being involved in the process of DNA replication. A possible way to further understand the role of this enzyme is to study a situation in which the control of replication is altered. In this paper we present evidence for an alteration of a nuclear DNA polymerase in a case of acute lymphoblastic leukemia.

MATERIALS AND METHODS

Leukemic cells purification. Twenty ml of venous blood was obtained from a 14 year-old child (H.R.M.) with untreated acute lymphoblastic leukemia. At the time of extraction the peripheral leukocyte count was 84,700 per mm^3; 95% of these cells were morphologically microlymphoblasts. The blood was mixed with 1.2 ml of 10% Dextran T500 (dissolved in 0.9% NaCl) and allowed to sediment by gravity for 20 min. The supernatant plasma was separated and centrifuged for 10 min at 1000 x g. The precipitate was resuspended in 10 ml of Eagle's Minimal Essential Medium, layered on a 10 ml Ficoll gradient (5 ml of 12% Ficoll, 3 ml of 50% Isopaque and 2 ml Phosphate Buffered Saline) and centrifuged 10 min at 480 x g in a Sorvall HB-4 rotor. The interphase was separated, diluted with Eagle's Minimal Essential Medium and centrifuged for 10 min at 1000 x g. The pellet containing 300 x 10^6 lymphoblasts was kept at -70° until used. Normal human lymphocytes were isolated as previously reported (16).

Enzyme preparation. The preparation of the cytoplasmic extract and nuclear soluble fraction as well as its chromatography on DEAE-cellulose was done as described (15). When concentrated enzymes were needed the fractions obtained from the DEAE-cellulose columns were dialyzed 4 h against 100 volumes of a buffer containing 10 mM Hepes, pH 7.2; 1 mM EDTA and 3 mM β-mercaptoethanol. The samples were lyophilized and dissolved in small volumes of bidistilled water (100-200 μl).

Measurement of DNA polymerase activity. Unless otherwise indicated the reaction mixture contained, in a final volume of 0.05 ml or 0.1 ml, enzyme, 50 mM Tris-HCl, pH 8.0; 5 mM $MgCl_2$; 0.9 mg/ml bovine serum albumin; 5 mM dithiothreitol; 620 μg/ml of activated calf thymus DNA; 0.1 mM each dCTP, dGTP, TTP and 0.01 mM [^{3}H]dATP (sp. act. 1000 cpm/pmole). The reaction mixture was incubated at 37° for 45 min and the radioactivity incorporated into a perchloric acid insoluble fraction was determined (16).

Protein determination. It was done according to Lowry et al. (17).

RESULTS

We have previously demonstrated (15) that in the cytoplasm from normal resting lymphocytes two peaks of DNA polymerase activity can be detected on DE-52 column chromatography, one eluting at 0.07 M NaCl (C I_n) and a second one eluting at 0.12 M NaCl (C II_n) while the nuclear extracts show only one peak of activity that does not adsorb to the column (N I_n). When the cells are stimulated with PHA a different distribution is observed with one peak in the cytoplasm eluting at 0.12 M NaCl (C I_s) and two in the nuclei, one that does not adsorb to the column (N I_s) and a second one eluting at 0.07 M NaCl (N II_s). The properties of C I_n, C II_n, C I_s and N II_s were very similar and correspond to what is currently denominated DNA polymerase-α. On the other hand N I_n and N I_s are equivalent to DNA polymerase-β.

DE-52 Column chromatography of leukemic cytoplasmic and nuclear extracts. In Fig. 1 A the chromatographic pattern of the cytoplasmic DNA polymerase is shown.

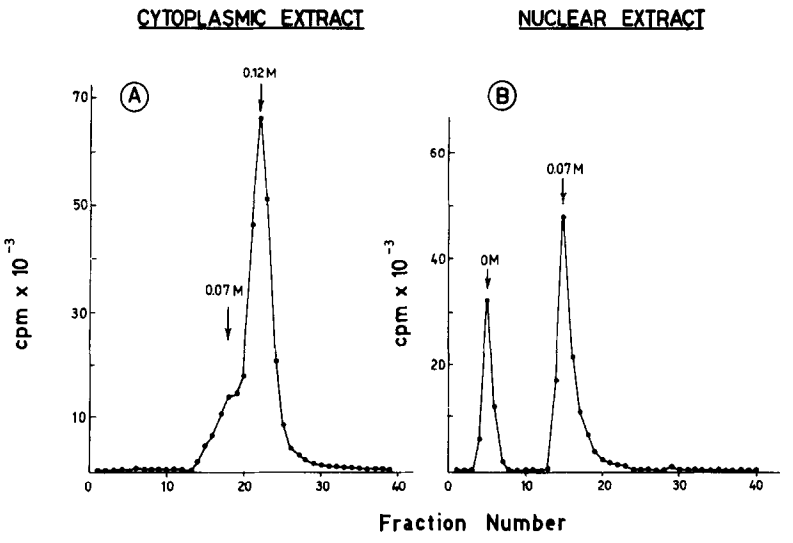

Fig. 1. DNA polymerase DNA dependent activities from leukemic cells. The cytoplasmic (A) and nuclear soluble fractions (B) were chromatographed on DE-52 cellulose. The enzyme activity was measured as described in Materials and Methods.

TABLE I. Subcellular Distribution of DNA polymerase activity of human lymphocytes

The isolation of the different fractions has been published (16). The enzymatic assay was done in a final volume of 0.1 ml as described in Materials and Methods.

Source of enzyme	PHA stimulated	Non-stimulated	Leukemic
	% Activity		
Total homogenate	100	100	100
Cytoplasmic extract	69	68	44
40,000 x g precipitate	2.4	4	5.3
Triton wash	14	16	15.4
Nuclear soluble fraction	9.4	9	32.8
Nuclear precipitate	0.9	2	2.4

A main peak of activity eluting from the column at 0.12 M NaCl (C II_L) with a small shoulder of activity eluting at a concentration of around 0.07 M NaCl (C I_L) are observed. In the nuclear extract (Fig. 1 B), two peaks are detected, one that is not adsorbed to the column (N I_L) and a second one eluting at a concentration of 0.07 M NaCl (N II_L). This pattern of DNA polymerase distribution is similar to that previously reported for PHA stimulated normal lymphocytes (15).

TABLE II. Requirements of the NII nuclear DNA polymerase

The enzymatic assay was done in a final volume of 0.1 ml as described in Materials and Methods. The enzymes used were the peak activities obtained after DEAE-cellulose column chromatography.

Reaction mixture	Normal enzyme	Leukemic enzyme
	% Activity	
Complete	100	100
Omit bovine serum albumin	0	1.9
Omit dithiothreitol	2.5	9.5
Omit dATP, dCTP, dGTP	27	39
Omit Mg^{++}, add 0.5 mM Mn^{++}	25	26
Plus 0.2 M KCl	31	112
Omit activated DNA, plus native DNA (62 μg)	5	6
Omit activated DNA, plus heated DNA (62 μg)	2	3

Subcellular distribution of DNA polymerase activity. As previously reported (16) DNA polymerase activity can be detected in the nuclei and cytoplasm from either resting or PHA stimulated normal human lymphocytes. Despite a different enzyme content and qualitative distribution between nucleus and cytoplasm, the subcellular distribution of total activity is very similar in both types of cells (Table I). As well as in other cells, the major part of DNA polymerase activity is found in the cytoplasmic extract. In the case of leukemic cells reported here a difference was observed in the subcellular distribution of the enzymes with respect to their normal counterpart. As seen in Table I, a much higher proportion of the total activity is found in the nuclear fraction, reaching about 33% of the cellular activity in contrast to the 9% found in normal cells. A corresponding decrease in the cytoplasmic fraction was observed.

Properties of nuclear DNA polymerase II. Some of the requirements of the N II DNA polymerase isolated from normal and leukemic cell nuclei were analyzed. The general properties of the enzymes are very similar with the sole exception of the KCl effect (Table II). While in the case of the normal enzyme 0.2 M KCl is strongly inhibitory, the enzyme isolated from the nuclei of leukemic cells is not affected by this concentration of salt. This salt effect suggested to us that a difference could exist between the normal and the leukemic enzyme.

The properties of the other leukemic DNA polymerases that we have analyzed were similar to the normal.

KCl dependence. Based in the results presented in Table II a more detailed study of the KCl effect was done, assaying the enzyme in a range of salt going from

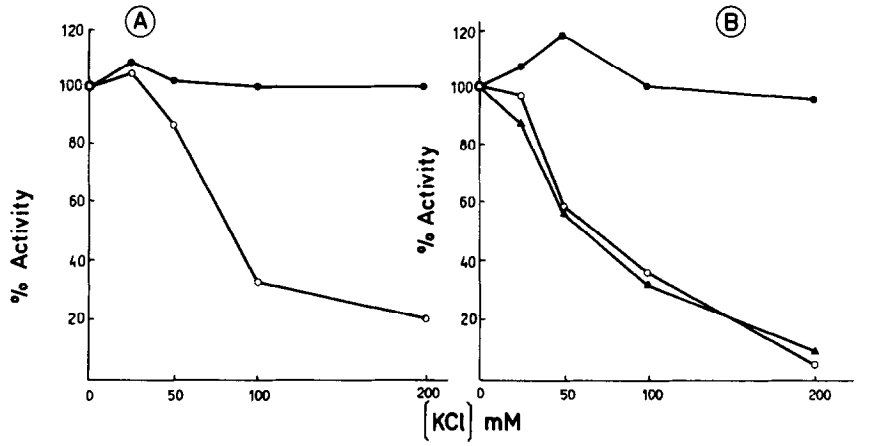

Fig. 2. Effect of KCl on DNA polymerase activity from normal, PHA stimulated and leukemic lymphocytes. Reactions were carried out as described in Materials and Methods using lyophilized enzymes. KCl was added at the indicated concentrations. A. Nuclear DNA polymerase: ○ , N II_s; ● , N II_L. B. Cytoplasmic enzymes: ● , C I_L; ◎ , C II_L; ▲ , C I_s.

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0 to 0.2 M KCl. A marked difference between the N II DNA polymerases from normal and leukemic cells is observed (Fig. 2 A).

It has been generally assumed that the high MW DNA polymerase found in the nuclei is the same enzyme than the high MW activity detected in the cytoplasm (18). However in the case of normal human lymphocytes we have always found that even though the high MW enzyme from cytoplasm and nuclei had very similar properties, they eluted from DE-52 cellulose columns at different ionic strength (15). As shown in Fig. 1 this is also true for the leukemic cells whose main cytoplasmic activity C II_L eluted at 0.12 M NaCl while the nuclear enzyme N II_L eluted at 0.07 M NaCl. Having observed an alteration in the nuclear leukemic enzyme with respect to its resistance to KCl, we used this parameter to study the relationship between the C II_L and the N II_L enzymes (Fig. 2 B). The C II_L DNA polymerase is fully inhibited by KCl in a manner similar to the cytoplasmic and nuclear enzyme isolated from normal cells. However when the small fraction of activity eluting at 0.07 M (C I_L) is analyzed, the salt response mimics that of the N II_L enzyme. This result indicates that in these leukemic cells the C II_L enzyme is different to the N II_L enzyme.

Temperature sensitivity. Another comparison between the normal and leukemic N II enzymes was done by analyzing their sensitivity at 48°. In Fig. 3 the inactivation pattern of these enzymes is shown. It can be seen that the normal enzyme is more resistant to heat than the leukemic enzyme since after 5 min at 48° it still retained 68% of the activity while the leukemic enzyme decreased to about 27%. This result might indicate an altered structure of the leukemic

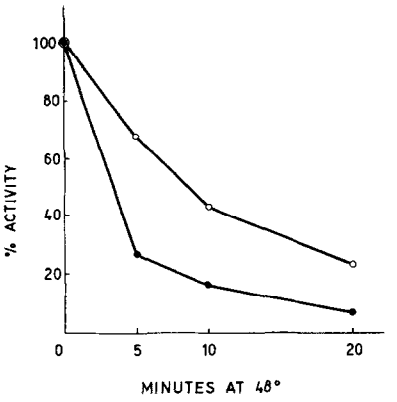

Fig. 3. Heat sensitivity of N II DNA polymerases. The lyophilized enzymes were preincubated at 48°C in 30 µl of a mixture containing 83 mM Tris-HCl buffer, pH 8; 1.5 mg bovine serum albumin and 8 mM $MgCl_2$. At the indicated times the enzymes were assayed increasing the volume to 50 µl and adjusting the assay mixture according to what was described under Materials and Methods. O , N II_S; ● , N II_L.

protein. We have observed that in general the leukemic enzyme is more unstable than the normal one, stored at -70° it lost activity in a shorter period of time. When chromatographed on Sephadex G-150 more than 90% of the activity was lost and for this reason we were unable to determine its MW.

Antibody inhibition. Antiserum against cytoplasmic DNA polymerase-α has been used by Spadari et al. (19) to show identity between cytoplasmic and nuclear DNA polymerase in HeLa cells. We have used the same antibody (kindly provided by Dr. A. Weissbach) to compare the normal and leukemic N II enzymes. The results obtained are presented in Fig. 4 and it can be seen that while the normal enzyme is 70% inhibited the leukemic enzyme lost only 20% of its activity. These data show that the leukemic enzyme differs antigenically from the normal enzyme.

DISCUSSION

The study of the physiological function of DNA polymerases is hampered by the difficulties in obtaining experimental mutants for DNA synthesis in higher cells. Another approach to achieve this goal is the finding of "natural" mutants in which some of the enzymes involved in DNA replication may be mutated. This seems to be the case with the results presented in this paper in which a very specific enzyme, N II_L, is altered in lymphoblasts of a patient with acute lymphoblastic leukemia. To our knowledge this is the first time in which an altered DNA polymerase can be correlated with a cell whose process of cell division is

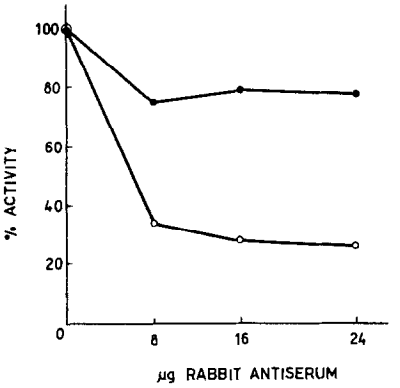

Fig. 4. Effect of antibody on N II DNA polymerases. The lyophilized enzymes were mixed with 130 µg bovine serum albumin and 80 nmoles Hepes buffer, pH 7.5, in a total volume of 15 µl and incubated 20 min at room temperature with varying amounts of antibody against HeLa cytoplasmic DNA polymerase-α . Afterwards, the enzymes were assayed for activity as described in Materials and Methods. O , N II_S; ● , N II_L.

uncontrolled. Previously, Springgate and Loeb (20) showed that cell free extracts from four patients with acute lymphatic leukemia had a DNA polymerase activity that copied a synthetic template with a higher level of infidelity than the normal enzyme. However, their results did not identify any cellular DNA polymerase that was actually modified. Even though we cannot say that the altered N II_L enzyme is responsible for the transformation process in the leukemic cell, it is suggestive that it seems to be precisely that DNA polymerase that has been related to DNA replication by several investigators. Since the results presented are drawn from a single patient it would be premature to assume that this is a general phenomenon in acute lymphoblastic leukemia. Until more cases are studied we assume that an uncontrolled cell duplication is a final effect which could arise as a consequence of alterations at several different levels of the cell division process, an alteration of the DNA polymerases being just one out of several possibilities.

The patient whose cells are being studied is at present under complete remission. If recurrency does occur the existence of a cellular marker will allow us to determine if the newly emerging sick cell will be the same as the original or if a new type of defective cell did appear.

The relationship between the large MW enzyme present in cytoplasm and nuclei has been analyzed by several investigators (2, 8, 15, 21). When the new nomenclature for mammalian DNA polymerase was recently presented (18) the large MW activity isolated from either cytoplasm or nuclei was designated as DNA polymerase-α , assuming that both were identical enzymes. In general, the study of

the cytoplasmic and nuclear enzymes indicated a very close similarity between these two activities. Spadari et al. (19), using antibody against HeLa DNA polymerase-α , obtained the same inhibitory effect with the cytoplasmic and nuclear enzymes. However, there are some tentative evidences presented in the literature suggesting that the two enzymes might be different proteins (3, 5). The data we have presented here suggest that the nuclear and cytoplasmic DNA polymerase-α in human lymphocytes are two distinct proteins. We have an altered nuclear enzyme with different properties than the cytoplasmic enzyme present in the same cell. In order to explain the discrepancy with the results indicating identity between the two proteins we might assume that all the high MW DNA polymerases have very similar properties and probably they share some peptides that could be forming part of the active site of the enzyme.

Based on the results obtained with the leukemic cells we could speculate that when normal lymphocytes start to replicate, the C I_n enzyme detected in the cytoplasm of resting cells enters to the nucleus and appears in stimulated cells as N II_s. This also implies that C I_n and C II_n are different enzymes.

Until a new nomenclature appears which differentiates between cytoplasmic and nuclear high MW DNA polymerases, we suggest to continue calling them DNA polymerase-α but indicating in each case its cytoplasmic or nuclear origin.

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