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Fidelity of Deoxyribonucleic Acid Polymerases from Normal and Leukaemic Human Cells in Polydeoxynucleotide Replication

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Although the relative incorporation of incorrect nucleotide (dCTP or dGTP) into poly(dA-dT)·poly(dA-dT) by partially purified 3–4S DNA polymerase from normal or leukaemic human cells was four to five times higher than that by the 6–7S DNA polymerase, no significant differences in the infidelity of these polymerases between normal and leukaemic cells were noted.

It has recently been suggested that DNA polymerases from human acute leukaemic lymphoblasts may be mutagenic (Springgate & Loeb, 1973). This suggestion is based on the finding that, with poly(dA-dT)·poly (dA-dT) as the template, nucleic acid-free crude extracts of acute leukaemic lymphoblasts polymerized ten times more dCTP than did extracts from phytohaemagglutinin-stimulated normal lymphocytes. Since such a difference between normal and leukaemic cells could have an important role in neoplasia the above findings were re-examined by using partially purified DNA polymerases from the above cells.

Materials and methods

Lymphocytes from leucocyte-rich normal blood (obtained by leucophoresis) were isolated and stimulated (72h) with phytohaemagglutinin-M (Difco Laboratories, Detroit, Mich., U.S.A.) as described by Han (1973). Lymphoblasts from a 32-year-old male patient in an active phase of acute lymphoblastic leukaemia were obtained by leucophoresis.

Normal or leukaemic cells (approx. 1g) were washed six times with phosphate-buffered saline. The extraction of the cells with buffer A (25mM-Tris–sulphate, pH8.3–1mM-$MgSO_4$–6mM-NaCl–4mM-dithiothreitol–0.1mM-EDTA) and the preparation of a 175000*g* supernatant and of purified chromatin have already been described (Srivastava & Minowada, 1973). The 175000*g* supernatant was chromatographed (Srivastava, 1974) on a column (1.3cm × 20cm) of DEAE-cellulose equilibrated with buffer A. The 6–7S DNA polymerase which was eluted at about 0.15M-NaCl was precipitated by 70% saturation with $(NH_4)_2SO_4$ and fractionated further by glycerol gradient centrifugation (10–30%, w/v, linear gradients prepared in buffer A containing 0.1M-NaCl). Peak fractions containing 6–7S DNA polymerase [sedimenting as a 10S aggregate under the conditions used, which were free of any 3–4S terminal deoxynucleotidyltransferase activity (Srivastava, 1974)] were used in the assays.

For the preparation of 3–4S DNA polymerase the purified chromatin was stirred (4h) with 25ml of 1M-NaCl dissolved in buffer B (0.01M-Tris–HCl, pH8.0, containing 0.01M-β-mercaptoethanol). The 1M-NaCl extract recovered by centrifugation was dialysed overnight against 2 litres of 0.14M-NaCl dissolved in buffer B. The precipitate formed was removed by centrifugation and the supernatant, after being adjusted to 0.25M-NaCl, was passed through a DEAE-cellulose column (1.3cm × 10cm) equilibrated with buffer B containing 0.25M-NaCl. The protein in the flow-through material was precipitated by 70% saturation with $(NH_4)_2SO_4$ and fractionated by glycerol gradient centrifugation as described above. Peak fractions containing 3–4S DNA polymerase [which sediments as 6–7S aggregate under the conditions used (Srivastava, 1974)] which were free of any detectable 3–4S terminal deoxynucleotidyltransferase activity were used in the assays.

To measure the fidelity with which poly(dA-dT)·poly(dA-dT) is copied by DNA polymerases from normal and leukaemic cells the incorporation of 'correct' nucleotides (dATP and dTTP) and 'incorrect' nucleotides (dCTP or dGTP) was determined in separate assays with the same enzyme preparation. For the determination of correct nucleotide incorporation the reaction mixture contained, in a final volume of 0.4ml, 16μM-[^{3}H]dTTP (1.1mCi/μmol), 18μM-dATP, 18μM-dCTP (or dGTP), 5mM-$MgCl_2$, 50mM-Tris–HCl, pH8.0, 10mM-dithiothreitol, 60μg of poly(dA-dT)·poly(dA-dT) (Miles Laboratories Inc., Elkhart, Ind., U.S.A.) and 50–100μl of enzyme. For the measurement of dCTP or dGTP incorporation, identical reaction mixtures were used except that they contained unlabelled dATP and dTTP and 18μM-[^{3}H]dCTP (23mCi/μmol) or 18μM-[^{3}H]dGTP (14mCi/μmol). After 1–2h incubation at 37°C,

Table 1. *Incorporation of correct ([^{3}H]dTTP and dATP) and incorrect ([^{3}H]dCTP or [^{3}H]dGTP) nucleotide with poly(dA-dT)·poly(dA-dT) as the template*

(*a*) and (*b*) refer to two different enzyme preparations. Incorporation of dATP is assumed to be equal to that of [^{3}H]dTTP. Reaction mixtures containing [^{3}H]dTTP, dATP and unlabelled dCTP or dGTP gave nearly the same amount of [^{3}H]dTTP incorporation and thus means of these two values are given for experiment (*b*). Blank values of 0.014pmol for zero-time controls have been subtracted from the values. Elimination of the template or the addition of 5μmol of EDTA also gave the same values as the blank.

Enzyme source	[^{3}H]dTTP incorporation (pmol)	Incorrect nucleotide incorporation (pmol)		Degree of infidelity	
		[^{3}H]dCTP	[^{3}H]dGTP	dCTP / dATP+dTTP	dGTP / dATP+dTTP
Phytohaemagglutinin-stimulated lymphocytes					
3–4S DNA polymerase	(*a*) 121	0.087	—	1/2780	—
	(*b*) 63	0.062	0.07	1/2032	1/1800
6–7S DNA polymerase	(*a*) 766	0.105	—	1/14590	—
	(*b*) 797	0.110	0.26	1/14490	1/6130
Acute leukaemia lymphoblasts					
3–4S DNA polymerase	(*a*) 142	0.085	—	1/3340	—
	(*b*) 246	0.170	0.25	1/2894	1/1968
6–7S DNA polymerase	(*a*) 526	0.075	—	1/14016	—
	(*b*) 367	0.055	0.09	1/13344	1/8154

during which the reaction was linear, 50μg of yeast RNA (plus 2mg of unlabelled dCTP and dGTP to tubes containing labelled dCTP and dGTP respectively) and 1ml of 10% trichloroacetic acid containing 3% sodium pyrophosphate were added. The precipitates were collected on B_6 nitrocellulose membrane filters (which had been presoaked overnight in saturated pyrophosphate solution), washed with 5% trichloroacetic acid, dried and counted in a toluene-based scintillation fluid (Srivastava, 1974).

Results and discussion

The data presented in Table 1 show that the degree of infidelity in terms of relative incorporation of incorrect nucleotide (dCTP or dGTP) into poly(dA-dT)·poly(dA-dT) by 3–4S DNA polymerase from normal or leukaemic cells was about four to five times higher than that by the 6–7S DNA polymerase. Moreover, both of these polymerases made more errors with dGTP than with dCTP. Low-molecular-weight DNA polymerase from regenerating rat liver has also been reported (Baril *et al.*, 1973) to make more errors in the copying of DNA than the high-molecular-weight DNA polymerase. Although both human cell DNA polymerases made considerably more errors (Table 1) than those reported for *Escherichia coli* DNA polymerase (Springgate & Loeb, 1973), the values for 6–7S DNA polymerase in Table 1 were similar to those for DNA polymerase from sea-urchin and bacteriophage T_4 (Loeb, 1973). The degree of infidelity obtained (with incorrect nucleotide dCTP) by Springgate & Loeb (1973) for DNA polymerase contained in crude extracts from normal cells was in between the values obtained here for 3–4S and 6–7S DNA polymerases. However, in contrast with the 10-times-higher infidelity values obtained by the above authors with extracts from leukaemic cells, no significant differences in the infidelity of 3–4S or 6–7S DNA polymerases between normal and leukaemic cells were noted in the present work whether dCTP or dGTP was the incorrect nucleotide used. Chromatin-associated terminal deoxynucleotidyltransferase, although detectable in phytohaemagglutinin-stimulated lymphocytes (Srivastava, 1974), is found in large amounts in acute leukaemic lymphoblasts (Srivastava, 1974; McCaffrey *et al.*, 1973). Although terminal deoxynucleotidyltransferase is 80–90% inhibited in the presence of more than one deoxyribonucleoside triphosphate (Srivastava, 1974), the fractions containing DNA polymerase from the glycerol gradients which were found to be contaminated with terminal deoxynucleotidyltransferase gave infidelity values as high as 1/822–1/236 in the present studies. Nevertheless, the high degree of infidelity obtained by Springgate & Loeb (1973) with DNA polymerase from leukaemic cells could result from contamination by terminal deoxynucleotidyltransferase, by the presence of another enzyme (which was lost during purification of 3–4S and 6–7S DNA polymerases) or other factors. Further examination of some of these alternatives is limited by the scarcity of the human material.

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