CHARACTERIZATION OF TERMINAL DEOXYNUCLEOTIDYLTRANSFERASE IN A CELL LINE (8402) DERIVED FROM A PATIENT WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Received June 2,1975

SUMMARY: High levels of terminal deoxynucleotidyltransferase comparable to the levels present in thymus gland were detected in a cell line (8402) derived from the blood cells of a patient with acute lymphoblastic leukemia. This cell line has previously been shown to exhibit some characteristics of T cells (12). We have purified the terminal transferase from these cells approximately 900 fold. The purified enzyme has a sedimentation value of 3.4S, a pH optimum of 7.8, a Mn^{2+} optimum of 0.1 mM, and a Mg^{2+} optimum of 7 mM. The purified enzyme efficiently utilizes a number of oligo or poly deoxynucleotide initiators and is similar in its properties to the terminal deoxynucleotidyltransferase purified from calf thymus and from the blast cells of some patients with chronic myelogenous leukemia.

Terminal deoxynucleotidyltransferase (EC 2.7.7.31, deoxyribonucleoside triphosphate: DNA nucleotidyl exotransferase) is an enzyme that catalyzes the polymerization of deoxyribonucleotide on the 3'-OH ends of oligo- or polydeoxyribonucleotide initiators in the absence of a template (1-3). High levels of this enzyme activity may normally be unique to the thymus gland (4), so the subsequent identification of this enzyme in amounts comparable to those in thymus tissue in certain types of human leukemia (5-11) suggested that the involved cell in these cases might be related to some primitive lymphoid cell. The detection of terminal transferase in human leukemia suggests that this enzyme can be used as a diagnostic marker for monitoring patients in remission and for the identification of types of cells that contain this enzyme. In order to develop *in situ* and radioimmunoassays for the detection of this enzyme, it is necessary to obtain antibodies against the human enzyme. Since it is difficult to obtain large amounts of human thymus tissue for the isolation of terminal

transferase required for the preparation of antibodies, we searched for alternative sources for this enzyme in established human cell lines.

Recently, lymphoid cell lines derived from peripheral blood of patients with acute lymphocytic leukemia, with some characteristics of T cells, have been established in tissue culture (12). Since they are now available in large quantities, they can be used to obtain large amounts of terminal transferase, if they contain this enzyme. Therefore, it is of some importance to determine whether these cells contain terminal transferase and whether this enzyme has biochemical and biophysical properties identical to terminal transferase from calf thymus and certain human leukemic cells (2,7-10). In this regard, cell lines with these characteristics have already been examined and one (Molt 4) has been reported to contain terminal transferase (13). In this report we describe the purification and characterization of terminal transferase from cell line 8402 . Like Molt 4 these cells do not synthesize immunoglobulins, but form rosettes with sheep red blood cells, and hence have been described as T cells (12).

MATERIALS AND METHODS

Materials: Tritium labeled deoxyribonucleoside triphosphates were obtained from Schwarz-Mann. Unlabeled deoxyribonucleoside triphosphates were obtained from Miles Laboratories and P.L. Biochemicals. Activated calf thymus DNA was prepared according to an earlier procedure (14).

Sucrose gradient analysis: An aliquot (0.2 ml) of the purified enzyme was layered on 4.5 ml of 5 to 20% (w/w) sucrose gradient in buffer (50 mM Tris·HCl (pH 7.5), 1 mM DTT, 0.5 M KCl) and centrifuged for 16 hrs. at 189,000 xg in a Spinco SW 50.1 rotor. Fractions (0.15 ml) were collected from the bottom of the tube and an aliquot (10 µl) was assayed for terminal transferase.

Enzyme assays: Terminal transferase activity was assayed at 37° for 1 hr. as described earlier (7,8) in a standard reaction mixture (0.05 ml) which contained 50 mM Tris·HCl buffer (pH 7.8), 50 mM KCl, 0.1 mM $MnCl_2$, 5 mM dithiothreitol (DTT), 100 µM of the labeled deoxyribonucleoside triphosphate, 2.5 µg of poly(dA) as a primer, and 5 µl of the enzyme fraction. The specific activity of the labeled deoxyribonucleoside triphosphates used was [^{3}H]dGTP (1500 cpm/pmole), and [^{3}H]dTTP (2200 cpm/pmole). The reaction was arrested by the addition of 50 µg of yeast tRNA and 2 ml of 10% trichloracetic acid, collected on millipore filters and counted in a scintillation counter (15).

Source of cells: 8402 is an established T cell line derived from the peripheral blood of a 16 year old girl with acute lymphoblastic leukemia (12). These cells do not synthesize immunoglobulins but form rosettes with sheep red blood cells (12). Fresh cells grown in tissue culture from this cell line were obtained from HEM Research Associates, Bethesda, Maryland.

Cell extractions: All cell extractions were carried out at 0-4°C. Cells (4 g) were washed with phosphate buffered saline (pH 7.4) twice, and suspended in 5 volumes of buffer A (10 mM Tris·HCl (pH 7.4), 10 mM KCl and 1.5 mM Mg^{2+}) and allowed to swell for 10 min. The cells were manually disrupted in a tight fitting stainless steel homogenizer and mixed with equal volume of buffer B (50 mM Tris·HCl (pH 7.5), 5 mM DTT, 1 M KCl, 1% triton X-100, 20% glycerol) and stirred in ice for 2 hrs. The soluble extract was removed, dialyzed against buffer C (50 mM Tris·HCl (pH 7.5), 5 mM DTT, 20% glycerol) containing 0.5 M KCl and subsequently against buffer C.

Enzyme Purification

DEAE cellulose chromatography: The dialyzed cell extract was made 0.3 M in KCl and then applied to a DEAE cellulose column (5 x 1.5 cm) equilibrated with buffer C containing 0.3 M KCl as described earlier (7). The column was washed with buffer C containing 0.3 M KCl. Fractions (3 ml) were collected and assayed for terminal transferase. Fractions containing the enzyme activity were pooled and dialyzed against buffer C. The dialyzed solution was then applied to a second DEAE cellulose column (5 x 1.5 cm) equilibrated with buffer C. The column was washed with 50 ml of buffer C containing 0.05 M KCl followed by 50 ml of buffer C containing 0.3 M KCl. Most of the terminal transferase activity was eluted with 0.05 M KCl. The enzyme activity which eluted with 0.05 M KCl was pooled and dialyzed against buffer C.

Phosphocellulose chromatography: The DEAE cellulose pooled enzyme was applied to a phosphocellulose column (P11, Whatman) (4 x 1.4 cm) equilibrated with buffer C (7). The column was first washed with buffer C and then developed with a 50 ml linear potassium chloride gradient from 0.05 M to 0.7 M in buffer C. Fractions (0.5 ml) were collected and assayed for terminal transferase as described above. The fractions containing the peak of enzyme activity were pooled, dialyzed against buffer C, and rechromatographed on a second phosphocellulose column as described above.

Hydroxyapatite chromatography: The phosphocellulose pooled enzyme was made 0.05 M in potassium phosphate buffer (pH 7.5) and applied to a hydroxyapatite column (4 x 1.4 cm) equilibrated with 0.05 M potassium phosphate buffer (pH 7.5) containing 1 mM DTT, 0.05% triton X-100 and 20% glycerol (buffer D). Hydroxyapatite was prepared according to an earlier procedure (16). The column was washed with 5 column volumes of buffer D and then developed with 50 ml linear gradient of buffer D and 0.8 M potassium phosphate buffer (pH 7.5) containing 1 mM DTT, 0.05% triton X-100 and 20% glycerol. Fractions (0.5 ml) were collected and assayed for terminal transferase as described above. Fractions containing the peak of enzyme activity were pooled and dialyzed against buffer C. The hydroxyapatite purified enzyme was used in the experiments described here.

RESULTS

Terminal deoxynucleotidyl transferase was purified from cell line 8402 by successive chromatography on DEAE cellulose, phosphocellulose and hydroxyapatite columns. Terminal transferase was eluted along with DNA polymerase β (17) from DEAE cellulose in 0.05 M KCl wash (7). On subsequent chromatography on a phosphocellulose column, terminal transferase activity was eluted around 0.2 M KCl and the low molecular weight DNA polymerase (DNA polymerase β) was eluted at higher salt (0.4 M KCl). Rechromatography of terminal transferase

from the first phosphocellulose column on a second phosphocellulose column gave a single peak of terminal transferase activity (figure 1A). In contrast the detection of two distinct peaks of terminal transferase in human leukemic cells and thymocytes has recently been reported (10). The enzyme eluted from the phosphocellulose column was further purified by chromatography on a hydroxyapatite column (figure 1B). On this column it eluted with 0.15 M phosphate buffer. The pooled terminal transferase activity was then dialyzed against

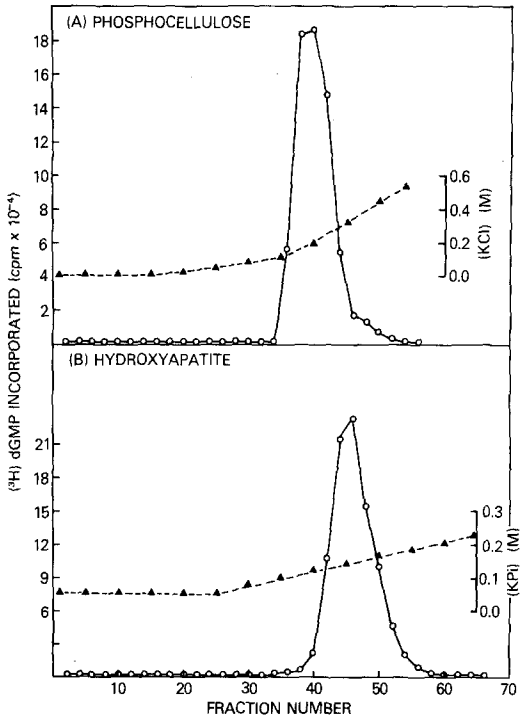

Figure 1 (A). Chromatography of terminal transferase on phosphocellulose. The phosphocellulose pool containing the terminal transferase activity was adsorbed on a second phosphocellulose column equilibrated with buffer C. The column was developed and assayed for terminal transferase activity as described in Materials and Methods. $(dA)_n$, (o—o); KCl, (▲--▲).

(B). Chromatography of terminal transferase on hydroxyapatite. The phosphocellulose pool containing the terminal transferase activity was chromatographed on a hydroxyapatite column and the fractions were assayed for terminal transferase as described in Materials and Methods. $(dA)_n$, (o—o); KPi, (▲--▲).

buffer C and used for further experiments. The purification of terminal transferase through various column chromatographic steps is summarized in table 1.

The effect of addition of unlabeled deoxyribonucleoside triphosphates on the polymerization of [^{3}H]dGTP catalyzed by purified terminal transferase is shown in table 2. Addition of one or more unlabeled deoxyribonucleoside triphosphates reduced the incorporation of [^{3}H]dGMP to approximately 10% of the control, a characteristic of terminal transferase (2,7) and distinct from the polymerizing property of template directed DNA polymerases.

The enzyme efficiently utilized oligo and polydeoxyribonucleotides as initiators as shown in table 3. In our experience oligo(dA) and poly(dA) are the most effective initiators. Poly(dG), oligo and polyribonucleotides, on the other hand, are very poor initiators confirming earlier results with terminal transferase from calf thymus and chronic myelogenous leukemia cells (2,7).

Terminal transferase purified to the hydroxyapatite chromatography step

Table 1. Purification of Terminal Deoxynucleotidyltransferase from Cell Line 8402.

Source of protein	Protein (mg)	Total activity*	Specific activity*	Purification (fold)
30,000 xg supernatant	48.3	170	3.5	1
DEAE cellulose (1st)	9.7	350	36	10
DEAE cellulose (2nd)	3.7	590	160	46
Phosphocellulose	2.0	420	210	60
Hydroxyapatite	0.3	940	3130	895

*Total activity is the total number of nanomoles of [^{3}H]dGMP incorporated into trichloracetic acid precipitable product in 30 min. with poly(dA) as the initiator Specific activity is the picomoles of [^{3}H]dGMP incorporated into trichloracetic acid precipitable product per 30 min. of assay per μg protein. The protein concentration of various enzyme fractions was determined by the procedure of Lowry et al. (19).

Table 2. Effect of the Addition of Deoxyribonucleoside Triphosphates on the Polymerization of [^{3}H]dGTP Catalyzed by Terminal Deoxynucleotidyltransferase from Cell Line 8402.

Substrate	[^{3}H]dGMP incorporated per reaction*	
	pmoles	%
[^{3}H]dGTP	37.5	100
[^{3}H]dGTP + dATP	4.1	11
[^{3}H]dGTP + dATP + dCTP	3.7	9.9
[^{3}H]dGTP + dATP + dCTP + dTTP	3.5	9.4

*Terminal deoxynucleotidyltransferase assays were carried out at 37° for 30 min. in the presence of 0.1 mM Mn^{2+} in a standard reaction mixture as described in Materials and Methods. Activated salmon sperm DNA was used as the initiator at a final concentration of 50 µg/ml. 20 µM of the labeled deoxyribonucleoside triphosphate [^{3}H]dGTP) and 80 µM of the unlabeled deoxyribonucleoside triphosphates were used where indicated.

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has a Mn^{2+} optimum of 0.1 mM, a Mg^{2+} optimum of 7 mM (figure 2A), pH optimum of 7.8 (figure 2B) and a KCl optima of 40 mM (figure 2C). The enzyme was inhibited more than 80% by 5 mM N-ethylmaleimide or 1 mM sodium pyrophosphate. The sedimentation value of the purified enzyme was 3.4S. This was estimated by sucrose density gradient centrifugation (18) with ovalbumin as a marker (figure 3). This value is similar to that obtained for terminal transferase from calf thymus and chronic myelogenous leukemia cells (2,7).

DISCUSSION

Terminal transferase, an enzyme first isolated from calf thymus was not detected in bone marrow, liver, lungs, lymph nodes and spleen, hence high levels were considered to be specific for thymus (4). Recent studies have shown that this enzyme is also present in relatively large amounts in the peripheral blood from many patients with acute lymphocytic leukemia (5,10)

Table 3. Response of Terminal Deoxynucleotidyltransferase from Cell Line 8402 to Various DNA and RNA Initiators.

Initiator	pmoles of [^{3}H]dGMP incorporated per reaction*	
	Mn^{2+}	Mg^{2+}
1. Deoxyribonucleotide		
$(dA)_{15}$	850	130
$(dC)_{15}$	380	20
$(dG)_{15}$	90	20
$(dT)_{15}$	180	20
$(dA)_n$	730	270
$(dC)_n$	70	40
$(dG)_n$	1	1
$(dT)_n$	290	7
2. Ribonucleotide		
$(A)_4$	9	1
$(A)_n$	7	2
$(C)_n$	3	0.3
$(G)_n$	2	1
$(U)_n$	4	1

*Terminal transferase assays were carried out at 37° for 30 min. as described in Materials and Methods in the presence of 0.1 mM Mn^{2+} or 7 mM Mg^{2+}. The initiator concentration used for the assays was 50 µg/ml. $(dN)_{15}$ represents an average chain length of 15 derived from oligo deoxyribonucleotides of chain length from 12 to 18.

and in some patients with acute myelomonocytic leukemia (9) or chronic myelogenous leukemia in acute "blast" phase of the disease (7,8,10). The presence of low levels of terminal transferase in normal bone marrow has also been recently reported (9,10).

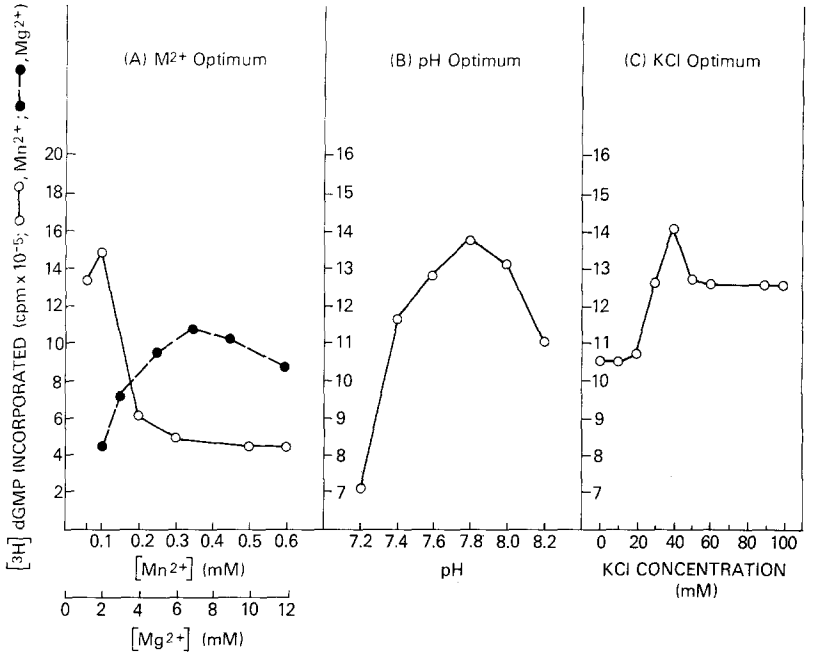

Figure 2. Determination of optimum conditions for terminal transferase with $(dA)_n$ as the initiator.

(A). Effect of divalent cation concentration on [^{3}H]dGMP incorporation.
(B). Effect of pH (Tris·HCl buffers) on [^{3}H]dGMP incorporation.
(C). Effect of KCl concentration on [^{3}H]dGMP incorporation.

Our present results show that a cell line (8402) derived from a patient with acute lymphocytic leukemia contains high levels of terminal transferase comparable to the levels observed in human and calf thymus gland and another T cell line, Molt 4. The biochemical properties of terminal transferase purified from these cells are similar to those reported for terminal transferase from calf thymus and from some patients with an acute (blast) phase of chronic myelogenous leukemia (2,7). Since insignificant levels of terminal transferase are characteristic of normal hematopoietic tissues, a marked increase in the levels of this enzyme in leukemia patients might be useful in determining the proliferation of a particular clone of cells. With the availability of T cell lines derived from the peripheral blood of patients with

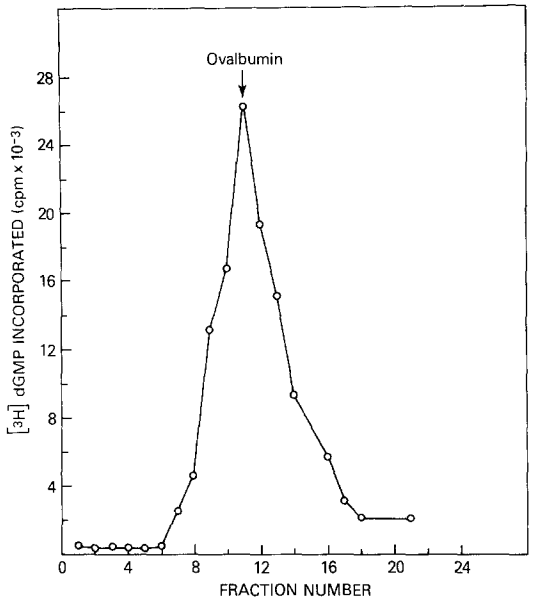

Figure 3. Sedimentation analysis of terminal transferase. An aliquot (0.2 ml) of the purified enzyme was layered on top of a 4.5 ml sucrose gradient (5-20%) and processed as described in Materials and Methods. Ovalbumin as a marker protein was centrifuged in a parallel gradient. [^{3}H]dGMP incorporation with $(dA)_n$ as the initiator (o—o).

acute lymphocytic leukemia containing high levels of terminal transferase, large quantities of this enzyme can now be isolated for more refined biochemical and biophysical studies. This may also make it possible to prepare antibodies to the human enzyme which are needed for development of a rapid and possibly *in situ* assay for terminal transferase in peripheral blood and bone marrow from leukemia patients in various phases of their disease and for determining which cells in a heterogeneous population of cells in bone marrow contain the enzyme.

ACKNOWLEDGEMENT

We thank Dr. Claude Klee (NIH) for a sample of hydroxyapatite and Bong Hee Ro for expert technical assistance.

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