TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE ACTIVITY IN A CELL LINE (MOLT-4) DERIVED FROM THE PERIPHERAL BLOOD OF A PATIENT WITH ACUTE LYMPHOBLASTIC LEUKEMIA*

B.I. Sahai Srivastava† and J. Minowada

Roswell Park Memorial Institute, Buffalo, N.Y. 14203

Received February 8,1973

SUMMARY. A terminal deoxynucleotidyl transferase having a sedimentation coefficient of 3-4S has been found associated with the chromatin from a cell line (Molt-4) derived from the peripheral blood of a patient with acute lymphoblastic leukemia.

INTRODUCTION

Recent work from several laboratories (1, 2) seems to indicate that chronic lymphocytic leukemia of man represents leukemia of the bone marrow-derived lymphocytes and acute lymphoblastic leukemia represents that of the thymus derived lymphocytes (T cell). This hypothesis is supported by the fact that the Molt-4 cell line which was derived from peripheral blood of a patient with acute lymphoblastic leukemia was of T cell origin (3). Since terminal deoxynucleotidyl transferase ("terminal transferase"), an enzyme which catalyzes the addition of deoxynucleotidyl residues from deoxynucleoside triphosphates to the 3'-hydroxyl group of preformed polynucleotide chains, occurs in thymus, but not in bone marrow (4), it was considered desirable to examine Molt-4 cells for the presence of this enzyme. The results are reported in this paper.

MATERIALS AND METHODS

Molt-4 cell line derived from peripheral blood of a patient with acute

---

* This work was supported by a grant from the National Cancer Institute, U.S.P.H.S. (CA-13038).

† To whom requests for reprints should be addressed.

lymphoblastic leukemia (3) was grown (5) in Roswell Park Memorial Institute 1640 medium containing 10% heat-inactivated fetal calf serum, penicillin (100 units/ml) and streptomycin (50 units/ml). The cells (ca lg) were recovered by centrifugation and washed 6 times with phosphate buffered saline (pH 7.2). The extraction of cells with Buffer A (25 mM tris-sulfate, pH 8.3; 1 mM $MgSO_4$; 6 mM NaCl; 4 mM dithiothreitol and 0.1 mM EDTA) and the preparation of 37,000 x g supernatant and of purified chromatin (obtained by ultracentrifugation through 1.7 M sucrose) has already been described (5). The 37,000 x g supernatant was centrifuged 1 hr. at 175,000 x g. To the supernatant obtained (cytoplasmic fraction) ammonium sulfate was added to 70% saturation (ammonium carbonate 1/50th of the concentration of ammonium sulfate was also added). The precipitate formed was recovered by centrifugation (25,000 x g, 15 min.) dissolved in ca 1.5 ml of Buffer A containing 0.1 M NaCl and 5% glycerol and dialyzed against the same buffer for 5-6 hr. at 1°C. The dialysate was clarified by centrifugation and aliquots (0.5 ml) from it were layered onto 4.6 ml of a linear [10 to 30% w/v] glycerol gradient prepared in Buffer A containing 0.1 M NaCl. The gradients were centrifuged at 40,000 rpm for 16 hr. at 2°C in the SW50L rotor in the Spinco model L centrifuge. Fractions (12 drops each) were collected by puncturing the bottom of the tube and assayed for DNA polymerase ("polymerase") and "terminal transferase" activity as given in the legend of Fig. 1.

The purified chromatin saved above was stirred (4 hr.) with 25 ml of 1 M NaCl (dissolved in 0.01 M Tris-HCl, pH 8.0, containing 0.01 M β-mercaptoethanol) and the 1 M NaCl extract, recovered by centrifugation (37,000 x g, 20 min.), was dialyzed overnight against 2 liters of 0.14 M NaCl (dissolved in 0.01 M Tris-HCl, pH 8.0, containing 0.01 M β-mercaptoethanol). The precipitate formed was removed by centrifugation (37,000 x g, 20 min.) and the supernatant (chromatin fraction) obtained was processed exactly as described above for the cytoplasmic fraction (175,000 x g supernatant). Peak fractions from the glycerol gradient containing chromatin-

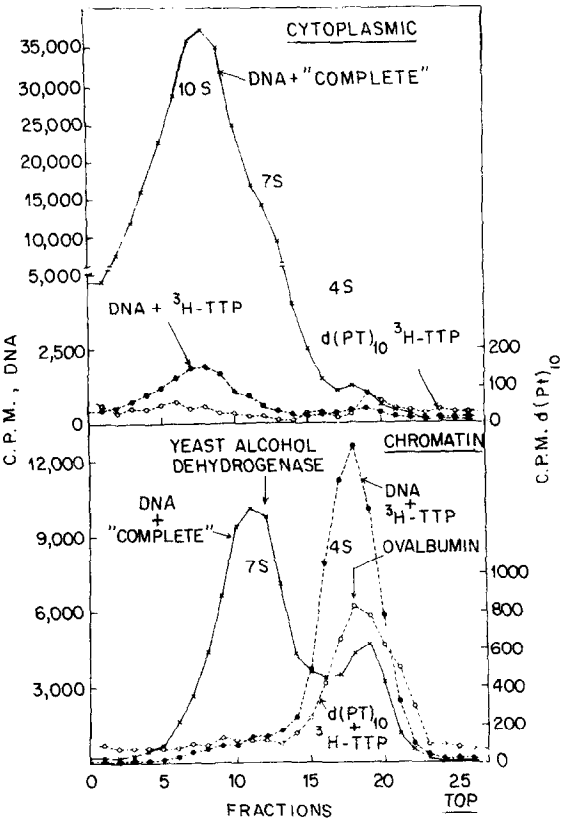

Figure 1. Glycerol gradient centrifugation of cytoplasmic and chromatin-associated "polymerase" and "terminal transferase" activity from Molt-4 cells.

The "complete" system for the assay of "polymerase" activity contained in a final volume of 0.2 ml: 10 μmoles Tris-HCl, pH 8.3; 1.2 μmoles magnesium acetate; 4 μmoles dithiothreitol; 12 μmoles of NaCl; 0.15 μmoles of each of dATP, dCTP and dGTP; 2 μC (^{3}H-methyl)-TTP (specific radioactivity 15.4 mC/μmole); 20 μg heat denatured calf thymus DNA (Worthington) and 50 μl aliquot from each fraction. For the assay of "terminal transferase" activity dATP, dCTP and dGTP were omitted and either 20 μg denatured DNA or 0.1 unit of d(pT)$_{10}$ (P.L. Biochemicals) were used as initiators. After 30 min. incubation at 37°C, 100 μg of yeast RNA and 1 ml of 10% trichloroacetic acid were added. The precipitates were collected on presoaked (overnight in saturated pyrophosphate solution) B_6 nitrocellulose membrane filters, washed with 5% trichloroacetic acid, dried and counted using toluene based scintillation fluid.

Ovalbumin (mol. wt. 45,000) and yeast alcohol dehydrogenase (mol. wt. 148,000) were simultaneously sedimented as external standards. Their position in the gradients were located by A276nm measurement of gradient fractions.

---

associated "terminal transferase" activity were pooled and used for the examination of some of the properties of "terminal transferase" as given in Tables 1 and 2.

Table 1

Effect of Deoxyribonucleoside Triphosphate Addition on

"Terminal Transferase" Activity

Reaction mixture	^{3}H-TMP incorporated, % of control
"Control"	100
⟫ plus dATP	20
⟫ plus dATP and dCTP	11
⟫ plus dATP, dCTP and dGTP	13

The reaction mixture ("control") for the assay of "terminal transferase" activity contained in a final volume of 0.2 ml: 10 μmoles Tris-HCl, pH 8.3; 1.2 μmoles magnesium acetate; 4 μmoles dithiothreitol; 12 μmoles NaCl; 2 μC (^{3}H-methyl)-TTP (specific radioactivity 15.4 mC/μmoles); 20 μg heat denatured calf thymus DNA and 50 μl of the chromatin enzyme from pooled "terminal transferase" peak fractions (17-19) of the gradient as in Figure 1; and where indicated 0.16 μmoles of each of dATP, dCTP and dGTP. After 30 min. incubation at 37°C, the trichloroacetic acid precipitable radioactive material was collected and counted as given in the legend of Figure 1. The "control" system gave 11,944 cpm.

RESULTS AND DISCUSSION

Since "terminal transferase" activity in the thymus tissue has been found in the cytoplasm (4,6), as well as in the chromatin (4,7,8), both of these fractions from Molt-4 cells were examined. The data presented in Fig. 1 show two peaks of "polymerase" activity in the cytoplasmic fraction at about 10S (fraction 8) and 4S (fraction 18-19), whereas the shoulder at 6-7S (fraction 11-12) could result from either of these polymerases. An examination of the assay profile where DNA or $d(pT)_{10}$ were used as primers in the absence of dATP, dCTP and dGTP suggest that "terminal transferase" activity (around 3-4S region) if present in the cytoplasmic fraction was very low. In the chromatin fraction, however, a "polymerase" activity peak was detected at 6-7S and an overlapping peak of residual 3-4S "polymerase" and "terminal transferase" at 3-4S. That most of the activity in the 3-4S region of the chromatin

Table 2

Effect of Changes in Reaction Mixture on "Terminal Transferase" Activity

	Reaction mixture	^{3}H-TMP incorporated, % of control
1.	"Control"	100
2.	1 minus denatured DNA	0
3.	2 + d(pT)$_{10}$, 0.1 unit	8
4.	1 + EDTA, 5 μmoles	0
5.	1 + pyrophosphate, 1 μmole	0
6.	1 + p-hydroxy mercuribenzoate, 0.15 μmole	15
7.	1 + N-ethylmaleimide, 1 μmole (preincubated with the enzyme for 10 min. at 37°C)	60

Composition of the reaction mixture ("control") for the assay of "terminal transferase" activity including the enzyme and the incubation time were the same as described in the legend of Table 1. The inhibitors (except for 7) were added at the same time as the other components of the reaction mixture. The "control" system gave 11,718 cpm.

fraction was due to "terminal transferase" is illustrated by the higher incorporation of TMP with DNA primer in the absence of dATP, dCTP and dGTP compared to the "complete" system, as well as by the utilization of d(pT)$_{10}$ as the initiator. The use of ^{3}H-CTP in place of ^{3}H-TTP with both cytoplasmic and chromatin enzymes gave essentially similar results as in Fig. 1. Although details about "polymerases" from several lymphoid cells will be published elsewhere (Srivastava, B.I.S., in preparation), it is important to note here for the purpose of clarification that cytoplasmic fraction from these cells has 2 major "polymerases", the high molecular weight of which sediments at 6-7S or 10S and the low molecular weight one sediments at 3-4S or 6-7S depending upon the salt concentration of the gradient. The chromatin has only one "polymerase" of 3-4S which at low salt as in Fig. 1 sediments predominantly at 6-7S. This behavior of low molecular weight "polymerase" was important as it permitted its separation from 3-4S "terminal transferase" in the chromatin fraction.

The data presented in Table 1 show that the optimum incorporation of TMP into acid insoluble material was obtained in the absence of other deoxyribonucleoside triphosphates and that the addition of one or more of these triphosphates strongly inhibited the reaction. Unlike the "terminal transferase" from mammalian chromatin (7, 8) but like the soluble "terminal transferase" of Bollum (9) the "terminal transferase" from Molt-4 cells could not catalyze the incorporation of AMP into DNA when ATP-8-^{14}C was used instead of ^{3}H-TTP.

The data presented in Table 2 show that "terminal transferase" activity was dependent on the presence of DNA or $d(pT)_{10}$ in the assay mixture and that the reaction was inhibited by EDTA, pyrophosphate, p-hydroxy mercuribenzoate and N-ethylmaleimide. The "terminal transferase" activity from other mammalian sources (7, 8, 10) is also strongly inhibited by EDTA. The product of the "terminal transferase" reaction from Molt-4 cells was also found to be sensitive to deoxyribonuclease 1 but not to boiled pancreatic ribonuclease A.

The data presented herein show that Molt-4 cells contain a "terminal transferase" which resembles in molecular size (4, 6) and other properties "terminal transferase" from other mammalian sources (6-10). The presence of "terminal transferase" in Molt-4 cells and its absence in several other lymphoid cell lines examined lends support to the argument (3) that the Molt-4 cell line is of T cell origin. This suggestion should, however, be interpreted with caution since "terminal transferase" activity in addition to its presence in thymus tissue has also been found in rat intestinal mucose (11), as well as tobacco tissue cultures (12). Whether the presence of "terminal transferase" in Molt-4 cells is a consequence of their being derived from cells of thymic tissue origin or because derepression of the genome coding for "terminal transferase" occurred in bone marrow originated cells during their passage through thymus remains unknown. Thus the significance of this finding as applied to the origin of acute leukemic lymphoblasts remains to be determined.

REFERENCES

1. Wilson, J.D. and Nossal, G.J., Lancet, ii, 788 (1971).
2. Preud'homme, J.L. and Seligman, M., Blood, 40, 777 (1972).
3. Minowada, J., Ohnuma, T. and Moore, G.E., J. Natl. Cancer Inst., 49, 891 (1972).
4. Chang, L.M.S., Biochem. Biophys. Res. Commun., 44, 124 (1971).
5. Srivastava, B.I.S. and Minowada, J., Cancer Res., 32, 2481 (1972).
6. Chang, L.M.S. and Bollum, F.J., J. Biol. Chem., 246, 909 (1971).
7. Krakow, J.S., Coutsogeorgopoulos, C. and Canellakis, E.S., Biochim. Biophys. Acta, 55, 639 (1962).
8. Wang, T.Y., Arch. Biochem. Biophys., 127, 235 (1968).
9. Bollum, F.J., J. Biol. Chem., 237, 1945 (1962).
10. Chang, L.M.S. and Bollum, F.J., Proc. Nat. Acad. Sci., 65, 1041 (1970).
11. Leung, F.Y.T. and Zbarsky, S.H., Canad. J. Biochem., 48, 537 (1970).
12. Srivastava, B.I.S., Biochem. Biophys. Res. Commun., 48, 270 (1972).