Purification of a High Molecular Weight Human Terminal Deoxynucleotidyl Transferase*

(Received for publication, February 21, 1979)

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Terminal deoxynucleotidyltransferase has been purified from lymphoblasts of leukemia patients. The enzyme has a molecular weight of approximately 62,000 as determined by gel filtration and non-denaturing gel electrophoresis and is not dissociated into subunits by sodium dodecyl sulfate. In contrast, the terminal transferase enzyme from calf thymus has a molecular weight of 42,000 as determined by gel filtration, and is dissociated into two subunits of $M_\text{r} = 30,000$ and 8,000 by sodium dodecyl sulfate. The enzyme has an isoelectric point of 8.2 and kinetic characteristics which are similar to those of calf thymus terminal transferase. The apparent $K_m$ for purine nucleotide polymerization at saturating initiator concentration with $Mg^{2+}$ is 0.2 mM and with $Mn^{2+}$ is 0.05 mM. Like calf terminal transferase, the reaction velocity is higher in the presence of $Mg^{2+}$ than $Mn^{2+}$. ATP inhibits the reaction catalyzed by terminal transferase isolated from human lymphoblasts due to mutual recognition of ATP and dATP by a common site on the enzyme. Preliminary experiments indicate that human terminal transferase may contain a small amount of carbohydrate. This report represents the first purification to near homogeneity of terminal transferase from a tissue source other than calf thymus.

Terminal deoxynucleotidyltransferase (deoxynucleoside triphosphate:oligodeoxynucleotide deoxynucleotidyltransferase, EC 2.7.7.31) is a DNA-polymerizing enzyme which does not utilize a template. The enzyme is generally found only in mammalian bone marrow and thymus and has heretofore been obtained in homogeneous form only from calf thymus. Very high levels of terminal transferase activity have been detected in lymphoblasts from most patients with acute lymphoblastic leukemia (1-6) and in leukemic cells from some patients with chronic myelogenous leukemia in blast crisis (7-10). Thus, the enzyme represents a useful marker of neoplastic cells in certain human diseases (5, 6, 8). While no definitive role for terminal transferase has been established, speculations that the enzyme participates as a somatic mutator in the generation of immunological diversity in the early stages of lymphocyte differentiation have been advanced (11, 12).

In this study, we present a purification procedure for human terminal transferase. Unlike earlier reports of partial purification of the human enzyme (13), our enzyme is very different in structure but remarkably similar in kinetic characteristics to calf terminal transferase. Both enzymes catalyze template-independent addition of deoxynucleotides to the 3'-OH group of oligo- or polydeoxynucleotides. However, the calf enzyme, a protein of approximately $M_\text{r} = 42,000$, is comprised of two subunits of differing size, 30,000 and 8,000, while, in contrast, human terminal transferase is a protein of $M_\text{r} = 62,000$ and is comprised of a single polypeptide chain.

The procedure reported in this paper results in a protein preparation which is highly purified. In the miniprint, we have included a rapid purification scheme which is suitable for small amounts of tissue (less than 100 g) and results in a preparation in which terminal transferase comprises about 50% of the total protein.

**EXPERIMENTAL PROCEDURES**

**Materials**—Radioactive deoxynucleoside 5'-triphosphates were purchased from New England Nuclear Corp. Unlabeled deoxynucleoside 5'-triphosphates were from Sigma Chemical Co. and were further purified (14). Affi-Gel Blue (100 to 200 mesh), acrylamide, bisacrylamide, Bio-Gel A 0.5m, Bio-Lytes (pH 3 to 10 and pH 8 to 10), and Bio-Gel HTP were obtained from Bio-Rad Laboratories. Agarose-hexane-2'-deoxyadenosine-5'-triphosphate (dATP-Sepharose) containing 1.6 pmol of dATP/ml of agarose was purchased from P-L Biochemicals, Inc. Cibacron Blue F3GA (free dye) was obtained from Polysciences, Inc. (Warrington, Pa.). CM-Sepharose C-50 was obtained from Pharmacia Fine Chemicals. Proteins used in molecular weight analyses were obtained from Sigma Chemical Co. and were further purified by gel filtration on Bio-Gel A 0.5m.

**Source of Human Lymphoblasts**—Human leukemic lymphoblasts were collected from patients undergoing therapeutic leukapheresis (15). This procedure is routinely performed at the University of Kentucky Medical Center and normally yields between 200 and 1000 g of cells per patient. The lymphoblasts were purified as previously described (6) and were stored at -80°C.

**Source of Homogeneous Calf Thymus Terminal Transferase**—Terminal deoxynucleotidyl transferase was purified according to the procedures of Chang and Bollum (16). The final preparation consisted of two subunits with molecular weights of 30,000 and 8,000 as judged by SDS-polyacrylamide gel electrophoresis according to the method of Weber and Osborn (17).

**A rapid purification procedure for small quantities of tissue (including Fig. 1S and Table 1S) is presented as a miniprint supplement immediately following this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 79M-293, cite author(s), and include a check or money order for $1.00 per set of photocopies.
**Enzyme Assays**—The assay of human terminal transferase has been described previously (18). The assay mixture in a final volume of 125 µl contained 0.2 M potassium cacodylate (pH 7.5), 1 mM 2-mercaptoethanol, 0.012 mM p(dA)₃, and 1 mM [³²P]dATP (100 cpm/pmol) with 1.5 mM MgCl₂, or 1 mM [³²P]dGTP (100 cpm/pmol) with 0.5 mM MnCl₂. The reactions were incubated at 30°C for varying time periods, and terminated by application of 25-1.11 aliquots onto GF/C glass fiber papers (Whatman) as previously described (19). One unit of enzyme activity is defined as 1 nmol of radioactive deoxynucleotide incorporated/h, and specific activity is expressed as units of activity/mg of protein.

**Protein Determinations**—Enzyme protein was measured by the method of Lowry et al. (20) and by the method of Kalb and Bernlohr (21).

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis**—SDS-gel electrophoresis was conducted according to the methods of Laemmli (22) and Weber and Osborn (17) using either a slab apparatus (model 4214 cell and model 4200 tank) manufactured by Ortec, Inc. or a cylindrical gel apparatus (model 150A) obtained from Bio Rad Laboratories. Protein samples, containing 10 mM 2 mercaptoethanol, 25% glycerol, 2% sodium dodecyl sulfate, 0.2% bromphenol blue, and the appropriate stacking gel buffer were immersed in a boiling water bath for 3 to 5 min. Electrophoresis was performed using an Ortec-pulsed constant power supply (model 4100) with initial settings of 300 pulses/s and 5 to 10 mA current/sample. A set of protein standards (20 to 40 µg/gel) were run simultaneously in separate gels. Gels were stained by incubation for 12 h in a solution of 0.2% Coomassie brilliant blue R-250 in 50% methanol and 10% acetic acid and destained in a solution containing 50% methanol and 10% acetic acid.

**Isoelectric Focusing**—Isoelectric focusing on polyacrylamide gels was performed using a model 150A Bio-Rad electrophoresis unit and an Ortec-pulsed constant power supply (model 4100). The component used in the polymerization of gels (0.5 cm in diameter by 8.0 cm in height) were: 9% acrylamide (acrylamide:bisacrylamide ratio of 37.5:1), 2% (w/v) ampholytes (pH 3 to 10), 0.5% (w/v) ampholytes (pH 8 to 10), 25% glycerol, and 3% riboflavin. The electrode solutions consisted of 0.1 M phosphoric acid and 0.15 M ethanolamine. Protein samples (5 to 25 µg) were applied in 50% glycerol and 1 mM 2-mercaptoethanol onto the upper gel surfaces (alkaline). Electrophoresis was conducted at constant power (300 V and 300 pulses/s) for up to 20 h. Methyl red and hemoglobin were completely focused in 3 h, but it was necessary to find independently an appropriate electrophoresing time for terminal transferase (by repeated experiments). The pH gradient generated by electrophoresis was determined by incubation of 2-mm gel sections in 0.5 ml of distilled water for 5 h, followed by lyophilization using a microelectrode. Terminal transferase was assayed following extraction of the activity from 2-mm gel slices.

**Enzyme Assays of Terminal Transferase in Polyacrylamide Gels**—Terminal transferase activity was determined by elution of enzyme from the gels in 3 volumes of 25 mM potassium phosphate, pH 7.2, 15% glycerol, and 1 mM 2-mercaptoethanol, followed by the standard assay as described above.

**Molecular Weight Determinations**—The molecular weight of terminal transferase was determined by gel filtration on Sephadex G-100 and Bio-Gel A-0.5m and non-denaturing polyacrylamide gel electrophoresis according to the method of Hedrick and Smith (23). Six slab gels, consisting of 5 through 12% acrylamide content were independently used for the electrophoresis of protein standards (20 µg/gel) and purified human terminal transferase (1 µg/gel). The acrylamide concentration in the gel and stacking gels were varied between 5 and 20% for the two patients. These were designated as follows: RP-3 and Atl-1. This purification procedure was also utilized to prepare terminal transferase from a separate lot of lymphoblasts from patient RP, which was designated RP-2. The purification of terminal transferase from a small lot of cells (RP-1) is presented in the miniprint. MOLT-4 cells, initiated into cell culture from a patient with acute lymphoblastic leukemia (26), were utilized for partial purification of terminal transferase.

**Preliminary Purification**—A suspension of packed lymphoblasts (300 g) was diluted with 3 volumes of extraction buffer (0.25 M potassium phosphate, pH 7.5, containing 1 mM 2-mercaptoethanol) and sonicated twice (15-s bursts). The resulting suspension was stirred at 4°C for 60 min and centrifuged at 25,000 x g for 60 min (Sorvall, GSA rotor) to yield Fraction I (supernatant). To minimize inactivation of terminal transferase in all subsequent steps, the temperature was maintained at 4°C. Furthermore, since terminal transferase from human lymphoblasts was found to be very susceptible to dilution inactivation, the pools of enzyme activity were concentrated immediately by ultrafiltration (PM-10 membrane, Amicon Corp.). The results of the entire purification scheme are summarized in Table I.

**Affi-Gel Blue Chromatography**—The binding of human terminal transferase to Affi-Gel Blue (Cibacron Blue F3GA-Sepharose) is probably a consequence of the recognition of the dye for oligodeoxynucleotide sites on the enzyme. This dye is a competitive inhibitor of p(dA)₅ (at fixed, saturating concentrations of dGTP) as shown in Fig. 1, although the degree of inhibition by Cibacron Blue F3GA is not linearly dependent on dye concentration as shown in the inset to Fig. 1. However, we conclude that Cibacron Blue F3GA binds primarily to the initiator site since the inhibition of dGTP polymerization (at fixed, saturating concentrations of dGTP) by the dye is noncompetitive (data not shown). Cibacron Blue F3GA-Sepharose has been used to form a complex with nucleotide-binding enzymes (27). In at least one instance, substantial purification was achieved by substrate elution (28). However, our attempt to use Cibacron Blue F3GA-Sepharose for specific binding and elution by either dGTP or p(dA)₅ was unsuccessful.

Fraction I (supernatant) was diluted with 3 volumes of 1 mM 2-mercaptoethanol and mixed with 580 ml of Affi-Gel Blue (equilibrated with 50 mM potassium phosphate, pH 7.2, containing 1 mM 2-mercaptoethanol, Buffer A) with gentle stirring for 60 min. Following the preparation of a column (7.5-cm diameter) with the suspension, the gel was washed with 4 liters of Buffer A. Terminal transferase activity was eluted by 3 liters of 0.3 M potassium phosphate, pH 7.2, containing 1 mM 2-mercaptoethanol, and immediately concentrated by ultrafiltration (model 2000, 150-mm membrane). Chromatography on Affi-Gel Blue was repeated once with...
Human Terminal Transferase

Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>Specific activity (units/mg)</th>
<th>Total activity (units)</th>
<th>Yield (%)</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Crude extract</td>
<td>600</td>
<td>22,190</td>
<td>(13.0) b</td>
<td>(300,230)</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>II. Affi-Gel Blue</td>
<td>120</td>
<td>3,720</td>
<td>(61.5) b</td>
<td>(228,575)</td>
<td>74.6</td>
<td>4.4</td>
</tr>
<tr>
<td>III. Hydroxylapatite-I</td>
<td>30</td>
<td>285</td>
<td>335</td>
<td>95,560</td>
<td>31.2</td>
<td>24.3</td>
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<tr>
<td>IV. CM-Sepharose-I</td>
<td>5.1</td>
<td>91</td>
<td>3,585</td>
<td>43,025</td>
<td>14.0</td>
<td>259.8</td>
</tr>
<tr>
<td>V. dATP-Sepharose</td>
<td>5.0</td>
<td>12</td>
<td>23,480</td>
<td>58,700</td>
<td>19.1</td>
<td>1,701</td>
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<tr>
<td>VI. Hydroxylapatite-II</td>
<td>1.0</td>
<td>2.5</td>
<td>62,160</td>
<td>29,750</td>
<td>9.4</td>
<td>2,304</td>
</tr>
<tr>
<td>VII. CM-Sepharose-II</td>
<td>0.65</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Represents polymerization of Mg²⁺dATP onto a synthetic initiator, p(dA)₅₀. One unit of enzyme activity is defined as 1 nmol of radioactive deoxynucleotide incorporated/h.
b Specific activity in crude extracts was determined by polymerization of Mn²⁺dGTP onto p(dA)₅₀. The Mg²⁺dATP specific activities were then obtained by use of dGTP/dATP activity ratios calculated in purified TdT preparations (16). The average ratio of dGTP/dATP activities in the purified preparation was determined to be 2.0.

FIG. 1. Effect of varying p(dA)₅₀ concentration at fixed, saturating concentrations of dGTP on the reaction rate of human terminal transferase: inhibition by Cibacron Blue F3GA. Standard assay conditions were employed except that the final concentration of reaction components included: 25 mM potassium phosphate, pH 7.2, 1 mM [³H]dGTP, 0.5 mM CoCl₂, 1 mM 2-mercaptoethanol, 20% glycerol, and variable p(dA)₅₀ as indicated. The enzyme was 14 pg of partially purified terminal transferase protein (approximate specific activity, 1000 units/mg). The replot of slopes versus dye concentration is shown in the inset. Fractions in the column load volume which possessed terminal transferase activity. Both terminal transferase concentrates were combined to yield Fraction II.

Hydroxylapatite Chromatography—Fraction II was diluted with 4 volumes of Buffer A and applied to a column of Bio-Gel HTP (7.5 × 5.0 cm). Following a wash with 1500 ml of Buffer A, terminal transferase activity was eluted with a linear gradient (2000 ml) from 50 to 350 mM potassium phosphate, pH 7.2, containing 15% glycerol and 1 mM 2-mercaptoethanol. Fractions containing terminal transferase activity were pooled and concentrated to yield Fraction III.

CM-Sephadex C-50 Chromatography—I—Fraction III was diluted with 4 volumes of 25 mM potassium phosphate, pH 7.2, containing 1 mM 2-mercaptoethanol (Buffer B) and applied to a column of carboxymethyl (CM)-Sephadex (2.5 × 15.0 cm). Following a wash with 950 ml of Buffer B, terminal transferase activity was eluted with a linear gradient (500 ml) from 25 to 500 mM potassium phosphate, pH 7.2, containing 1 mM 2-mercaptoethanol. Fractions containing terminal transferase activity were pooled and concentrated to yield Fraction IV.

dATP-Sepharose Chromatography—Fraction IV was diluted with 9 volumes of 1 mM 2-mercaptoethanol and applied to a column of agarose-hexane-2'-deoxyadenosine-5'-triphosphate (dATP-Sepharose, 2.5 × 11.0 cm). The column was washed with 150 ml of Buffer B containing 2 mM dGTP and 0.5 mM MnCl₂, followed by 75 ml of Buffer B alone. Considerable protein, but not terminal transferase activity, was eluted from the column by this wash. Finally, terminal transferase activity was eluted from the dATP-Sepharose column with a linear gradient (300 ml) from 25 to 300 mM potassium phosphate, pH 7.2, containing 1 mM 2-mercaptoethanol. Fractions containing terminal transferase activity were pooled and concentrated by ultrafiltration to yield Fraction V.

Hydroxylapatite Chromatography—II—Fraction V was diluted with 3 volumes of 1 mM 2-mercaptoethanol and applied to a column of Bio-Gel HTP (hydroxylapatite, 1.2 × 3.5 cm). The column was washed with 50 ml of 50 mM potassium phosphate, pH 7.2, containing 10% glycerol, and 1 mM 2-mercaptoethanol (Buffer C). Terminal transferase activity was eluted from the column with a linear gradient (200 ml) from 50 to 300 mM potassium phosphate, pH 7.2, containing 10% glycerol and 1 mM 2-mercaptoethanol. Fractions containing terminal transferase activity were combined and concentrated by ultrafiltration to yield Fraction VI.

CM-Sephadex C-50 Chromatography—II—Fraction VI was diluted with 9 volumes of Buffer B containing 10% glycerol and applied to a column of CM-Sephadex (1.2 × 4.0 cm). The column was washed with 15 ml of Buffer B containing 10% glycerol, and terminal transferase activity was eluted with a linear gradient (150 ml) from 50 to 300 mM potassium phosphate, pH 7.2, containing 10% glycerol and 1 mM 2-mercaptoethanol.
Human Terminal Transferase

Kinetic Constants—Kinetic constants of purified human terminal transferase

**Table II**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Substrate (varied)</th>
<th>Metal ion</th>
<th>$K_{m}$ (μM)</th>
<th>$V_{max}$ (pmol/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dATP</td>
<td>dGTP</td>
<td>Mg$^{2+}$</td>
<td>163.6 ± 26.2</td>
<td>895.9 ± 116.4</td>
</tr>
<tr>
<td>dATP</td>
<td>dGTP</td>
<td>Mn$^{2+}$</td>
<td>59.8 ± 7.1</td>
<td>537.4 ± 42.2</td>
</tr>
<tr>
<td>dATP</td>
<td>dGTP</td>
<td>Mg$^{2+}$</td>
<td>244.1 ± 20.6</td>
<td>952.9 ± 69.0</td>
</tr>
<tr>
<td>dATP</td>
<td>dGTP</td>
<td>Mn$^{2+}$</td>
<td>43.9 ± 4.9</td>
<td>218.7 ± 14.7</td>
</tr>
<tr>
<td>dGTP</td>
<td>dATP</td>
<td>Mg$^{2+}$</td>
<td>0.3 ± 0.0</td>
<td>404.4 ± 15.1</td>
</tr>
<tr>
<td>dGTP</td>
<td>dATP</td>
<td>Mn$^{2+}$</td>
<td>0.1 ± 0.0</td>
<td>322.1 ± 13.4</td>
</tr>
<tr>
<td>dATP</td>
<td>dATP</td>
<td>Mn$^{2+}$</td>
<td>0.6 ± 0.1</td>
<td>543.4 ± 54.8</td>
</tr>
<tr>
<td>dATP</td>
<td>dATP</td>
<td>Mn$^{2+}$</td>
<td>1.8 ± 0.3</td>
<td>151.3 ± 17.6</td>
</tr>
</tbody>
</table>

**FIG. 3.** Estimation of molecular weight of human terminal transferase. Left, molecular weight estimation of human terminal transferase by nondenaturing polyacrylamide gel electrophoresis utilizing the procedure of Hedrick and Smith (23). Standards included carbonic anhydrase (I), carboxypeptidase A (2), ovalbumin (3), inorganic pyrophosphatase (4), bovine serum albumin (5), bovine serum albumin dimer (6), and lactate dehydrogenase (7). Each protein was subjected to electrophoresis at several gel concentrations between 5 and 12% acrylamide. The protein bands were stained with Coomasie brilliant blue G-250 according to the method of Holbrook and Leaver (24) as described under “Experimental Procedures.” The negative slope of each standard protein as well as terminal transferase was calculated by linear regression analysis of the line generated by plotting the following variables: 100 log (Rn × 100) versus the acrylamide gel concentration. Subsequently, a standard curve of negative slopes versus molecular weight for the marker proteins was calculated by linear regression. For terminal transferase activity, a negative slope of 5.31 was obtained, which corresponds to a molecular weight of 60,000. Right, estimation of the molecular weight of human terminal transferase by chromatography on Sephadex G-100. The standards were chromatographed individually as follows: 1) blue dextran 2000 (Vc); 2) bovine serum albumin; and 3) carbonic anhydrase. Human and calf thymus terminal transferase enzyme activities were assayed as described under “Experimental Procedures.”

**FIG. 4.** SDS-polyacrylamide gel electrophoresis. Purified human terminal transferase and several standard protein markers were subjected to SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (22), except that the stacking and separating gel concentrations were 6 and 12%, respectively. Methods utilized for this experiment, including staining of protein bands, were discussed under “Experimental Procedures.” A, 5 μg of ovalbumin (45,000) and 40 μg of myoglobin (17,000); B, 10 μg of bovine serum albumin (67,000) and 5 μg of carbonic anhydrase (30,000); and C, 10 μg of purified human terminal transferase.
FIG. 5. Demonstration of the absence of human serum albumin in our purified enzyme preparation (A) and a terminal transferase activity profile (B). A, Human terminal transferase (10 ng in Tracks 1 and 2) and human serum albumin (10 ng in Tracks 3 and 4) were subjected to polyacrylamide gel electrophoresis according to the method of Laemmli (22), except that SDS was omitted, and the 7% polyacrylamide slab gel was polymerized in the presence of 4 M urea.

B, Terminal transferase activity eluted from 2-mm sections of a nondenaturing polyacrylamide gel (9%) prepared and subjected to electrophoresis according to the method of Laemmli (22). Fraction VII terminal transferase (1 ng) was applied to the gel. The relative migration of albumin on a control gel was $R_m = 0.86 \pm 0.05$.

Discussion

Human terminal deoxynucleotidyltransferase was isolated from lymphoblasts of two patients with acute lymphoblastic leukemia. The specific activity of the enzyme in leukemic cells is relatively high (5 to 800 units/mg) compared to calf thymus (5 to 20 units/mg). Thus, these cells are an excellent source of terminal transferase. The purification steps included binding to Affi-Gel Blue, hydroxylapatite, agarose-hexane-2-deoxynucleotidyltransferase.
Human Terminal Transferase 8639

adenosine-5'-triphosphate, and carboxymethyl-Sephadex. The final step results in an overall 6000-fold purification of human terminal transferase. The enzyme appears to be near homogeneity since the specific activity of the enzyme eluting from the carboxymethyl-Sephadex II column is constant across the peak, the specific activity is similar to that reported by Chang and Bollum (16) for the homogeneous calf thymus enzyme, and the protein is judged to be >95% pure by SDS-gel electrophoresis. For the final stages of enzyme purification, dATP or dCTP polymerization, and not dGTP polymerization, are preferable for monitoring enzyme activity. The assays using dGTP polymerization were designed specifically for relatively impure enzyme preparations of terminal transferase because aggregation of poly(dG) protects the product against exonuclease activities. However, this feature of dGTP polymerization makes it undesirable for assays of the highly purified enzyme. Polymerization with Mg\(^{2+}\)-dATP is linear to greater than 80% of substrate utilization (29) and has been used to monitor protein purification in this report.

The kinetic analyses of polymerization of purine nucleotides with human terminal transferase using p(dA)\(_2\) as initiator show a remarkable similarity to analyses of calf thymus terminal transferase. Both enzymes demonstrate only a terminal addition reaction. For the purified human enzyme, Mg\(^{2+}\)-purine nucleotide polymerization results in a higher reaction velocity than does Mn\(^{2+}\)-purine nucleotide polymerization, in contrast to our previous findings with less pure enzyme preparations (18). The apparent \(K_m\) of both the monomer and initiator are lower in the presence of Mn\(^{2+}\) than Mg\(^{2+}\). Why the reaction velocities are higher with Mn\(^{2+}\) as divalent cation in impure enzyme preparations is not understood.

It has been suggested that ATP may play a unique role in the regulation of terminal transferase activity in vivo (30). However, we find that ATP inhibition is competitive for both dATP and dGTP, and the apparent \(K_i\) is roughly equal to the apparent \(K_m\) for both substrates, indicating that affinities of all these nucleotides for the enzyme are low and nearly equivalent. These results are in disagreement with Modak (30) and Bhalia et al. (31) who report a lower apparent \(K_i\) for ATP, but in agreement with Kato et al. (29) who find an apparent \(K_i\) similar to that reported here.

The striking differences between the human and calf enzymes are in the apparent molecular weight and subunit structure of the protein. Human terminal transferase appears to be a single polypeptide chain with a molecular weight of approximately 62,000. In contrast, the calf thymus enzyme has two subunits and a native molecular weight of 42,000. In addition, a small amount of periodic acid-Schiff's staining material co-migrates with human terminal transferase but not with calf terminal transferase on SDS-gel electrophoresis. No carbohydrate was detected in hydrolyzed samples of calf terminal transferase analyzed by gas chromatography. The identity, quantitation, and confirmation of carbohydrate material associated with human enzyme awaits accumulation of larger amounts of purified protein.

Terminal deoxynucleotidyltransferase from calf thymus has been purified to homogeneity by Chang and Bollum (16) and the physical properties of the protein have been carefully documented. While no in vivo function has been unequivocally established for terminal transferase, its unusual location in mammalian thymus and bone marrow has suggested a role in immunological programming (11, 12). An intracellular cytoplasmic location of thymic terminal transferase was established by subcellular fractionation studies (32) and by immunofluorescence detection of the enzyme in small thymocytes from adult animals (33). More recent immunofluorescence studies have demonstrated that terminal transferase in bone marrow cells, in immature thymocytes (34), and in leukemic lymphoblasts (35) is present exclusively in nuclei. As a consequence, it has been suggested that the cytoplasmic terminal transferase in thymic tissue may be undergoing a post-translational cleavage and may be enzymatically active in vivo, but not functionally active in vivo (34). Therefore, it is possible that the homogeneous enzyme prepared from calf thymus tissue represents a modified form of the protein.

In human lymphoblasts, terminal transferase is located in the nucleus. Its presence in such cells has become an important clinical tool for differential diagnosis (6, 9, 36). Immunocytochemical techniques have been developed for detection of the human enzyme utilizing antibody developed against calf thymus terminal transferase (35, 37-39). Purification of the human enzyme from a nontoxic source is desirable to furnish antigen from another tissue and species for antibody production. Such an antibody would expand the battery of immunocytochemical reagents for use in the differential diagnosis of adult and childhood leukemia and lymphoma.

The purification scheme we report is the first which results in highly purified human terminal transferase. Recently, Bollum and Brown (40) have presented supporting evidence for a high molecular weight form of the enzyme as shown by immunoprecipitation of terminal transferase from a cultured human lymphoblastoid line. Whether differences in structure between the human and calf enzymes reflect only a species variation or intracellular post-translational modifications resulting in two forms of the enzyme is the subject of current investigation.

Acknowledgments—We wish to thank Dr. F. J. Bollum for his helpful discussions and Dr. O. Nelson for providing the clinical material.

REFERENCES


M. R. Deibel, Jr., and M. S. Coleman, unpublished data.

Supplement

10. PURIFICATION OF A HIGH MOLECULAR WEIGHT HUMAN TERMINAL DEOXYNUCLEOTIDYLTRANSFERASE

BY: MARTIN K. DESHEL, JR. AND MARTY SEE COLEMAN

RESULTS

Small scale purification of human terminal transferase. A suspension of pooled lymphocytes from patients with acute lymphocytic leukemia (107) was grown in a volume of extraction buffer (0.05 M potassium phosphate, pH 7.5, containing 1 mM -mercaptoethanol) and incubated twice (15 second bursts) at 37°C with a Host System Saturator (Newark, N.J.). The suspension was stirred at 66 rpm for 60 minutes followed by centrifugation at 25,000 x g for 1 hour (25°C, 12,000 rpm). The supernatant fluid was dialyzed against 10 liters of buffer A (10 mM potassium phosphate, pH 7.5, containing 1 mM -mercaptoethanol (Buffer A)). Substantial precipitation, but not terminal transferase activity, was eluted from the column by a wash with 1 liter of 10 mM ATP in Buffer A, as determined by SDS-polyacrylamide gel electrophoresis (data not shown). Following a second wash with 1 liter of Buffer A, terminal transferase activity was eluted from a single column (200 ml) of DEAE-Sephadex A-50 (phosphate form) with a linear gradient (200 ml) from 0 to 300 mM potassium phosphate, pH 7.5, containing 10 mM ATP and 1 mM -mercaptoethanol. Fractions containing terminal transferase activity were pooled and concentrated to yield Fraction III.

Fraction III was diluted to 10 ml with 0.5 M potassium phosphate, pH 7.5, containing 10% glycerol and 1 mM -mercaptoethanol (Buffer B) and applied to a column of agarose-immobilized 12-mercaptobenzoic acid (5 ml)/12-mercaptopropionate (3 ml). The column was washed with 100 ml of Buffer B, and terminal transferase activity was eluted using 100 ml of 0.5 M potassium phosphate and 0.5 M MgCl2 in Buffer B. To remove excess phosphoglycerate-1-6-diphosphate from the eluate, the column was eluted with 300 ml of Affigel Blue. Following a wash of this Affigel Blue column with 100 ml of Buffer B, terminal transferase activity was eluted from a single column (200 ml) of DEAE-Sephadex A-50 (phosphate form) with a linear gradient (200 ml) from 0 to 300 mM potassium phosphate, pH 7.5, containing 10 mM ATP and 1 mM -mercaptoethanol. Fractions containing terminal transferase activity were pooled and concentrated to yield Fraction IV.

Table 1

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>Specific Activity (units/mg)</th>
<th>Total Activity (units)</th>
<th>Purification Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Crude Extract</td>
<td>245</td>
<td>6,275</td>
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<td>2. Affigel Blue</td>
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<td>3. DEAE-Sephadex</td>
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<td>4. Hydroxylapatit</td>
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<td>4.8</td>
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<td>39,456</td>
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<td>6. Immunologic</td>
<td>*</td>
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</table>

* Values not determined

This represents purification of a specific activity on a specific inhibitor, the unit of activity is defined as 1 mole of radioactive IDP incorporated per hour.

Figure 1-5: Sodium dodecyl sulfate polyacrylamide gel electrophoresis of Fraction VI human lymphoblast transferase. The gel was prepared according to methods described by Weber and Osborn (11), except that the polymerization was conducted in the presence of 10% sucrose. Gel A contained approximately 30 µl of Fraction VI protein and gel B contained binding assay effluent and radioactive eluates, incubated with radioactive analysis of 87,500 and 30,000, respectively.
Purification of a high molecular weight human terminal deoxynucleotidyl transferase.
M R Deibel, Jr and M S Coleman


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