TERMINAL DEOXYRIBONUCLEOTIDYL TRANSFERASE ACTIVITY OF GERMINATING WHEAT EMBRYO

Teresa BRODNIEWICZ-PROBA and Jerzy BUCHOWICZ
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-532 Warsaw, Poland

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1. Introduction

Since 1962, when terminal deoxyribonucleotidyl transferase (TDT) activity was described for the first time [1], the enzyme has been extensively purified and characterized [2-5]. Its physiological role, however, still remains completely unknown and the elucidation of its possible physiological functions has not received sufficient attention.

The enzyme would be considered to play a part in DNA biosynthesis if it were widely distributed. Unfortunately however, data on its occurrence are scant. According to present opinion [1–7], the occurrence of TDT is limited, mainly if not exclusively, to thymus gland. Recently, similar enzyme preparations have been isolated from other mammalian sources [8,9], but even in these cases the possibility that the enzyme originated in the thymus gland is difficult to exclude. This difficulty can be circumvented by using plant tissues, which do not have secretory glands. To our knowledge there is only one report on TDT of plant origin, that given by Srivastava [10] for cultured tobacco tissues.

Extending the search for TDT of non-mammalian origin, we describe here the isolation of a similar enzyme from germinating wheat embryo. A possible involvement of the enzyme in new DNA strand formation is suggested.

2. Materials and methods

Wheat grains (*Triticum aestivum*, cv. Kutnowska) were germinated under optimal conditions for 24 h. Embryos were then separated and used for the isolation of TDT activity. The enzyme was isolated essentially according to the procedure of Bhattacharyya [9]. A sample of 500 embryos, about 2.1 g, was homogenized with 10 ml of 0.1 M Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.25 M sucrose and the homogenate centrifuged at 18 000 *g* for 20 min at 4°C. The crude supernatant was fractionated with ammonium sulfate. The fraction that precipitated between 40 and 65% saturation was collected, desalted on a Sephadex G-25 column and applied to a DEAE-cellulose (DE-52) column (10 × 2 cm). The column was then eluted with about 50 ml of 60 mM NaCl in the Tris-HCl buffer. Fractions of 0.5 ml were collected and tested for TDT activity. The enzyme was eluted as a single peak corresponding to fractions 27–30. The peak fractions were pooled and used as the enzyme source. The enzyme was quite stable at this purification stage and could be stored at −20°C for several weeks without appreciable loss of activity.

The standard assay contained in a final volume of 0.1 ml: 50 mM Tris-HCl (pH 7.8), 5 mM dithiothreitol, 0.6 mM $MnCl_2$, 50 mM KCl, 2 μCi of [^{3}H-methyl]-thymidine triphosphate (specific radioactivity 30 Ci/mmole, Amersham), 20 μg of calf thymus DNA (Sigma), and 40 μl of the enzyme preparation containing approx. 30 μg of protein. The mixture was incubated at 37°C for 30 min. The acid-precipitable material was then collected on a filter paper disk [2] and used for the measurement of radioactivity with a Packard liquid scintillation counter.

Pyrimidine clusters were isolated according to the procedure of Shapiro and Chargaff [11]. An excess of non-labelled DNA (1 mg) was added to the standard enzyme assay at the end of the incubation period. The mixture was then treated with 2 volumes of

ethanol. The precipitate was hydrolysed with 0.1 M H_2SO_4 at 100°C for 35 min and the resulting pyrimidine oligonucleotides were separated by two-dimensional paper chromatography [11].

3. Results

The purification procedure resulted in obtaining 2.3 mg of enzyme preparation from 500 wheat embryos, of specific activity approx. 25-fold higher than that of the crude extract (table 1).

The purified preparation catalysed the effective incorporation of [^{3}H]dTTP into the acid-insoluble fraction (table 2). The reaction was strongly inhibited by dATP, dGTP and dCTP, and depended on the presence of Mn^{2+} ion and a polynucleotide primer. Among the primers, commercial DNA was the most effective. Considerable incorporation was, however, observed also when total RNA of wheat germ was used. These characteristics indicate that the enzyme preparation isolated is similar to TDT in respect of its catalytic activity.

Assuming the molecular weight of the added DNA to be 10^7, 1.53 thymidylate residues on average were added to each primer molecule. In attempts to assess the actual size of the oligo(dT) formed, the reaction product was depurinated and hydrolysed to obtain polypyrimidine clusters. When the hydrolysis products were separated by paper chromatography, considerable radioactivity was found in all oligonucleotides containing thymidylate residues (table 3). However, the longer the polythymidylate run the higher the observed relative frequency of the labelled product. The ratio of radioactive to non-radioactive pyrimidine clusters clearly indicated that the primer was enriched in oligo(dT) tracts of various chain lengths. The longest of them could not be resolved by paper chromatography.

Table 1
Summary of the purification procedure

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)
Crude extract	160.0	780	4.9
$(NH_4)_2SO_4$ precipitate	105.0	650	6.2
Sephadex G-25	82.6	540	6.5
DE-52 chromatography	2.3	285	123.9

One unit of the activity corresponds to the amount of enzyme which incorporates 1 pmol of dTTP into acid-precipitable product under the standard assay conditions (Materials and methods).

Table 2
Requirements of terminal deoxyribonucleotidyl transferase from wheat embryo

Reaction mixture	Nucleotide incorporation (pmoles/assay)
Complete	3.05
+ dATP	0.81
+ dGTP	1.12
+ dCTP	1.34
+ dATP + dGTP + dCTP	0.15
– [^{3}H]dTTP + [^{3}H]UTP	0.50
– $MnCl_2$	0.06
– DNA	< 0.01
heated DNA used	2.00
– DNA + poly(rA)·$(dT)_{10}$	0.90
– DNA + oligo(dT)	0.95
– DNA + poly(rA)	<0.01
– DNA + yeast tRNA	0.02
– DNA + wheat total RNA	0.90
– Enzyme	< 0.01
Complete, zero time	< 0.01

Complete reaction mixture is given under Materials and methods. The concentrations of nonlabelled nucleoside triphosphates were 100 μM. [^{3}H]UTP and polynucleotides were used in amounts equal to [^{3}H]dTTP (radioactivity) and DNA (weight), respectively.

4. Discussion

The ability to catalyse polynucleotide-primed polymerization of dTTP in a reaction strongly inhibited by other deoxyribonucleoside triphosphates indicates that the enzyme preparation isolated from wheat embryos is similar to TDT purified from calf thymus gland by Bollum and collaborators [1–3,5]. Preferential utilization of dTTP over UTP makes it possible to distinguish the preparation obtained from a related terminal nucleotidyl transferase activity, reported by Krakow et al. [12] to occur also in the same gland.

In considering the possible physiological functions

of TDT, the following features should be emphasized: (a) its occurrence is not limited to thymus gland and related mammalian sources since it may also be isolated from wheat embryo; (b) long polypyrimidine tracts occur in eukaryotic DNAs [13,14] and may be thought to arise from a reaction catalysed by this enzyme; (c) the enzyme is able to use RNA as a primer and, therefore, may be involved in an initial stage of new DNA strand formation which is known to be primed by RNA [15,16]; and (d) the apparent cytoplasmic localization of this enzyme and the fact that it can work at the absence of DNA make it possible to understand why in vivo thymidine is incorporated into wheat embryo cytosol polydeoxyribonucleotides at an early germination stage, when nuclear DNA replication was not yet been triggered [17].

All these observations make us believe that TDT may collaborate in cellular DNA synthesis, especially at initial stages of this process.

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Table 3
Distribution of radioactivity in pyrimidine oligonucleotides resulted from acid hydrolysis of the TDT reaction product

Isostich	Composition	% of total thymine	% of total radioactivity	Frequency ratio (radioactive: non-radioactive)
1	T	18.4	8.1	0.4
2	CT	5.7	2.9	0.5
2	T_2	9.2	6.3	0.7
3	C_2T	2.3	1.1	0.5
3	CT_2	5.9	3.6	0.6
3	T_3	5.2	4.0	0.8
4	C_3T	1.0	0.3	0.3
4	C_2T_2	3.5	2.7	0.8
4	CT_3	4.1	3.3	0.8
4	T_4	3.0	3.2	1.1
5	C_3T_2	1.8	0.8	0.4
5	C_2T_3	3.1	2.6	0.8
5	CT_4	2.7	2.3	0.9
5	T_5	1.6	2.5	1.6
6	C_4T_2	0.9	0.4	0.4
6	C_3T_3	1.8	1.6	0.9
6	C_2T_4	1.4	3.0	2.1
6	CT_5	1.0	2.3	2.3
6	T_6	0.4	1.8	4.5
> 6	(unresolved)	15.0	36.0	2.4

Experimental conditions were as described in Materials and methods. The oligonucleotide composition is indicated by the symbols for the nucleosides.

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