ASSOCIATION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE ACTIVITY WITH CHROMATIN FROM PLANT TISSUE*

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SUMMARY. A terminal deoxynucleotidyl transferase activity has been found associated with chromatin from tissue cultures of tobacco. To the knowledge of this author, this is the first example of the presence of this enzyme in plant tissues.

INTRODUCTION

Although a number of enzymes have been found to be associated with chromatin from mammalian cells (1-8) such reports are rather limited for plant tissues (9-11). During an investigation of DNA polymerases in normal and crown gall tumor tissue cultures of tobacco a terminal deoxynucleotidyl transferase (TDT) activity was found associated with the chromatin. Presumably this is the first time the occurrence of this enzyme in plant tissues is described since so far this enzyme has been found only in thymus tissue from animals (12).

MATERIALS AND METHODS

Three-week-old cultures of stem tissue of tobacco (Nicotiana tabacum cv. Wisconsin 38) maintained on Murashige and Skoog's (13) medium were used. The chromatin was prepared and purified by ultracentrifugation through 1.7 M sucrose, essentially as described by Huang and Bonner (14). The chromatin pellet obtained by ultracentrifugation was washed by suspension in 0.05 M Tris-HCl, pH 8.0, and centrifugation at 30,000 x g for 30 min. The washed chromatin pellet was dispersed in 0.01 M Tris-HCl, pH 8.0, and used for TDT assays as described under Table 1.

In order to solubilize TDT, chromatin was stirred (2 hr.) with 1 M NaCl

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Table 1

Effect of deoxynucleotide triphosphate addition on TDT activity.

Reaction mixture	^{3}H-TTP incorporated, % of control
Complete system (control)	100
≫ plus dATP	3.4
≫ plus dATP and dCTP	2.0
≫ plus dATP, dCTP and dGTP	2.6

The complete system contained in a final volume of 0.2 ml: 10 μm Tris-HCl, pH 8.3; 1.2 μm MgAc; 4 μm dithiothreitol; 1.2 μm NaCl; 2 μC (^{3}H-methyl)-TTP (specific radioactivity 7.7 mC/μm); 20 μg heat denatured calf thymus DNA and chromatin equivalent to 5 μg of DNA; and where indicated 0.16 μm of each of dATP, dCTP and dGTP. After 30 min. incubation at 37°C, 100 μg of yeast RNA and 1 ml of 10% TCA were added. The precipitates were collected on pre-soaked (overnight in saturated pyrophosphate solution) B_6 membrane filters, washed with 5% TCA, dried and counted using toluene based scintillation fluid. The complete system gave 5034 cpm.

(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 (30,000 x g, 20 min.) was dialyzed (5) for four hours against six volumes of water. The precipitate formed was removed by centrifugation and the supernatant obtained was used for the TDT assays.

Chromatin DNA content was measured by the diphenylamine procedure (15) after previous hydrolysis in 5% trichloracetic acid (TCA) for 15 min at 90°C.

RESULTS AND DISCUSSION

The data presented in Table I show that optimum incorporation of ^{3}H-TTP into acid insoluble material was obtained in the absence of other deoxynucleotide triphosphates and that the addition of one or more of these triphosphates strongly inhibited the reaction. Unlike the TDT from mammalian chromatin (5, 16) but like the soluble TDT of Bollum (17) the TDT from tobacco chromatin could not catalyse the incorporation of ribonucleotide into DNA when ATP-8-^{14}C was used instead of ^{3}H-TTP.

DNA in chromatin was a relatively poor primer for endogenous TDT since

Table 2

Effect of changes in reaction mixture on TDT activity

Reaction mixture	^{3}H-TTP incorporated, % of control
1. Complete system (control)	100
2. 1 minus denatured DNA	58
3. 2 + native DNA, 20 μg	120
4. 2 + $(pT)_{10}$, 0.2 units	107
5. 1 + actinomycin D, 20 μg	96
6. 1 + pyrophosphate, 1 μm	1
7. 1 + EDTA, 5 μm	105
8. 1 + p-chloromercuribenzoate, 0.5 μm	120
9. 1 + N-demethyl rifampicin, 50 μg	40
10. 1 + boiled ribonuclease A, 10 μg	102
11. 1 + deoxyribonuclease I, 10 μg	7

Composition of the complete system and incubation time were the same as described in the legend of Table 1. The inhibitors were added at the same time as the other components of the reaction mixture. Where indicated, the incubated mixture was heated at 100°C for 10 min., and then treated with RNase or DNase at 37°C for 20 min. The complete system gave 3865 cpm.

Table 3

Response of solubilized TDT to various primers

Primer	^{3}H-TTP incorporated, % of control
Denatured DNA, 20 μg (control)	100
Native DNA, 20 μg	110
$(pT)_{10}$, 0.2 units	97
No primer added	3
Denatured DNA, 20 μg, but with 0.16 μm of each of dATP, dCTP, and dGTP	4

The composition of the reaction mixture for TDT assays and the incubation time were the same as those described in the legend of Table 1, except that solubilized TDT instead of chromatin and primers were used. Control gave 1950 cpm.

the activity was increased on adding exogenous primer (Table 2). The activity of the solubilized enzyme was, however, completely dependent upon the addition of primer (Table 3). It is important to note that $(pT)_{10}$ was as effective as DNA for ^{3}H-TTP incorporation both with whole chromatin (Table 2) and the solubilized enzyme (Table 3) indicating a lack of template requirement, a

characteristic of TDT. A greater stability and the lack of inhibition of TDT activity by actinomycin D, EDTA, and p-chloromer curibenzoate (Table 2) further distinguishes this enzyme activity from DNA polymerase activities in tobacco tissue. The lack of inhibition of tobacco TDT activity by EDTA is in contrast to the inhibition of mammalian TDT (5, 16, 18) by this agent.

In an analogous study, the details of which would be reported elsewhere, the TDT activity in the tobacco crown gall tumor tissue cultures was found to be about 1/2 that of normal tobacco tissue cultures. This was in sharp contrast with the DNA polymerase activities which were 10-20 fold higher in the tumor tissue than in the normal tissue. The biological role of TDT in the tobacco tissue, as in mammalian systems, remains unknown.

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