# MULTIPLE DNA POLYMERASE ACTIVITY SOLUBILIZED FROM HIGHER PLANT CHROMATIN

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DNA polymerase preparations solubilized from chromatin of unwashed and washed sugar beet storage tissue exhibit multiple activity peaks when separated by DEAE-Sephadex chromatography. Activity peaks isolated from unwashed and washed tissue required $Mg^{2+}$, all four deoxynucleoside triphosphates and added DNA. Activity from peaks isolated from washed tissue was completely sensitive to pancreatic DNase and insensitive to RNase. Enzyme activity was increased in the tissue during the washing period. In addition, there was a drastic change in template specificity from single-stranded primer in unwashed tissue to double-stranded DNA in washed tissue.

Recent investigations of DNA polymerase activity in whole eukaryotic tissues and in different cellular locations have indicated the presence of multiple DNA polymerases having distinct properties[1-4]. In addition, the cellular concentration of certain of these enzymes change during specific stages of normal or induced growth[5-7]. In an attempt to elucidate the controls of nucleic acid synthesis in higher plants, we have chosen sugar beet storage tissue as an experimental system. Following slicing of the tissue, washing for extended time periods in sterile phosphate buffer results in a dramatic increase in nucleic acid synthesis[8,9]. To investigate the possible presence and properties of multiple DNA polymerases in plants, the isolation of chromatin from unwashed and washed sugar beet tissue was selected as a starting point and a source of the enzyme(s).

In this communication we report the separation of four peaks of DNA polymerase activity by DEAE-Sephadex chromatography of enzyme preparations from both unwashed and washed tissue. Two of the peaks from washed tissue eluted at the same salt concentration as two of the peaks from unwashed tissue. Furthermore, three of the four peaks from washed tissue exhibited a marked

preference for double-stranded template, whereas a preference for single-stranded DNA was observed with activities from unwashed tissue.

## Materials and Methods

Sliced sugar beet roots were prepared and washed in sterile phosphate buffer, pH 6.5, as described previously.[10]

Chromatin was isolated by a modification of a procedure described by Huang and Bonner[11] from 900g lots of unwashed or 20-hour-washed tissue. DNA polymerase activity was solubilized from chromatin and desalted by passage through Sephadex G-50 as previously described for RNA polymerase.[12] Sugar beet DNA was isolated by a modification of a procedure[13] which included SDS-EDTA-SSC fractionation, pronase and ribonuclease treatment and hydroxylapatite column chromatography.

The soluble DNA polymerase assay contained in a total volume of 0.4 ml: 40 μmoles tris-HCl, pH 8.0; 2 μmoles $MgCl_2$; 2 μmoles dithiothreitol; 0.1 μmole each of dATP, dCTP, dGTP; 0.004 μmole TTP, 0.0006 μmole of $^3$H-TTP (specific radioactivity 18.8 mc/μmole); 20 μg DNA and 0.05 ml enzyme (corresponding to 10-15 μg protein). The reaction was initiated with the addition of enzyme, run for 30 minutes at 37° and stopped with the addition of 3 ml of 40 mM sodium pyrophosphate in 10% TCA. The assay mixtures were filtered through glass filters (Whatman GF/A) which were then washed repeatedly with 5% TCA, dried, and the radioactivity determined by liquid scintillation. Protein determination was by the Lowry method.[14]

## Results

Extract. Following desalting of the enzyme preparation through a Sephadex G-50 column (2.5 x 7.5 cm), the extract was partially characterized. DNA polymerase activity isolated from unwashed and washed tissue was dependent on $Mg^{2+}$, added DNA and all four deoxynucleoside triphosphates (Table 1). Activity was linear through 30 minutes and proportional to added protein up to 50 μg.

DEAE-Sephadex chromatography. Extracts (from unwashed and washed tissue)

Table 1. Properties of DNA polymerase activity in extract solubilized from chromatin.

| | Unwashed tissue | Washed tissue |
|---|---|---|
| | relative activity | |
| Complete* | 100 | 100 |
| -$Mg^{2+}$ + 12 mM EDTA | <1 | <1 |
| -dATP | 18 | 6 |
| -dATP, dCTP | 6 | 3 |
| -dATP, dCTP, dGTP | <1 | 2 |
| -DNA | 1 | 2 |
| -assay DNA + native C. T. DNA | 32 | 40 |

*Complete: unwashed tissue = 25 pmoles TMP incorporated/mg protein/ 30 minutes (denatured calf thymus DNA as template).
washed tissue = 75.2 pmoles TMP incorporated/mg protein/ 30 minutes (activated calf thymus DNA as template).

were separately adsorbed to columns of DEAE-Sephadex A-25 (1 x 20 cm) previously equilibrated with TGMED buffer[15], pH 7.9. The columns were washed with 15 ml of 0.032 M $(NH_4)_2 SO_4$ in TGMED and then eluted with an 80 ml linear gradient of $(NH_4)_2 SO_4$ (0.032 to 0.08 M) in TGMED (Figure 1). Four major activity peaks were observed from unwashed and washed tissues. Peaks I and II of both tissues appeared to elute from the columns at the same salt concentration. Peak III from washed tissue, a major activity peak, appeared to be absent in unwashed tissue. Although similar amounts of protein were solubilized from both tissues, washing resulted in a tremendous increase in DNA polymerase activity (Figure 1). Activity peaks from unwashed tissue were less stable than those from washed tissue. Activity was reduced by 50% after one week in liquid nitrogen for peaks

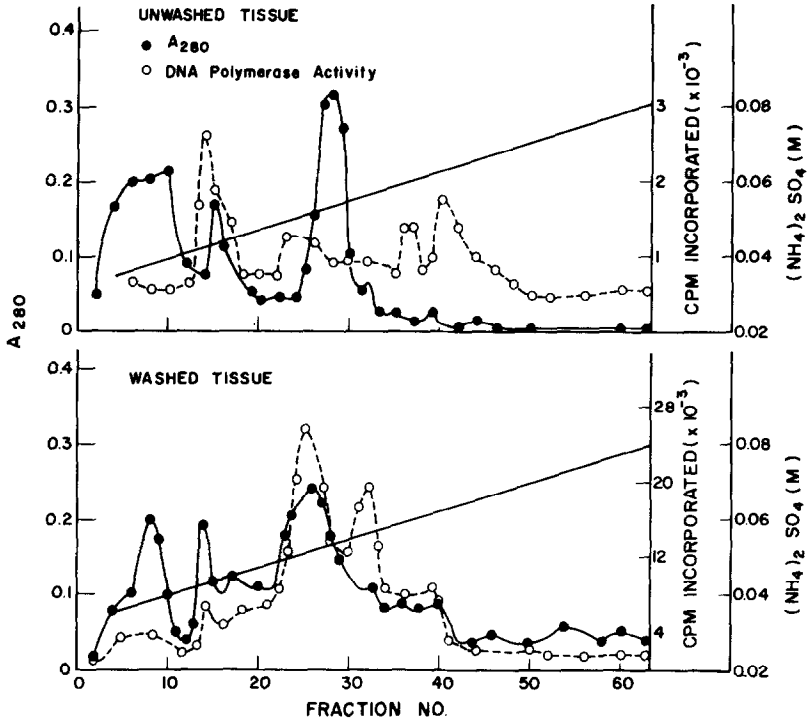


Figure 1. DEAE-Sephadex chromatography of DNA polymerase activity solubilized from chromatin of unwashed and washed sugar beet tissue. Unwashed tissue profile: Peak I = tubes 14-16, Peak II = tubes 23-25, Peak III = tubes 36,37 and Peak IV = tubes 40-42. Washed tissue profile: Peak I = tubes 14-16, Peak II = tubes 24-26, Peak III = tubes 31-34 and Peak IV = tubes 38-40.

isolated from unwashed tissue. With the exception of peak I, the activity from washed tissue was reduced only 10% after two weeks with repeated freezing and thawing. Other attempts at purification using DEAE-Sephadex in batch columns with two salt steps followed by chromatography with linear gradients on the salt step fractions have resolved four activity peaks at the salt concentrations observed in Figure 1.

Properties of DNA polymerase activities. Peak tubes of the four major activity peaks from both tissues were separately pooled and the DNA polymerase activity characterized (Table 2). Activity in each peak was dependent on all four deoxynucleoside triphosphates, $Mg^{2+}$, added DNA and enzyme. Activity was linear through 45 minutes and proportional with respect to enzyme concentration

Table 2. Properties of DNA polymerase activity peaks following DEAE-Sephadex chromatography.

| | Unwashed tissue | | | |
|---|---|---|---|---|
| | Peak | | | |
| | I | II | III | IV |
| Complete | 15.5* | 38.9 | 11.3 | 20.3 |
| -$Mg^{2+}$ + 12 mM EDTA | 0.5 | 0.9 | 1.0 | 0.2 |
| -dNTP | 1.1 | 0.8 | 0.0 | 1.5 |
| -DNA | 0.5 | 0.3 | 0.2 | 0.2 |
| -enzyme | 0.2 | 0.1 | 0.0 | 0.1 |
| | Washed tissue | | | |
| | I | II | III | IV |
| Complete | 72.6 | 1936.5 | 1054.2 | 668.1 |
| -$Mg^{2+}$ + 12 mM EDTA | 0.0 | 0.0 | 0.0 | 6.0 |
| -dNTP | 3.0 | 4.1 | 1.0 | 0.6 |
| -DNA | 0.0 | 1.0 | 1.6 | 0.8 |
| -enzyme | 0.2 | 0.0 | 0.1 | 0.7 |

*Activity expressed as pmoles/mg of protein in 30 minutes employing saturating levels of template yielding the most activity for each peak (see Table 3).

(up to 40 μg in assay). Further characterization of the activity of each peak was possible due to the high activity in peaks isolated from washed tissue. Activity in all four peaks was completely sensitive to actinomycin D (40 μg/ml). The products of the reaction employing each peak separately were completely sensitive to pancreatic DNase (20 μg/ml) and insensitive to RNase (20 μg/ml). Activity was approximately 80% of that observed with activated calf thymus DNA when poly (dA-T) was employed as template. No activity was observed when

poly (rA-dT) was employed as the primer/template for any of the activity peaks.

Template specificity. The four activity peaks from both tissues were separately studied to determine (1) whether the peaks have different template requirements and (2) whether this specificity was altered during the washing procedure. As can be seen in Table 3, there is an extensive shift in overall

Table 3. Template Specificity of DNA polymerase activity peaks.

| UNWASHED TISSUE | DEAE-SEPHADEX PEAK | | | |
|---|---|---|---|---|
| TEMPLATE | I | II | III | IV |
| ACTIVATED C.T. DNA | 12.8* (100) | 8.1 (100) | 11.3 (100) | 0.4 ( 100) |
| NATIVE C.T. DNA | 0.0 ( 0) | 13.0 (161) | 0.0 ( 0) | 2.6 ( 628) |
| DENATURED C.T. DNA | 0.0 ( 0) | 38.9 (482) | 7.2 ( 63) | 20.3 (4842) |
| DENATURED S.B. DNA | 15.5 (120) | 4.5 ( 56) | 0.0 ( 0) | 0.0 ( 0) |
| NATIVE S.B. DNA | 10.0 ( 78) | 2.0 ( 25) | 1.3 ( 11) | 0.0 ( 0) |
| YEAST RNA | 0.0 ( 0) | 0.0 ( 0) | 1.3 ( 10) | 0.0 ( 0) |
| WASHED TISSUE | | | | |
| ACTIVATED C.T. DNA | 23.5 (100) | 1936.5 (100) | 1054.2 (100) | 668.1 ( 100) |
| NATIVE C.T. DNA | 72.6 (309) | 258.3 ( 13) | 119.5 ( 11) | 18.5 ( 3) |
| DENATURED C.T. DNA | 2.4 ( 10) | 107.4 ( 6) | 37.3 ( 4) | 7.1 ( 1) |
| DENATURED S.B. DNA | 8.4 ( 4) | 22.2 ( 1) | 11.6 ( 1) | 31.5 ( 5) |
| NATIVE S.B. DNA | 31.9 (136) | 134.7 ( 7) | 35.3 ( 3) | 23.7 ( 4) |
| YEAST RNA | 0.0 ( 0) | 0.0 ( 0) | 0.0 ( 0) | 1.5 ( 1) |

*Pmoles/mg of protein for 30 minutes. Numbers in parenthesis represent percentage of control activity based on employment of activated calf thymus DNA as template. S.B. = Sugar beet.

template specificity during the washing period. In unwashed tissue there is a preference for single-stranded DNA, especially noticeable in peaks II and IV. In washed tissue, however, double-stranded template is preferred, primarily due to the strong preference for activated calf thymus DNA exhibited by peaks II, III and IV.

## Discussion

The slicing and washing of sugar beet storage tissue results in increased DNA synthesis as evidenced by an increase in DNA polymerase activity. At this time we do not know if protein synthesis is necessary for the observed increase in polymerase activity. If enzyme activation is involved, increased activity could result from alteration of existing polymerase molecules or by the simple removal of an inhibitor during the washing period. Based on the DEAE-Sephadex profiles and the various peak characteristics, the increased activity may have resulted from de novo synthesis, enzyme activation or both. Peaks I and II from both tissues elute at the same salt concentrations and have similar properties and, therefore, may be the same proteins. Peak III of washed tissue, however, is not detectable in unwashed tissue and its activity could be a result of de novo synthesis. This does not, however, preclude the possibility of an alteration of an existing protein.

The interesting change in template specificity following the washing period may also have resulted from protein alteration or an induction of DNase activity and its association with the polymerase activities. The preference for double-stranded template in washed tissue does not agree with DNA polymerase activity from corn[16] and from various algae[17,18] in which single unfractionated enzymes were found to prefer denatured templates. Although it is possible that the high activity of sugar beet polymerase observed with activated calf thymus DNA was primarily a result of the exposure of 3' hydroxyl groups[19], high activity was also observed when native calf thymus or native sugar beet DNA was employed as template.

The product of the DNA polymerase reaction as isolated from corn[16] was specified by the base composition of the native primer. Employing denatured template, however, an increased incorporation of dAMP was observed. In our studies further purification of the activity peaks from unwashed and washed sugar beet tissue and further characterization of the reaction products should indicate whether or not the change in template preference results in products of altered base composition. In addition, studies are underway to determine if the activity peaks are all unique enzymes, if enzyme alterations occur during washing and, ultimately, the function of each of the enzymes in repair or replication of the plant genome.

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## References

1. L. M. S. Chang and F. J. Bollum, J. Biol. Chem. 246, 5835 (1971).
2. D. Spencer and R. R. Whitfeld, Arch. Biochem. Biophys. 132, 477 (1969).
3. R. L. Momparler, M. Rossi and A. Labitan, J. Biol. Chem. 248, 285 (1973).
4. A. Matankage, E. W. Bohn and S. H. Wilson, Fed. Proc. 32: 451 (1973).
5. L. M. S. Chang and F. J. Bollum, J. Biol. Chem. 247: 7948 (1972).
6. J. Chiu and S. C. Sung, Biochem. Biophys. Acta. 269: 364 (1972).
7. P. Grippo and A. Lo Scavo, Biochem. Biophys. Res. Commun. 48: 280 (1972).
8. C. J. Leaver and J. Edelman, Biochem. J. 97: 27 (1965).
9. C. T. Duda and J. H. Cherry, Plant Physiol. 47: 262 (1971).
10. V. L. Dunham, B. C. Jarvis, J. H. Cherry and C. T. Duda, Plant Physiol. 47: 771 (1971).
11. R. C. C. Huang and J. Bonner, Proc. Natl. Acad. Sci. U.S. 48: 1216 (1962).
12. V. L. Dunham and J. H. Cherry, Phytochem. 12 (1973) in press.
13. J. Marmur, J. Mol. Biol. 3: 208 (1961).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. Biol. Chem. 193: 265 (1951).
15. R. G. Roeder and W. J. Rutter, Proc. Natl. Acad. Sci. U.S. 65: 675 (1970).
16. E. R. Stout and M. Q. Arens, Biochem. Biophys. Acta. 213: 90 (1970).
17. O. Th. Schonherr and H. M. Keir, Biochem. J. 129: 285 (1972).
18. S. J. Keller, S. A. Biedenbach and R. R. Meyer, Biochem. Biophys. Res. Commun. 50: 620 (1973).
19. A. Kornberg, Science 163: 1410 (1969).