Poly(A) polymerase and poly(ADP-ribose) polymerase activities in normal and crown gall tumor tissue cultures of tobacco

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Received 3 May 1985

Normal and crown gall tumor tissue cultures of tobacco had poly(ADP-ribose) polymerase associated with the chromatin and two poly(A) polymerases (separable by DEAE-Sephadex A-25 chromatography) with the soluble fraction. The two poly(A) polymerases resembled mammalian enzymes in molecular mass (65–70 kDa), K_m^{ATP} (0.1–0.2 mM), primer and substrate preference (poly(A) and ATP, respectively), pH optimum (8–8.5), cation requirement (0.25 mM Mn^{2+}/1 mM Mg^{2+} = 5–15), inhibition by 3′-dATP and spermine and lack of inhibition by α-amanatin. Likewise the tobacco tumor tissue contained 4-times higher activities of both poly(A) polymerases and 6-times higher activity of poly(ADP-ribose) polymerase as compared to the normal tissue indicating their enhanced function in transformed cells.

Poly(A) polymerase *Poly(ADP-ribose) polymerase* *Crown gall tumor* *Tobacco tissue*

1. INTRODUCTION

There are only a few reports on poly(A) polymerase and poly(ADP-ribose) polymerase in plant cells [1–7] which carry out post-transcriptional addition of AMP residues from ATP to 3′-OH end of m-RNA and poly ADP-ribosylation of proteins [8–9], respectively. The former enzyme is important for the generation of functional m-RNA and the latter enzyme has been postulated to play a role in the regulation of DNA repair and replication, gene expression, differentiation and cellular transformation [9]. Increased activities of these enzymes in leukemic cells compared to normal cells have been reported from this and other laboratories [10–14]. Likewise we report here a much higher activity of these enzymes in crown gall tumor cultures as compared to the normal tissue cultures of tobacco. The crown gall tumors carry the integrated tumor inducing plasmid which is transferred by *Agrobacterium tumefaciens* to host plant cell upon cellular transformation [15].

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2. MATERIALS AND METHODS

2.1. *Plant material*

Proliferating cultures of normal and bacteria-free crown gall tumor tissue of tobacco (*Nicotiana tabacum* cv. Wisconsin 38) maintained as in [16] were used.

2.2. *Partial purification and assay of poly(A) polymerases*

This was carried out to separate the 2 poly(A) polymerases and remove any components which may interfere in their assay. Normal or tumor tissue (40–60g) was homogenized in a chilled Waring blender with buffer A (50 mM Tris-HCl, pH 8.0, 250 mM sucrose, 1 mM $MgCl_2$, 1 mM DTT (dithiothreitol), 1 mM phenylmethylsulfonyl

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

fluoride). The homogenate was filtered through cheesecloth and Miracloth, and the filtrate was centrifuged at 30000 × *g* for 30 min.

The proteins in the supernatant were precipitated by 75% saturation with ammonium sulfate, dissolved in buffer B (50 mM Tris-HCl, pH 8.0, 1 mM DTT, 10% glycerol), dialysed against the same buffer, clarified by centrifugation and passed through a DEAE-Sephadex A-25 column (30 × 2.8 cm) equilibrated with buffer B. Part of the poly(A) polymerase activity was obtained in flow through and buffer B wash from the column (polymerase I), whereas the remainder (polymerase II) was step eluted with buffer B containing 0.4 M $AmSO_4$. Fractions containing poly(A) polymerase activity were pooled, concentrated by negative pressure dialysis, and used in various studies. Sucrose (5–25%) gradient centrifugation (40000 rpm, 48 h, Beckman SW-50 rotor) and Sephadex G-100 column (1.8 × 50 cm) chromatography with 50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.3 M KCl, 10% glycerol buffer were used to determine the molecular masses with reference to alcohol dehydrogenase, bovine serum albumin and ovalbumin markers. The standard assay for poly(A) polymerase consisted of 10–60 μl enzyme, 50 mM Tris-HCl, pH 8.0, 25 mM KCl, 0.05 mM ATP, 0.25 mM $MnCl_2$, 4 μCi[^{3}H]ATP (23.3 Ci/mmol), and 180 μg poly(A) primer in a final volume of 0.2 ml. Incubation was at 37°C usually for 30 min and linearity was established with respect to time and amount of enzyme. The reaction was terminated by adding 0.1 ml of 3% sodium pyrophosphate, 50 μg yeast RNA and 1.0 ml chilled 12% trichloroacetic acid. The precipitates were collected on GF-C glass filters, washed with cold trichloroacetic acid solubilized with NCS solubilizer and counted using toluene-based scintillation fluid.

2.3. *Chromatin isolation and assay of poly(ADP-ribose) polymerase activity*

The chromatin was isolated and purified by ultracentrifugation through 1.7 M sucrose as described [17]. The assay mixture contained 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM DTT, 2 μCi [^{3}H]NAD (spec. act. 1 Ci/mmol), 18 μM cold NAD and chromatin equivalent to 10–60 μg DNA in a final volume of 200 μl. After 10 min incubation at 37°C the reaction was terminated with cold 20% trichloroacetic acid and precipitates counted for radioactivity as given above for poly(A) polymerase. Addition of 10 mM cyclic AMP [poly(ADP-ribose) glycohydrolase inhibitor], 10 mM NaF (phosphodiesterase inhibitor) [14] or 10 μg pancreatic deoxyribonuclease I showed no increase in enzyme activity. The DNA in chromatin was estimated as in [17].

3. RESULTS AND DISCUSSION

Tobacco tissue cultures had poly(ADP-ribose) polymerase activity associated with the chromatin whereas poly(A) polymerase activity was recovered in the soluble fraction. Extracts from both normal and tumor tissue on DEAE-Sephadex A-25 chromatography revealed only 2 poly(A) polymerase activities, one which was not retained (polymerase I) and the other which was bound by DEAE-Sephadex A-25 and eluted by 0.4 M ammonium sulfate (polymerase II). Although the ratio of poly(A) polymerase I to II was about 1:4.5 in both normal and tumor tissues, both of these activities were 4-times higher in the tumor tissue compared to the normal tissue (table 1). Similarly poly(ADP-ribose) polymerase activity was 6-times higher in the tumor tissue compared to the normal tissue (table 1). This indicates that plant tumor tissue like neoplastic mammalian tissues [10–14] have higher activities of these enzymes as compared to the normal tissue. To characterize further poly(A) polymerase I and II other than their behavior on DEAE-Sephadex A-25 these enzymes from tobacco tissue were examined as given below.

The K_m^{ATP} values for poly(A) polymerase I and II for normal tissue were 91 and 85 μM and for tumor

Table 1

Poly(A) polymerase I, poly(A) polymerase II and poly(ADP-ribose) polymerase activity of normal and tumor tissue of tobacco

Tissue	Poly(A) polymerase activity (pmol ATP used/h per g tissue)		Poly(ADP-ribose) polymerase activity (pmol NAD used/h per mg DNA in chromatin)
	I	II	
Normal	230	1492	1500
Tumor	1326	5832	9000

Table 2

Substrate specificity of poly(A) polymerase I and II from crown gall tumor tissue cultures of tobacco

Substrate	Poly(A) polymerase I		Poly(A) polymerase II	
	pmol substrate used	% incorporation	pmol substrate used	% incorporation
(1) 0.7 μM [^{3}H]ATP	1.77	100	1.41	100
(2) 1 + 50 μM cold ATP	97.00		324.20	
(3) 1 + 700 μM GTP	0.1	6	0.43	30
(4) 1 + 700 μM CTP	0.18	10	0.81	57
(5) 2 + 700 μM UTP, 700 μM GTP and 700 μM CTP	0.05	3	0.15	11
(6) 1, − [^{3}H]ATP + 0.7 μM [^{3}H]UTP	0.27	15	0.17	12
(7) 1, − [^{3}H]ATP + 0.7 μM [^{3}H]CTP	0	0	0	12
(8) 1, − [^{3}H]ATP + 0.7 μM [^{3}H]GTP	0	0	0	0

Assay as in Section 2.

tissue 98 and 200 μM, respectively. These values are comparable to those reported for poly(A) polymerase from rat liver (70 μM) [8] and human leukemic cells (200 μM) [10]. Both poly(A) polymerase I and II required an exogenous primer for activity. Poly(A) was the preferred primer, tRNA only 20–40% as good as poly(A), and poly(U), poly(G), poly(C) and native or denatured DNA were not used. The data in table 2 show that neither poly(A) polymerase I nor II used [^{3}H]CTP or [^{3}H] GTP whereas [^{3}H]UTP was used very poorly (10–15%) compared to [^{3}H]ATP as the substrate. The utilization of [^{3}H]ATP by polymerase I and II was strongly inhibited by cold GTP, CTP or CTP + GTP + UTP, although this inhibition was less for polymerase II. Both enzymes had an M_r of 65 000–70 000 and optima for 900 μg/ml poly(A), 0.25 mM Mn^{2+}, 1 mM Mg^{2+}, pH 8.0–8.5 and Mn^{2+}/Mg^{2+} activity ratio of 5 for I and 15 for II. These properties are similar to those of enzymes from animal sources [8]. Likewise, the plant poly(A) polymerase I and II were 90% inhibited by 0.75 mM cordycepin triphosphate but not by 6 μg/ml α-amanatin. Like the mammalian poly(A) polymerase [18] the poly(A) polymerase I and II from tobacco tissue were also 80% inhibited by 0.25 or 0.75 mM spermine by complexing with the primer poly(A) used in the assays. At 0.25 mM spermine the increase of poly(A) from 125 to 900 μg/ml completely reversed the inhibition but not at 0.75 mM spermine. Visible precipitate was noted on adding spermine to the assay mixtures and the determination of absorbance at 260 nm of the supernatant after removal of precipitate by centrifugation indicated that at 900 μg/ml poly(A) only 35% poly(A) was precipated at 0.25 mM spermine but > 70% at 0.75 mM spermine. Although many characteristics of poly(A) polymerase I and II discussed above are not known for other plant tissues [1,5,6] the 'cytoplasmic' and 'chloroplast' poly(A) polymerases from wheat leaves [4] separable on DEAE-cellulose had similar a pH optimum of 8.0 and the cytoplasmic polymerase which represented the major activity in wheat leaves [4] also resembled poly(A) polymerases described here in its preference for poly(A), ATP and Mn^{2+} over Mg^{2+}. The chloroplast polymerase from wheat [4] however, differs from both tobacco enzymes reported here in its use of poly(A), poly(U), poly(C) poly(G) and RNA as primer, equal activity with Mn^{2+} and Mg^{2+}, and lack of inhibition of ATP utilization by CTP or UTP. Moreover, the chloroplast poly(G) polymerase from wheat leaves which used poly(G) primer and GTP as substrate was not detected in tobacco tissues examined here even though these

tissues were cultured under continuous light and contained chloroplasts.

Our results indicate that normal and crown gall tumor tissues of tobacco contain poly(A) polymerase activities which resemble in molecular mass, K_m^{ATP}, primer and substrate preference, pH, and cation requirement the enzymes from mammalian sources. Like mammalian neoplastic tissues, the tobacco tumor tissue contains several-fold higher activity of poly(A) polymerases and poly(ADP-ribose) polymerase as compared to the normal tissue. These results together with an earlier report of high DNA polymerase activity in crown gall tumor tissue compared to normal tissue [19] indicate stimulation of several nucleic acid polymerases on cellular transformation.

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