SENSITIVITY TO α-AMANITIN OF MULTIPLE DNA-DEPENDENT RNA POLYMERASES FROM EXPERIMENTAL TUMORS

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SUMMARY. The distribution and properties of DNA-dependent RNA polymerases separated by DEAE-Sephadex chromatography from extracts obtained after lysis of transplanted tumor nuclei were compared with those from rat spleen and kidney nuclei. The effect of α-amanitin has been investigated on the multiple forms of the enzyme isolated from normal and neoplastic extracts. The results show that while from rat spleen and kidney only two peaks containing polymerase activity were obtained, from the nuclei of four experimental tumors investigated, three distinct peaks were separated. On the basis of the degree of inhibition by α-amanitin, it was possible to classify the first two activities from tumor tissue as belonging to Class A and the third to Class B.

INTRODUCTION

The existence of multiple forms of DNA-dependent RNA polymerase in normal and neoplastic eukaryotic cells has been reported from several laboratories (1-13). Concerning the separation of the enzymes from tumor tissues and their resistance to amanitin, Tsai and Saunders (9) found two forms of RNA polymerase in lymphocytes of patients with chronic lymphocytic leukemia, one of which was sensitive to the drug, while multiple activities were separated from Ehrlich ascites tumor cells (10), from mouse myeloma (11) and from Hela cells (12), two of which resulted amanitin sensitive. Moreover, Froehner and Bonner (13) isolated three forms of polymerase from Novikoff ascites tumor, but the effect of amanitin was not investigated.

In the present work we applied a method (1) based on the chromatographic resolution on DEAE-Sephadex of the enzymatic extracts obtained after lysis of nuclei isolated from four

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transplanted tumor nuclei and compared the results there obtained with rat spleen and kidney nuclei. In order to classify, according to Chambon (14), the type of enzyme separated, we tested the inhibitory power of α-amanitin on the separated forms in the presence of either Mn^{2+} or Mg^{2+} and analogous DNA.

MATERIALS AND METHODS

Dithiothreitol, nucleotides and Tris buffer were supplied by Sigma Chemical Company (U.S.A.); DEAE-Sephadex and Sephadex by Pharmacia, Uppsala (Sweden); [^{14}C]UTP by Amersham (England); 2-mercaptoethanol by Mann Research Lab. Inc. New York (U.S.A.); all other reagents were from Carlo Erba, Milano (Italy). Protein was determined according to Lowry method (15).

The extracts used in this work were: rat spleen and kidney, Walker carcinoma, Morris hepatoma 7793, Oberlin-Guérin myeloma and Yoshida ascites sarcoma transplanted in rats by subcutaneous transplant of bits of solid tumor on the back or on the thigh, or by abdominal injection in the case of ascites tumor. The nuclei, isolated from tissue as previously described (16), were lysed in an alkaline medium and incubated at 37° C for 45 min in order to solubilize the enzyme as already described (1). The active extract was applied to a A-25 DEAE-Sephadex column, previously equilibrated with 0.05 M $(NH_4)_2SO_4$ dissolved in a medium composed of 50 mM Tris-HCl buffer (pH 7.9), 1 mM dithiothreitol, 0.5 mM EDTA and 30 % (v/v) glycerol. After washing the column with the same solution containing 0.1 M $(NH_4)_2SO_4$, a linear gradient 0.1-0.6 M $(NH_4)_2SO_4$ was applied. Fractions of 1.8 ml each were collected, and pooled on the basis of the results of activity assays (see Figs. 1 and 2). Each pool, after dialysis against a medium of the same composition, omitting glycerol, was concentrated on Diaflo ultrafiltration membrane and passed through a G-25 Sephadex column in order to eliminate $(NH_4)_2SO_4$. The eluates, after addition of glycerol and albumin, were stored at -30° C and used for the experiments with α-amanitin reported in Tables I-III.

Preparation of homologous DNA from different tissues was performed according to Bernardi et al. (17), except that the first extraction with 0.15 M NaCl-0.1 M EDTA (pH 8.0) was carried out from nuclei purified as already described (16). The purity of the preparation was determined by UV ratio absorption at 260-280 nm. The incubation mixtures for polymerase assays contained in 0.3 ml the following components: 25 μmoles of Tris-HCl buffer (pH 8.0); 10 μmoles of KCl; 12 μmoles of mercaptoethanol; 0.3 μmoles of ATP, CTP, GTP each; 0.005 μmoles of [^{14}C]UTP; 0.045 μmoles of cold UTP; 20 μg of DNA. $MgCl_2$ or $MnCl_2$

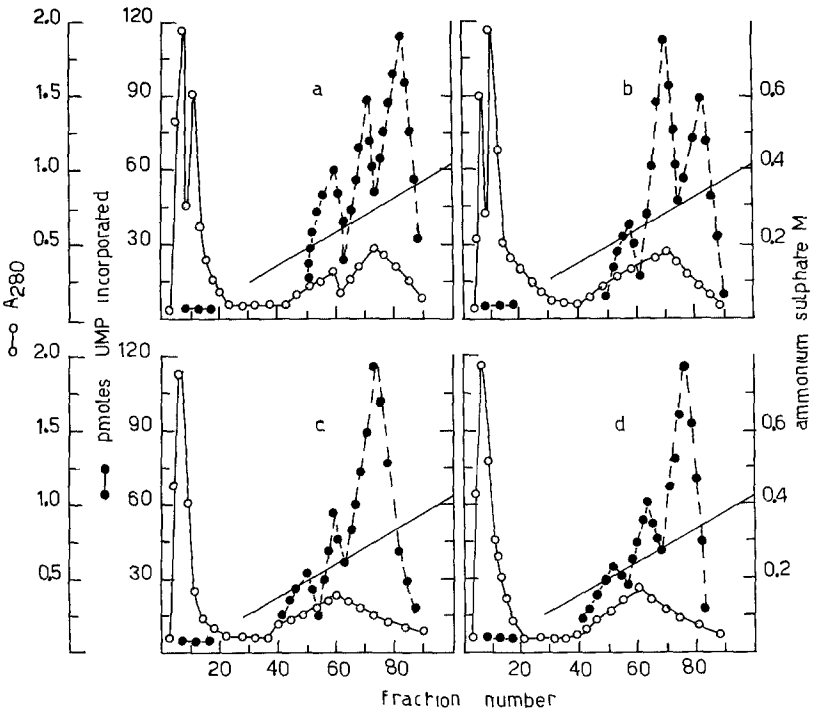

Fig. 1 - Chromatographic separation of RNA polymerases solubilized from tumor nuclei by homogenization and incubation in an alkaline medium at 37° C for 45 min. Elution with linear gradient of $(NH_4)_2SO_4$. 0.1 ml of eluate was incubated in the presence of 4 mM $MnCl_2$ at 37° C for 15 min. a) Walker carcinoma; b) Morris hepatoma 7793; c) Oberlin-Guérin myeloma; d) Yoshida ascites sarcoma.

were added as indicated in the Figs. and Tables. The ionic strength, for the optimal activity of the enzyme, was obtained by adding to the assay mixtures suitable concentration of $(NH_4)_2SO_4$. After incubation, the reaction was stopped by adding 2 ml of 10 % ice-cold TCA; the precipitate was collected on a Millipore filter and washed according to Travers and Burgess (18); the radioactivity was measured in a Beckman liquid scintillation apparatus DMP-100. The results are expressed as pmoles of UMP incorporated/mg protein (see Tables).

RESULTS AND DISCUSSION

The elution profiles of the chromatographic separation reported in Fig. 1 for the tumors investigated show the presence

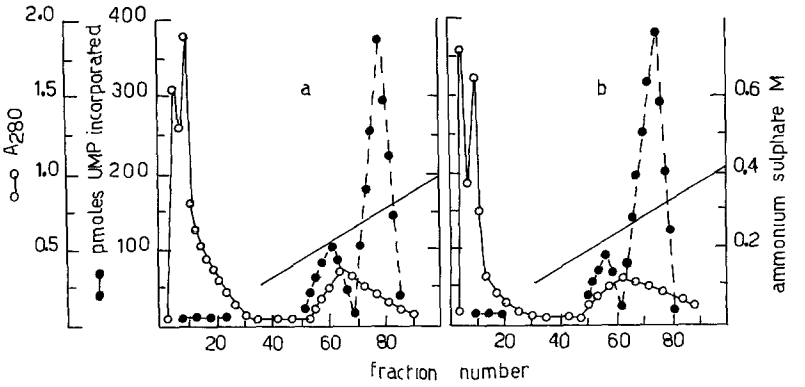

Fig. 2 - Chromatographic separation of RNA polymerases solubilized from Kidney (a) and spleen (b) rat nuclei. For the experimental details, see Fig. 1.

of three peaks containing polymerase activity independent of the type of tumor: Walker carcinoma (1a); Morris hepatoma (1b); Oberlin-Guérin myeloma (1c) and Yoshida ascires sarcoma (1d). The results show that the three activities were separated approximately at the same gradient concentration. On the contrary, the results in Fig. 2, for rat spleen (2a) and kidney (2b) show that only two fractions containing activity were separated at comparable gradient concentrations.

The sensitivity to α-amanitin was tested on the isolated enzymes in the presence of either Mn^{2+} or Mg^{2+} and reported in Tables I-III. The cation concentration and the ionic strength reported in the Tables represent the optimal concentration of $MnCl_2$ or $MgCl_2$ and $(NH_4)_2SO_4$ determined in preliminary experiments. The results in Table I and II show that the first two activities isolated from tumors were not inhibited by the drug at concentration 10^{-3} M (about 1 mg/1 ml) in the presence of both cations. On the contrary, amanitin at concentration 10^{-7} M strongly inhibited the third form of the enzyme in the presence of $MnCl_2$, and less in the presence of $MgCl_2$. With regard to the two activities separated from normal tissues, the data in Table III show that the first enzymatic form is not affected by the

TABLE I. Sensitivity to α-amanitin of RNA polymerase activity from tumors in the presence of $MnCl_2$

α-amanitin M	Tumor	AI		AII		B	
		specific activity	Δ%	specific activity	Δ%	specific activity	Δ%
-	Walker	2290		3150			
10^{-3}	carcin.	2250	- 2	2880	- 6		
-						7850	
10^{-7}						810	- 90
-	Morris	1990		4020			
10^{-3}	hepatoma	1870	- 6	3770	- 6		
-						5460	
10^{-7}						420	- 92
-	Myeloma	1810		2230			
10^{-3}		1890	+ 4	3160	- 3		
-						8840	
10^{-7}						730	- 92
-	Yoshida	1590		3340			
10^{-3}	ascites	1640	+ 3	3220	- 3		
-						7910	
10^{-7}						540	- 93

The experiments were carried out at different ionic strength and salt concentration (see text): I = 0.17 for polymerase of Class A, 0.40 for Class B; $MnCl_2$, 0.6 mM for polymerase of Class A, 0.4 mM for Class B; $MgCl_2$, 0.8 mM for all enzymes. Incubation at 37° C for 30 min. Specific activity expressed as pmoles of UMP incorporated/mg protein.

higher concentration of amanitin, while the activity of the second is almost completely inhibited by 10^{-7} M amanitin.

Consistent with the suggestion of Chambon (14) that the sensitivity of RNA polymerase to α-amanitin constitutes the criterion for the differentiation of the enzymatic forms, the data in Tables I and II suggest that the first two activities

TABLE II. Sensitivity to α-amanitin of RNA polymerases activity in the presence of $MgCl_2$.

α-amanitin M	Tumor	AI		AII		B	
		specific activity	Δ%	specific activity	Δ%	specific activity	Δ%
-	Walker	2970		3230			
10^{-3}	carcin.	2910	- 2	2990	- 6		
-						1030	
10^{-7}						470	- 54
-	Morris	2460		4130			
10^{-3}	hepatoma	2350	- 3	3980	- 4		
-						2030	
10^{-7}						810	- 60
-	Myeloma	1970		2450			
10^{-3}		1920	- 2	2380	- 3		
-						2550	
10^{-7}						1220	- 52
-	Yoshida	1810		3170			
10^{-3}	ascites	1770	- 2	3040	- 4		
-						2060	
10^{-7}						1130	- 45

The experiments were carried out as described in Table I.

separated from tumor tissues belong to Class A (AI and AII), while the third activity, the only one inhibited by the drug, belongs to Class B. Similarly, the results in Tables III suggest that the first form separated from rat spleen and kidney belongs to Class A, while the second to Class B.

Comparison of the data for neoplastic and normal tissues, respectively, show that the total tumor polymerase activity in Class A is two fold greater than in rat spleen and kidney.

It is well known that in eukaryotic cells RNA polymerases

TABLE III. Sensitivity to α-amanitin of RNA polymerases activity from normal tissue.

α-amanitin M	Tissue	Cation	A		B	
			specific activity	Δ%	specific activity	Δ%
-	Spleen	$MnCl_2$	2420			
10^{-3}			2380	- 2		
-					10450	
10^{-7}					910	- 91
-		$MgCl_2$	3680			
10^{-3}			3570	- 3		
-					3010	
10^{-7}					490	- 84
-	Kidney	$MnCl_2$	2460			
10^{-3}			2340	- 5		
-					12370	
10^{-7}					860	- 93
-		$MgCl_2$	2920			
10^{-3}			3010	+ 3		
-					3980	
10^{-7}					500	- 87

The experiments were carried out as described in Table I.

possess different transcriptive functions and that the enzymes of Class A, of nucleolar origin, are responsible for ribosomal RNA synthesis, while the enzymes of Class B, located in nucleoplasm, synthesize messenger RNA. Our results confirm the increased ribosomal synthesis in tumor cells, probably necessary for the high protein synthesis in rapidly growing tissues. This suggestion agrees with the observation of Rutter et al. (19) according to which in normal tissues the polymerase of Class A transcribes less than 0.1 % of total RNA, while in neoplastic

tissues the ribosomal RNA represents about 50 % of the total RNA synthesized.

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