DECREASED ACTIVITY OF A SOLUBLE DNA-DEPENDENT RNA POLYMERASE FROM THYMUS OF RATS INJECTED WITH A THYMOLYTIC STEROID

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Summary. The Mg^{2+}- and Mn^{2+}- catalyzed DNA-dependent RNA polymerase activity of rat thymic nuclei has been solubilized by three procedures. The soluble preparation required addition of DNA for activity, indicating that endogenous DNA had been separated from the enzyme. Enzymatic activity solubilized from thymocytes of rats injected with either cortisol or fluocinolone acetonide 3 hr prior to sacrifice was significantly lower than that from cells of control rats. This decreased DNA-dependent RNA polymerase activity is due, at least in part, to an alteration in the enzymatic component of the DNA-RNA polymerase complex.

Progress has been made in the purification of mammalian DNA-dependent RNA polymerases from rat liver (1-4) and prostate (5), calf thymus (1, 6-9), bovine lymphosarcoma (8), and human placenta (10). Moreover, the RNA polymerase activity of either whole nuclei or the aggregate enzyme from liver (11,12), uterus (13,14) and prostate (15) is increased when exposed in vivo to cortisol (or cortisone), estradiol and testosterone, respectively, while that of thymus is decreased by cortisol both in vivo (16-18) and in vitro (19).

Assessment of the relative hormonal effects on RNA polymerase and on DNA template requires separation of these two bound components. A soluble DNA-dependent RNA polymerase preparation, essentially free of DNA, has been obtained from thymocytes of control rats and from cells previously exposed to a thymolytic steroid in vivo. Enzymatic activity is negligible in the absence of added calf thymus DNA. Moreover, the activity is significantly lower, per unit of soluble protein, in fractions from thymocytes of rats injected with a thymolytic steroid.

MATERIALS AND METHODS

Chemicals - Calf thymus DNA, RNase, UTP, ATP, GTP, CTP, phosphoenolpyruvate and pyruvate kinase were purchased from Sigma or Calbiochem; ^{3}H-UTP (25 Ci/mmole) and ^{3}H-GTP (1 Ci/mmole) from Schwarz BioResearch. Cortisol was purchased from Steraloids,

Pawling, N.Y., fluocinolone acetonide (6α,9α-difluoro-11β,16α,17,21-tetrahydroxy pregna-1,4-diene-3,20-dione 16,17-acetonide) was a gift of Syntex and actinomycin D of Merck, Sharp and Dohme.

Animals and Treatment - Male rats of the Sprague-Dawley strain, weighing 100 to 120 g were purchased from Manor Research, Puerto Rico at least one week before use and maintained on Rockland rat chow and water ad libitum. Each rat was injected intraperitoneally with either 5 mg of cortisol or 1.0 mg of fluocinolone acetonide per 100 g body weight. The steroids were suspended in 0.9% NaCl (15 mg of cortisol/ml; 2.5 mg of fluocinolone acetonide/ml). Control rats received an equal volume of saline. Animals were killed by decapitation 3 hr following injection.

Preparation of Soluble RNA Polymerase - An aggregate enzyme was obtained from the nuclei of two to four pooled thymi (20). RNA polymerase activity was solubilized from the aggregate complex by one of three procedures conducted at 0-4°C.

I. The aggregate preparation was suspended in 2 ml of 0.05 M Tris-Cl (pH 7.8), 0.005 M $MgCl_2$, 0.002 M dithiothreitol and 0.5% Triton X-100 and sonicated in a Branson sonifier for 15 sec at setting No. 3. The mixture was centrifuged at 15,000 X g for 5 min and the supernatant fraction then centrifuged at 105,000 X g for 30 min. The supernatant solution was used for the RNA polymerase assay.

II. As an additional procedure for solubilization of RNA polymerase activity, the washed aggregate enzyme preparation was suspended in a known volume of 0.05 M Tris-Cl (pH 7.8), 0.005 M $MgCl_2$, 0.002 M dithiothreitol and 20% glycerol. The mixture was homogenized with 2 gm of washed fine glass beads (21) in a Waring Blendor at top speed for 2 min. The mixture was then centrifuged twice as above and the clear supernatant fraction used for polymerase assay.

III. A third preparative procedure was identical with II, above, except for the presence of 0.2 M $(NH_4)_2SO_4$ during homogenization. Due to instability of the soluble RNA polymerase, each preparation and assay of the enzyme was always carried out within a four to five hour period.

Enzyme Assay - The RNA polymerase assay system contained 0.25 ml of the extract (200-300 μg of protein), 250 μg of calf thymus DNA dissolved in 0.1 ml of 0.05 M Tris-Cl (pH 7.8), and 0.25 ml of a solution containing one μmole each of ATP, GTP, CTP, UTP and phosphoenolpyruvate, 10 μg of pyruvate kinase, 50 μmoles of Tris-Cl (pH 7.8) and 6 μmoles of 2-mercaptoethanol. The assay mixture also contained either 10 μmoles of $MgCl_2$ or 4 μmoles of $MnCl_2$ and 2 μCi of a tritiated nucleoside triphosphate; the latter replaced the corresponding unlabeled triphosphate. The specific activity of each of the labeled substrates was 0.8 Ci/mmole. The incubation period was 10 min at 37°C. At the end of 10 min, each tube was chilled in an ice bath and 1 mg

of bovine serum albumin added, followed by 3 ml of cold 10% trichloroacetic acid (TCA) containing 0.02 M soidum pyrophosphate. The precipitate obtained by centrifugation was dissolved in 0.5 ml of cold 0.2 M NaOH and 3 ml of 10% TCA-0.02 M sodium pyrophosphate added. The centrifuged precipitate was again dissolved and precipitated as above. The precipitate was then hydrolyzed at 90°C for 20 min in 0.3 ml 5% TCA. Both the TCA supernatant fraction and precipitate were counted in a vial containing 15 ml of scintillation fluid (10 g of butyl - PBD and 125 g of naphthalene/liter of dioxane), using a Packard liquid scintillation counter. Counts obtained were corrected for 0 min incubation. Linearity of enzymatic activity with time was maintained significantly beyond the period of incubation. Employing the 10 min assay, the rate of utilization of ^{3}H-UTP or ^{3}H-GTP was also linear with varying concentrations of enzyme extract (100-600 µg protein). Protein concentration was determined by the Lowry method (22).

RESULTS

Some properties of the soluble RNA polymerase from rat thymocytes are shown in Table 1. Less than 2% of the total activity is apparent in the absence of added DNA, suggesting that the enzyme has been separated from template. Maximal rate of incorporation into RNA occurs only in the presence of either Mg^{2+} or Mn^{2+} and all four nucleoside triphosphates. Also this rate is markedly decreased in the presence of RNase or of actinomycin D.

Table 1. Some properties of the Mg^{2+} - Mn^{2+} -catalyzed soluble RNA polymerase activity of rat thymic nuclei[a]

Assay system	µµmoles ^{3}H-UMP incorporated per mg protein			
	Mg^{2+}	% Change	Mn^{2+}	% Change
Complete	148	-	369	-
-DNA	2	-99	6	-98
-GTP	12	-92	21	-94
+50 µg RNase	6	-96	42	-89
+30 µg actinomycin D	13	-91	35	-91

[a]The soluble enzyme fractions were extracted from aggregate preparation utilizing 0.2 M $(NH_4)_2SO_4$ (see Materials and Methods).

The higher total RNA polymerase activity solubilized in the presence of 0.2 M $(NH_4)_2SO_4$ (Table 2) cannot be due entirely to the stimulatory effect of salt on polymerase activity. The soluble enzyme obtained in the absence of salt showed only a small increase in activity on addition of $(NH_4)_2SO_4$ to the assay system (data not presented). These observations suggest that solubilization of enzymatic activity is increased when salt is added to the homogenization medium. The enzyme obtained by all three methods of solubilization has the properties indicated in Table 1.

The data in Table 2 demonstrate that enzymatic activity is significantly less when

Table 2. Decreased activity of soluble RNA polymerase preparations from rat thymocytes exposed to thymolytic steroids in vivo

Method of solubilizing enzyme	Steroid injected	Labeled precursor	Cation	μmoles labeled nucleotide incorporated/mg protein		% change[a]
				Control	Steroid treated	
Sonication[b]	Cortisol	^{3}H-UTP	Mg^{2+}	4(4)[c]	3(4)	-25± 1.4
Homogenization[d]	Cortisol	^{3}H-UTP	Mg^{2+}	14(8)	11(8)	-21± 1.9
Homogenization[d]	Cortisol	^{3}H-UTP	Mn^{2+}	28(5)	25(5)	-11± 3.0
Homogenization[d]	Cortisol	^{3}H-GTP	Mg^{2+}	14(2)	11(2)	-21± 2.0
Homogenization[d]	Fluocinolone acetonide	^{3}H-UTP	Mg^{2+}	14(2)	12(2)	-14± 3.5
Homogenization[e]	Fluocinolone acetonide	^{3}H-UTP	Mg^{2+}	61(9)	52(9)	-15± 1.4
Homogenization[e]	Fluocinolone acetonide	^{3}H-UTP	Mn^{2+}	244(10)	185(10)	-24± 2.0
Homogenization[e]	Fluocinolone acetonide	^{3}H-GTP	Mg^{2+}	85(3)	67(3)	-21± 1.0
Homogenization[e]	Fluocinolone acetonide	^{3}H-GTP	Mn^{2+}	143(4)	113(4)	-21± 2.5

[a] These values are means ± S.E.; all values in this column are significant at a "p" value of <0.01 (paired comparisons).

[b] See Materials and Methods, Procedure I.

[c] Numbers in parentheses are number of experiments.

[d] Homogenization of aggregate enzyme preparation in absence of $(NH_4)_2SO_4$ (see Materials and Methods, Procedure II).

[e] Homogenization of aggregate enzyme preparation in presence of 0.2 M $(NH_4)_2SO_4$ (see Materials and Methods, Procedure III).

obtained from thymocytes exposed to a thymolytic steroid in vivo for 3 hr. These observations reveal an influence of the steroid on the enzymatic component of the DNA-enzyme complex. Two possible loci of hormonal action may be suggested from the present study: 1) on the enzyme molecules and/or 2) on a factor or factors associated with the enzyme. These possibilities are now being examined by further purification of the enzymes from thymic nuclei of both control and steroid-treated animals.

Fig. 1 shows the time course of incorporation of a nucleoside triphosphate into RNA by the soluble RNA polymerase preparation from the thymic nuclei of control and fluocinolone acetonide injected animals. Utilization of ^{3}H-GTP by the control enzyme remains relatively linear for 60 min of incubation, whereas the rate declines somewhat earlier with the preparation from cells previously exposed to steroid. This earlier decline in the activity of the latter preparation may be due to decreased stability of the enzyme; this point requires further study.

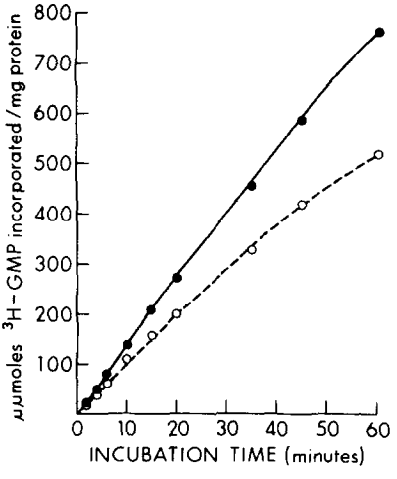

Fig. 1. Time course of utilization of ^{3}H-GTP by a soluble RNA polymerase preparation from Thymic nuclei (see Materials and Methods). The assay system contains 4 μmoles of $MnCl_2$. ●—●, enzyme from control cells; O---O, enzyme from cells exposed to fluocinolone acetonide in vivo (see Materials and Methods).

DISCUSSION

The preparation of a soluble essentially template-free RNA polymerase from rat thymocytes has permitted the first demonstration of a decrease in activity of a non-particulate DNA-dependent RNA polymerase resulting from the action of a hormone. The data indicate that the decreased RNA polymerase activity in thymic nuclei exposed to a thymolytic steroid in vivo is to a major degree a result of an alteration in the enzymatic component or of

factors other than DNA which may be associated with the enzyme. The possible presence of a factor in soluble polymerase preparations affecting enzymatic activity has been examined in initial experiments (data not presented). When soluble polymerase preparations from thymocytes of control animals and animals exposed to fluocinolone acetonide were aged at -20°C for one week, more than 80% of the original activities were lost. However, the magnitude of the steroid effect, based upon the residual activity, was not altered. Addition of an aliquot of the steroid-exposed aged enzyme to a freshly prepared extract of control thymic nuclei did not inhibit the activity of the latter. Experiments are presently in progress designed to examine possible effects of a thymolytic steroid on the turnover rate and the physical and chemical properties of the soluble RNA polymerase.

The demonstration of reduced activity of a DNA-free RNA polymerase from thymocytes previously exposed to a thymolytic steroid would appear to eliminate DNA template per se as the prime site of action of this steroid. However, the influence of the steroid on polymerase activity cannot be explained solely on the basis of an alteration in the enzyme. The magnitude of the hormonal effect is consistently smaller in the soluble RNA polymerase preparation than in nuclei (16) or the aggregate preparation (20) from thymocytes exposed to steroid. It has previously been shown that the decrease in RNA polymerase activity resulting from incubation of rat thymocytes with cortisol in vitro is only partially prevented by inhibitors of protein synthesis (19). Thus in the present system also, the action of cortisol may have at least two components.

The data available at the present time, including unpublished studies from our own laboratory with α-amanitin, do not permit decision as to whether there are multiple polymerases in rat thymic nuclei as have been described in other mammalian tissues (1,3,6,9). Purification studies are in progress in order to examine this and other questions. This approach should provide additional insight into the underlying basis for the decreased activity of the soluble DNA-dependent RNA polymerase of rat thymocytes which have been exposed to a thymolytic steroid.

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