Cortisone acetate does not change free and chromatin-bound RNA polymerase II activities early after injection

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Received 20 August 1982; revision received 29 September 1982

Cortisone acetate *RNA polymerase II* *Chromatin-bound RNA polymerase II*
Template engaged activity *Poly (dA–dT)* *Actinomycin D*

1. INTRODUCTION

It has been reported that peritoneal injection of cortisone stimulates nucleolar RNA polymerase I activity but not nucleoplasmic RNA polymerase activity in rat liver [1,2]. Later, injection of cortisone acetate was reported to stimulate both free and chromatin-bound RNA polymerase I activities through rapid protein turnover [3], consistent with [4,5]. Both forms of RNA polymerase II activities remained unchanged by this treatment [3]. However, in [6] free RNA polymerase II activity was reported to be drastically increased (2.5–3.5-fold of control activity) and template-engaged enzyme activity was considerably decreased after cortisone injection.

To compare directly the finding in [3] with that in [6], the early effect of cortisone on free and chromatin-bound RNA polymerase II activities was analyzed under the same conditions as those used in [6]. Here, evidence is presented that solubilized free and bound RNA polymerase I activities were stimulated at 1.5 h after cortisone treatment, while both forms of RNA polymerase II activity remained unchanged. Reasons for the discrepancy between these results and those in [6] will be discussed by pointing out assay problems of free and template-engaged RNA polymerases.

2. MATERIALS AND METHODS

2.1. *Cortisone treatment and isolation of nuclei*

Isolation of nuclei from rat liver was performed as in [7] with only slight modification. Five male Sprague-Dawley rats (~150 g body wt) were sacrificed at 1.5 h after intraperitoneal injection of 5 mg cortisone acetate (Fluka). Five control animals were injected with saline. All the following steps were performed at 4°C. The liver was minced with scissors and homogenized in 2 vol. 2.1 M sucrose containing 3 mM $MgCl_2$ with 12 strokes in a Potter homogenizer. After filtration of the homogenate through 4 layers of gauze, 8 vol. of the same solution was added and mixed. The nuclear pellet was obtained by centrifugation at 28000 rev./min for 60 min in a Hitachi RP-30 rotor.

2.2. *Differential solubilization of free and chromatin-bound RNA polymerase in nuclei*

Free RNA polymerase was prepared by a slight modification of the method in [8]. Nuclei were suspended in 2 ml 0.34 M sucrose/g wet wt liver and homogenized by hand in a Potter homogenizer. The homogenate was cenrifuged at $1000 \times g$ for 10 min; and the pellet was resuspended in the same volume of 0.34 M sucrose and recentrifuged at $1000 \times g$ for 10 min. The supernatants were combined, salted out by the addition of 2 vol. saturated $(NH_4)_2SO_4$ (pH 7.9), and centrifuged at 16000 rev./min for 15 min in a Beckman JA-20 rotor. The pellet was dissolved in 2 ml TGMED buffer (50 mM Tris–HCl (pH 7.9), 20% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM dithiothreitol). Chromatin-bound enzyme activity was assayed in a pelleted fraction from nuclei prepared by the sonication method in [9]. Both free and bound enzyme

Published by Elsevier Biomedical Press
001457593/82/0000—0000/$2.75

preparations were dialyzed against TGMED buffer containing 40 mM $(NH_4)_2SO_4$.

2.3. *DEAE-Sephadex A-25 chromatography of RNA polymerases*

The crude preparations of free or bound RNA polymerase were applied to a DEAE-Sephadex A-25 column (1.8 × 15 cm) equilibrated with TGMED buffer containing 40 mM $(NH_4)_2SO_4$ and eluted with TGMED buffer containing a linear gradient of from 40–400 mM $(NH_4)_2SO_4$. For the assay of enzyme activity, 0.1 ml of each column fraction was used.

2.4. *Assay for RNA polymerase activity*

RNA polymerase activity was determined as in [10]. Furthermore, total RNA polymerase activity in whole nuclei was measured by using a nuclear suspension in 0.34 M sucrose (70 μg DNA/assay mixture). The activity of free RNA polymerase in whole nuclei was assayed using poly(dA–dT) (6.25 A_{260}/ml) as template. The DNA content of nuclei was determined as in [11].

3. RESULTS AND DISCUSSION

Free and chromatin-bound RNA polymerase II activities and the ratio of the enzyme activities were not changed at 1.5 h after the cortisone treatment (table 1). This result is different from that in [6] where free RNA polymerase II activity was drastically increased (2.5-fold of control activity) and the ratio of the enzyme activities was greatly altered. In order to confirm this finding, enzymes from control and cortisone-treated animals were purified by DEAE-Sephadex A-25 chromatography. Although free and bound RNA polymerase I activities were considerably increased, both RNA polymerase II activities remained unchanged (fig. 1).

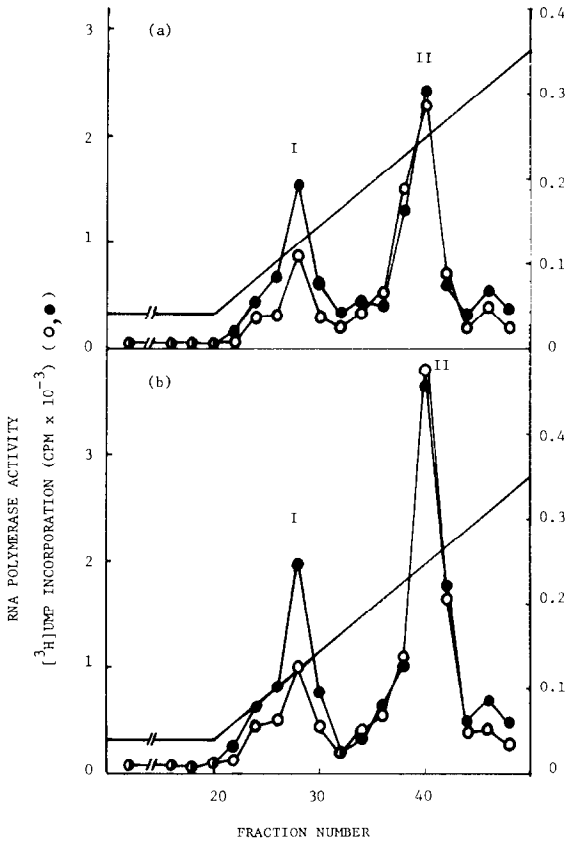

Fig. 1. DEAE-Sephadex A-25 column chromatography of free and chromatin-bound RNA polymerases from control and cortisone-treated animals. The crude free (a) and chromatin-bound (b) preparations from control (○) and cortisone-treated (●) animals were chromatographed on Sephadex A-25 columns as in section 2.

Table 1

Free and chromatin-bound RNA polymerase II activity

Form	[^{3}H]UMP Incorporation (cpm/g liver)		
	Control	+ Cortisone	+ Cortisone / control
Free	12202 ± 625	11836 ± 506	0.97 (2.51)
Bound	32745 ± 448	33169 ± 619	1.01 (0.91)
Free/bound	0.37 (0.54)	0.36 (1.50)	

Each reported activity represents the mean and standard error of 5 animals. The value in parentheses is that from [6]

This discrepancy seems to be derived from the technical problems involved in assaying the nuclear free and chromatin-bound (engaged) enzyme activities. In [6] free enzyme activity was measured by the poly(dA–dT)-actinomycin D technique [5] and template-engaged enzyme activity by RNA polymerizing activity of whole nuclei. The poly-(dA–dT) technique is based on the finding that

actinomycin D intercalates into G:C basepairs or between guanine and its neighbouring residues [12], allowing poly(dA–dT)-dependent enzyme activity to be assayed in the presence of actinomycin D. However, this evaluation of free enzyme activity is not complete:

(i) Natural DNA has a saturation level for the strong actinomycin D binding to G:C pairs or nucleotides around the guanosine [13];

(ii) Only 60% of G:C basepairs found in crab poly (dA–dT)·poly(dT–dA) are binding sites for actinomycin D [14].

Therefore, actinomycin D cannot mask endogenous template completely. In fact, even very high concentrations of actinomycin D (> 400 μg/ml) cannot completely repress RNA polymerase II activity; in our work considerable background enzyme activity remained (not shown). In addition, free enzyme activity in the nuclear system detected by the poly(dA–dT) technique was strongly affected by the ionic strength of the assay system. The free enzyme activity was apparently increased in the presence of relatively low ionic strength. This overestimation of free enzyme activity seems to be due to enzyme which was only loosely bound to chromatin and which utilized the poly(dA–dT) template [15].

Furthermore, the engaged enzyme activity cannot be determined by the whole nuclear assay system for the following reasons:

(i) This assay cannot discriminate chromatin template availability from the chromatin-bound enzyme itself.

(ii) Endogenous template-dependent RNA synthesis in the whole nuclei assay system using < 100 mM KCl as in [6] represents not only template-engaged enzyme activity but also the reinitiation activity of free or loosely chromatin-bound enzymes [15].

(iii) There are various inhibitory and stimulatory factors affecting RNA synthesis in the crude nuclear system.

The enzymes must be separated from these factors by procedures such as differential enzyme solubilization or DEAE-Sephadex chromatography. RNA polymerase II activity/mg liver DNA determined by two methods are compared in table 2. One set of activities was assayed using differentially solubilized enzymes, and the other determined using whole nuclei. The results of these methods differ considerably from each other, and the corresponding ratios of free and bound enzyme activities measured by the two techniques are vastly different.

Table 2

RNA polymerase II activities as measured by the solubilization or by the whole nuclear assay methods

Form	[^{3}H]UMP incorporation (cpm/mg DNA)	
	Solubilization	Nuclear assay
Free	6720	11867
Bound	14832	11046
Free/bound	0.45	1.07

Each value represents the average of triplicate assays

Therefore, the nuclear assay system in [6] might not correctly evaluate the activity of free and template-engaged RNA polymerase II.

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