SIMULTANEOUS ASSAY OF RNA POLYMERASE I AND II IN NUCLEI ISOLATED FROM RESTING AND GROWING RAT LIVER WITH THE USE OF α-AMANITIN

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Received 18 March 1970

1. Introduction

The existence of multiple forms or "activities" of RNA polymerase (nucleoside triphosphate-RNA nucleotidyltransferase, EC 2.7.7.6) in rat liver nuclei has been postulated [1,2]. More recently, at least two different forms of the enzyme have been separated from rat liver [3, 4] and from calf thymus [5]. These polymerases, called I and II [4], respectively A and B [5] differ by a number of properties and for their localization, the I being present in the nucleolus and the II in the nucleoplasm. A polymerase III is present in sea urchin eggs and possibly in rat liver [4].

α-Amanitin, a toxin from *Amanita phalloides* [6], is a potent inhibitor of nuclear RNA polymerase activity [7] and acts by binding to the enzyme [5, 8-10]. Kedinger et al. [5] reported that α-amanitin inhibits polymerase B from calf thymus without affecting polymerase A, and it was observed that the toxin inhibits completely polymerase II from rat liver, but not polymerase I (Rutter, personal communication). These observations suggested that α-amanitin could be a useful tool to estimate by difference the activity of polymerase I and II without extracting them from nuclei. This was done with the experiments described in this paper: the optimal conditions for the assay of these enzyme activities in isolated rat liver nuclei were selected, and the activity of the two polymerases was determined in nuclei from rats at various ages, and in regenerating liver.

2. Experimental procedure

Rats were of the Wistar strain, fed *ad libitum* on a commercial diet. Male rats were used, except for experiments with animals less than 30 day old, which were of both sexes. Partial hepatectomy was performed according to Higgins and Anderson [11] under ether anaesthesia.

Chemicals were from the same source and basic assay conditions were as in previous work [12], with the modifications described. DNA was determined by the method of Burton [13].

3. Results

3.1. Assay of RNA polymerase I and II

The differential assay of RNA polymerase I and II was based on the addition of a relatively high concentration of α-amanitin, which should inhibit completely polymerase II without affecting polymerase I. Thus the activity measured at low ionic strength in the presence of α-amanitin should be due to polymerase I, whereas the activity of polymerase II could be obtained from the difference measured in the presence and in the absence of α-amanitin, in a high ionic strength medium.

With this system, the optimal pH and Mg^{2+} concentration for polymerase I were 8.0 and 3-5 mM, respectively. When Mn^{2+} was substituted for Mg^{2+} the optimum concentration of the ion was 1–2 mM, but the maximum activity observed was slightly less than with Mg^{2+}. Under optimum conditions of pH and of Mg^{2+} concentration the system was saturated by nu-

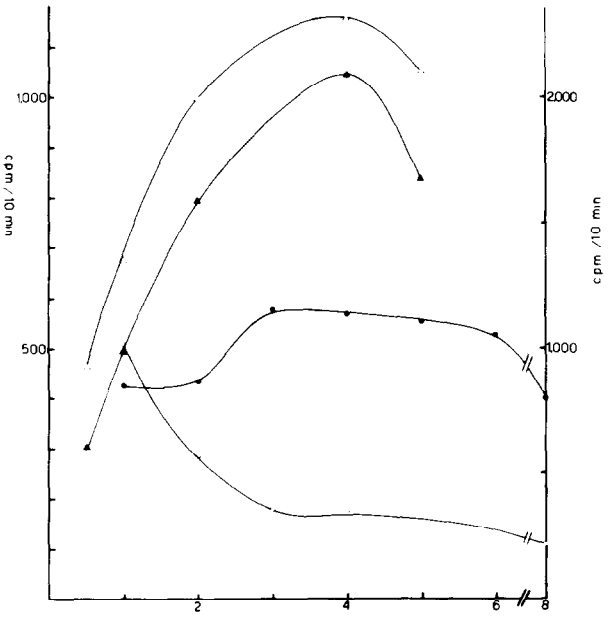

Fig. 1. Effect of Mg^{2+} and Mn^{2+} concentration on the activity of RNA polymerase. The activity was assayed at low ionic strength in the presence of α-amanitin with Mg^{2+} (•) or Mn^{2+} (○) (left scale), or at high ionic strength with Mn^{2+}, before (△) and after (▲) subtraction of values obtained in the presence of α-amanitin (right scale). Other experimental conditions were as in table 1.

cleotides at the concentration used in previous work [12].

The optimal pH for polymerase II was also 8.0 and the optimal concentration of Mn^{2+} was 4 mM (fig. 1), in the presence of 0.23 M ammonium sulphate (which gave maximal activity of RNA polymerase). The reaction system for RNA polymerase II was saturated by a concentration double than that saturating RNA polymerase I, if the reaction was stopped at 10 min, but the reaction proceeded for a longer time only with higher concentrations of nucleotides.

The conditions selected for the final assay of polymerase I and II were as reported in the footnote to table 1, in which the average results obtained with normal rats are also given.

3.2. Modifications of RNA polymerase I and II with age and after partial hepatectomy

The method described above was used to determine the activity of the two RNA polymerases in the liver of rats at different age and in regenerating liver (table 2). Both activities increased after birth, the I more than the II, until at one month they were 140% and 38%, respectively, above birth level. After this age the activity of polymerase I remained constant throughout the period examined, whereas the activity of polymerase II was somewhat lower in 12-month old rats. As a consequence of these changes, the ratio of the activities of polymerase I and II was higher than 3 until the 15th day of age, and decreased to approximately 2 at 1-2 months and to 1.4 at 12 months of age.

The activity of both polymerases, and mainly of polymerase I, increased during the early phases (12-24 hr) of liver regeneration, and that of polymerase I returned toward normal values more rapidly than that of polymerase II.

4. Discussion

The validity of the differential assay of the polymerase I and II by using α-amanitin is based upon the results obtained with the purified enzymes and on the assumption that α-amanitin affects RNA polymerase II in isolated nuclei in the same way as purified enzyme. This seems to be largely justified by the results obtained previously [5,7-10] and further support is given by the results of present experiments. Thus the fact that the activity of the RNA polymerase assayed at high ionic strength in the presence of α-amanitin is very close to the activity assayed at low ionic strength confirms that the value subtracted in the assay of polymerase II represents the incorporation due to polymerase I.

Our results indicate also that the optimum pH is 8.0 for both polymerases and that Mg^{2+} is a slightly better activator than Mn^{2+} for polymerase I. The concentration of ammonium sulphate giving optimum activity of polymerase II is higher than that optimum for the purified enzyme [4] and is very close, if not identical, to that reported previously as optimal for isolated nuclei [12]. It is possible that the presence of protein and of nuclear structures accounts for the difference. The same, and also the presence of a different concentration of ammonium sulphate may account for the higher concentration of Mn^{2+} required for optimum activity of polymerase II in nuclei as compared with purified preparations. It is confirmed

Table 1

Activity of RNA polymerase I and II as assayed with the use of α - amanitin.

	AMP incorporated (pmoles/10 min/mg of DNA)			
	RNA polymerase I			RNA polymerase II
System	1	2	3	4
	Mg^{2+}, low ionic strength	Mn^{2+}, high ionic strength		(3-2)
α - amanitin	present	absent	present	
	324 ± 35.9	1107 ± 103.8	324 ± 34.7	783 ± 87.6

Data are mean values ± S.E.M. of 5 determinations on normal male rats. The assay system contained, in a final volume of 0.5 ml: for RNA polymerase I: 50 μmoles of tris-HCl buffer, pH 8.0, 2 μmoles of $MgCl_2$, 7 μmoles of mercaptoethanol, 3 μmoles of NaF, 0.3 μmole each of CTP, GTP and UTP, 0.015 μmole of non-radioactive ATP, 0.005 μmole of 8 - ^{14}C-ATP and 1 μg of α - amanitin and 0.1 ml of nuclear suspension (containing approximately 200 μg of DNA); for RNA polymerase II: 50 μmoles of tris-HCl buffer, pH 8.0, 2 μmoles of $MnCl_2$, 0.14 mmole of $(NH_4)_2SO_4$, 0.9 μmole each of CTP, GTP and UTP, 0.045 μmole of non-radioactive ATP, and 0.015 μmole of 8 - ^{14}C-ATP, 1 μg of α - amanitin (when present) and 0.1 ml of nuclear suspension. Incubation was at 37° for 10 min.

Table 2

Activity of RNA polymerase I and II in the liver of rats at various age and in regenerating liver.

Animals	RNA polymerase I (pmoles of AMP incorporated/10 min/mg of DNA)	RNA polymerase II (pmoles of AMP incorporated/10 min/mg of DNA)
less than 1 day old (2)	151	482
3 days (4)	109 ± 7.4	392 ± 58.6
5 days (4)	152 ± 16.3	520 ± 57.3
15 days (5)	195 ± 14.4	597 ± 72.7
30 days (4)	351 ± 25.5	651 ± 66.2
2 months (5)	349 ± 21.0	721 ± 98.8
1 year (6)	312 ± 13.2	434 ± 78.9
Controls * (9)	330 ± 22.0	683 ± 70.2
Regenerating 12 hr (4)	746 ± 88.5	1046 ± 175.3
Regenerating 24 hr (6)	682 ± 56.8	1026 ± 100.1
Regenerating 48 hr (4)	400 ± 76.5	942 ± 141.0
Regenerating 72 hr (4)	364 ± 33.1	942 ± 38.1

Data are mean values ± S.E.M. The numbers of experiments are given in parentheses. The enzyme activities were assayed as described in the text, with the reaction mixtures as in table 1.

* 4 of these controls were sham-operated 24 hr before killing. These values were not different from those obtained with 5 normal rats, and therefore the results were pooled.

also that high ionic strength does not stimulate polymerase I, even in the nuclei.

Our results on RNA polymerase activity agree with those obtained with different methods with the liver of growing rats [14] and with regenerating rat liver [15-16]. The interpretation of the changes occurring during growth or liver regeneration is difficult, since the role of the two RNA polymerases is still not clarified. It has been observed that the RNA synthesized *in vitro* by nuclei in a high ionic strength medium

(presumably by polymerase II) is more DNA-like (messenger RNA?) [17-19], although a similar RNA was produced by the three polymerases solubilized from sea urchin eggs [4]. However, if one puts together the fact that a) during liver regeneration (i) the activity of polymerase I increases more than that of polymerase II (present data); (ii) the activity of the nuclear polymerase increases more than that of the extranucleolar enzyme [16]; (iii) a larger amount of rRNA is synthesized with b) the reported distribution of RNA polymerases I and II [23-24], the hypothesis that polymerase I is within the nucleolus and makes rRNA receives a strong support.

Acknowledgements

We are grateful to Professor E. Bonetti for his interest in this research and to Professor T. Wieland for generous gifts of α-amanitin. The work was aided by a grant from Consiglio Nazionale delle Ricerche, Rome.

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