NOVEL FEATURES OF DNA-DEPENDENT RNA POLYMERASE FROM PROTOZOAN *TETRAHYMENA PYRIFORMIS*

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1. Introduction

The existence of multiple forms of DNA-dependent RNA polymerase (EC 2.7.7.6) with different intranuclear localization and function has been reported in a wide variety of eukaryotic organisms [1–9]. In rat liver, for example, polymerase I (nucleolar) and II (extra-nucleolar nuclear) are engaged in the synthesis of ribosomal and DNA-like RNA, respectively [10]. Also, polymerase III has been discovered in several organisms [1, 6]. Recently Horgen and Griffin demonstrated that polymerase III of aquatic fungus *Blastocladiella emersonii* was of mitochondrial origin and suggested that this enzyme was functioning in the synthesis of mitochondrial RNA [11]. In view of the evidence obtained with DNA-dependent RNA polymerase of prokaryotes [12, 13], the appearance of multiple forms of the enzyme in eukaryotic cells might reflect some drastic change in the RNA synthesizing system during the course of evolution. Since ciliated protozoa *Tetrahymena*, although classified as an animal in the current taxonomy, was shown to possess cytochrome *c* of bacterial type [14], analysis of RNA polymerase of this organism is of much interest from the viewpoint of evolution and differentiation of RNA synthesizing system. The similarity of *Tetrahymena* and mammalian RNA polymerases [15] and the existence of two RNA polymerase activities in the isolated nuclei of *Tetrahymena* were anticipated previously [15, 16]. We report here the identification of multiple forms of RNA polymerase in the isolated nuclei of *Tetrahymena pyriformis* together with novel properties that have not been described thus far.

2. Methods

Tetrahymena pyriformis GL (amicronucleate strain) was grown in proteose–peptone medium at 28° with gentle shaking. Nuclei were isolated by the Nonidet procedure [17] with some modification utilizing sucrose medium containing 10 mM $MgCl_2$ [18, 19]. Extraction of RNA polymerase was performed essentially as described by Roeder and Rutter [2]: the solubilized enzyme obtained by sonicating the isolated nuclei in a buffer of high ionic strength and by centrifuging down the chromatin material was concentrated by the addition of solid ammonium

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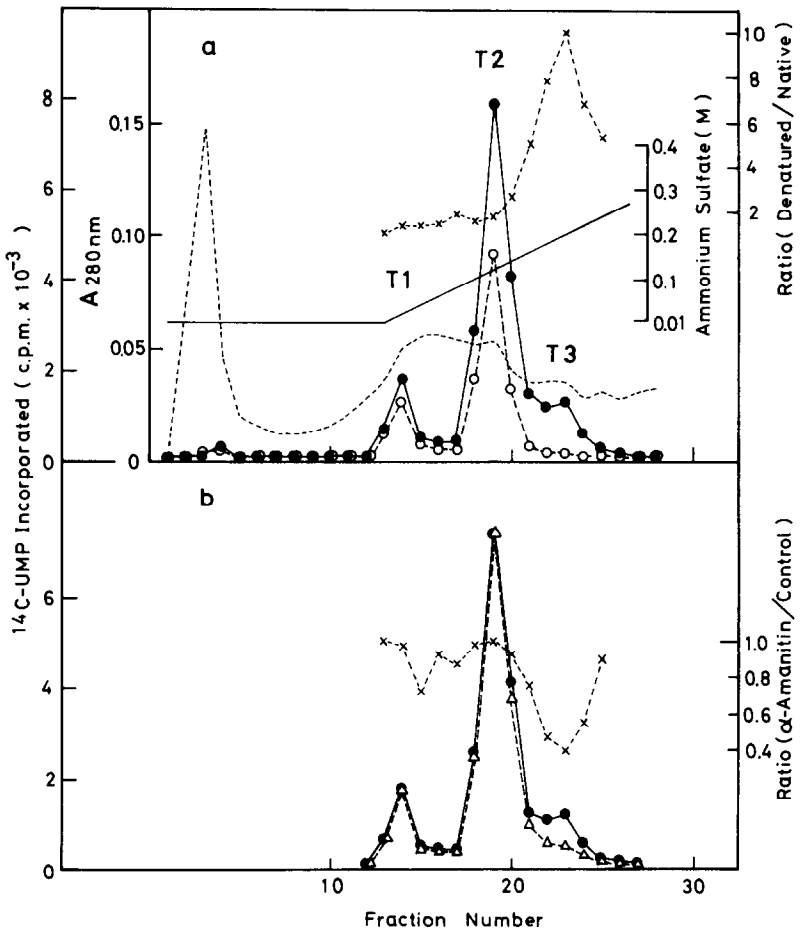

Fig. 1. a) Elution profile of DNA-dependent RNA polymerase from *Tetrahymena pyriformis* chromatographed on DEAE-Sephadex A-25. (●—●—●) Activity with heat-denatured DNA; (○—○—○) activity with native DNA; (- - - - - - -) absorbancy at 280 nm; (× - - × - - ×) ratio of activity with heat-denatured DNA to that with native DNA. The molarity of ammonium sulphate of each fraction was monitored by measuring the refractive index (n_D) of each. b) Assay of peak fractions in the presence of α-amanitin (2 μg/assay). (●—●—●) Control (activity with heat-denatured DNA); (△ - - △ - - △) activity with 2 μg per assay of α-amanitin; (× - - × - - ×) ratio of activity with α-amanitin to control.

sulphate. The precipitate formed was collected by high-speed centrifugation, dissolved in a few ml of TGMED (0.05 M Tris-HCl (pH 7.9) – 30% (v/v) glycerol – 5 mM $MgCl_2$ – 0.1 mM EDTA – 0.5 mM dithiothreitol) and dialyzed against 0.01 M ammonium sulphate in TGMED. The dialysate was centrifuged at 160,000 *g* for 1 hr to remove any particulate material which contained little activity. The resulting supernatant fraction, which corresponds to Fraction IV of Roeder and Rutter [2] and is found to be completely freed from endogenous template, was charged to the column (0.7 cm × 10 cm) of DEAE-Sephadex A-25 equilibrated with 0.01 M ammonium sulphate in TGMED. The column was washed with 5 ml of 0.01 M ammonium sulphate in TGMED followed by elution with a linear gradient of 0.01–0.4 M ammonium sulphate in the same buffer. Each fraction was assayed for the RNA polymerase activity as described previously except that the incubation was carried out at 28° [21].

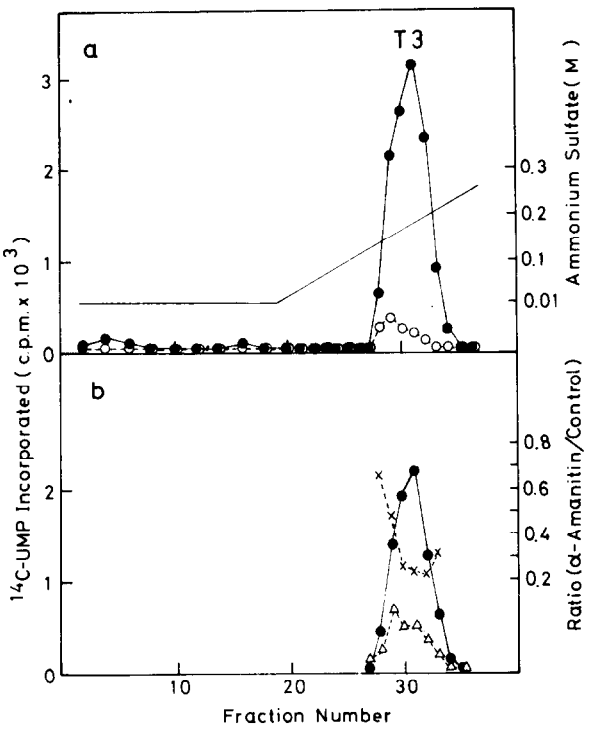

Fig. 2. a) Elution profile of RNA polymerase T3 rechromatographed on DEAE-Sephadex A-25. (●—●—●) Activity with heat-denatured DNA; (○ - - ○ - - ○) activity with native DNA. b) Assay of peak fractions in the presence of α-amanitin (2 μg/assay). (●—●—●) Control (activity with heat-denatured DNA); (△ - - △ - - △) activity with 2 μg per assay of α-amanitin; (X - - X - - X) ratio of activity with α-amanitin to control.

3. Results and discussion

As shown in fig. 1a, the solubilized enzyme was resolved by DEAE-Sephadex chromatography into three peak fractions, termed T1, T2 and T3, eluting at approx. 0.05 M, 0.15 M and 0.2 M ammonium sulphate, respectively. The peak T3 was detectable only when heat-denatured DNA was used as template. The preference of T3 enzyme for denatured DNA is evident from the ratio (denatured/native) shown in fig. 1a. T1 and T2 also transcribe denatured DNA more efficiently, but the degree of preference for denatured template was much smaller than that of T3. The relative activities of T1 and T2 were variable from experiment to experiment, and the same Fraction IV (see Methods) gave different proportions of these two enzyme activities. This observation, coupled with the behaviour towards α-amanitin and the optimal ionic strength for the activities of T1 and T2, mentioned below, raises the possibility that T1 and T2 are interconvertible forms of a single enzyme, although the conversion seems to be brought about at sites other than active. α-Amanitin, a bicyclic peptide of toadstool *Amanita phalloides* and a potent inhibitor of RNA polymerase II of various origins [3, 4, 7, 8, 20], inhibited only T3 enzyme at 2 μg per assay while T1 and T2 were resistant to the action of this toxin (fig. 1b).

In order to confirm these points, peak T3 region was pooled and rechromatographed in the same manner. As shown in fig. 2a, T3 was eluted at around 0.2 M ammonium sulphate as originally noted (fig. 1). The preferential transcription of the denatured DNA by T3 enzyme and the inhibition by α-amanitin were much more evident; the slight activities observed with native template may be due to contaminating T2 enzyme (note the peak point shifted to the left when native template was used). This situation was reflected by the lower inhibition ratio at fraction 28 and 29 (fig. 2b). Fig. 3 presents the effect of ionic strength on the activities of T1, T2 and T3 polymerase. All three enzymes, even T3, are progressively inhibited by increasing the ionic strength of the assay mixture, a finding compatible with that of Kurtz and Pearlman although in their case the enzyme was not chromatographically resolved into multiple species [16]. This property of T3 is in contrast to the results obtained so far in that the activity of α-amanitin sensitive enzyme, corresponding to polymerase II of rat liver, is stimulated by the addition of ammonium sulphate [1, 8]. Whether this finding represents an intermediate nature of T3 polymerase in the course of evolution is yet to be answered.

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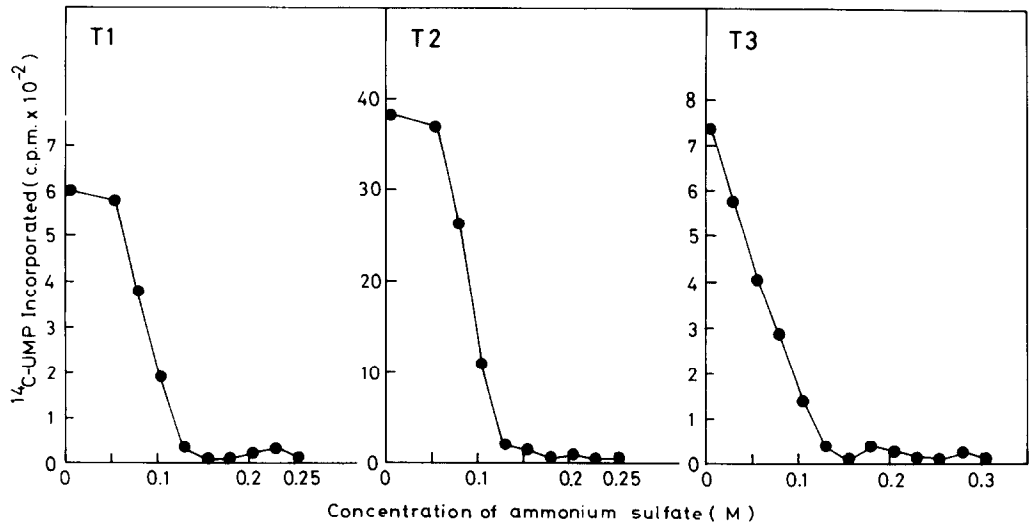

Fig. 3. Effect of ionic strength on RNA polymerases. T1 and T2 polymerase from fig. 1, and T3 polymerase rechromatographed as shown in fig. 2 were dialyzed against 0.01 N ammonium sulphate in TGMED in the presence of bovine serum albumin (1 mg/ml) They were assayed for the polymerase activities with varying concentration of ammonium sulphate as indicated on the abscissa.

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