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# RNA POLYMERASES AND THEIR STIMULATING FACTOR FROM LEUKEMIA L1210 ASCITES CELLS*)

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## ABSTRACT

RNA polymerases I and II were partially purified from L1210 ascites cells by sonic disruption, batchwise treatment with diethylaminoethyl (DEAE) cellulose, ammonium sulfate-fractionation, and subsequent linear KCl gradient chromatograpy on DEAE cellulose column. The RNA polymerases from L1210 cells showed similar enzymatic properties as in cases of Ehrlich carcinoma-RNA polymerases. RNA polymerase-stimulating factor was obtained by carboxymethyl (CM) cellulose column chromatography from L1210 cell lysate. The factor enhanced both of RNA polymerases I and II when native calf thymus DNA was used as a template, although it failed to casuse any stimulating effect when poly dAT was employed as a template DNA.

## INTRODUCTION

Multiple forms of RNA polymerases [nucleosidetriphosphate: RNA nucleotidyltransferase (DNA dependent), EC 2. 7. 7. 6] have been reported in mammalian normal[1-3] and neoplastic[4-9] tissues. It has been also evidenced that RNA polymerase-stimulating factors play some important roles in trancription of ribosomal RNA by polymarase I[10,11] and of DNA-like RNA by polymerase II[12-17]. Thus, DNA-dependent RNA polymerases and their stimulating factors may be effective in regulation of gene expression, particularly in tissue differentiation and in cancer. Leukemia L1210 is known as to be extremely malignant neoplasm with extraordinary higher frequency of metastasis than other experimental tumors and cancers[18]. Therefore, it is of interest to know the transcriptional feature of this type of leukemia cells at enzyme level. In this study, we attempted to evaluate some enzymatic nature of RNA polymerases I and II, and of their stimulating factor from L1210 ascitic cells.

## MATERIALS AND METHODS

*Assay for RNA polymerase activity.* Unless otherwise stated, 0.25 m*l* of the reaction mixture, containing 40 mM Tris-HCl (pH 7.9), 4 mM $MgCl_2$, 2 mM $MnCl_2$, 50 mM ammonium sulfate, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 3 mM mercaptoethanol, 0.5 mM dithiothreitol, 10% glycerol, 15 $\mu$g calf thymus DNA, 0.4 mM each of ATP, CTP, and GTP, 0.04 mM $^3$H-UTP (100 mCi/mmol), and 100 $\mu l$ of enzyme solution, was incubated at 37°C for 60 min. The reaction was stopped by chilling

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in ice and a 50 $\mu l$ aliquot of the reaction mixture was directly applied to Whatman No. 52 filter paper (2×2 cm square). The filter paper was rinsed five times with 5% trichloroacetic acid, twice with 95% ethanol, and air dried. Radioactivity was estimated in Packard Tri-Carb liquid scintilation counter. This assay method is essentially the same as described by D'Allesio et al[19].

*Purification of RNA polymerases.* Packed cells (2 m$l$) of L1210 ascites cells harvested from L1210-transplanted $BDF_1$ male mice (purchased from Shizuoka Union for Experimental Animals, Shizuoka) were homogenized in 4 m$l$ of buffer-A (50 mM Tris-HCl, pH 7.9, containing 5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 25% glycerol) and then sonicated in the presence of 0.3 M ammonium sulfate. The resulting cell lysate was diluted with 2.5 vol. of buffer-A and centrifuged at 120,000×g for 2.5 h. The supernatant was added with equal vol. of buffer-A and mixed with 12 g of DEAE cellulose. After washing the DEAE cellulose twice with 50 m$l$ of buffer-A, RNA polymerase was eluted with 70 m$l$ of buffer-A containing 0.55 M KCl. The resulting eluate was subjected to ammonium sulfate fractionation. In this case, RNA polymerase was precipitated at 23 to 73% saturation of ammonium sulfate. The resultant precipitate was dissolved in 10 m$l$ of buffer-A and dialyzed against the same buffer containing 0.05 M KCl. The dialyzed fraction was then applied on DEAE cellulose column chromatography with KCl-linear gradient (0.05–0.7 M). As shown in Fig. 1, RNA polymerases I and II were eluted from the column at 0.3 and 0.38 M KCl, respectively. Small amount of RNA polymerase activity was also found to be eluted at 0.5 M KCl (presumably, RNA polymerase III).

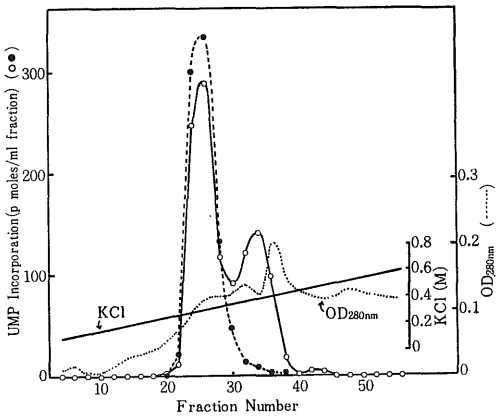


**Fig. 1.** Elution profile of RNA polymerases from DEAE cellulose column. Crude enzyme preparation obtained by ammonium sulfate fractionation was subjected to DEAE cellulose column chromatography as described in "Materials and Methods". Each eluted fraction was examined for RNA polymerase activity in the absence (○) and presence (●) of 2 $\mu$M of $\alpha$-amanitin.

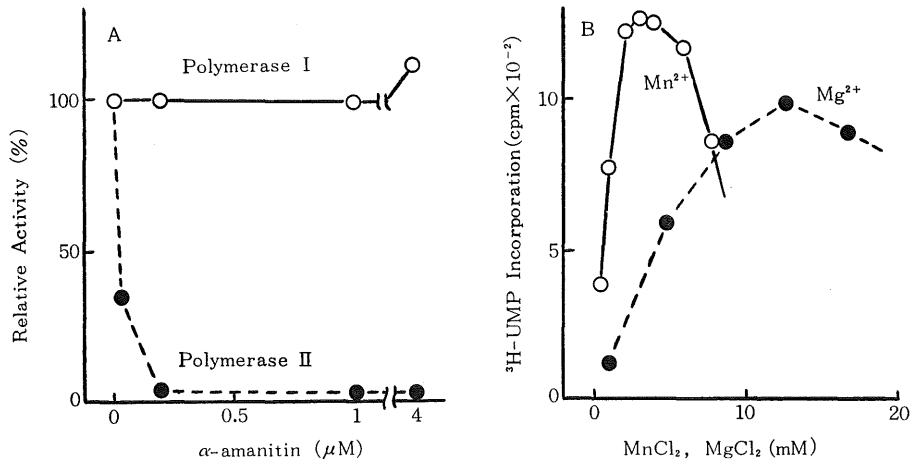


**Fig. 2.** Some enzymatic properties of L1210 RNA polymerases. (A). Effect of $\alpha$-amanitin on the activity of L1210 RNA polymerases I and II. (B). Optimum concentrations of $Mn^{2+}$ and $Mg^{2+}$ ions for the activity of L1210 RNA polymerases. Dose response curves of $MnCl_2$ (○) and $MgCl_2$ (●) were studied using the mixed fraction of RNA polymerases I and II (in a ratio of 1 : 1) as an enzyme source.

## RESULTS

As shown in Fig. 2, $\alpha$-amanitin caused a 70% inhibition of RNA polymerase II-activity even at 0.02 $\mu$M and an almost complete inhibition at the concentrations of higher than 0.2 $\mu$M. On the other hand, RNA polymerase I was insensitive to $\alpha$-amanitin even at 4 $\mu$M dose. These findings are well consistent with the observation by Lindell et al[20]. Optimum concentrations of $Mn^{2+}$ and $Mg^{2+}$ ions for the activity of these two enzymes were approximately 3 and 12 mM (Fig. 2). Actinomycin D and pluramycin caused about 88 and 82% inhibition of the activity of these enzymes, respectively, while rifampicin caused only about 35% inhibition, when these antibiotics were added to the reaction mixture at 4 $\mu$g/m*l*. each When various DNA preparations were used as template, relative UMP incorporation by the enzymes (the mixture of RNA polymerses I and II in equal amount) were as follows; calf thymus DNA (native), 100%; calf thymus DNA (heat-denatured), 78%; salmon spleen DNA (native), 70%; poly dAT, 228%.

Unadsorbed fraction (32 m*l*) of DEAE cellulose-batchwise treatment of L1210 cell lysate (see "MATERIALS AND METHODS") was applied on CM cellulose column equilibrated with buffer-B (0.01 M potassium phosphate, pH 7.5, containing 0.1 mM EDTA, 1 mM dithiothreitol, and 15% glycerol). The column (1.6 $\times$6 cm) was washed with 50 m*l* of buffer-B and then adsorbed proteins were eluted with a 120-m*l* linear gradient of 0 to 0.5 M KCl/buffer-B. The resulting elution pattern is indicated in Fig. 3. RNA polymerase-stimulating factor was found to be eluted out with 0.17 M KCl. Using the peak fraction of this

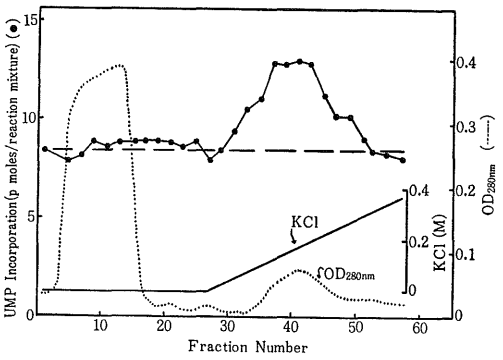


**Fig. 3.** CM cellulose column chromatography of RNA polymerase-stimulating factor from L1210 cells. Unadsorbed fraction obtained by DEAE cellulose-batchwise treatment of L1210 cell lysate was applied on CM cellulose column, and eluted as mentioned in the text. The eluted fraction (50 $\mu$*l*) was added to the reaction mixture of RNA polymerase, and studied for the enhancing effect on RNA synthesis (●). In this case, the mixed fraction of RNA polymerases I and II (in a ratio of 1 : 1) was employed as an enzyme source.

**Table 1.** Enzyme and Template Specificity of RNA Polymerase-Stimulating Factor from L1210 Cells*

| RNA Polymerase | DNA | Stimulating Factor | $^3$H-UMP Incorporation (cpm) | Stimulation Ratio (%) |
|---|---|---|---|---|
| Polymerase-I | NCT-DNA | − | 286 | |
| | | + | 372 | 38 |
| Polymerase-II | NCT-DNA | − | 484 | |
| | | + | 686 | 41 |
| Polymerase-I+II** | Poly dAT | − | 1462 | |
| | | + | 1305 | −11 |

* RNA Polymerase activity was assayed as mentioned in "Materials and Methods" except that indicated DNA was added to each reaction mixture (total 0.25 m*l*) at 12.5 $\mu$g dose. Into each incubation mixture, 75 $\mu$*l* of buffer-B or fraction 41 of CM cellulose column chromatography (as a RNA polymerase-stimulating factor source; see text) was added.

** The mixed fraction of RNA polymerases I and II in a ratio of about 1 : 2 was used as enzyme source.

Abbreviation; NCT-DNA is native calf thymus DNA.

activity (Fr. 41), some enzymatical properties of this factor were examined. As shown in Table 1, the factor enhanced RNA polymerases I and II in a similar manner (1.38 and 1.41 fold stimulation, respectively), when calf thymus DNA was employed as a template. On the contrary, when poly dAT was used as a tepmlate DNA, the factor failed to cause any stimulation of RNA synthesis, but showed a slight inhibition.

## DISCUSSION

Enzymatic properties of L1210-RNA polymerases I and II were almost the same as of Ehrlich carcinoma-RNA polymerases reported by Blair[9], on the basis of the susceptibility to $\alpha$-amanitin and optimum concentration of $Mg^{2+}$ and $Mn^{2+}$ ions for the enzyme activity. Moreover, template specificity of these two enzymes were similar as reported by Natori et al[5]. in the case of RNA polymerase II from Ehrlich ascites tumor cells. The present RNA polymerase-stimulating factor obtained from L1210 cells enhanced the activity of both of RNA polymerases I and II at 30 to 40% ratio. This stimulating ratio is comparable to the values reported in cases of RNA polymerase-stimulating factors from rat liver[11], Ehrlich ascites cells[16], and chiken myloblastosis cells[17]. It may be noted that the present RNA polymerase-stimulating factor from L1210 cells can enhance both of RNA polymerases I and II at the same ratio. Similar enzyme-specificity has been observed in the case of "from I factor" purified from rat liver[10]. It may be also noteworthy that L1210-derived RNA polymerase-stimulating factor could not augment RNA synthesis when poly dAT was used as template DNA. Similar template specificity of the stimulating factors has been reported in cases of RNA polymerase I-stimulating factors from rat liver[10] or RNA polymerase I-stimulating factor of Ehrlich ascites cells[16]. However, this feature is considerably different from that described in most cases of RNA polymerase II-stimulating factors such as those from mouse myeloma[8], and from chicken myeloblastosis cells[17], which could enhance RNA synthesis using poly dAT as template DNA.

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