ANTIGENIC HOMOLOGY OF EUKARYOTIC RNA POLYMERASES

C.J. Ingles

Banting and Best Department of Medical Research
University of Toronto, Toronto, Canada M5G 1L6

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SUMMARY:

Facilitated by an improved enzyme purification procedure, antisera to calf thymus DNA-dependent RNA polymerase II was prepared in hens. Using immunoprecipitation and inhibition of enzymatic activity the immunological properties of several eukaryotic RNA polymerases were examined. Purified calf thymus and rat liver polymerase II exhibited antigenic homology. The partially purified amphibian (Xenopus laevis) and protozoan (Tetrahymena pyriformis) polymerase II had reduced cross-reactivities. Calf thymus polymerase I also shared antigenic homology with the form II enzymes.

INTRODUCTION:

The multiple forms of eukaryotic DNA-dependent RNA polymerase exhibit different catalytic, chromatographic, (1,2) and structural properties (3,4,5) and specific intracellular localizations (6). Indirect studies indicate a role for polymerase I in synthesizing ribosomal RNA (2,7,8). These data have been interpreted in terms of different transcriptive specificities for each form of RNA polymerase. Furthermore the levels of RNA polymerase activities vary in different physiological (2,9) or developmental states (10). In an effort to further explore the role of these enzymes in regulating RNA synthesis, antiserum to purified calf thymus RNA polymerase II has been prepared. Using this antiserum, the immunological properties of several eukaryotic RNA polymerases have been investigated.

METHODS:

Calf thymus RNA polymerase II was prepared by a modification of the methods of Weaver et al. (5) and Kedinger and Chambon (4). 100 g of frozen calf thymus was suspended in 200 ml of

TGMED* containing 0.1 mM phenylmethylsulfonyl fluoride and briefly homogenized in a Waring blender. After addition of 24 ml 4.0 M $(NH_4)_2SO_4$ and further homogenization in a Sorval Omnimix (3 x 20 sec, top speed), the homogenate was diluted with 2 volumes of TGMED, and centrifuged (13,000 g, 30 min). RNA polymerase activity in the supernatant was precipitated with $(NH_4)_2SO_4$ (209 g/l), collected by centrifugation (13,000 g, 45 min) and dissolved in a volume of TGMED such that the $(NH_4)_2SO_4$ concentration was 0.12 M for loading on a DEAE Sephadex A-25 column (2.5 x 50 cm). This column was eluted with 200 ml TGMED containing 0.1 M $(NH_4)_2SO_4$ and then a linear gradient 600 ml of 0.1 to 0.5 M $(NH_4)_2SO_4$ in TGMED. The pooled polymerase II activity was dialyzed against six volumes TGMED for 16 hours, and chromatographed on a phosphocellulose P-11 column (1.5 x 25 cm), pre-equilibrated with TGED (no $MgCl_2$) and eluted with a linear gradient, 160 ml, 0.05 to 0.5 M $(NH_4)_2SO_4$ in TGED containing 1 mM $MgCl_2$ and 0.5 mg/ml bovine serum albumin. After adjusting the $MgCl_2$ concentration to 5.0 mM and then assaying, polymerase II activity could be frozen and stored at -70°. The final purification step was sucrose gradient sedimentation (5) with $(NH_4)_2SO_4$ concentration in the gradient at 0.15 M. The purity of each preparation was monitored by SDS gel electrophoresis (5).

Partially purified calf thymus polymerase I was prepared after similar extraction, by $(NH_4)_2SO_4$ fractionation and chromatography on Biogel A-15 m, DEAE-Sephadex and phosphocellulose. Purified rat liver RNA polymerase II was prepared as described by Weaver et al. (5), omitting the chromatography on DNA-Sepharose. Partially purified RNA polymerase II from adult Xenopus laevis

*TGMED is .05 M tris-HCl, pH 7.9, 25% (v/v) glycerol, 5.0 mM $MgCl_2$, 0.1 mM EDTA and 1.0 mM dithiothreitol.

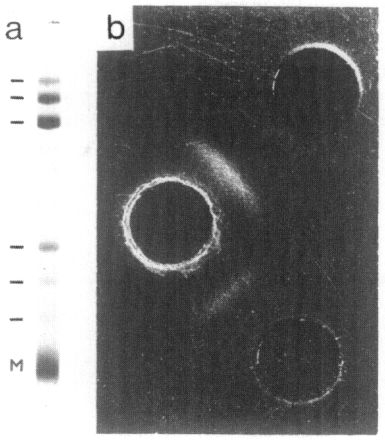

FIGURE 1. (a) SDS-polyacrylamide gel electrophoresis of purified calf thymus RNA polymerase II. 30 µg of the sucrose gradient purified polymerase II and 5 µg of a marker, horse heart cytochrome c, were electrophoresed in 5% acrylamide gels (5). (b) Immunoprecipitation of purified calf thymus and rat liver RNA polymerase II preparations by hen anti-calf thymus polymerase II serum. The left well contained .025 ml antiserum, the upper right well 13 µg calf thymus polymerase and the lower right well 10 µg rat liver enzyme. The gel was 0.5% agarose containing 1.5 M KCl, 1 mM $MgCl_2$, 10 mM triethanolamine (pH 7.5) and 0.01% sodium azide.

liver and from Tetrahymena pyriformis were prepared through the DEAE chromatography step by the procedures described by Roeder et al. (11) and Kurtz (12) respectively. Incomplete and variable inhibition by α-amanitin of different Tetrahymena pyriformis polymerase II preparations may indicate contamination of polymerase II by an α-amanitin resistant RNA polymerase.

Adult hens were immunized at 8-10 day intervals by 4 administrations, given subcutaneously and intramuscularly, each containing in a total volume of 1.0 ml, 500-800 µg purified calf thymus polymerase II emulsified with Freunds adjuvant.

RESULTS AND DISCUSSION:

To obtain antibodies to mammalian RNA polymerase, a relatively simple purification scheme was developed which greatly increased the yield of calf thymus RNA polymerase II. The procedure differs from that described by Weaver et al. (5) in that frozen tissue was used; the nuclei purification step was omitted, and an

ammonium sulfate fractionation and modified DEAE-Sephadex chromatography were introduced. The yield of enzyme from 100 g tissue is in the range 500-1200 µg, which compares to the 35 µg/100 g tissue reported by Weaver et al. (5) and 300 µg/100 g tissue reported by Kedinger and Chambon (4). As isolated from sucrose gradients, with specific activity in the range 210-300 units/mg protein (5), the enzyme appears a nearly equal mixture of the two components of the enzyme (IIA and IIB). SDS gel electrophoresis, Figure 1(a), indicates only subunits of molecular weight 200,000, 170,000, 140,000, 40,000, 30,000 and 21,000. This enzyme is therefore similar in subunit composition to the enzyme(s) from calf thymus isolated by Kedinger and Chambon (4), although minor differences in the assigned molecular weight values are apparent. Despite addition of a proteolytic inhibitor to these preparations (5), this procedure yields two components (IIA, IIB) of polymerase II. The purified rat liver polymerase II was also a mixture of the two components described by Weaver et al. (5), Chesterton and Butterworth (13) and Mandel and Chambon (14), and contained subunits of similar molecular weight to those of the calf thymus enzyme.

Repeated attempts to obtain antisera in either rabbits or guinea pigs to the form II enzymes were unsuccessful. Polymerase II purified to apparent homogeneity from both calf thymus and rat liver, and an RNA polymerase-DNA complex (15) was used as antigen in these initial studies. Potent antiserum was obtained after administration of calf thymus polymerase II to hens. As shown in Figure 1(b) serum, from immunized hens contains antibodies which precipitate both the purified calf thymus and rat liver RNA polymerase II preparations. Figure 2(a) indicates that increasing amounts of the antiserum preincubated with calf thymus polymerase I

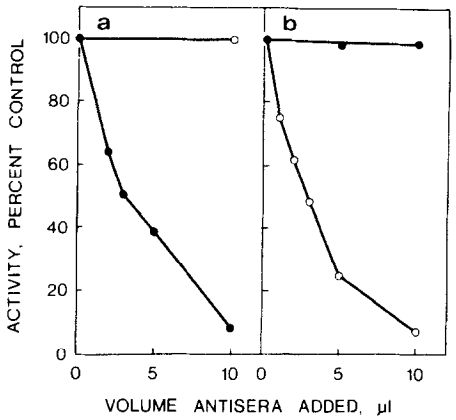

FIGURE 2. Inhibition of RNA polymerase activities by specific antisera. 0.01 aliquots of either sucrose gradient purified calf thymus polymerase II ●—● or E. coli RNA polymerase o—o (17) were assayed (2) after preincubation for 45 min at 0° of .02 ml of each enzyme (.006 units calf thymus II and .01 units E. coli enzyme) in TGME containing .04 mM dithiothreitol, 0.1 M $(NH_4)_2SO_4$ and 0.5 mg/ml bovine serum albumin with .01 ml serum containing the increasing proportion of specific antisera. (a) Hen anti-calf thymus RNA polymerase II serum, (b) rabbit anti-E. coli RNA polymerase serum. Activity is expressed as the percent of activity of each enzyme preincubated in control serum.

progressively inactivate this enzyme. The same antiserum is without effect on the activity of E. coli RNA polymerase. An analogous specificity is observed with rabbit anti-E. coli RNA polymerase serum. The latter while inhibiting the E. coli enzyme is without effects on the calf thymus polymerase (Figure 2b). The inhibition of activity is therefore clearly due to the specific antibodies and not serum nucleases or other trivial explanations. Specificity of the hen anti-calf thymus polymerase II antibodies to eukaryotic enzymes is also indicated.

This more sensitive immunoassay allowed a wider study of other eukaryotic RNA polymerases. As indicated in Table I the purified form II enzymes of calf thymus and rat liver and the partially purified polymerase II from Xenopus laevis and the protozoan species, Tetrahymena pyriformis, are all inhibited. The extent of inhibition of the purified mammalian enzymes is similar, while

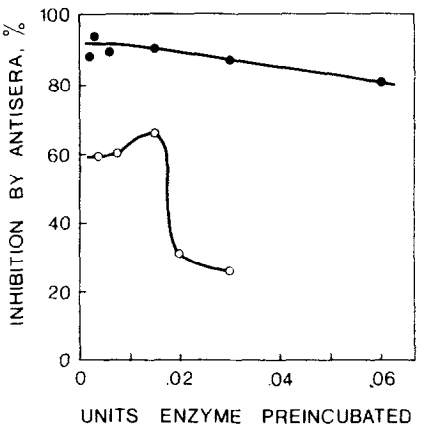

FIGURE 3. Inhibition of calf thymus polymerase I and II by anti-calf thymus polymerase II serum. Varying concentrations of purified calf thymus polymerase II, ●——●, or partially purified polymerase I, o——o, in .02 ml were preincubated with .01 ml anti-calf thymus polymerase II serum or control serum and the percent inhibition of activity by the antiserum determined (see Figure 2 for details).

somewhat reduced cross-reactivity is indicated in the case of the Xenopus and Tetrahymena enzymes. The similarity of many eukaryotic form II RNA polymerases, already demonstrated in terms of their chromatographic properties, salt and divalent metal ion requirements, inhibition by α-amanitin and subunit structure, clearly extends to their immunological properties.

The form I polymerase from calf thymus is inhibited up to 66% by anti-polymerase II serum (Figure 3). With an increasing concentration of polymerase I, however, antigen excess conditions prevail and reduced inhibition is seen. As the specific activities of each of the purified calf thymus polymerases are similar (3,4), it appears that only a fraction of the antibodies to polymerase II cross-react with polymerase I. Antigenic homology between Saccharomyces cerevisiae RNA polymerases I and II has also been reported (16). The putative subunit compositions of the two forms of calf thymus polymerase, I and II, indicate that at best these

TABLE I

Inhibition of RNA polymerase II preparations by hen anti-calf thymus polymerase II serum

Source of Enzyme	Units of Enzyme preincubated ($x10^3$)	Percent Inhibition
Calf thymus	3	94.1
	60	80.8
Rat liver	5	91.3
	60	82.1
Xenopus laevis liver	2	60.5
Tetrahymena pyriformis	9	63.2

Sucrose gradient purified calf thymus and rat liver polymerase II, and the partially purified polymerase II from *Xenopus* liver and *Tetrahymena*, each in a volume of .02 ml, were preincubated with either .01 ml anti-calf thymus polymerase II serum or control serum and assayed (see Figure 2 for details).

two enzymes may have only two low molecular weight subunits in common. This antigenic homology between the mammalian polymerases I and II may thus reside in the small subunits of similar molecular weight which both enzymes appear to contain (3,4). It might also indicate more extensive homology in the larger subunits.

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