RNA Polymerases from Avian Liver. Isolation and Fractionation by Heparin-Sepharose Chromatography*

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Optimal conditions for isolation of rooster liver nuclei were studied for the purification of RNA polymerases I and II by varying the concentrations of sucrose, magnesium chloride and spermine in the isolation medium. Addition of spermine and/or magnesium chloride to the hypertonic sucrose medium was found to be advantageous for the purification. Heparin-Sepharose chromatography can be recommended for purification of RNA polymerases when used in a stepwise manner. Furthermore, gradient elution in the heparin-Sepharose chromatography was found to separate RNA polymerases I and II. RNA polymerase III was eluted with RNA polymerase I. A few minor peaks of RNA polymerase II activity were detected with gradient elution. Factors influencing the affinity of RNA polymerases towards heparin-Sepharose are discussed.

Eukaryotic cells contain three classes (I, II and III) of DNA dependent RNA polymerases, EC 2.7.7.6. (for a review, see Ref. 1). It has been shown that the polymerases exist in two functional states in the nuclei. One is active toward an endogenous chromatin template (engaged enzyme) while the other is inactive and can be measured only with an exogenous template (free enzyme).[2] It is known that the composition of the initial homogenization medium (e.g. hypertonic sucrose) influences the recovery of RNA polymerases during isolation of the nuclei.[3,4] Polyamines and magnesium chloride, when added to the homogenization medium, have been reported to influence nucleolar morphology,[5] RNA polymerase activity [6] and base composition of the RNA synthesized.[7] We have examined the effect of magnesium chloride and spermine on the recovery and activity of RNA polymerases I and II using avian liver nuclei as a source of enzymes.

Sternbach et al.[8] have described a chromatographic method using heparin-Sepharose for the purification of RNA polymerase from E. coli in high yield and purity. The present study also deals with the use of heparin-Sepharose in purification and fractionation of eukaryotic RNA polymerases. In the course of this study others have also reported the use of heparin-Sepharose in the partial purification of eukaryotic RNA polymerases from different sources.[9–14] We describe now, that in addition to its use in a stepwise purification, heparin-Sepharose can also be used for fractionation of eukaryotic RNA polymerases I and II.

EXPERIMENTAL

Animals and chemicals. White Leghorn roosters weighing about 1.0 kg were obtained from a local hatchery and fed *ad libitum*. To increase activity of liver RNA polymerases for the chromatographic experiments, the roosters were estrogenized in the following manner. Estradiol-17β benzoate was dissolved in sesame oil and injected (20 mg/kg) in two portions into leg muscles. Roosters were decapitated 24 h after estrogen injection. Estradiol-17β benzoate (estra-1,3,5(10)-triene-3,17β-diol 3-benzoate) was from Nutritional Biochemicals Corp. [5-³H]Uridine 5'-triphosphate, sodium salt (2 Ci/mmol) was purchased from The Radiochemical Centre, Amersham. Unlabeled ribonucleoside triphosphates and α-amanitin were from Boehringer Mannheim Biochemicals. Calf thymus DNA and bovine

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serum albumin were from Sigma Chemical Co., and dithiothreitol (DTT) from Calbiochem. Sucrose and ammonium sulfate (special enzyme grade) were obtained from Schwarz/Mann. Glycerol was purchased either from E. Merck AG (87 %) or from BDH Chemicals Ltd (glycerol anhydrous). DEAE-Sephadex A-25 and CNBr-activated Sepharose 4B were from Pharmacia Fine Chemicals, Inc. Heparin (152 000 IU/g), U.S.P. XVIII, was from Leo Pharmaceutical Products. DE-81 filters were supplied by Whatman Ltd.

Isolation of nuclei. Nuclei were isolated by a modification of the methods of Chauveau *et al.*[15] and Lin *et al.*[4] Roosters were decapitated, bled and the livers were quickly removed, weighed and chilled in cold saline. All subsequent operations were performed at $0-4\,°C$. The following hypertonic homogenization media were used: medium (1) 2.2 M sucrose, 0.25 mM spermine, 15 mM $MgCl_2$; medium (2) 2.2 M sucrose, 0.25 mM spermine, 3 mM $MgCl_2$; medium (3) 2.2 M sucrose, 15 mM $MgCl_2$; medium (4) 2.2 M sucrose, 3 mM $MgCl_2$; medium (5) 2.2 M sucrose, 0.25 mM spermine. Pieces of liver were added to seven volumes of the homogenization medium, minced with scissors and homogenized in a glass-Teflon homogenizer with three strokes. The homogenate was filtered through two layers of cheesecloth. After centrifugation at 46 000 g_{av} for 90 min the nuclear pellets were suspended in a small volume of a buffer containing 0.05 M Tris-HCl (pH 7.9 at 20 °C), 25 % (v/v) glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT (TGMED buffer) plus 25 mM 2-mercaptoethanol. The isotonic homogenization medium contained 0.25 M sucrose, 0.05 M Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 25 mM KCl and 0.25 mM spermine where indicated. Pieces of liver were added to six volumes of the isotonic medium, homogenized and filtered as above. The homogenate was then centrifuged twice at 1000 g_{max} for 7 min. The nuclear pellets were further purified by homogenization and centrifugation in the hypertonic medium as described above.

Extraction and concentration of RNA polymerases. The nuclei were disrupted by sonication in 0.3 M ammonium sulfate.[16] For ammonium sulfate precipitation, 0.313 g of solid ammonium sulfate was added per ml of solution. The precipitate was collected by centrifugation at 48 000 g_{max} and resuspended in TGMED buffer plus 25 mM 2-mercaptoethanol. After dialysis for 4 h against TGMED buffer the dialysate was centrifuged at 105 000 g_{av} for 60 min. The supernatant was divided into small aliquots and frozen immediately in liquid nitrogen and stored at $-80\,°C$.

Assay of RNA polymerase activity. RNA polymerase activity was determined by measuring the incorporation of radioactivity from [³H]UTP into RNA which was collected on DE-81 ion exchange filters (DEAE-cellulose paper discs, 2.3 cm in diameter). The standard

reaction mixture contained the following components in 50 μl: 50 mM Tris-HCl (pH 7.9), 20 μg calf thymus DNA, 0.6 mM ATP, GTP and CTP, 0.5 μCi [³H]UTP, 1.25 mM dithiothreitol and 12 % glycerol. For RNA polymerase I, the standard mixture contained also 10 mM $MgCl_2$ and 1 $\mu g/ml$ α-amanitin. For polymerase II, 2 mM $MnCl_2$ and 100 mM $(NH_4)_2SO_4$ were added and the activity was calculated as a difference between the values obtained in the absence and presence (1 $\mu g/ml$) of α-amanitin. The reaction was started by addition of 10 μl of the enzyme in TGMED buffer. The incubation was for 5 min at 37 °C. The reaction was stopped by transferring a 50 μl aliquot from each tube to a DE-81 filter disc. The filters were washed [17] and counted for radioactivity. The counting efficiency of [³H]UMP incorporated into RNA and bound to the filter was 21 %. Conditions for RNA polymerase assays in column fractions are described below.

DEAE-Sephadex A-25 chromatography. DEAE-Sephadex was treated as described by Schwartz *et al.*[18] The ion exchange material was equilibrated with TGMED buffer containing 0.08 M ammonium sulfate. The sample (supernatant after chromatin removal) was adjusted to 0.08 M ammonium sulfate and applied to a column of DEAE-Sephadex (2.9 × 10.5 cm). The enzymes were eluted with 140 ml of a linear gradient containing 0.08 to 0.5 M ammonium sulfate in TGMED buffer. Fractions of 4.8 ml were collected at a rate of 0.3 ml per min and cm² of column area. The enzyme activity was determined from 10 μl aliquots of column fractions in the presence of 1 $\mu g/ml$ of α-amanitin and 10 mM $MgCl_2$ and in the absence of α-amanitin with 2 mM $MnCl_2$ replacing $MgCl_2$. The absorbance of the eluate was determined at 280 nm with an LKB 2089 Uvicord III absorbance monitor. Fractions containing RNA polymerase I and II activity were further analyzed by heparin-Sepharose chromatography.

Heparin-Sepharose chromatography of RNA polymerases I and II. Heparin was covalently coupled to CNBr-activated Sepharose 4B according to Iverius.[19] The amount of sulfate bound to the gel was measured by the method of Antonopoulos.[20] The sulfate content was $5-8$ $\mu g/mg$ of freeze-dried gel. To study the elution characteristics of the RNA polymerases on heparin-Sepharose, RNA polymerases I and II were purified and fractionated by DEAE-Sephadex chromatography as described in the previous section. For chromatography of DEAE-Sephadex peak I (RNA polymerase I) on heparin-Sepharose, the gel was equilibrated with TGMED buffer containing 0.3 M ammonium sulfate and 2 mg/ml of bovine serum albumin. The pooled fractions from DEAE-Sephadex chromatography were transferred into a 50 ml-graduated cylinder containing 2.5 ml of heparin-Sepharose, mixed gently

for 60 min and transferred to a small column (0.9 × 8 cm) filled with heparin-Sepharose. The column was washed with the equilibrating buffer and RNA polymerase I eluted with 20 ml of a linear gradient containing 0.3 to 0.8 M ammonium sulfate in TGMED buffer plus 2 mg/ml of bovine serum albumin. Fractions of 2 ml were collected at a rate of about 0.4 ml per min and cm² of column area. For chromatography of DEAE-Sephadex peak II (RNA polymerase II) on heparin-Sepharose, the gel was equilibrated with TGMED buffer containing 0.1 M ammonium sulfate and 2 mg/ml of bovine serum albumin. The linear gradient was from 0.1 to 0.8 M ammonium sulfate. Otherwise the procedure was as described above for RNA polymerase I. The column fractions were immediately frozen in liquid nitrogen and stored at −80 °C until analyzed for RNA polymerase activity. The incubation medium contained 2 mM MnCl₂ and the activity determinations were performed in the presence (1 µg/ml) and absence of α-amanitin.

Purification and fractionation of total RNA polymerase activity from rooster liver on heparin-Sepharose. For purification of RNA polymerases in a stepwise manner the column (2.2 × 6 cm) was equilibrated with TGMED buffer containing 0.1 M ammonium sulfate, 0.1 % Triton X-100 and 10 mM thioglycerin. The sample (supernatant after chromatin removal) was applied to the column, washed with the equilibrating buffer and with a buffer containing 0.15 M ammonium sulfate. RNA polymerases were eluted from the column with 65 ml of a buffer containing 0.5 M ammonium sulfate. Fractions of 5 ml were collected at a rate of 0.15 ml per min and cm² of column area. RNA polymerase activity was determined as in the previous heparin-Sepharose chromatography. For rechromatography, fractions containing enzyme activity were concentrated by membrane ultrafiltration (Amicon PM-10) and

the ammonium sulfate concentration was reduced to approximately 0.1 M.

For fractionation of RNA polymerases, the column (0.9 × 10 cm) was equilibrated as above. The ultrafiltrate was applied to the column and subsequently washed as in the stepwise chromatography. RNA polymerases were eluted as above with 50 ml of a linear gradient containing 0.15 to 0.5 M ammonium sulfate in the same buffer plus bovine serum albumin (1 mg/ml). Fractions of 2 ml were collected at a rate of about 0.4 ml per min and cm² of column area. The incubation medium for RNA polymerase activity determinations contained 2 mM MnCl₂ and 0, 1 or 166 µg/ml α-amanitin. From the activity data the elution profiles of RNA polymerases I, II, and III were calculated.

Other methods. Nucleic acids were determined as described by Ashwell,[21] RNA after alkaline digestion (1 M KOH, 1 h, 37 °C) and DNA from a hot trichloroacetic acid extract. Protein was determined by the method of Lowry et al.[22] with bovine serum albumin as a standard. From chromatographic fractions, protein was determined either by measuring absorbance at 280 nm or by the Coomassie Blue method [23,24] with bovine serum albumin as a standard. The ammonium sulfate concentration of a given sample was determined by taking a 50 µl aliquot, mixing with 10 ml of distilled water and measuring the conductivity at room temperature with a Philips PW 9501/01 conductivity meter.

RESULTS

Isolation of nuclei in various homogenization media. Hypertonic sucrose is known to prevent the leakage of RNA polymerases from the nuclei during isolation.[3,4] We have studied

Table 1. Recoveries of protein and nucleic acid during purification of nuclei in various homogenization media. Liver samples (about 14 g) were added to seven volumes of a given medium, homogenized and sedimented at 46 000 g_{av} for 90 min. The nuclear pellets were suspended in a small volume of the buffer containing 0.05 M Tris-HCl (pH 7.9), 25 % (v/v) glycerol, 5 mM MgCl₂, 0.1 mM EDTA, 5 mM DTT (TGMED buffer) plus 25 mM 2-mercaptoethanol. Protein and nuclei acids were determined as described in "Experimental".

| Medium | Addition to the 2.2 M sucrose medium | | Composition of the nuclei | | |
	MgCl₂/mM	Spermine/mM	RNA/mg g⁻¹ of wet tissue	DNA/mg g⁻¹ of wet tissue	Protein/mg g⁻¹ of wet tissue
1	15	0.25	0.204	1.278	2.027
2	3	0.25	0.198	1.159	2.281
3	15	—	0.251	1.378	2.670
4	3	—	0.231	1.374	2.699
5	—	0.25	0.210	1.566	2.740

Table 2. RNA polymerase I and II activities in the nuclei isolated in various homogenization media. RNA polymerases were extracted from the nuclei by sonication in 0.3 M ammonium sulfate and precipitated by 55 % ammonium sulfate saturation at 0 °C. The precipitates were dissolved in TGMED buffer, dialyzed and subjected to activity determinations as described in "Experimental". For other details see the legend to Table 1.

| Medium | RNA polymerase I | | RNA polymerase II | | RNA polymerase I [a] |
	pmol/(mg DNA/5 min)	pmol/(mg prot./5 min)	pmol/(mg DNA/5 min)	pmol/(mg prot./5 min)	RNA polymerase II
1	53.6	111.6	39.7	82.8	1.35
2	50.9	93.1	45.6	83.4	1.12
3	55.7	112.0	40.4	81.4	1.38
4	45.3	87.5	42.7	82.4	1.06
5	33.0	73.3	35.3	78.4	0.93

[a] The ratio was calculated from the pmol/mg DNA/5 min data.

whether the sucrose concentration and additional agents in the isolation medium have an effect on the yields of RNA polymerases. The results are presented in Tables 1 and 2. The recovery of RNA polymerases from the nuclei was calculated with respect to DNA in purified nuclei. The recovery of RNA polymerase I was best in the medium containing, in addition to 2.2 M sucrose, 15 mM $MgCl_2$. For RNA polymerase II the recovery was best in the medium containing 3 mM $MgCl_2$ and 0.25 mM spermine. Also the specific activity of RNA polymerase I was highest with 15 mM $MgCl_2$ and that of RNA polymerase II with 3 mM $MgCl_2$ and 0.25 mM spermine. The activity ratio (RNA polymerase I/RNA polymerase II) was highest in the medium containing 15 mM $MgCl_2$ and lowest in the medium containing 0.25 mM spermine. Although the recovery of DNA per gram of wet tissue was highest in the medium containing 0.25 mM spermine, the yields of RNA polymerases were lowest in this medium.

With the isotonic homogenization medium the specific activity of RNA polymerase I was about half of that obtained with the hypertonic medium (results not shown). With respect to RNA polymerase II the ratio between isotonic and hypertonic medium was about 0.7. Spermine when added to the isotonic medium had no effect on the specific activity of RNA polymerase I. For polymerase II the ratio (− spermine/ + spermine) was about 0.8.

Elution of RNA polymerases I and II in DEAE-Sephadex and heparin-Sepharose chro- matography. In pilot experiments with heparin-Sepharose we observed that there were differences in the binding affinities of RNA polymerases I and II when the ammonium sulfate concentration was elevated from zero to 0.3 M.
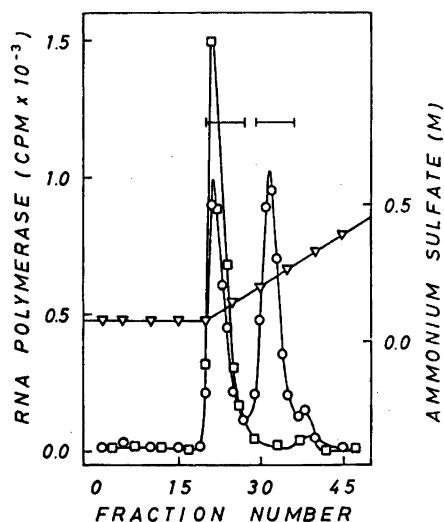
Fig. 1. DEAE-Sephadex chromatography of RNA polymerases extracted from avian liver nuclei. The column (2.9 × 10.5 cm) was equilibrated and washed as described in "Experimental". The linear gradient contained 0.08 to 0.5 M ammonium sulfate in TGMED buffer. The enzyme activity was determined in the presence of 10 mM $MgCl_2$ and 1 μg/ml of α-amanitin (□) or 2 mM $MnCl_2$ and with no α-amanitin (○). ▽, ammonium sulfate concentration. The horizontal bars indicate fractions which were separately pooled.
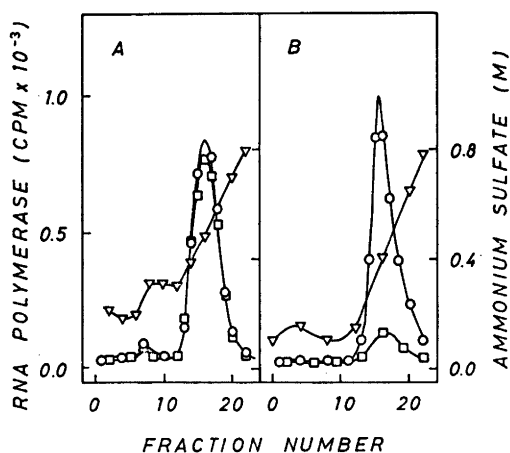
Fig. 2. Elution positions of RNA polymerases I and II from a heparin-Sepharose column (0.9 × 8 cm) A. The sample consisted of pooled fractions from DEAE-Sephadex chromatography (Fig. 1) containing RNA polymerase I activity. B. The sample consisted of pooled fractions from DEAE-Sephadex chromatography containing RNA polymerase II activity. For chromatographic details see "Experimental". The enzyme activity was determined in the presence of 2 mM $MnCl_2$ and 1 μg/ml of α-amanitin (□) or with no α-amanitin (O). ▽, ammonium sulfate concentration.

To test the binding affinities in more detail RNA polymerases I, II and III were isolated by DEAE-Sephadex chromatography (Fig. 1). RNA polymerases I and II separated well in the conditions used but RNA polymerase III was only partially separated from RNA polymerase II. The separated peaks were subse-

quently chromatographed on heparin-Sepharose. These results are shown in Fig. 2. RNA polymerases I and II eluted from heparin-Sepharose columns in reversed order with respect to DEAE-Sephadex, at 0.5 and 0.4 M ammonium sulfate, respectively. The degree of purification and recoveries of RNA polymerases I and II on DEAE-Sephadex and heparin-Sepharose are shown in Table 3. The final recoveries of RNA polymerases I and II were 32.1 and 50.7 %, respectively. During DEAE-Sephadex chromatography the specific activities increased about 4- and 16-fold. The specific activities could not be determined after heparin-Sepharose chromatography because bovine serum albumin was added to the gradient solutions.

Purification of RNA polymerases I and II by stepwise heparin-Sepharose chromatography. In the course of the previous experiments we observed that stepwise elution gave best results in purification of avian liver polymerases both with respect to total and DEAE-Sephadex fractionated activities. The results of a representative experiment are shown in Fig. 3. RNA polymerase I and II activities coeluted in the stepwise elution scheme. The purification data are presented in Table 4. It can be seen that heparin-Sepharose chromatography removed about 90 % of protein. The specific activities of RNA polymerases I and II increased about 5- and 11-fold. The recoveries were 62 and 122 %, respectively.

Fractionation of RNA polymerases I and II by heparin-Sepharose chromatography. The results of a fractionation experiment without

Table 3. Purification and recoveries of RNA polymerases I and II in DEAE-Sephadex and heparin-Sepharose chromatography. RNA polymerases derived from livers of estrogenized roosters were treated as described in Figs. 1 and 2. RNA polymerase determinations were as described in "Experimental".

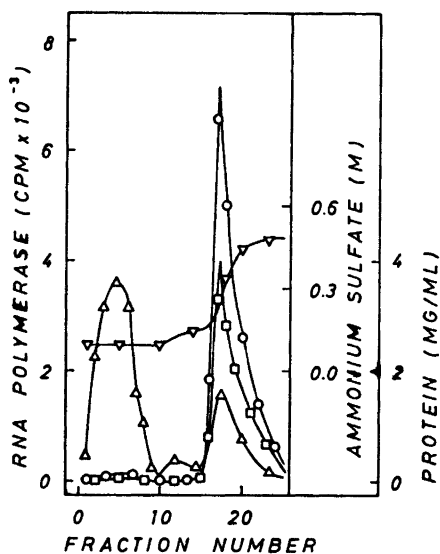| Purification step	Class of RNA polymerase	Specific activity pmol/(mg prot./5 min)	Total activity pmol/5 min	Recovery %
Supernatant after	I	56.4	3933	100.0
chromatin removal	II	30.8	2152	100.0
DEAE-Sephadex	I	208.1	2247	57.1
chromatography	II	501.4	2808	130.5
Heparin-Sepharose	I	—	1262	32.1
chromatography	II	—	1091	50.7

Fig. 3. Purification of total RNA polymerase activity from rooster liver nuclear extract by a stepwise heparin-Sepharose chromatography. The column (2.2 × 6 cm) was equilibrated and washed as described in "Experimental". The enzyme activity was eluted with the equilibration buffer containing 0.5 M ammonium sulfate. △, protein, mg/ml, other symbols are as in Fig. 2.
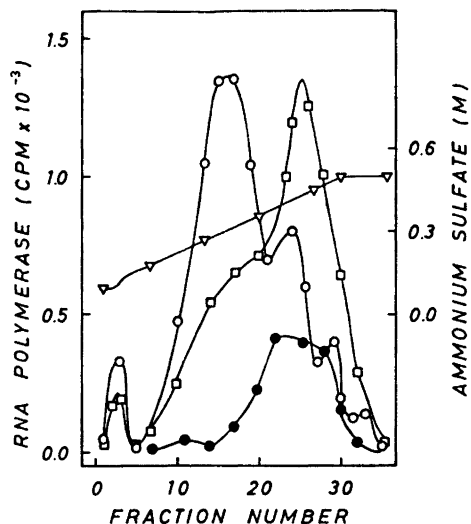

Fig. 4. Fractionation of RNA polymerases on heparin-Sepharose with linear gradient elution. The enzyme activity (from Fig. 3) was eluted with 50 ml of a linear gradient containing 0.15 to 0.5 M ammonium sulfate in the TGMED buffer plus bovine serum albumin (1 mg/ml). The enzyme activity was determined in the presence of 2 mM MnCl$_2$.○, difference between the assays without α-amanitin and with 1 μg/ml α-amanitin, □, activity in the presence of 166 μg/ml α-amanitin; ●, difference between the assays with 1 and 166 μg/ml of α-amanitin; ▽, ammonium sulfate concentration.

previous DEAE-Sephadex chromatography are shown in Fig. 4. To separate RNA polymerases I and II a shallow gradient must be used which leads, however, to dilution and partial inactivation of the enzymes. RNA polymerases I and II can be partially separated by heparin-Sepharose chromatography but polymerases I and III are not separated. There were shoulders in the RNA polymerase II

activity profile and asymmetry in the RNA polymerase I profile which suggest the presence of subspecies of these RNA polymerases in avian liver.

In repeated experiments, where RNA polymerases I and II were first separated on DEAE-Sephadex, recombined, and subsequently chro-

Table 4. Purification and recoveries of RNA polymerases I and II in heparin-Sepharose chromatography with a stepwise gradient. For the procedure see the legend for Fig. 3. Fractions containing RNA polymerase activity were pooled, concentrated by ultrafiltration and subjected to polymerase activity determinations.

Purification step	Class of RNA polymerase	Specific activity pmol/(mg prot./5 min)	Purification fold	Total activity pmol/5 min	Recovery %
Supernatant after chromatin removal	I	56.9	—	15188	100.0
	II	15.1	—	4020	100.0
Ultrafiltrate	I	308.9	5.4	9423	62.0
	II	161.1	10.7	4915	122.3

matographed on heparin-Sepharose using a linear gradient of $(NH_4)_2SO_4$, the activity of RNA polymerase I almost totally disappeared. Nevertheless, it was found that the small remaining peak of activity was eluted separately from RNA polymerase II as in Fig. 4.

Characterization of the polymerases from the individual fractions after chromatography by polyacrylamide gel electrophoresis or isoelectric focusing did not give satisfactory results because of the large excess of albumin present in the fractions and the large molecular weight and lability of the enzymes.

DISCUSSION

Our results are in general agreement with previous reports [3,4] in that hypertonic sucrose is important for the isolation of nuclei for purification of liver RNA polymerases. The difference between isotonic and hypertonic sucrose was found to be more pronounced with respect to RNA polymerase I than RNA polymerase II (see also Ref. 4). The present results show that addition of magnesium chloride or spermine into the hypertonic sucrose medium during isolation of nuclei has a relatively small effect on recoveries and specific activities of RNA polymerases. Here also, the differences were more pronounced with respect to RNA polymerase I. The presence of 15 mM $MgCl_2$ in the homogenization medium appears to favour the reaction catalyzed by RNA polymerase I (ribosomal RNA synthesis) which is in accord with the results of Moulé.[7] Thus, if one is interested only in RNA polymerase I, addition of 15 mM $MgCl_2$ with or without spermine in the homogenization medium is advantageous. With respect to the RNA polymerase II, addition of 3 mM $MgCl_2$ and 0.25 mM spermine can be recommended. For both RNA polymerases a compromise must be made with respect to the best homogenization medium. We have not tested the effect of the different homogenization media on the activity of RNA polymerase III. Some RNA polymerase III activity is probably present in the assay where RNA polymerase I activity was determined, since we did not routinely use a high α-amanitin concentration to determine RNA polymerase III activity separately.

The presence of spermine in the isotonic homogenization medium had a preserving effect on RNA polymerase II activity. In addition, the nuclei sedimented better in hypertonic sucrose when the tissue was initially homogenized in isotonic sucrose containing 0.25 mM spermine (unpublished observations).

When RNA polymerases I and II previously separated by DEAE-Sephadex chromatography were chromatographed on heparin-Sepharose, they eluted at 0.5 and 0.4 M ammonium sulfate, respectively. The elution order is the same as with cation exchangers in general.[1] Although heparin is an inhibitor of RNA polymerases [25-27] one can ask whether heparin-Sepharose chromatography with ammonium sulfate elution represents true affinity or cation exchange chromatography or both. Smith and Braun [10] eluted RNA polymerase II from Physarum polycephalum at about 0.4 M ammonium sulfate. Others have eluted RNA polymerases I and III from Acanthamoeba castellanii with 0.5 to 0.7 M ammonium sulfate.[11,12] In contrast to the present results, RNA polymerase III from KB-cells has been reported to be eluted very early, at 0.1 to 0.25 M ammonium sulfate.[9]

Using a stepwise elution scheme we purified RNA polymerases I and II from avian liver nuclei about 5- and 11-fold. This agrees with a recent result of Pflugfelder and Sonnenbichler [14] who reported a 11-fold purification of rat liver RNA polymerase II by a similar procedure. With RNA polymerases from Escherichia coli [8] and from lower eukaryotes [10] 80- and 60-fold purification has been reported. In other studies about 10-fold purification has been reported.[9,11]

Although the degree of purification obtained naturally depends on the stage in the purification scheme where affinity chromatography is used, we feel that heparin-Sepharose is more suitable for purification of RNA polymerases from bacteria and lower eukaryotes than from higher eukaryotes. This may also be due to a greater stability of RNA polymerases from lower organisms.

As to the method of elution, a stepwise elution [9,14] together with a low elution speed [11,12] can be recommended for purification. A shallow linear gradient leads to dilution and instability of the enzymes. The recovery of RNA polym-

erases I and II was 62 and 122 %. The results in the literature vary between 57 and over 100 %.[9–14] The recovery depends, e.g., on the age of the animal,[14] growth status of the tissue [28] and the amount of tissue.[29] We have used estrogen treatment which increases the activities of RNA polymerases in avian liver.[30,31]

In addition to the use of heparin-Sepharose in a stepwise manner, we found that heparin-Sepharose can be used to fractionate RNA polymerases I and II. This is the first report on the use of heparin-Sepharose for this purpose. Several minor peaks were observed in the elution profiles of RNA polymerases on heparin-Sepharose. They may represent true subforms (e.g. various phosphorylated forms) or they may, perhaps, result from the method of activity determination (ammonium sulfate concentration is not constant due to the gradient). It is also possible that subunits have detached from the enzymes during ultrafiltration or fractionation. Krebs and Chambon [32] observed only one form of RNA polymerase II (BIIb) in hen liver but three forms in hen oviduct. We used estrogenized rooster liver as the enzyme source and the hormone treatment may have an influence on the presence of subforms of RNA polymerases in the chromatography. Estrogenized chick liver has been reported to contain two subforms of RNA polymerase I and estradiol treatment changes their activity ratio.[33] We found that the elution profile of RNA polymerase I is asymmetrical on heparin-Sepharose. This may indicate partial separation of subforms.

Heparin-Sepharose did not separate RNA polymerase I and III activities (Fig. 4). This is in accord with the data of Spindler et al.,[11,12] who studied RNA polymerases I and III from Acanthamoeba castellanii. Chromatography on DEAE-Sephadex appears to be the most effective means for resolution of the various eukaryotic RNA polymerases in a single chromatographic step. Chromatography on DEAE-cellulose does not separate RNA polymerases I and III and chromatography on phosphocellulose does not separate RNA polymerases II and III well.[1] The elution profiles of the RNA polymerases I, II and III on CM-Sephadex [1] and heparin-Sepharose (present study) resemble each other. The advantage of the

use of heparin-Sepharose is, perhaps, its larger capacity with respect to the ion exchange materials mentioned above.

In summary, heparin-Sepharose can be used in a stepwise manner to purify RNA polymerases from avian liver. In addition, linear gradient elution will separate RNA polymerases I and II although fractionation with a shallow gradient leads to dilution of the enzymes. This can be possibly avoided by replacing ammonium sulfate with a more specific elution agent.

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