Separation and Characterization of the Subunits of Ribonucleic Acid Polymerase*

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SUMMARY

This paper presents studies on the subunit structure of Escherichia coli DNA-dependent RNA polymerase. Phosphocellulose column purified enzyme is denatured and its polypeptide chains separated by gel filtration on Sephadex G-200 in the presence of 1% sodium dodecyl sulfate. The amino acid compositions, molecular weights, NH₂ terminal amino acids, and molar ratios of these polypeptide chains are presented. It is concluded that phosphocellulose enzyme has the structure C₅b₃' where the molecular weights of the chains α, β, and β' are 39,000 ± 5%, 155,000 ± 10%, and 165,000 ± 10%, respectively. In addition, a small protein component, γ, with molecular weight of 9,000 ± 10% is observed. These subunits are not joined together by disulfide linkages. From these data one can compute a molecular weight of 400,000 ± 10% for the monomeric form of the phosphocellulose purified polymerase.

Molecular weights in the range of 360,000 to 440,000 daltons have been reported for the 13S form of DNA-dependent RNA polymerase from Escherichia coli (14). It has been observed that treatment of the enzyme with high pH (5) or urea (6) results in dissociation into material sedimenting at about 3 S. Starch gel electrophoresis of the enzyme in 6 M urea reveals two major and possibly six minor protein bands (5). It thus appears that this large enzyme is composed of several, possibly different, polypeptide chains. Measurement of the size and stoichiometry of these chains composing the enzyme will provide a basis for determining the functions performed by these chains and thus contribute to the understanding of the mechanism of action of the enzyme. Such studies require the dissociation of the enzyme into its individual polypeptide chains and the separation and characterization of these chains. This has been achieved and is described below.

The enzyme used for these studies was purified by the phosphocellulose column method described in the accompanying paper. It has been previously reported that a protein component normally associated with polymerase is removed by this method (7). As a result, the enzyme contains fewer polypeptide chains than enzyme purified by other methods. However, it is still able to synthesize RNA from a DNA template and is chosen for study because it represents a relatively defined protein, possibly the minimal RNA polymerase enzyme.

MATERIALS AND METHODS

Reagents

Urea (Merck) was recrystallized by dissolving 1 pound in 1 liter of boiling 95% ethanol, filtering through a boiled Millipore type AA filter, and crystallizing overnight with stirring at 4°. The crystals were collected, washed with 100 ml of ice cold absolute ethanol on a Buchner funnel, and dried in a vacuum. Urea solutions were deionized immediately before use by stirring 1 liter of an 8.6 M solution with 50 ml of mixed bed resin (Bio-Rad AG 501-X8, 20 to 50 mesh) for 1 hour at 25°.

Sodium dodecyl sulfate was obtained from Matheson Coleman and Bell and was recrystallized as follows. SDS¹ (250 g) was added to 4 liters of 95% ethanol and heated to 70° on a hot plate. It was then filtered through Whatman No. 1 filter paper on a previously heated Buchner funnel. The solution was allowed to cool to room temperature with stirring and then stirred overnight at 4°. Crystals were collected at 4° on a Buchner funnel cooled beforehand to −20° and were dried as much as possible with suction, then dried thoroughly by lyophilization. The yield was about 70%.

Guanidine hydrochloride, 2,4-dinitrofluorobenzene, and Coomassie brilliant blue R-250 were purchased from Mann. Acrylamide and N,N'-methylene bisacrylamide were purchased from Eastman. Ethylenimine was purchased from K and K Laboratories.

All other chemicals were reagent grade and were used without further purification. All solutions were made with double distilled or deionized water.

Enzyme

DNA-dependent RNA polymerase from E. coli K-12 was purified by chromatography on phosphocellulose, as described in the accompanying paper, including the final passage through the A-1.5m agarose column at 1.0 M KCl.

¹ The abbreviation used is: SDS, sodium dodecyl sulfate.
Polyacrylamide Gel Electrophoresis

Polyacrylamide gels at pH 8.7 were prepared by the general methods of Ornstein (8) and Davis (9) with added 8 M urea as described by Jovin, Chrombooth, and Naughton (10). These gels routinely contained 7.5% acrylamide. A 10- to 20-μg sample of protein in 100 μl of 8 M urea containing 3% 2-mercaptoethanol and 0.0002% bromophenol blue tracking dye was applied directly to the gel.

Gels containing 0.1% SDS were prepared and run as described by Maizel (11) and Shapiro, Viñuela, and Maizel (12). A 10- to 20-μg sample was added to 100 μl of an application buffer containing 0.1% SDS, 1% 2-mercaptoethanol, 0.01 M sodium phosphate, pH 7.2, 10% glycerol, and 0.002% bromophenol blue, and was applied directly to the gel.

Gels were stained for at least 2 hours in a 0.2% solution of Coomassie brilliant blue in methanol-acetic acid-water (5:1:5). They were then soaked in 7.5% acetic acid-5% methanol for 30 min, destained electrophoretically, and stored in this same solvent. With this method it is possible to detect as little as 0.1 μg of protein in a sharp band.

Molecular weights of polypeptide chains were estimated by the procedure of Shapiro et al. (12) with the use of polyacrylamide gels containing 0.1% SDS. Accurate estimates of the molecular weights of β', β, α, and ω could not be made on gels of one acrylamide concentration because of the wide range of molecular weights involved. As a result β', β, and α were estimated on 5% acrylamide gels, and α and ω on 15% gels.

Chromatography

DEAE-cellulose (Whatman No. DE-52, 1.0 meq per g, dry weight) was washed with 0.5 M HCl, then with 0.5 M NaOH, rinsed with distilled water to pH 8, and equilibrated with the buffer to be used. Sephadex G-200 was obtained from Pharmacia. Polyamide thin layer chromatography sheets (15 × 15 cm) were obtained from Gallard-Schlesinger Chemical Manufacturing Company, Carle Place, New York. Hydroxylapatite was prepared by the method of Tiselius, Hjerten, and Levin (18). SDS Sephadex G-200 Column—A 10 mg per ml polymerase solution (3 ml) was brought to 1% SDS by the addition of 1% SDS. It was dialyzed extensively against 1% SDS and subjected to gel filtration chromatography on Sephadex G-200 at 25°C. The column (2.5 × 95 cm) was packed and eluted at a hydrostatic pressure of 20 cm with unbuffered 1% SDS solution. The flow rate of the column was 20 ml per hour. Fractions, 3 ml each, were collected and monitored by optical density at 280 nm.

NH₄-terminal Amino Acid Determination

Lyophilized protein was suspended in 5.7 N HCl in a constricted tube, the tube was evacuated for 10 min with vigorous vortexing, sealed, and placed in an oven at 110°C for 22 to 72 hours. The hydrolysate was taken rapidly to dryness at 60°C on a Buchler Rotary Evapo-Mix. The amino acids were dissolved in 1 ml of 0.2 N citrate buffer, pH 2.2, and analyzed on a Spinco model 120C amino acid analyzer by the method of Spackman, Moore, and Stein (18). Tryptophan was estimated spectrophotometrically by the method of Beaven and Holiday (19). Cysteine was determined as cysteic acid (20).

Ultracentrifugal Analysis

A Spinco model B analytical ultracentrifuge equipped with the RTIC temperature control unit and both schlieren and Rayleigh interference optical systems was employed.

Velocity sedimentation was carried out at 20°C with the use of either a single or double sector 12-mm filled Epon centerpiece in an An-D rotor. The schlieren patterns were photographed on Kodak metallocraphic plates and the plates measured on a Guerier microcomparator. All sedimentation coefficients were converted to α values with corrections for KCl solutions from the International Critical Tables (14) and for urea solutions from the data of Kawahara and Tanford (15).

Sedimentation equilibrium ultracentrifugation molecular weights for the separated subunits were determined by the high speed or meniscus depletion method of Yphantis (16) employing either a 12-mm filled Epon double sector centerpiece or a 12 mm six-channel Yphantis centerpiece. Solution densities were measured in 10-ml Gay-Lussac pycnometers. Partial specific volumes (V) were calculated from the amino acid composition by the method of Cohn and Edsall (17). Interference patterns were photographed on Kodak spectrographic plates, emulsion type II-G. The interference fringes were measured on a Guerier microcomparator and the weight average molecular weight (Mw) was calculated as described by Yphantis (16).

Amino Acid Analysis

Lyophilized protein was suspended in 5.7 N HCl in a constricted tube, the tube was evacuated for 10 min with vigorous vortexing, sealed, and placed in an oven at 110°C for 22 to 72 hours. The hydrolysate was taken rapidly to dryness at 60°C on a Buchler Rotary Evapo-Mix. The amino acids were dissolved in 1 ml of 0.2 N citrate buffer, pH 2.2, and analyzed on a Spinco model 120C amino acid analyzer by the method of Spackman, Moore, and Stein (18). Tryptophan was estimated spectrophotometrically by the method of Beaven and Holiday (19). Cysteine was determined as cysteic acid (20).
lyophilized (20).

subjected to electrophoresis on 0.1% SDS, pH 7.2, gels and 0”. The reaction mixture was diluted 1:9 with water and allowed to proceed 2 hours at 25°. 50 µl were added per ml of the protein solution and the reaction was allowed to proceed 2 hours at 0°. The reaction mixture was diluted 1:9 with water and lyophilized (20).

Protein was dissolved in 98% formic acid at about 1 mg per ml. Of performic acid (30% H2O2-98% formic acid, 1:9, incubated for 2 hours at 25°) 50 µl were added per ml of the protein solution and the reaction was allowed to proceed 2 hours at 0°. The reaction mixture was diluted 1:9 with water and lyophilized (20).

RESULTS

Separation of Subunits

Fig. 1 shows the gel patterns obtained when polymerase is subjected to electrophoresis on 0.1% SDS, pH 7.2, gels and on 8 M urea, pH 8.7, gels. The bands will be referred to as β', β, α, and ω, in order of increasing mobility and decreasing molecular weight. Since SDS gels separate proteins according to size (12), the positions of the bands indicate significant differences in the molecular weights of the polypeptide chains of RNA polymerase.

Fig. 2 shows the schlieren patterns of polymerase dissociated with 1% SDS or 8 M urea and subjected to analytical ultracentrifugation. In both cases, two major peaks are observed, again indicating that the chains have different sizes. These results suggested the use of gel filtration to separate the subunits. Since 1% SDS is a convenient and effective denaturing agent, gel filtration chromatography on Sephadex G-200 was performed in 1% SDS as suggested by Rutner and Lane (24). The column profile in Fig. 3 shows two major peaks, A and B, and a minor peak, C. Fig. 4 shows gels that were run on material from those peaks. It is clear that Peak A contains β' and β, Peak B contains α, and Peak C contains ω.

Separation of the polypeptide chains was also attempted by ion exchange chromatography on DEAE-cellulose in 8 M urea. A KCl gradient was applied and several peaks of protein were eluted as is shown in Fig. 5. The subunits present in each peak were determined by gel analysis and are indicated above each peak. Although the column does not completely resolve α and β, it is useful for separating the mixture of β' and β obtained from the SDS Sephadex G-200 columns.

RNA polymerase binds to phosphocellulose even though it...
FIG. 3. Separation of the polypeptide chains of RNA polymerase by chromatography on Sephadex G-200. A column of Sephadex G-200 equilibrated with 2% SDS was employed as described in the text. $\beta' + \beta$ eluted in Peak A, $\alpha$ in Peak B, and $\omega$ in Peak C. Peak fractions were analyzed on the acrylamide gels shown in Fig. 4.

FIG. 4. Polyacrylamide gel electrophoresis of the separated polypeptide chains of RNA polymerase. Fractions from the column shown in Fig. 3 were analyzed on SDS gels and on urea gels. a, SDS gel of 20 $\mu$g of total enzyme; b, SDS gel of 20 $\mu$g of Peak A protein ($\beta'$ and $\beta$); c, SDS gel of 8 $\mu$g of Peak B protein ($\alpha$); d, urea gel of 20 $\mu$g of total enzyme; e, urea gel of 16 $\mu$g of Peak A protein ($\beta'$ and $\beta$); f, urea gel of 10 $\mu$g of Peak B protein ($\omega$); g, urea gel of 6 $\mu$g of Peak C protein ($\omega$).

has a net negative charge at pH 8, suggesting a positively charged region on the enzyme. If this region is localized on one polypeptide chain it might provide a way to separate the chains. This was attempted by ion exchange chromatography on phosphocellulose in 4 M urea. The $\alpha$ and $\omega$ chains did not bind to the column and flowed through. Elution with a KCl gradient from 0 to 0.2 M and with buffer steps containing 0.5 and 2.0 M KCl failed to elute $\beta$ or $\beta'$. While this column does not provide a useful separation, it does show that both the $\beta$ and $\beta'$ chains are able to bind very tightly to phosphate groups, and thus may be involved in binding polynucleotides.

Of the separation methods tested, gel filtration on Sephadex G-200 in 1% SDS is by far the most satisfactory. The remainder of the work presented here utilizes this method. As a result, the large polypeptide chains, $\beta$ and $\beta'$, are studied as a mixture ($\beta + \beta'$). The $\omega$ chain is recovered in such small amounts that it has not been well characterized.

Integrity of Polypeptide Chains

Before characterizing the subunits $\alpha$, $\beta$, $\beta'$, and $\omega$, it is necessary to show that each of these subunits is a single, unique polypeptide chain which cannot be further dissociated. Aggregation that has been observed for some proteins on SDS gels can be prevented by heating the sample to 65° for 10 min in the presence of 1% 2-mercaptoethanol to reduce disulfide linkages (12). RNA polymerase exhibits the pattern shown in Fig. 1a whether or not it has been heated or reduced. This indicates that the subunits are not joined by disulfide linkages. To exclude the possibility that these conditions are not severe enough to disrupt strong aggregates, enzyme was reduced with 2% 2-mercaptoethanol and then subjected to electrophoresis on a gel containing 1% SDS which had been run previously with 0.1 M glutathione. The mobilities of $\alpha$, $\beta$, $\beta'$, and $\omega$ relative to marker proteins were not changed. Under all of these conditions, no differences in the relative amounts of $\alpha$, $\beta$, $\beta'$, and $\omega$ were observed, indicating that no breakdown from $\beta$ or $\beta'$ to $\alpha$, or from $\alpha$ to $\omega$ had occurred.

Although these results indicate the contrary, it is possible that the double bands, $\beta$ and $\beta'$, are artifacts of the gel and that only one of these exists before the gels are run. It seems unlikely that just half of the material would be altered however. In order
FIG. 6. SDS gel analysis of oxidized and aminoethylated RNA polymerase. a, 10 μg of β' + α obtained from Peak A of the column shown in Fig. 3 which was reduced and aminoethylated. b, 20 μg of untreated enzyme; c, 15 μg of RNA polymerase which was reduced and aminoethylated; d, 15 μg of RNA polymerase which was oxidized with performic acid.

FIG. 7. SDS gels of separated β' and β chains. a, 7.5 μg each of separated β' and β obtained from a DEAE-cellulose column similar to that shown in Fig. 5 which were mixed prior to putting on the gel; b, 5 μg of β; c, 5 μg of β'.

to exclude this possibility, Peak A material, containing β' and β from the SDS Sephadex G-200 column, was chromatographed on a DEAE-cellulose column equilibrated with 8 M urea as described above. Two peaks of protein were eluted and were analyzed on SDS gels as is shown in Fig. 7. Each peak exhibits a single band and a mixture of material from the two peaks had the normal β' + β pattern. This shows that both β' and β are present in RNA polymerase.

Characterization of Separated Subunits

Molecular Weights

Polyacrylamide Gel Electrophoresis in SDS—Molecular weights have been estimated for β', β, α, and ω by the method of Shapiro et al. (12). The mobilities of a number of polypeptide chains of known molecular weight were determined on both 5 and 15% gels and are shown in Fig. 8. From these curves and the mobilities of β', β, α, and ω, molecular weights of 165,000 ± 15,000, 155,000 ± 15,000, 39,000 ± 2,000, and 9,000 ± 2,000 daltons are estimated. In order to obtain reliable estimates for β' and β, it was necessary to reduce the acrylamide concentration in the gels to 5% so that these chains would move well into the gel. Since very few large molecular weight markers are readily available and their molecular weights are not known with great accuracy, the resulting estimates for β' and β are subject to greater uncertainty than the estimate for α. The validity of this method for molecular weight determination has been further verified by Weber and Osborn (33).

Sedimentation Equilibrium Ultracentrifugation—An independent measure of the molecular weights of the polypeptides of RNA polymerase was obtained by sedimentation equilibrium ultracentrifugation. The results obtained under a variety of conditions are summarized in Table I. Figs. 9 and 10 show representative sedimentation equilibrium data. An average value of 38,400 daltons was obtained for the α chain which agrees well with the value obtained on SDS gels. An average value of 130,500 daltons obtained for the β + β' mixture is somewhat lower than the 160,000 predicted from SDS gels. This sedimentation equilibrium value might be expected to be lower for several reasons. A 5 to 10% breakdown of β + β' to smaller heterogeneous material in the molecular weight range 60,000 to 130,000 if often observed when material is taken out of the model E cell after an equilibrium run and examined on SDS gels. This sedimentation equilibrium value might be expected to be lower for several reasons. A 5 to 10% breakdown of β + β' to smaller heterogeneous material in the molecular weight range 60,000 to 130,000 if often observed when material is taken out of the model E cell after an equilibrium run and examined on SDS gels. This breakdown may have occurred during the long dialyses prior to centrifugation or during the centrifugation itself. The presence of such material would cause underestimation of the weight average molecular weight, especially when higher protein concentrations are used in a run, for in this case data cannot be taken near the bottom of the model E cell and must be taken nearer the meniscus where the contribution of the smaller molecular weight material is greater. This effect can be seen in

FIG. 8. Molecular weight estimation by SDS polyacrylamide gels. Proteins of known molecular weight were electrophoresed on 5% acrylamide gels (O—O) and 15% acrylamide gels (●—●) and their mobilities relative to the tracking dye determined. The marker proteins are bovine fibrinogen (run without reduction by 2-mercaptoethanol), molecular weight 340,000 (25); bovine thyroglobulin, molecular weight 165,000 (26); E. coli β-galactosidase, molecular weight 130,000 (27); bovine serum albumin, molecular weight 67,500 and dimer 135,000 (28); chicken ovalbumin, molecular weight 45,000 (29); E. coli aspartate transcarbamylase subunits, molecular weights 33,000 and 17,000 (30); bovine trypsin, molecular weight 23,800 (31); horse heart cytochrome c, molecular weight 13,400 (32). The mobilities of β', β, α, and ω are indicated by arrows.
**Table I**

Summary of molecular weight determinations

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Weight average, molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>α chain</strong></td>
<td></td>
</tr>
<tr>
<td>4 M urea-0.2 M KCl-0.01 M Tris, pH 7.9-0.1 M dithiothreitol</td>
<td></td>
</tr>
<tr>
<td>1. 0.5 mg per ml</td>
<td>38,700</td>
</tr>
<tr>
<td>2. 0.12 mg per ml</td>
<td>38,700</td>
</tr>
<tr>
<td>5. 0.1 mg per ml</td>
<td>38,700</td>
</tr>
<tr>
<td>8 M urea-0.1 M KCl-0.01 M Tris, pH 7.9b</td>
<td></td>
</tr>
<tr>
<td>1. 0.5 mg per ml</td>
<td>38,000</td>
</tr>
<tr>
<td>2. 0.33 mg per ml</td>
<td>39,300</td>
</tr>
<tr>
<td>1% SDSb</td>
<td></td>
</tr>
<tr>
<td>5. 0.1 mg per ml</td>
<td>37,200</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>38,400</td>
</tr>
<tr>
<td><strong>β + β′ chains</strong></td>
<td></td>
</tr>
<tr>
<td>8 M urea-0.1 M KCl-5 mm Tris, pH 7.9-0.5 mm dithiothreitol</td>
<td></td>
</tr>
<tr>
<td>1. 0.3 mg per ml</td>
<td>121,500</td>
</tr>
<tr>
<td>1% SDSb</td>
<td></td>
</tr>
<tr>
<td>2. 0.2 mg per ml</td>
<td>134,000</td>
</tr>
<tr>
<td>8 M guanidine hydrochloride-5 mm Tris, pH 7.9b</td>
<td></td>
</tr>
<tr>
<td>3. 0.2 mg per ml</td>
<td>137,000</td>
</tr>
<tr>
<td>6 M guanidine hydrochloride-0.05 x Tris, pH 7.9</td>
<td></td>
</tr>
<tr>
<td>4. 0.5 mg per ml</td>
<td>122,000</td>
</tr>
<tr>
<td>5. 0.2 mg per ml</td>
<td>135,000</td>
</tr>
<tr>
<td>8 M urea-0.075 M KCl-5 mm Tris, pH 7.9</td>
<td></td>
</tr>
<tr>
<td>6. 0.22 mg per ml</td>
<td>131,000</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>130,500</td>
</tr>
</tbody>
</table>

* The data from this run are shown in Fig. 9.
† Protein was aminoethylated before subunit separation.
‡ The data from this run are shown in Fig. 10.
* Run in a Yphantis six-channel centerpiece. A sample of *E. coli* β-galactosidase, run in the same cell in the most centripetal position exhibited a weight average molecular weight of 136,000 when a value of \( P = 0.74 \) was used.

Table I in runs 4 and 5 of the β + β′ chains in 6 M guanidine hydrochloride. The measured molecular weight is lower at the higher protein concentration. If this concentration dependence is taken into account, the sedimentation equilibrium values for \( \beta + \beta' \) are closer to 140,000 to 145,000, and thus closer to the values obtained on SDS gels. However, because of the small amount of breakdown of the \( \beta + \beta' \) chains resulting in this concentration dependence and to the fact that corrections have not been made for possible preferential binding of components of the solvents, the sedimentation equilibrium molecular weight values for \( \beta + \beta' \) are only approximate. Nevertheless, they do strengthen the argument that the molecular weights of the \( \beta + \beta' \) chains are in the range 130,000 to 170,000.

**Amino Acid Composition**

The amino acid composition of total enzyme, \( \alpha \), and \( \beta + \beta' \) were determined and are shown in Table II. The composition of chain \( \alpha \) is significantly different from that of chain \( \beta + \beta' \) or the total protein. It should be noted that in almost every case the mole per cent of a given amino acid residue in the total enzyme is intermediate between the mole percentages in the separated subunits, as would be expected if the total were a mixture of \( \alpha \) and \( \beta + \beta' \). Partial specific volumes calculated from these amino acid compositions for use in the molecular weight determinations are 0.742 ml per g for \( \alpha \), 0.737 ml per g for \( \beta + \beta' \), and 0.738 ml per g for total enzyme.

**NH₂-terminal Amino Acid Groups**

Determination of the NH₂-terminal amino acid by the method of Sanger (21) revealed only methionine for the total enzyme, although another dinitrophenyl amino acid residue of 10% the amount of methionine would not have been detected. Separated \( \alpha \) and \( \beta + \beta' \) also showed only methionine NH₂ termini. No end groups were obtained on separated \( \alpha \) and \( \beta + \beta' \) which had been aminoethylated prior to separation, apparently because of the destruction of the methionine by the excessive amount of ethylenimine used during aminoethylation. Finding only methionine is not surprising since about 40% of *E. coli* proteins have NH₂-terminal methionines (34). The analysis of bovine serum albumin by this method gave only aspartic acid, in agreement with published data (35). This determination was qualitative and did not allow quantitation of the methionine residues present. However, the amount of protein analyzed should have given approximately equimolar amounts of the end groups of \( \alpha \), \( \beta + \beta' \), and bovine serum albumin (about 20 μmole). The intensity of the dinitrophenyl amino acid spots on the chromatograms was similar for all three proteins, suggesting that the NH₂ termini are not blocked.
Fig. 10. Sedimentation equilibrium ultracentrifugation of β' + β chains. β' + β chain mixture, which had been aminoethylated prior to chromatography on SDS Sephadex G-200, was precipitated with 80% acetone and dialyzed extensively against 8 m guanidine hydrochloride-5 μ Tris, pH 7.9. The protein was centrifuged at a concentration of 0.2 mg per ml for 22 hours at 27,690 rpm and 20°. A weight average molecular weight of 137,000 was calculated using a partial specific volume of 0.737 ml per g and a measured solvent density of 1.209 g per ml.

Weight Ratio of Chains

The weight ratio of the polypeptide chains present in RNA polymerase was estimated by several methods. The results are summarized in Table III.

The stained bands observed on SDS gels were scanned spectrophotometrically in the manner described by Berg (38). The optical density was recorded and the area under each peak assumed to represent the amount of protein in each band. It has been shown for Coomassie brilliant blue by Fazekas de St. Groth, Webster, and Datyner (39) that the amount of dye bound is proportional to the amount of protein present with a variation of less than 10% among the different proteins tested. The average weight ratio of (β' + β)/α determined on three separate SDS gels was 4.1.

The amount of protein in the two major peaks from the SDS Sephadex G-200 column in Fig. 3 was estimated by the method of Lowry et al. (36) and by measuring the difference (A_{15} - A_{21}) as described by Waddell (37). These two methods gave average weight ratios of 4.4 and 4.2, respectively. About 95% of the total protein applied to the column was recovered in the two major peaks so it was assumed that no significant preferential loss of one subunit occurred.

From the amino acid compositions of α and β + β' given in Table II, a weight ratio of β + β' to α can be calculated which best reproduces the amino acid content of the total enzyme. For each amino acid residue, \[ 1(α) + x_i(β + β') = (1 + x_i) \text{(total enzyme)} \]

where \( x_i \) is the weight ratio and \( (α) \), \( (β + β') \), and \( \text{(total enzyme)} \) are the mole percentages of Residue \( i \). It follows that \( x_i = ((α) - \text{(total enzyme)})/(\text{(total enzyme)} - (β + β')) \). Such residue weight ratios were calculated for those residues which showed significant differences as both numerator and denominator (aspartic acid, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine). These yielded an average weight ratio of 3.6.
Subunit Model

The above methods give weight ratios of $\beta + \beta'$ to $\alpha$ of 3.6 to 4.4. Since the molecular weight of $\beta + \beta'$ is 3.4 to 4.1 times that of $\alpha$, it seems likely that the molar ratio of $\beta + \beta'$ to $\alpha$ is 1:1. It also appears that $\beta$ and $\beta'$ are present in equimolar amounts as shown by stain intensities on SDS gels. This suggests that RNA polymerase has the structure $\alpha_2\beta\beta'$. To rule out the possibility that the enzyme being studied is not $\alpha_2\beta\beta'$ but a nearly equal mixture of $\alpha_2\beta_3$ and $\alpha_2\beta\beta'$, the enzyme was absorbed to a hydroxylapatite column and eluted with a linear gradient of increasing phosphate concentration. Hydroxylapatite chromatography was reported by Richardson (1) and Pettijohn and Kamiya (40) to separate polymerase into two peaks of active protein. The profile seen in Fig. 11 shows two peaks of protein. Fractions from these two peaks were analyzed on SDS gels. No detectable change in the ratio of $\beta$ to $\beta'$ was observed. The difference in the two peaks was that the second lacked the low molecular weight $\omega$ chain. A similar result was observed when RNA polymerase was eluted with a shallow KCl gradient from a phosphocellulose column. Again two peaks were obtained which exhibited no change in the $\beta$ to $\beta'$ ratio on SDS gels. Again the second peak appeared to lack $\omega$. These results suggest either that polymerase is $\alpha_2\beta\beta'$ or that a complex, $\alpha_2\beta_3\alpha_2\beta\beta'$, exists which is not resolved under these chromatographic conditions. The latter seems unlikely since the two peaks are eluted from the phosphocellulose column at KCl concentrations (0.32 and 0.34 M) known to dissociate this enzyme into its 13 S form.

More evidence pertaining to the subunit composition of RNA polymerase can be obtained by considering intermediates observed on partial denaturation of the enzyme. It has been observed that upon aging, RNA polymerase breaks down to inactive material sedimenting at 7 to 9 S (41). When RNA polymerase is exposed to mild dissociating conditions such as pH 10.6 or 2 M urea, it does not dissociate directly from the 13 S form of the enzyme into the 2 to 3 S mixture of completely denatured polypeptide chains. An intermediate breakdown product is observed which is 7 to 8 S and may correspond to the 7 to 9 S material observed upon aging. Until the molecular weight of this intermediate is determined, a precise description of the steps involved in the dissociation of polymerase cannot be made. However, if the 7 to 8 S material is predominantly globular, it would have a molecular weight around 200,000 daltons and might represent the result of the dissociation of $\alpha_2\beta\beta'$ to $\alpha_2\beta$ and $\alpha_2\beta'$.

Molecular Weight of Enzyme

Attempts were made to obtain a molecular weight for phosphocellulose purified RNA polymerase directly by sedimentation equilibrium centrifugation. Even at very low protein concentrations severe aggregation occurred unless high salt conditions (0.5 to 1.0 M KCl) were employed. Difficulty in correctly interpreting the data in the three-component system caused by the presence of the high salt and the possibility that the high salt conditions might be causing yet further dissociation of the enzyme, makes such a direct determination of the molecular weight highly unreliable. However, from the subunit composition of phosphocellulose-purified RNA polymerase, $\alpha_2\beta\beta'$, and the molecular weights of the individual chains, it is possible to calculate the molecular weight of the enzyme. Using the values 39,000, 155,000, and 165,000 for $\alpha$, $\beta$, and $\beta'$, the molecular weight calculated for the enzyme is 400,000 ± 40,000 daltons.

It has been shown that phosphocellulose-purified enzyme has lost a protein component which is normally associated with polymerase purified by most other methods (7). This component, $\sigma$, has a molecular weight of about 95,000 and is present in a complex which has a composition, $\alpha_2\beta\sigma$ (42). This complex would have a molecular weight of 495,000 ± 50,000, which is only slightly above the range of values obtained for the 13 S form of the enzyme thought to be the functional unit of transcription.

DISCUSSION

The polypeptide chains of RNA polymerase have been separated and studied. The data presented are consistent with a subunit model of $\alpha_2\beta\sigma$ for the phosphocellulose-purified enzyme. Although $\beta$ and $\beta'$ have been separated they have not yet been studied individually in any detail. The conclusions obtained from studying the mixture of $\beta$ and $\beta'$ are nevertheless valid. While it can be argued that the amino acid composition of $\beta$ is identical with that of $\alpha$ and that the whole difference between $\beta + \beta'$ and $\alpha$ lies in $\beta'$, this seems unlikely. It is possible that $\beta$ and $\beta'$ are quite similar, especially if they evolved from duplicated genes. Further characterization of these two chains will answer this question.

It has not been shown that both $\alpha$ chains are identical, but if different $\alpha$ sequences exist, they are very similar in size and charge.

The $\omega$ chain has not been considered in the studies presented here. It appears to be present in all enzyme preparations except those fractionated on hydroxylapatite or a gradient eluted phosphocellulose column as mentioned earlier. It does not appear to be necessary for activity, although it cannot be ruled out that it has some role in the complete process of accurate transcription. Its molecular weight has been estimated from SDS gels to be about 8,000 to 10,000. Comprising less than 5% of the total protein, it is present as one or two chains per enzyme molecule. It is interesting to note that its molecular weight is approximately equal to the difference in molecular weights of $\beta$ and $\beta'$. Whether this is coincidence, the result of

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure11}
\caption{Hydroxylapatite column chromatography of RNA polymerase. A column of hydroxylapatite equilibrated with 0.04 M sodium phosphate, pH 7.5-8.5, 5% glycerol, 0.1 mM dithiothreitol was employed as described in the text.}
\end{figure}
a splitting of a $\beta'$ gene to $\beta + \omega$ in past evolution, or the result of
development of $\beta$ into $\beta$ and $\omega$ after synthesis of $\beta'$, is not known.
Neglecting the contribution of $\omega$ to the amino acid composition of
the total enzyme does not change the basic conclusions, but
may explain the slight differences for certain residues between
the amino acid content calculated from $\alpha$ and $\beta + \beta'$ and the
content of the total enzyme.

Little is known about the functions of the various chains in
the action of the total enzyme. The only available information is
the unusual affinity of $\beta$ and $\beta'$ for the phosphate groups of
the phosphocellulose column even in the presence of $4 \times$ urea.
This observation does not prove that $\beta$ and $\beta'$ are involved in
polyanucleotide binding but it does suggest it, for $4 \times$ urea is not a
particularly effective denaturing agent in some cases and it
might leave a nonspecific polyanion binding site undisrupted.

The subunit structure of RNA polymerase has also been studied
by Fuchs, Palm, and Zillig who have obtained gel patterns similar to those presented here. Walter, Seifert, and Zillig (43) and Bautz and Dunn (44) have recently presented evidence suggesting that some modification in the $\alpha$ chain occurs after infec-
tion of the cell by the phage T4. Whether this change in the en-
zyme results in the alteration of specificity needed for transcription
of the "late" phage genes or in the alteration of the response to other elements controlling specificity is not known.

The drug rifampicin inhibits the initiation of RNA synthesis
and binds to RNA polymerase (45-47). Drug resistant bac-
terial mutants produce enzyme which is not as susceptible to
other elements controlling specificity is not known.

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