tively short sequence of 15 amino acids which was apparently comprised of collagenous and noncollagenous sequences. The relationship of the aggregate isolated in our studies to those isolated in the above studies remains to be determined.

In summary, the data on the high molecular weight, disulfide-bonded, collagenous aggregate suggest that the $40B_1$ and $40B_2$ components form separate helical segments with the majority of the helicity derived from segments formed by the $40B_1$ chains. In contrast, the 40A subunit does not participate in the formation of helical structures. Further, each helical segment contains disulfide bridges between chains participating in the helix, and all major elements of the structure are linked through intermolecular disulfide bonds, including the attachment of relatively short noncollagenous chains to each of the major polypeptides. This latter conclusion is supported by the observation that on reduction and alkylation under denaturing conditions, the aggregate is completely dissociated to yield the three major polypeptides plus a number of small molecular weight, noncollagenous peptides (Furuto & Miller, 1980). Furthermore, as discussed above, disulfide linkages between the chains involved in helix formation would contribute to the thermal stability of the native, unreduced aggregate. This in turn would render the helices relatively resistant to proteolysis with pepsin even at relatively high temperatures provided disulfide linkages were present. Resistance to proteolysis on exposure to collagenase, on the other hand, is likely to be conferred by the presence of disulfide-linked, noncollagenous components in the unreduced protein.

Although numerous roles of the native aggregate might be suggested at this time, the fact that the aggregate can be reproducibly isolated following limited pepsin proteolysis of placenta and other tissues (Chung et al., 1976) certainly suggests that is represents a portion of a unique macromolecule or a highly specialized junction region connecting different segments of several distinct macromolecules which have been fragmented by limited pepsin digestion. The apparent complexity of the aggregate favors the latter possibility.

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Procedure for Purification of *Escherichia coli* Ribonucleic Acid Synthesis Termination Protein ρ†

Lawrence R. Finger* and John P. Richardson

ABSTRACT: An improved purification procedure is described for the ρ transcription termination factor of *Escherichia coli*. The method involves lysozyme–sodium deoxycholate lysis, Polymin P fractionation, and chromatography on phosphocellulose, poly(uridylic acid)–Sepharose, and AMP–agarose. The method yields up to 9 mg of electrophoretically pure protein from 200 g of *E. coli* MRE 600. From quantitative amino acid analysis ρ is calculated to have an $E_{280nm}^{1\%}$ of 3.7 ± 0.3. The purified ρ has an ATPase specific activity of 32 nmol of P_i released min^{-1} μg^{-1} when poly(cytidylic acid) is used as a cofactor, and it functions effectively in termination of T7 DNA transcription. A subunit molecular weight of 48 000 for ρ was determined by phosphate-buffered sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The amino acid composition and circular dichroism spectrum in the far-ultraviolet for ρ are presented.

The activity of the DNA[1]-dependent RNA polymerase isolated from *Escherichia coli* can be modulated by several protein factors (Losick, 1972). One of these, called ρ, causes termination of RNA synthesis at specific sites on the DNA template and release of the termination RNA molecules (Roberts, 1969, 1976; Adhya & Gottesman, 1978). The highly purified ρ is also an RNA-dependent nucleoside triphosphate phosphohydrolase (NTPase). Its NTPase activity is required for termination; however, ρ can catalyze the NTPase reaction in the absence of termination (Lowery-Goldhammer & Richardson, 1974).

For elucidation of the mechanism of action and physical properties of ρ, large amounts of the enzyme will be required.

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[1] Abbreviations used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; poly(C), poly(cytidylic acid); poly(U), poly(uridylic acid); DNase, deoxyribonuclease; DEAE, diethylaminoethyl; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid; ATPase, adenosine 5′-triphosphatase; ATP, adenosine 5′-triphosphate; GTP, guanosine, 5′-triphosphate; UTP, uridine 5′-triphosphate; CTP, cytidine 5′-triphosphate.

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The original procedure by Roberts (1969) gives relatively low yields, ~1 mg of ρ/100 g of *E. coli* cells, and requires two ultracentrifugation steps that are not well suited for processing large quantities of cells (200 g or more). Carmichael (1975) has reported a simple procedure for isolating large amounts of ρ by using affinity chromatography on poly(C)–cellulose. However, urea is necessary to elute the ρ from the column, and although ρ can be renatured from urea solutions, there is the possibility that the protein might be modified by contaminants in the urea. The procedure detailed in this paper can yield 9 mg of ρ/200 g of *E. coli* cells and combines techniques for cell lysis and for separation of ρ from contaminating proteins and nucleic acids that can be used with large quantities of cells. A key step in the purification is the use of poly(U)–Sepharose, from which tightly bound ρ can be eluted with a nondenaturing eluant. In addition, we present several physical and chemical properties of ρ, including its subunit molecular weight, extinction coefficient, amino acid analysis, and circular dichroism spectrum in the far-ultraviolet.

Materials and Methods

(*A*) *Materials.* *E. coli* MRE 600 cells grown to late log phase in an enriched medium were purchased from Grain Processing Corp., Muscatine, IA. ATP, GTP, CTP, and UTP were obtained from Boehringer-Mannheim Corp. [^{3}H]UTP was purchased from ICN Pharmaceuticals Inc. Rifampicin was a gift from Ciba-Geigy Corp. Polymin P, poly(C), and poly(U) were obtained from Miles Laboratories. Sodium deoxycholate, phenylmethanesulfonyl fluoride, lysozyme, beef liver catalase, and AMP–agarose [N^6-[[(6-aminohexyl)carbamoyl]methyl]-5′-AMP–agarose] were from Sigma Chemical Co. Cyanogen bromide and *N,N,N′,N′*-tetramethylethylenediamine were purchased from Eastman Kodak Co. Electrophoresis grade acrylamide, sodium dodecyl sulfate, *N,N′*-methylenebis(acrylamide), and ammonium persulfate were obtained from Bio-Rad Laboratories. Ovalbumin and pig heart fumarase were from Schwarz/Mann Laboratories. Bovine serum albumin was purchased from Pentex, Inc., and merthiolate was from Eli Lilly. Pancreatic DNase, electrophoretically purified, was obtained from Worthington Biochemical Corp., and Sepharose 4B was from Pharmacia. Whatman P-11 phosphocellulose was precycled as described in the Whatman pamphlet on ion-exchange celluloses. RNA polymerase purified from *E. coli* MRE 600 by the method of Burgess & Jendrisak (1975) was purified further on DEAE-cellulose (Chamberlin & Berg, 1962). T7 DNA was purified as previously described by Thomas & Abelson (1965). All other chemicals were reagent grade.

(*B*) *Buffers.* All buffers were prepared from distilled and deionized water, and pH was measured at 21 °C. Grinding buffer contained 0.05 M Tris-HCl (pH 7.5), 0.25 M KCl, 2.0 mM EDTA, 0.1 mM dithiothreitol, 1.2 mM β-mercaptoethenol, 130 μg/mL lysozyme, 23 μg/mL phenylmethanesulfonyl fluoride, and 5% (v/v) glycerol. Buffer TGD used for the phosphocellulose column contained 0.2 M Tris-HCl (pH 7.9), 0.1 mM dithiothreitol, and 5% (v/v) glycerol. Buffer U used for the poly(U)–Sepharose column contained 0.05 M Tris-HCl (pH 7.9), 10 mM $MgOAc_2$, 1.0 mM EDTA, 0.1 mM dithiothreitol, and 10% (v/v) glycerol. Buffer A used for the AMP–agarose column contained 0.05 M Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 0.1 mM dithiothreitol, and 10% (v/v) glycerol. Storage buffer contained 0.05 M triethanolamine hydrochloride (pH 8.0), 0.1 M KCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 50% (v/v) glycerol.

(*C*) *Poly(U)–Sepharose.* Poly(U)–Sepharose was either purchased from Pharmacia or prepared by a modification of the procedure described by Poonian et al. (1971). Basically, 75 mL of Sepharose 4B was washed with 20 volumes of deionized water and resuspended in 75 mL of deionized water at 22 °C. After the pH was adjusted to 11 with 10 N NaOH, cyanogen bromide (19 g dissolved in 19 mL of dioxane) was added with mixing. As the reaction proceeded, the pH of the solution was maintained at 11 by addition of 10 N NaOH from a buret, and the temperature was maintained below 30 °C by the addition of ice. The reaction was complete in ~10 min as indicated by a lack of change of the pH. After addition of 100 g of ice, the resin was filtered and washed with 20 volumes of ice-cold 0.05 M potassium phosphate buffer (pH 8.0). The activated resin was allowed to react with poly(U) by suspending it in 75 mL of a 0.2% solution of poly(U) in 0.05 M potassium phosphate buffer (pH 8.0). After gentle mixing for 48 h at 4 °C, the poly(U)–Sepharose was washed with 20 volumes of 0.05 M potassium phosphate buffer (pH 8.0), suspended in 75 mL of 0.1 M ethanolamine (pH 8.0), and mixed further for 2 h at 4 °C. The ethanolamine solution was removed by filtration, and the resin was washed with 20 volumes of 0.05 M potassium phosphate buffer (pH 8.0). Finally it was washed in a column with 2 M NaCl in 0.05 M Tris-HCl (pH 7.9) at 4 °C until there was no absorbance at 260 nm detected in the wash. By mild alkaline hydrolysis (Wagner et al., 1971), the amount of poly(U) bound to the resin was found to be approximately ~0.5 mg/mL of resin. The poly(U)–Sepharose was stored in buffer containing 0.05 M Tris-HCl (pH 7.5), 0.7 M KCl, 10 mM EDTA, 25% formamide, and 0.025% merthiolate. Before applying fraction IV, the column was equilibrated with buffer U containing 0.05 M NaCl.

(*D*) *ρATPase Assay.* Since none of the buffers used contain orthophosphate, ATPase activity can be determined by measuring the amount of P_i released from ATP with a colorimetric assay (Ames & Dubin, 1960). A 100-μL reaction mixture contained 0.04 M Tris-HCl (pH 7.9), 0.05 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 10 μg/mL poly(C), and 1 mM ATP. For location of the peak, 5-μL samples were assayed by incubation with this mixture for 10 min. Once the peak was located, 0.5-μL samples were assayed with a 10-min incubation (phosphocellulose column) or with a 2-min incubation [poly(U)–Sepharose and AMP–agarose columns] to determine which fractions to pool. Incubation was at 37 °C and enzymatic activity was terminated by adding 900 μL of a solution containing 1 part of 10% ascorbic acid, 2 parts H_2O, and 6 parts 0.42% ammonium molybdate tetrahydrate in 1 N H_2SO_4. After further incubation for 20 min at 45 °C, the absorbance of the sample was measured at 820 nm in a spectrophotometer. The release of 100 nmol of P_i resulted in an A_{820} value of ~1.6.

(*E*) *ρ Termination Assay.* Termination was assayed by the inhibition of RNA synthesis in a standard RNA polymerase reaction mixture by the following procedure: 0.9 μg of RNA polymerase and 4 μg of T7 DNA were preincubated for 5 min at 37 °C in 45 μL of 0.053 M Tris-HCl (pH 8.0), 0.067 M KCl, 6.6 mM $MgCl_2$, and 0.18 mM dithiothreitol. After addition of up to 0.3 μg of ρ in 20 μL of ρ dilution buffer [0.01 M Tris-HCl (pH 8.0), 0.1% bovine serum albumin, and 0.1 mM dithiothreitol], RNA synthesis was initiated by adding 0.1 μmol each of ATP, GTP, and CTP, 5 nmol of [^{3}H]UTP (40 Ci/mol), and 0.5 μg of rifampicin in 35 μL of 0.055 M Tris-HCl (pH 8.0), 0.059 M KCl, and 5.9 mM $MgCl_2$ which had been prewarmed at 37 °C. Samples were incubated 30 min at 37 °C, and RNA synthesis was measured by the incorporation of labeled [^{3}H]UTP into acid-insoluble material

Table I: Summary of Purification[a]

fraction	stage of purification	volume (mL)	total protein[b] (mg)	total act. (units)[c]	sp act. (units/mg)	yield (%)
I	cell extract	800	33 900	407	0.012	100
II	low-speed supernatant	650	16 000	273	0.017	67
III	polymin P fraction	142	7400	326	0.044	80
IV	phosphocellulose pooled peak	14.6	87	385	4.43	95
V	poly(U)-Sepharose pooled peak	28.0	9.98	280	28.1	69
VI	AMP-agarose pooled peak	2.67	7.18	230	32.0	57

[a] From 200 g of *E. coli* MRE 600 cells. [b] Protein was determined by the method of Bensadoun et al. (1976). [c] ρATPase activity was assayed by measuring the release of $^{32}P_i$ from [γ-^{32}P]ATP as described by Lowery & Richardson (1977). For fractions I-III, activities are from initial rates of hydrolysis and are corrected from ATPase activity measured in the absence of poly(C). One unit is equal to the release of 1 μmol of P_i/min. [d] One unit corresponds to the release of 1 μmol of P_i from ATP/min.

(Lowery & Richardson, 1977).

(*F*) *Amino Acid Composition Analysis.* Amino acid composition analysis was done on samples dialyzed exhaustively against 1 N acetic acid and lyopholized in 13 × 100 mm test tubes. To each tube, 1 mL of double-distilled 6 N HCl was added together with two drops of phenol solution. The tubes were evacuated, sealed, and heated at 110 °C for 24, 48, and 72 h. The hydrolysate was dried by vacuum desiccation and redissolved in 0.2 N sodium citrate buffer (pH 2.2). Samples were analyzed on a Beckman Model 121-M amino acid analyzer by using the chromatographic techniques developed by Spackman et al. (1958). The analyzer was connected to a Beckman System AA computing integrator. For determination of tryptophan, samples were hydrolyzed for 24 h in 3 N *p*-toluenesulfonic acid (Liu & Chang, 1971). After hydrolysis, the solution was titrated to pH 2 with 10 N NaOH and diluted with 0.2 N sodium citrate buffer (pH 2.2) before chromatographing on the Beckman analyzer.

(*G*) *Circular Dichroism Measurement.* Circular dichroism measurements were performed on a Jasco ORD/UV-5 spectropolarimeter fitted with the Sproul-Scientific SS-10 CD modification. Each sample was scanned 3 times from 245 to 200 nm at 25 °C in a 1.0-mm pathlength cell. ρ concentrations were 100, 200, and 400 μg/mL in 0.04 M Tris-HCl (pH 7.9) and 0.05 M KCl. Analysis of the circular dichroism spectra from 245 to 205 nm was by the method of Greenfield & Fasman (1969), using the reference spectra of Chen et al. (1974). A value of 114 for the mean residue molecular weight of ρ was used as determined from its amino acid composition.

Purification Procedure

All steps were carried out at 4 °C unless otherwise noted. A summary of the purification is shown in Table I and Figure 2.

(*A*) *Cell Disruption.* Frozen *E. coli* MRE 600 cells (200 g) were broken into small pieces and placed in a 1.5-L beaker with 600 mL of grinding buffer. The cells were blended at low speed with a Virtis homogenizer until they were completely suspended. After standing for 30 min at room temperature, 10 mL of 4% (v/v) sodium deoxycholate was added, and the mixture was blended for 30 s at low speed. The suspension was left for 15 min at room temperature, followed by chilling for 15 min in the cold room. $MgCl_2$ was added to a final concentration of 0.01 M, followed by 3 mg of DNase. After ~45 min when the viscosity of the cell extract had decreased (fraction I), it was centrifuged for 45 min at 8500 rpm, and the supernatant solution was retained (fraction II).

Alternatively, cell lysis can be carried out as described for the purification of *E. coli* RNA polymerase by Burgess & Jendrisak (1975). However, this modified procedure yields a variable amount of ρ protein (2–9 mg of ρ/200 g of cells). The critical step appears to be in the shearing of the DNA in fraction I, with mechanical shearing of the DNA not being as effective as digestion by DNase.

(*B*) *Polymin P Fractionation.* Fraction II was placed in a graduated cylinder, and a 10% (v/v) solution of Polymin P (pH 7.9) was added slowly with stirring to a final concentration of 0.61% (6.5 mL of Polymin P/100 mL of fraction II). After stirring was continued for 10 min, the mixture was centrifuged 15 min at 6000 rpm.

The amount of Polymin P needed to precipitate proteins is a function of both the nucleic acid and protein content of fraction II, which may vary with different batches of cells. Therefore, Polymin P titration curves are routinely done on 1-mL portions of fraction II. Various amounts of Polymin P are added, mixed for 5 min, and centrifuged at top speed for 5 min in a clinical centrifuge. The concentration of Polymin P used for precipitation is 0.05% above the concentration which results in a clear supernatant in the titration.

(*C*) *Ammonium Sulfate Precipitation.* The supernatant solution from Polymin P fractionation was placed in a graduated cylinder, and 39 g of ammonium sulfate/100 mL was slowly added while stirring. One drop of 1 N NaOH/10 g of ammonium sulfate was also added. After 1 h, the suspension was centrifuged for 45 min at 8500 rpm. The pellet was suspended in 60 mL of buffer TGD containing 0.05 M KCl and dialyzed overnight against this buffer. The dialyzate was centrifuged at 6000 rpm for 10 min and the supernatant solution saved (fraction III).

(*D*) *Phosphocellulose Column.* Fraction III was pumped on a 400-mL column of Whatman P-11 phosphocellulose (5 × 20.3 cm), and the column was washed with 400 mL of buffer TGD containing 0.05 M KCl. ρ was eluted at ~0.3 M KCl with a 1.5-L linear gradient from 0.05 to 0.55 M KCl in buffer TGD at a flow rate of 0.24 mL/min. Nine peak fractions containing 15 mL each were pooled and precipitated with ammonium sulfate as described under Ammonium Sulfate Precipitation. The pellet was suspended in 12 mL of buffer U containing 0.05 M NaCl and dialyzed overnight against this buffer (fraction IV).

(*E*) *Poly(U)-Sepharose Column.* Fraction IV was applied to a 45-mL column of poly(U)-Sepharose (1.5 × 25.5 cm), and the column was washed successively with 75 mL of buffer U containing 0.05 M NaCl and 75 mL of buffer U containing 0.2 M NaCl. ρ was eluted at ~0.6 M NaCl with a 250-mL linear gradient from 0.05 to 1.5 M NaCl in buffer U at a flow rate of 0.5 mL/min (Figure 1). Peak fractions were pooled and dialyzed against buffer A containing 0.05 M NaCl overnight (fraction V). If the poly(U)-Sepharose column is too small, a variable fraction of ρ ATPase activity is detected in

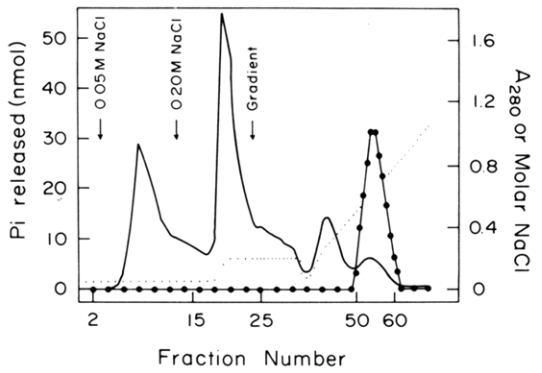

FIGURE 1: Poly(U)–Sepharose chromatography. Fraction IV was applied to a 45-mL poly(U)–Sepharose column as described in the text; 7.6-mL and 3.6-mL fractions were collected before and after starting the gradient, respectively. All fractions were assayed for absorbance at 280 nm (—), poly(C)-dependent ρATPase activity (●), and NaCl concentration by conductivity measurements (···).

the 0.20 M NaCl step, although no activity is seen in the 0.05 M NaCl wash.

(*F*) *AMP–Agarose Column.* Fraction V was applied to a 9.5-mL column of AMP–agarose (0.7 × 24.8 cm), and the column was washed with 15 mL of buffer A containing 0.05 M KCl. ρ was eluted with buffer A containing 0.40 M KCl at a flow rate of 0.24 mL/min. Six peak fractions containing 1.2 mL each were pooled and dialyzed against storage buffer (fraction VI). The AMP–agarose column removes minor impurities and serves to concentrate the ρ.

Alternatively, the ρ protein isolated from the poly(U)–Sepharose column is sufficiently pure for most experimental work and could be concentrated on a small phosphocellulose column by the following procedure. Pooled peak fractions from poly(U)–Sepharose were displayed against buffer TGD containing 0.05 M KCl overnight and applied to a 4-mL phosphocellulose column (0.9 × 10.4 cm). After the column was washed with 8 mL of buffer TGD containing 0.05 M KCl, ρ was eluted with buffer TGD containing 0.55 M KCl at a flow rate of ~0.2 mL/min. Peak fractions were pooled and dialyzed against storage buffer.

Comments on the Purification Procedure

(*A*) *Yield.* Fractions from various stages of the purification were analyzed by gel electrophoresis (Figure 2) and assayed for protein content and enzymatic activity (Table I). The yield from this preparation was 7.18 mg of ρ from 200 g of *E. coli* MRE 600 cells, and in general up to 9 mg of ρ can be readily purified from similar preparations. The overall yield of ρ was 57% based on the estimates of ρATPase activity in crude extracts.

ρ antibody studies suggest that there may be as much as 40 mg of ρ present in 200 g of *E. coli* cells (Shigesada & Imai, 1978). By assumption of this value, the overall yield would be 21%. In any case, the yields obtained in this procedure are a substantial improvement over the yields obtained by the procedure of Roberts (1969), which gives ~2 mg of ρ/200 g of *E. coli* cells.

(*B*) *Purity.* Fraction VI of ρ protein is electrophoretically pure (Figure 2) and has an A_{280}/A_{260} ratio of 1.7 which suggests that it is substantially free of nucleic acid. No DNA endonuclease activity in the ρ preparation could be detected after incubating 6 μg of T7 DNA and 6 μg of ρ together in Buffer TKMD [0.05 M Tris-HCl (pH 8.0), 0.05 M KCl, 5 mM $MgCl_2$, and 0.1 mM dithiothreitol] for 2 h at 37 °C as analyzed by the migration of the T7 DNA in a 20–5% (w/v) alkaline sucrose gradient (Richardson, 1966). No RNA endonuclease activity in the ρ preparation could be detected after

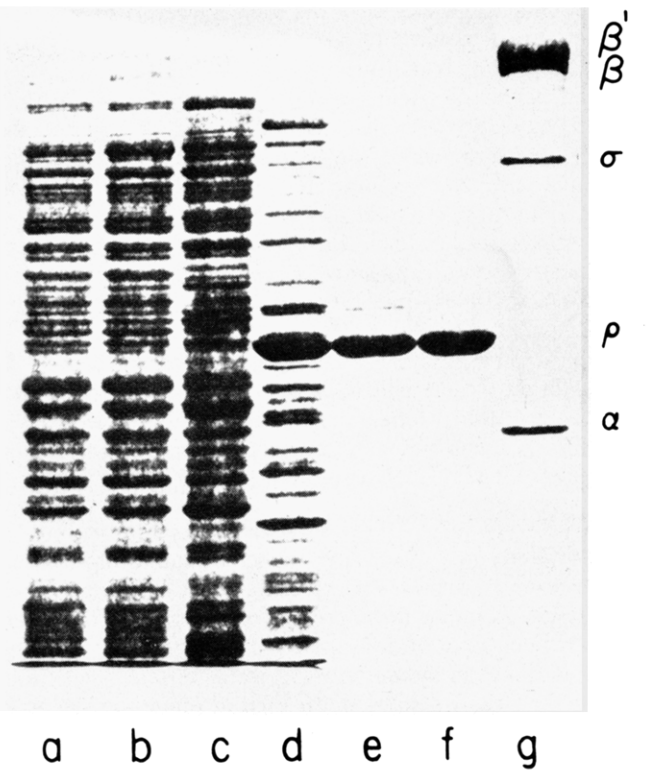

FIGURE 2: Tris–glycine sodium dodecyl sulfate–10% polyacrylamide gel of various fractions of purification: (a) fraction I (40 μg of total protein); (b) fraction II (40 μg); (c) fraction III (40 μg); (d) fraction IV (20 μg); (e) fraction V (5 μg); (f) fraction VI (5 μg); (g) *E. coli* RNA polymerase (3 μg) as a marker. The gel system used was as developed by Laemmli (1970).

incubating 9 μg of T7 [^{3}H]RNA and 9 μg of ρ together in buffer TKMD for 2 h at 37 °C as analyzed by the migration of the T7 [^{3}H]RNA in a 4% polyacrylamide–7 M urea gel (Maizels, 1973). Also no RNase activity in the ρ preparation could be detected after incubating 0.035 μg of [^{3}H]poly(C) and 0.035 μg of ρ together for 2 h at 37 °C as analyzed by trichloroacetic acid precipitation of the labeled poly(C).

Fraction V of ρ protein is usually over 90% pure as seen after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Figure 2) and has a A_{280}/A_{260} ratio of 1.6, which suggests that is substantially free of nucleic acid. The common protein impurities have a molecular weight of 60 000 and 100 000. In most instances there has been no enzymatic contamination in fraction V, so this ρ can be used for many studies without further purification.

(*C*) *Activity.* The ATPase activity for the highly purified ρ protein is completely dependent on an RNA cofactor. When the protein is assayed with poly(C), the specific activity is 32 nmol of ATP hydrolyzed min^{-1} μg^{-1}. Although Lowery-Goldhammer & Richardson (1974) reported a value that is ~3 times higher for MRE 600 ρ isolated by the procedure of Roberts (1969), they used an assumed extinction coefficient of $E_{280nm}^{1\%} = 10$ for determining ρ concentrations in the assay. From our determination of the extinction coefficient (vide infra), their protein concentrations were underestimated by a factor of 2.7, and, thus, their specific activity is overestimated by that same factor. Hence, ρ isolated by this procedure does have a specific activity as measured in the poly(C)–ATPase assay that is nearly identical with that of ρ isolated by the procedure of Roberts (1969).

The ρ is also active in the termination of T7 DNA transcription. Under conditions described under Materials and Methods, saturating amounts of ρ decrease the incorporation

of ribonucleotides to 8% of the control level. The minimum amount of ρ required to saturate transcription in this assay is 0.1 μg, which is equivalent to approximately 1 ρ hexamer/6 RNA polymerase molecules or 1 ρ hexamer/RNA chain synthesized. Studies (Richardson & Macy, 1981) indicate that ρ-terminated T7 RNA transcripts made by using ρ purified by this procedure are discrete, short RNA molecules very similar to those reported by Darlix (1974).

The ρ purified by the method described in this paper appears to be as enzymatically active as ρ isolated by the procedure of Roberts (1969). In addition, the yield of ρ is 4-fold higher and possibly has greater purity compared to the ρ isolated by the procedure of Roberts (1969). This new method can be used for preparing small amounts of ρ from *E. coli* (20 g) that might be necessary for enzymatic studies as well as for isolating milligram quantities of ρ which are required for protein chemistry studies such as those presented in this paper.

Physical–Chemical Properties

(*A*) *Extinction Coefficient.* The absolute ρ protein concentrations were measured by using quantitative amino acid analysis. Relating these protein concentrations to the absorbance of ρ at 280 nm, the $E_{280}^{1\%}$ of ρ is 3.7 ± 0.3 when measured in storage buffer. This value differs significantly from the assumed value of 10 used in some previous work (Richardson, 1970; Lowery & Richardson, 1977).

For measurement of low ρ concentrations, it is convenient to use a modified Lowry colorimetric assay (Bensadoun & Weinstein, 1976). The values determined by this procedure using bovine serum albumin as a standard should be multiplied by 0.94 to correct for differences in reactivities of ρ and bovine serum albumin with the Lowry reagents.

(*B*) *Subunit Molecular Weight.* The subunit molecular weight of ρ protein was determined by analysis of its relative mobility upon electrophoresis through 5.0, 6.25, 7.5 and 9% (w/v) polyacrylamide gels in a potassium phosphate buffer containing sodium dodecyl sulfate (Weber & Osborn, 1975). The relative mobilities of ρ and several marker proteins were determined at the different acrylamide gel concentrations. From these data, the negative retardation coefficient ($-K_r$) for each protein was calculated as the change in the logarithm of relative mobility per unit change in acrylamide gel concentration. The value of the retardation coefficient for the ρ subunit of 0.0725 corresponds to M_r = 48 000, when compared with bovine serum albumin [M_r = 66 296 (Brown, 1976)], catalase [M_r = 58 000 (Weber & Osborn, 1975)], fumarase [M_r = 49 000 (Weber & Osborn, 1975)], ovalbumin [M_r = 43 000 (Castellino & Barker, 1968)], and the α subunit of *E. coli* RNA polymerase [M_r = 36 512 (Ovchinnikov et al., 1977)]. This particular procedure (Ferguson, 1964; Frank & Rodbard, 1975) was chosen because it minimizes the error introduced from differences in the apparent free mobility of proteins. The retardation coefficient presumably reflects the relative extent to which a protein is retained due to the sieving properties in acrylamide gels and thus should reflect the size of the polypeptide chain. On the basis of evidence that ρ has a hexameric structure (Oda & Takanami, 1972; Finger & Richardson, 1979), the M_r of nondenatured ρ protein would be 288 000.

On the basis of a difference in their mobilities during sodium dodecyl sulfate polyacrylamide gel electrophoresis, Ratner (1976) suggested that the ρ protein from K strains of *E. coli* is ~5% larger than the ρ protein from B strains. However, we have failed to detect a difference in mobilities of ρ proteins from our B (MRE 600) and K (derivatives of W3110) strains in either phosphate-buffered (Weber & Osborn, 1975) or Tris–glycine-buffered (Laemmli, 1970) gels. Thus, our results suggest that there is no difference in size of ρ proteins isolated from these two strains. We do not know the reason for the discrepancy between our results and those of Ratner.

Table II: Amino Acid Composition of ρ

amino acid	mol %[a,b]	residues/ρ molecule[f]
Lys	6.97 ± 0.07	29
His	1.87 ± 0.08	8
Arg	7.05 ± 0.07	30
Asx	10.24 ± 0.12	43
Thr[b]	5.24 ± 0.03	22
Ser[b]	5.42 ± 0.14	23
Glx[c]	12.05	51
Pro	3.92 ± 0.08	15
Gly	7.28 ± 0.06	31
Ala	7.43 ± 0.07	31
1/2-Cys[e]	+	+
Val[c]	6.16	26
Met[b]	4.03 ± 0.19	17
Ile[c]	6.57	28
Leu	10.25 ± 0.15	43
Tyr	1.57 ± 0.05	7
Phe	3.56 ± 0.06	15
Trp[d]	0.39	2
		total 421

[a] These values are averages of data obtained from the analyses of two separate ρ samples after 24, 48, and 72 h of hydrolysis, unless otherwise indicated. Standard deviations of the means are shown. [b] Extrapolated to zero time from 24, 48, and 72 h HCl hydrolysis. [c] Values reached after 72 h of HCl hydrolysis. [d] Determined after 24 h of hydrolysis with 3 N *p*-toluenesulfonic acid. [e] Too small to measure accurately. [f] Calculated by using a ρ subunit molecular weight of 48 000.

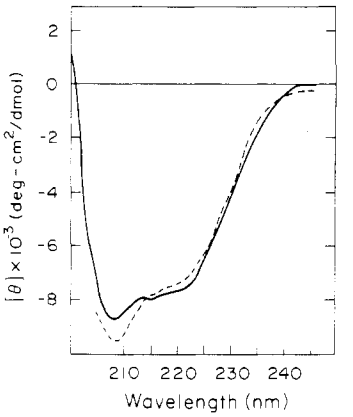

FIGURE 3: Circular dichroism spectrum of ρ protein at 200 μg/mL. The observed spectrum (—) was determined as described in the text. A model spectrum (---) was calculated by a linear combination of circular dichroic curves for 24% α helix, 22% β structure, and 54% random coil from 205 to 245 nm (Chen et al., 1974; Greenfield & Fasman, 1969).

(*C*) *Amino Acid Composition.* The amino acid composition of ρ protein is presented in Table II. Since the p*I* for ρ is ~9.0 (Blumenthal et al., 1976), a substantial fraction of Glx and Asx must be Gln and Asn, respectively. The low amount of tryptophan in ρ is consistent with its relatively low extinction coefficient. The ρ amino acid composition presented here differs in many residues from that obtained by Darlix (1975).

(*D*) *Circular Dichroism.* The circular dichroism spectrum of ρ protein at 200 μg/mL is shown in Figure 3. From this spectrum the secondary structure composition of ρ can be estimated by generating a similar curve through a linear combination of the spectra from proteins with known contents of α helix, β sheet, and random coil (Chen et al., 1974). The best-fit composite curve for ρ (Figure 3) represents a secondary

structure composition of approximately 24% α helix, 22% β sheet, and 54% random coil assuming the reference proteins are valid standards. Spectral measurements taken at 100 and 400 μg of ρ/mL gave similar results.

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