SEDIMENTATION PROPERTIES OF E. COLI RNA POLYMERASE AND ITS COMPLEXES WITH POLYURIDYLIC ACID*

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Both Fuchs et al. (1964) and Colvill et al. (1966) reported the preparation of RNA polymerase from Escherichia coli having a sedimentation coefficient of 24-25s; a smaller sedimentation coefficient was detected after aging of the enzyme (Fuchs et al., 1964) or by varying the method of preparation (Colvill et al., 1966). Using sedimentation velocity and sedimentation equilibrium studies, Richardson (1966) showed that at low ionic strength E. coli RNA polymerase has a sedimentation coefficient of 21-24s and a molecular weight of about 880,000. At high ionic strength, the enzyme dissociated into subunits (13s, MW = 440,000), and the dissociation process was reversible.

Studies of the sedimentation properties of E. coli RNA polymerase prepared in our laboratory are reported in this paper. The results show, in agreement with those of Richardson (1966), that the sedimentation coefficient of the enzyme is 13s at high ionic strength and 22s at low ionic strength. In addition, single peaks of active enzyme with intermediate S values are found at intermediate ionic strengths. Sedimentation coefficients were also found to depend on sucrose concentration. Studies of complexes of the enzyme and polyuridylic acid (poly U) suggest that both forms of the enzyme bind poly U and that the 13s enzyme has a molecular weight of approximately 390,000.

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Experimental--RNA polymerase was prepared from E. coli B by a modification of the method of Stevens and Henry (1964). The final enzyme preparation used in the experiments reported here appeared more than 90% pure as examined by centrifugation in the analytical ultracentrifuge and by starch gel electrophoresis. The ratio of absorbance at 280 mμ to that at 260 mμ was 1.5-1.6. The specific activity of the preparations was 350 using the assay of Stevens and Henry (1964) with calf thymus DNA (Worthington Biochemicals

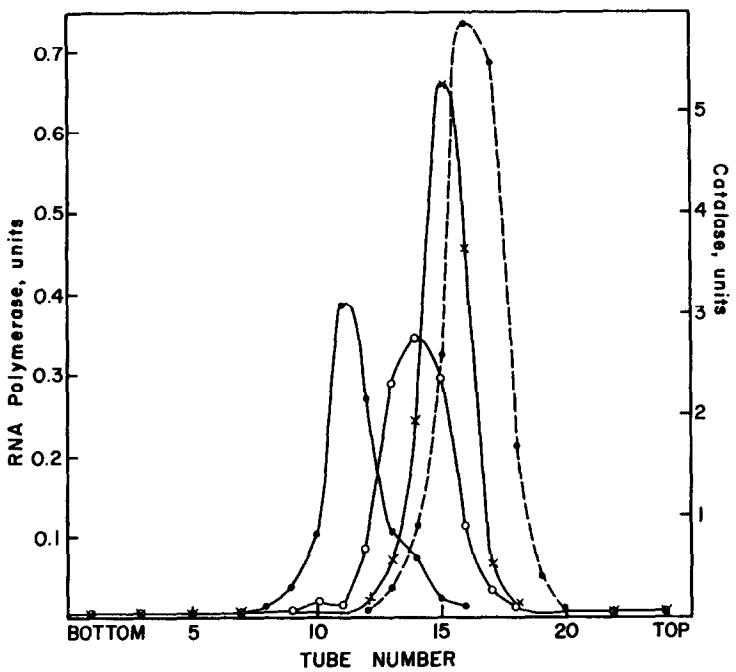

Figure 1--Sedimentation of RNA polymerase in sucrose density gradients at different NH_4Cl concentrations. RNA polymerase, 45 μg, and 60 μg of catalase in a final volume of 0.1 ml were layered on 5-20% sucrose gradients (4.6 ml). After centrifugation for 17 hours at 24,000 rpm (0°), fractions were collected and aliquots (50 μl) assayed for RNA polymerase activity (Stevens and Henry, 1964). Catalase activity was measured on a 20 μl aliquot as described by Martin and Ames (1961). One unit of catalase activity corresponded to a change in absorbancy at 240 mu of 0.02 per minute. Recovery of RNA polymerase from the gradients was about 65% at the lower NH_4Cl concentrations and 90% at the higher. ●—●—●, RNA polymerase in 10 mM Tris buffer, pH 7.8, containing 10 mM $MgCl_2$, 5 mM mercaptoethanol, and 20 mM NH_4Cl; o—o—o, same buffer, but with 50 mM NH_4Cl; x—x—x, same buffer, but with 75 mM NH_4Cl; ----------, catalase.

Corp.) as template. ^{3}H-Enzyme was prepared by growing E. coli B in a medium containing a reconstituted hydrolysate of ^{3}H-amino acids (Schwarz BioResearch Inc.). ^{3}H-Poly U was prepared from ^{3}H-UDP with polynucleotide phosphorylase from Micrococcus lysodeikticus (Singer and Guss, 1962). The average chain length of the poly U was measured by determination of the ratio of ^{3}H-uridine-3' (2')- monophosphate to ^{3}H-uridine following alkaline hydrolysis. (The chain length determined in this manner would be a maximum chain length since some chains might terminate in a 3'-phosphate.) A value of 400 was obtained by this method. The sedimentation coefficient of the ^{3}H-poly U was 6-7s, corresponding to a chain length of about 300 (Martin and Ames, 1962). Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Catalase was obtained from Worthington Biochemicals Corp.

Results--The distribution of RNA polymerase activity in three sucrose gradients containing different concentrations of NH_4Cl is shown in Fig. 1 (solid lines). Catalase ($S_{20,w}$ = 11.3) was used in each gradient as a standard protein. Its position is shown by the dashed line in Fig. 1. As the concentration of NH_4Cl was increased, RNA polymerase sedimented more slowly. At each concentration, a single peak of active enzyme was found. (The enzyme peaks were usually quite symmetrical.). The different sedimenting forms were readily interconvertible on changes in NH_4Cl concentration.

Table I presents a summary of the sedimentation coefficients found using different conditions of centrifugation. In (a) are shown the values obtained using 5-20% sucrose gradients at different NH_4Cl concentrations. The lowest value found was 13.4s and the highest, 20.5s. No lower value was found even when the enzyme was centrifuged in 1 M NH_4Cl or 10% saturated $(NH_4)_2SO_4$. Addition to the sucrose gradients of the four ribonucleoside triphosphates (0.25 mM each) did not alter the sedimentation values. NH_4Cl, NaCl, and LiCl were equivalent as salts. Thus, it appears that changes in ionic strength bring about the changes in sedimen-

tation rate. Centrifugation at 20 mM and 200 mM NH_4Cl in 5-20% glycerol gradients gave S values of 18.9 and 13.1 respectively. In 15-30% sucrose gradients, the enzyme sedimented as a 13-14s peak even at the lowest ionic strength tested (no NH_4Cl). In (b) of Table I are shown sedimentation coefficients obtained when the enzyme was centrifuged at a protein concentration.

Table I. Sedimentation Coefficients of RNA Polymerase at Different Ionic Strengths

		NH_4Cl, mM	$S_{20,w}$
(a)	Centrifugation in 5-20% Sucrose Gradients	0	20.5
		20	19.5
		50	16.1
		75	14.0
		200	13.4
(b)	Centrifugation in the Analytical Ultracentrifuge	20	22.1
		50	21.7
		100	18.1
		200	15.3
		400	14.3

All centrifugation media contained 10 mM Tris buffer, pH 7.8, 10 mM $MgCl_2$, 5 mM mercaptoethanol and NH_4Cl at the concentration shown. For the measurements in (a), 45μg of RNA polymerase and 60μg of catalase in a final volume of 0.1 ml were centrifuged. Determinations of enzyme activity were carried out as described in Fig. 1. $S_{20,w}$ values were calculated as described by Martin and Ames (1961). For the measurements in (b) centrifugation was carried out at 59,780 rpm at 5°. Sedimentation values were corrected using a partial specific volume of 0.73.

of 1.5 mg per ml in the analytical ultracentrifuge. The highest sedimentation coefficient found was 22.1s and the lowest 14.3s. The ionic strength required for the change in sedimentation rate is of greater magnitude than in the sucrose gradients. The absence of sucrose may account for these results or possibly, the change in protein concentration may influence the S values. The effect of protein concentration on the sedimentation coefficient of the enzyme is currently under investigation.

The sedimentation coefficients of 22s at low ionic strength and 13s at high ionic strength are similar to those reported by Richardson (1966). The finding of single peaks of active enzyme at intermediate ionic strengths suggests

that the two forms of the enzyme are in rapid equilibrium (Schachman, 1959). However, further results are necessary to clarify the findings since considerable differences are found with enzyme preparations in different laboratories. Richardson (1966) found that at 50 mM KCl in sucrose gradients the enzyme sedimented as a 24s particle, while we find a 16s fraction using almost exactly the same conditions. He did report that aged enzyme gave a value of 16s. The two enzyme preparations must vary in some unknown manner to account for these differences.

We have examined the effect of ionic strength, sucrose, and glycerol on the activity of the RNA polymerase (using the assay of Stevens and Henry, 1964). NH_4Cl has little effect on the activity until a concentration of 400 mM is reached. Then, it inhibits 75% if added before enzyme, DNA, and ribonucleoside triphosphates, but only 30% if added after mixing the latter three components. As reported by Anthony (1966), ionic strength appears to have a greater effect on the binding of enzyme to DNA than on the polymerization reaction. Glycerol at concentrations of 10% and 25% inhibits the activity of the enzyme by 40% and 85%, respectively. Sucrose at a concentration of 20% inhibits the activity of the enzyme by 40%. While the results suggest that conditions favoring monomeric (13s) enzyme inhibit the enzyme activity, it is not possible to assess the activity of the two forms on the basis of these experiments. Besides the enzyme, other aspects of the reaction may be affected.

Studies of complexes of enzyme and poly U suggest that both the 13s and the 22s enzyme bind poly U. The sedimentation pattern of a mixture of ^3H-poly U and enzyme is shown in Fig. 2. Under the conditions shown, free enzyme sedimented as a 20s peak, its position being shown by the arrow on the left. Free ^3H-poly U is the radioactive peak at the top of the tube. Two peaks of fast-sedimenting ^3H-poly U are found. One sediments as a 24-25s fraction, the other as a 15-16s fraction. Both peaks catalyzed the formation of ^{14}C-polyadenylic acid when incubated with ^{14}C-ATP (dashed line, Fig. 2),

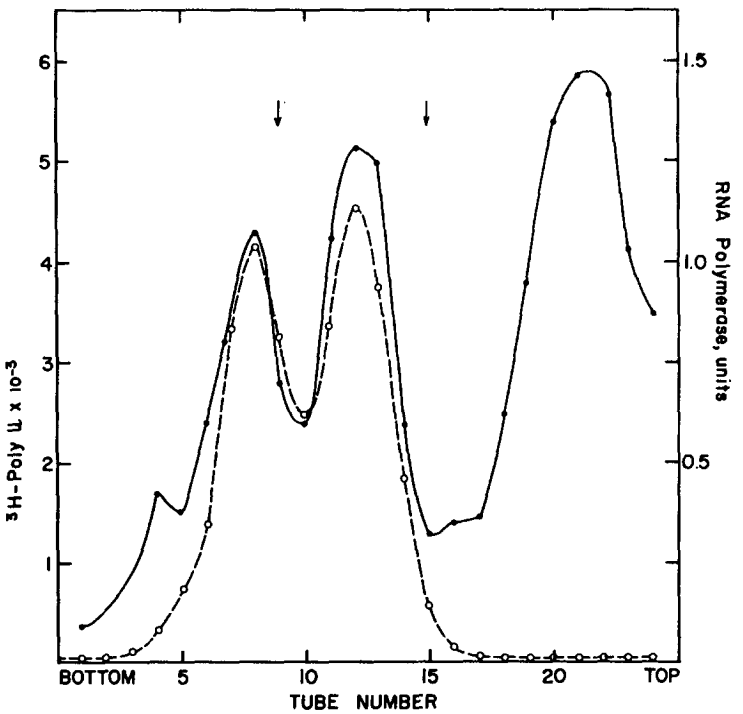

Figure 2--Sedimentation of mixtures of ^{3}H-poly U and RNA polymerase in sucrose density gradients. ^{3}H-Poly U, 20 mμ moles as UMP, 9.4 x 10^{4} cpm, and RNA polymerase, 40 μg, were mixed at 0° in 0.2 ml of solution containing 20 mM Tris buffer, pH 7.8, 2.5 mM $MnCl_2$, and 50 mM mercaptoethanol. After 10 minutes at 0°, the mixture was layered on a 5-20% sucrose gradient containing 10 mM Tris buffer, pH 7.8, 2 mM $MnCl_2$, 20 mM $MgCl_2$, and 5 mM mercaptoethanol. After spinning for 17 hours at 28,000 rpm (0°), fractions were collected from the bottom of the tube and assayed for ^{3}H-poly U and RNA polymerase. ^{3}H-Poly U was measured by counting an aliquot of each fraction in Bray's solvent (Bray, 1960) in a Packard Tri-Carb scintillation counter. Values are plotted per 0.2 ml fraction. Enzyme activity was measured by addition of an aliquot (0.1 ml) of each fraction to 0.1 ml of a reaction mixture containing ^{14}C-ATP, 6.7 x 10^{5} cpm/μ mole, 1.2 mM; Tris buffer, pH 7.8, 40 mM; $MnCl_2$, 5 mM; and mercaptoethanol, 50 mM. After incubating 20 minutes at 37°, the 5% trichloroacetic acid-insoluble material was collected on millipore filters and counted in a Nuclear Chicago end-window counter. One unit of enzyme activity corresponds to the incorporation of 1 mμ mole of ^{14}C-ATP into acid-insoluble material in 20 minutes. Values are plotted per 0.1 ml fraction. ●—●—●, ^{3}H-Poly U; o—o—o, RNA polymerase, units. The arrow at the left indicates the position of 20s enzyme, the one at the right, 13s enzyme.

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indicating that they are enzyme-poly U complexes. Using ^{3}H-enzyme and ^{3}H-poly U, we have determined the mμ moles of poly U bound per μg of enzyme in the 15s complex. Table II shows values obtained in four experiments with

the average value being 2.6×10^{-3}. From this value, the molecular weight of the protein capable of reacting with one poly U molecule can be calculated to be 386,000. This value for the molecular weight suggests that the 15s complex is composed of 13s enzyme and one poly U molecule. The value of 386,000 is in fair agreement with that of 440,000 reported by Richardson (1966) for the 13s enzyme. In preliminary experiments we have analyzed the 25s complex for its content of poly U and enzyme and the results suggest that it has the same ratio of poly U to enzyme as the 15s complex. It is possible that it is a complex of 22s enzyme and two poly U molecules. Further studies of these complexes are in progress.

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Table II. Determination of the Amount of Poly U Bound to Enzyme in the 15S Complex

Expt. No.	^{3}H-Poly U/ ^{3}H-Enzyme
	mμ moles x 10^{-3}/ μg
(1)	2.7
(2)	2.2
(3)	2.5
(4)	3.1
Average	2.6

^{3}H-Poly U, 20 mμ moles as UMP, containing 9.4×10^4 cpm, and ^{3}H-RNA polymerase, 44 μg, 150 cpm/μg, were mixed and centrifuged as described in Fig. 2. The ^{3}H-poly U and ^{3}H-enzyme content of each fraction was determined. In Expt. (1), ^{3}H-enzyme was measured by determination of 5% trichloroacetic acid-insoluble radioactivity following treatment of an aliquot of each fraction with an excess of pancreatic RNase. In Expts. (2), (3), and (4), ^{3}H-enzyme was measured by determination of acid-insoluble radioactivity following heating of an aliquot of each fraction for 30 minutes at 95° in 5% trichloroacetic acid. Acid-insoluble precipitates were collected on Millipore filters and counted in a toluene solvent in a Packard Tri-Carb scintillation counter. ^{3}H-Poly U was measured by counting aliquots of each fraction as described in Fig. 2 and subtracting the ^{3}H-enzyme activity. (The ^{3}H-enzyme radioactivity was very low in comparison to the poly U radioactivity.) The mμ moles of poly U were calculated using a value of 400 for the chain length of a poly U molecule.

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