Optical Rotatory Dispersion and Circular Dichroism Studies on *Escherichia coli* Ribonucleic Acid Polymerase*

Robert L. Novak† and Paul Doty

ABSTRACT: From its $[\theta]_{220}$ (13,000) and from its $[m]_{232'}$ (3900) the phosphocellulose-purified *Escherichia coli* ribonucleic acid (RNA) polymerase was estimated to contain 32% helix. The reversible dissociation of the polymerase in 2 M urea into subunits takes place with a 10% net decrease in the helical content of the subunits. A similar decrease in helical content occurred in 1 M urea, but the polymerase retained one-third of its enzyme activity. Dissociation of the RNA polymerase in 0.1% sodium dodecyl sulfate resulted in no change in its $[\theta]_{220}$, but in an increase in the magnitude its $[\theta]_{208}$. No decrease in the $[\theta]_{220}$ of the polymerase was found from pH 3 to 9; a 10% decrease in magnitude at pH 10 and 11; and a 40% decrease at pH 12. No change occurred in the $[\theta]_{220}$ of RNA polymerase when bound to double-stranded calf thymus DNA. However, thermal optical rotatory dispersion and enzyme activity studies from 25 to 60° show the polymerase enzyme to be much less stable in the DNA complex than in the free state. This thermal instability was not found in an analogous complex formed with single-stranded DNA or in the same complex after the start of RNA synthesis.

A new procedure for the large-scale isolation of pure *Escherichia coli* RNA polymerase (Burgess *et al.*, 1969; Burgess, 1969a) now makes possible a detailed study of the structure of the RNA polymerase molecule. The phosphocellulose-purified enzyme (PC-RNA polymerase) differs from previous *E. coli* RNA polymerase preparations in that it lacks the σ-initiation subunit. The *E. coli* polymerase readily transcribes native calf thymus DNA without this subunit, but shows little activity on native T4 or T5 DNAs (Burgess *et al.*, 1969). The PC-RNA polymerase contains three subunits: α (mol wt 39,000), β (mol wt 155,000), and β' (mol wt 165,000) subunits (Burgess, 1969b). In order to learn more about the secondary structure of the PC-RNA polymerase, we have studied the optical rotatory dispersion and the circular dichroism of the polymerase at various temperatures and pH values, in the presence of chemical denaturants, and during the binding and transcription of DNA.

Materials Methods

Preparation of PC-E. coli RNA Polymerase. The polymerase enzyme was isolated according to procedure developed by Burgess (1969a). Frozen (200 g) 0.75 log *E. coli* (Grain Processing, Muscatine, Iowa) and Superbrite 100 glass beads (3M Co.) (600 g) in 200 ml of BG[1] buffer were homogenized in a Waring blender at full speed for two 3-min periods. An additional 40 ml of BG buffer was added, and the homogenization was repeated three more times. The temperature of the homogenate was always kept below 10°. DNase I (1 mg) (Worthington Biochemical) was added and homogenate was allowed to stand for 15 min at 4°. All subsequent steps were also performed at 4°. The supernatant was then decanted and the glass beads were repeatedly washed with 50–100-ml aliquots of BG buffer until the volume of the combined decantations was 500 ml. This extract was centrifuged at 100,000*g* for 2 hr. Solid $(NH_4)_2SO_4$ was added to the decanted 100,000*g* supernatant until the solution was 33% saturated. After stirring for 15 min the precipitate was removed by centrifugation for 10 min at 30,000*g*. More $(NH_4)_2SO_4$ was added to the clear supernatant to give a 50% saturated solution. The resulting 30,000*g* precipitate was washed once each with 200 and 100 ml of a 47% saturated $(NH_4)_2SO_4$–BA buffer solution. The polymerase remains in the supernatant after centrifugation of the 36% saturated solution for 10 min at 30,000*g*.

The polymerase was reprecipitated from this solution by the addition of 28 ml of saturated $(NH_4)_2SO_4$ (pH 7.0) per 100 ml of supernatant and dissolved in 1 l. of BA buffer. This solution was applied to an 8 (diameter) × 8 cm Whatman DE-52 DEAE-cellulose column which had been equilibrated with BA buffer. The column was washed with 50 ml of BA buffer and then 300 ml of 0.13 M KCl–BA buffer, and the RNA polymerase finally eluted with 300 ml of 0.23 M KCl–BC buffer.

The pooled polymerase fractions were then applied directly to a 2 (diameter) × 12.5 cm Whatman P-11 phosphocellulose column which had been previously equilibrated with 0.1 M KCl–BC buffer. The column was washed with one column volume of 0.1 M KCl–BC and then with two column volumes of 0.3 M KCl–BC. The polymerase was eluted with 0.4 M KCl–BC buffer. All chromatography was carried out at 4°.

Finally, the pooled polymerase fractions were precipitated in 50% $(NH_4)_2SO_4$ and applied in a small amount of 1.0 M KCl–BC to a 2 (diameter) × 40 cm (200–400 mesh) Bio-Gel A-1.5m (Bio-Rad Laboratories) column. The column was equilibrated and eluted with the same buffer. The RNA

* From the Department of Chemistry, Harvard University, Cambridge, Massachusetts. *Received September 30, 1969.* Supported by Grant HD-01229 of the U. S. Public Health Service.

† Present address: DePaul University, Department of Chemistry, Chicago, Ill. 60614.

[1] The following buffer systems were used: BG, 0.05 M Tris (pH 7.5)–0.01 M $MgCl_2$–0.2 M KCl–0.1 mM Cleland's reagent–0.1 mM EDTA–5% glycerol; BA, 0.05 M Tris (pH 7.9)–0.01 $MgCl_2$–0.1 mM EDTA–0.1 mM Cleland's reagent–5% glycerol; BC, 0.05 M Tris (pH 7.9)–0.1 mM EDTA–0.1 mM Cleland's reagent–5% glycerol.

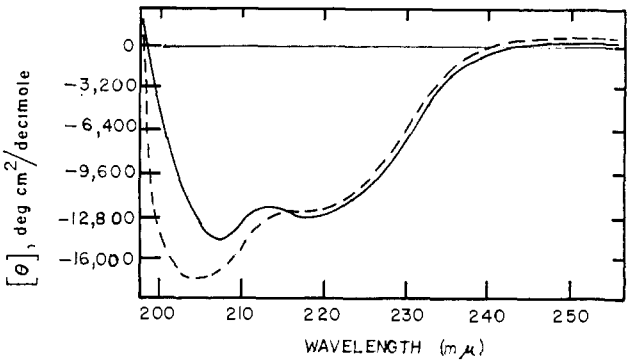

FIGURE 1: Circular dichroism of the *E. coli* PC-RNA polymerase. (——) Polymerase in 0.01 M Tris buffer (pH 7.5); (---) polymerase in 0.1% sodium lauryl sulfate–0.01 M Tris buffer (pH 7.5).

polymerase was the major protein peak which was eluted after a small protein impurity peak, which material passed directly through the column with the void volume.

The *E. coli* PC-RNA polymerase showed the same three major bands during 0.1% sodium dodecyl sulfate disc gel electrophoresis as reported by Burgess (1969b) and had a specific enzyme activity of 0.5 unit/μg. One activity unit was defined as the amount of enzyme incorporating 1 mμmole of [^{14}C]ATP into 10% trichloroacetic acid insoluble material in 10 min (37°) with excess native calf thymus template. The 0.5-ml polymerase reaction mixture used in these assays contained 100 mM Tris-HCl buffer (pH 7.5), 4.6 mM $MgCl_2$, 0.07 mM EDTA, 5.8 mM mercaptoethanol, 0.8 mM GTP, UTP, and CTP, 0.8 mM [^{14}C]ATP (1 mCi/mole), 200 mM KCl, 100 μg of native calf thymus DNA (Worthington Biochemical), and the specified amount of RNA polymerase enzyme.

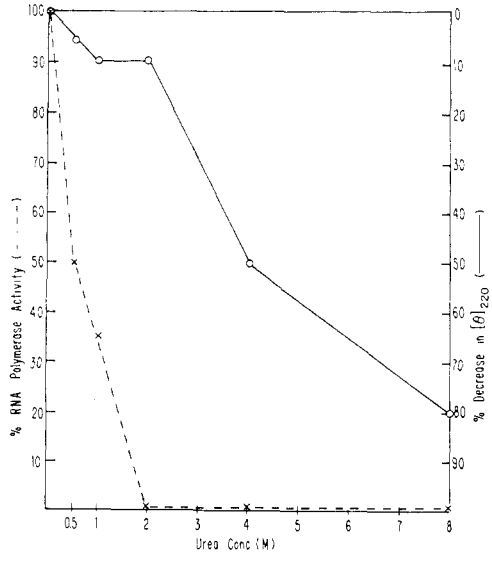

FIGURE 2: Effect of urea on the $[\theta]_{220}$ and on the enzyme activity of the PC-RNA polymerase. All urea solutions contained 0.1 M Tris (pH 7.5). Urea was removed from the polymerase samples by dialysis against BC buffer (4°) before the polymerase assays were performed.

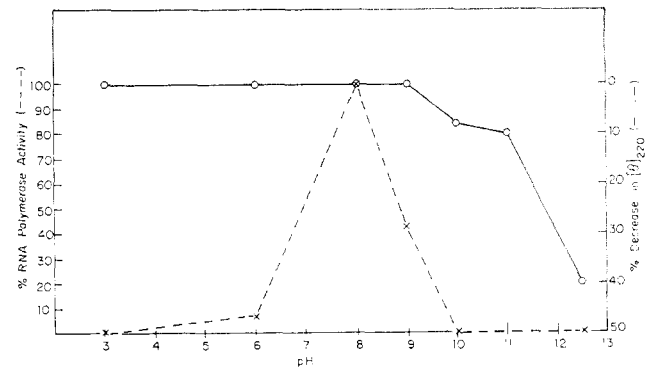

FIGURE 3: Effect of pH on the $[\theta]_{220}$ and on the enzyme activity of the PC-RNA polymerase. The desired pH values were achieved by titrating the RNA polymerase (in BC, pH 7.9) with either hydrochloric acid or with ammonium hydroxide. The polymerase solutions were incubated for 30 min (25°) at their respective pH values before above measurements were taken. All polymerase assays were performed at pH 7.5.

Preparation of the DNA–RNA Polymerase Complexes. A 1:1.5 weight ratio of calf thymus DNA (Worthington Biochemical) to PC-RNA polymerase was used in the formation of all the polymerase complexes. The binary DNA–PC-RNA polymerase complexes (Richardson, 1969) were prepared by mixing the DNA and the enzyme in either 0.1 M KCl–0.1 M potassium phosphate buffer (pH 7.5) or in 0.1 M KCl-0.1 M Tris buffer (pH 7.5). The complex gave identical thermal optical rotatory dispersion patterns in the two buffers. The single-stranded DNA complexes were prepared with calf thymus DNA, which was heated to 100° for 10 min, and then quickly cooled in an ice–water bath. The DNA–RNA polymerase–RNA complexes (Bremer and Konrad, 1964; Hayashi, 1965) were isolated by Sephadex G-200 chromatography from a 3 min (37°) polymerase reaction mixture (Novak, 1967). The DNA:RNA polymerase:RNA ratio in these complexes was 10:15:1. The DNA–RNA polymerase–(RNase resistant) RNA complex (Hayashi, 1965) was prepared by treating the above-mentioned complex with pancreatic RNase (weight ratio RNase:polymerase = 1:2) for 30 min (25°). The RNase in 0.15 M NaCl (pH 5.0) was heated to 80° for 10 min before use to inactivate any contaminating DNase. Five per cent of the RNA in the complex was resistant to RNase.

Optical Rotatory Dispersion and Circular Dichroism Measurements. The optical rotatory dispersion and circular dichroism measurements were made on a Cary 60 recording spectrophotometer equipped with a Model 6001 circular dichroism attachment. For the thermal optical rotatory dispersion measurements the 1-, 2-, or 10-mm path-length cells were housed in a water-jacketed copper block. The temperature of the block was increased in 5° increments by manually increasing the temperature of a Tamson circulating water bath. The dwell time between each rise in temperature and the subsequent optical rotatory dispersion recording was 30 min. The mean residue molecular weight and the $\epsilon^{1\%}_{1\,cm}$ at 280 mμ of PC-RNA polymerase used in calculating $[m']$ and $[\theta]$ were 110 and 6.5, respectively (Burgess, 1969b; Richardson, 1966).

Sedimentation Measurements. The sedimentation constants for the RNA polymerase were determined by moving-bound-

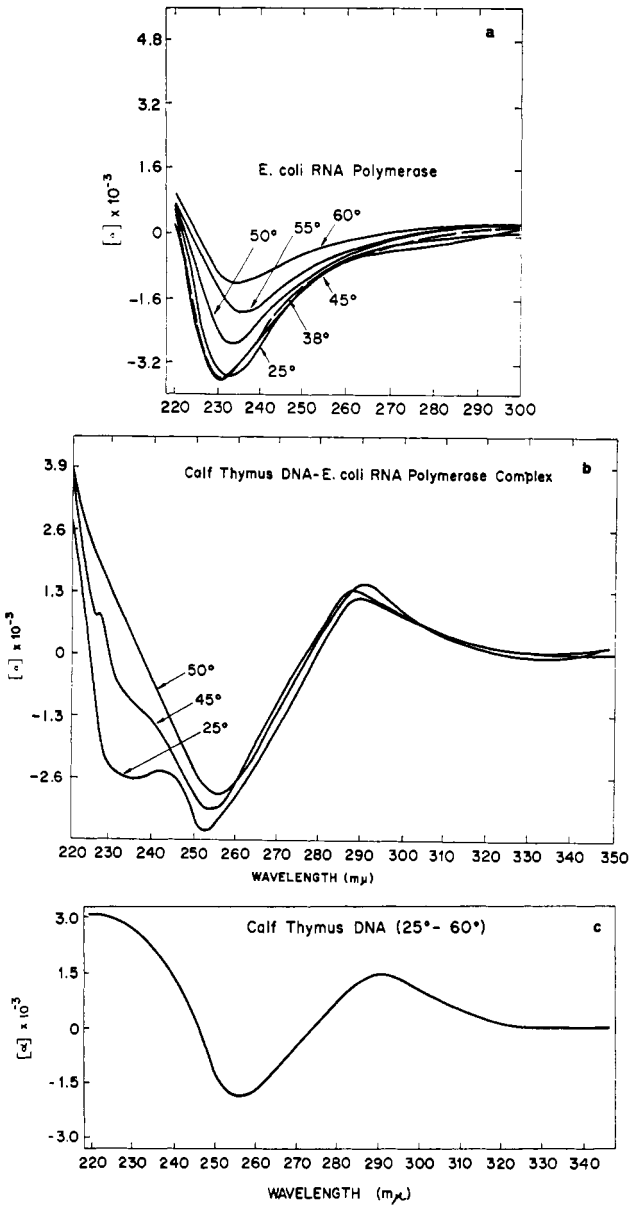

FIGURE 4: Thermal optical rotatory dispersion curves for the free (a) and for the native DNA-bound (b) PC-RNA polymerase. RNA polymerase concentrations in (a) and in (b) were 495 and 390 μg per ml. No change in optical rotatory dispersion curve for native calf thymus (300 μg/ml) was found between 25 and 60° (c). All the samples were run in a 0.1 M KCl–0.1 M Tris buffer (pH 7.5). Dwell time between sequential temperature increase was 30 min. Samples were removed at each reading and frozen for subsequent enzyme assay. The results of these assays are presented in Figure 5. The units of [α] were in deg ml/dm g of protein (DNA in part c).

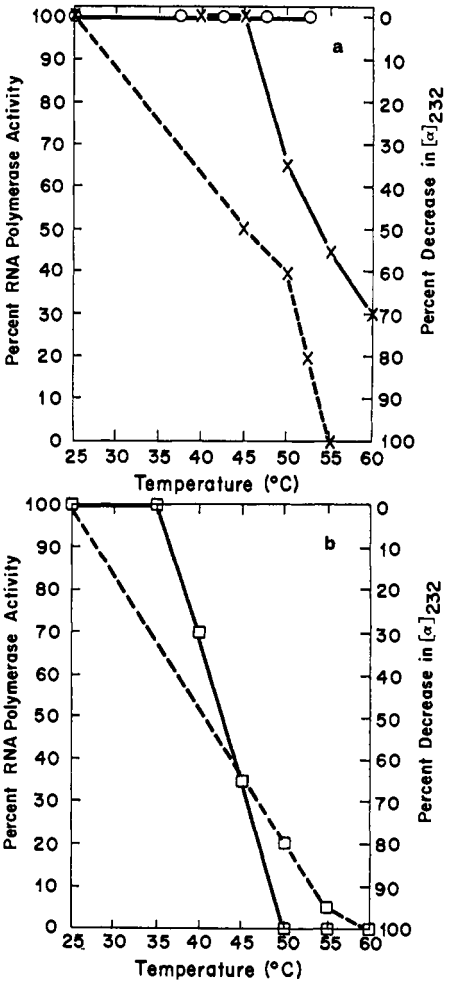

FIGURE 5: Effect of temperature on the $[\alpha]_{232}$ and on the enzyme activity of free and of DNA-bound RNA polymerases. The optical rotatory dispersion data were obtained from Figure 4. Polymerase samples were taken at each temperature and frozen for subsequent assay at 37°. (X——X) $[\alpha]_{232}$ of free RNA polymerase; (X--X) activity of the free polymerase; (O—O) $[\alpha]_{232}$ of free native calf thymus DNA; (□—□) $[\alpha]_{232}$ of the DNA-bound RNA polymerase; (□---□) polymerase activity of the DNA-bound RNA polymerase.

ary velocity sedimentation in a Spinco Model E analytical ultracentrifuge equipped with a monochrometer. All runs were monitored at 280 mμ. The resulting films were scanned with a Joyce-Loebl microdensitometer.

Results

From its molecular ellipticity at 220 mμ, $[\theta]_{220} = 13 \times 10^3$ (Figure 1) and from its reduced mean residue rotation at 232 mμ, $[m]'_{232}$ 3.9×10^3 the *E. coli* PC-RNA polymerase dimer was estimated to be 32% helical (Holzwarth and Doty, 1965; Simmons *et al.*, 1961). The same values were also obtained in 1.0 M KCl–BC buffer for the PC-RNA polymerase monomer. With increasing concentrations of urea the $[\theta]_{220}$ of the polymerase decreased in magnitude, showing a small decrease from 0 to 1 M urea, and then a rather large decrease at urea concentrations above 2 M (Figure 2). The polymerase dimer retained its 23S value in 1 M urea, and despite its 10% decrease in helicity was partially active after the removal of the urea. As the urea concentration was rasied to 2 M no further decrease in helicity occurred, but the polymerase dissociated into inactive subunits, the largest of which was 9 S, in good agreement with the 9S, 2.5S, and 1.5S subunits reported by Ishihama and Hurwitz (1969). No change in the $[\theta]_{220}$ was observed when the polymerase was dissociated in 0.1% sodium lauryl sulfate, but an increase was observed in the magnitude of the $[\theta]_{208}$ (Figure 1). No change

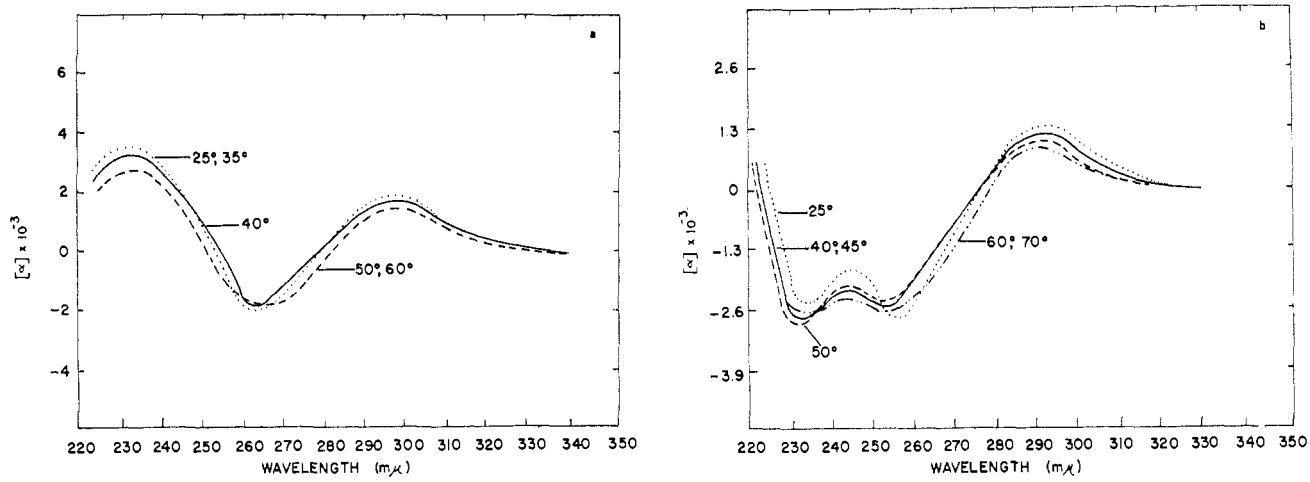

FIGURE 6: Thermal optical rotatory dispersion studies on the single-stranded calf DNA–RNA polymerase complex (a). The corresponding thermal optical rotatory dispersion curves for single-stranded calf DNA are shown in part b. The DNA and the DNA complex were run in 0.1 M KCl–0.1 M Tris buffer (pH 7.5). The units of [α] were in deg ml/dm g of protein (DNA in part b).

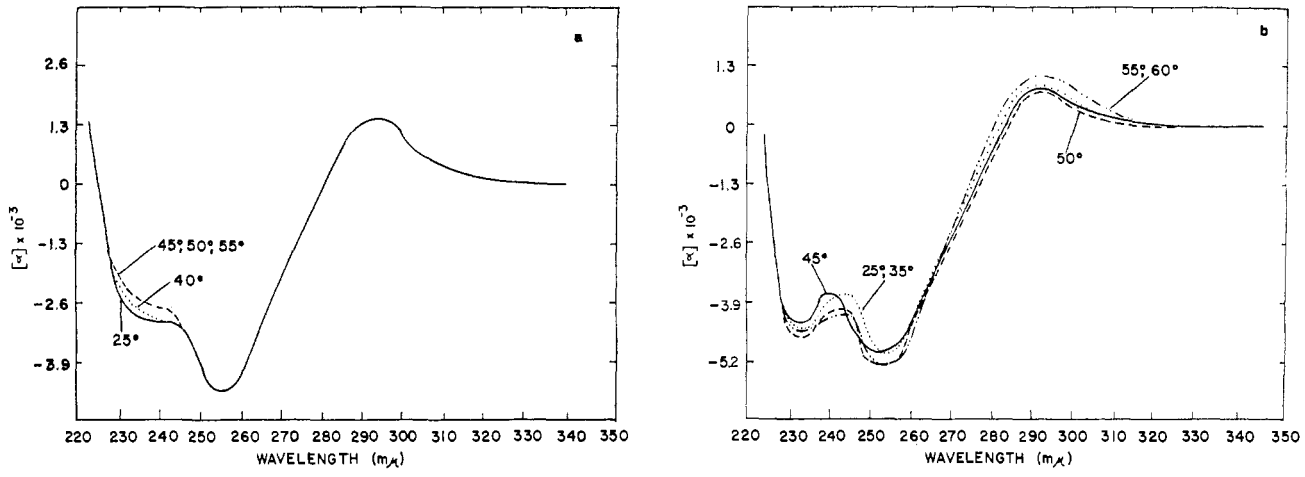

FIGURE 7: Thermal optical rotatory dispersion studies on the DNA–RNA polymerase–RNA (a) and on the DNA–RNA polymerase–(RNase resistant) RNA complexes (b). The complexes were isolated in a 0.1 M KCl–0.1 M Tris buffer (pH 7.5). The thermal optical rotatory dispersion curves for the DNA–RNA polymerase–RNA complex (Figure 7a) were identical except in the region around 230 mμ. In part b no correction was made for the RNase (weight ratio of polymerase:RNase = 1:2) present with the RNase-resistant complex. Thus the increase of the $[\alpha]_{232}$ from 4.5 (a) to 6.5. The units of [α] were in deg ml/dm g of polymerase protein.

was found in the $[\theta]_{220}$ of the polymerase protein from pH 3 to 9 (Figure 3). A 10% decrease in $[\theta]_{220}$ was found at pH 10–11. At pH 6 the polymerase aggregated, and at pH 10 the polymerase dimer dissociated into 13S monomers.

The binding of RNA polymerase to double-stranded calf DNA did not result in the disruption, but rather in the straining, of large helical regions in the polymerase. The optical rotatory dispersion and the circular dichroism curves for the DNA–RNA polymerase complex at 25° were the sum of the curves of the individual components. However, thermal optical rotatory dispersion studies (Figures 4 and 5) showed that the helical regions in the DNA-bound PC-RNA polymerase were more susceptible to thermal disruption than they were in the free polymerase. No change in the $[\alpha]_{232}$ of native calf thymus DNA was found over the temperature range studied (Figure 4 inset). A parallel thermal inactivation study (Figure 5) showed the polymerase to be also enzymatically less stable in the DNA complex than in the free state. Such a large decrease in thermal stability of the polymerase helix was not found for the polymerase in the single-stranded DNA complex (Figure 6) or for the polymerase in the native DNA–RNA polymerase–RNA or native DNA–RNA polymerase–(RNase resistant) RNA complexes (Figure 7). Only a small decrease in thermal stability was noted in the latter complexes, while the polymerase in the denatured DNA complex was even more stable than the free RNA polymerase.

Discussion

Dissociation of the PC-RNA polymerase in 2 M urea and in 0.1% sodium lauryl sulfate involves only a small change in the helical content of the polymerase subunits (Figures 1

and 2). This finding is in good agreement with the reported reversible dissociation in 2 M urea, and the irreversible dissociation at higher concentrations of urea (Ishihama and Hurwitz, 1969). The only partial inactivation of polymerase after treatment with 0.5 and with 1 M urea presents possible opportunities for studying various aspects of the polymerase reaction using partially unfolded polymerase molecules. The pH studies suggest that future chemical reactions can be performed on the polymerase in the pH range from 3 to 11 without drastically changing the helical content of the protein.

The thermal optical rotatory dispersion and inactivation studies (Figure 5) show both the helical regions and the enzyme activity, respectively, to be less stable in the double-stranded DNA–RNA polymerase complex than in the free RNA polymerase. Recent enzyme stability studies suggest that this strain of the polymerase helix does not occur in the presence of σ factor (Khesin *et al.*, 1967; Novak and Doty, 1968). Much of this strain on the PC-polymerase is relieved after the onset of RNA synthesis, as judged from the thermal optical rotatory dispersion curves of the DNA–RNA polymerase–RNA complex (Figure 7). A possible source of this strain might be the localized strand separation effected by the PC-polymerase prior to RNA synthesis. This strain would be relieved after the onset of RNA synthesis, because the resulting DNA–RNA hybrid would then support all further localized strand separation.

Acknowledgments

We gratefully acknowledge the technical assistance of Miss Joan Harrington during the later stages of this study. We also wish to thank Dr. Richard Burgess for making available to us the polymerase isolation procedure before its publication.

References

Bremer, H., and Konrad, M. W. (1964), *Proc. Natl. Acad. Sci. U. S. 51*, 801.
Burgess, R. R. (1969a), *J. Biol. Chem. 244*, 6160.
Burgess, R. R. (1969b), *J. Biol. Chem. 244*, 6169.
Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. R. F. (1969), *Nature 221*, 43.
Hayashi, M. (1965), *Proc. Natl. Acad. Sci. U. S. 54*, 1736.
Holzwarth, G., and Doty, P. (1965), *J. Amer. Chem. Soc. 87*, 219.
Ishihama, A., and Hurwitz, J. (1969), *Fed. Proc. 28*, 659.
Khesin, R. B., *et al.* (1967), *Mol. Biol. 1*, 617.
Novak, R. (1967), *Biochim. Biophys. Acta 149*, 593.
Novak, R., and Doty, P. (1968), *J. Biol. Chem. 243*, 6068.
Richardson, J. P. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 1616.
Richardson, J. P. (1969), *Progr. Nucleic Acid Mol. Biol. 9*, 99.
Simmons, N. S., *et al.* (1961), *J. Amer. Chem. Soc. 83*, 4766.