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**Physicochemical studies on interactions between DNA and RNA polymerase. Ultraviolet absorption measurements \***

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**ABSTRACT**

The interaction between *Escherichia coli* RNA polymerase and a restriction fragment of coliphage T7 DNA containing four promoter sites for the *coli* enzyme has been studied by difference uv absorption spectroscopy in a low ionic strength buffer containing 10 mM MgCl<sub>2</sub> and 50 mM KCl. The binding of the enzyme to the DNA is accompanied by a hyperchromic shift which shows a maximum around 260 nm, and increases with increasing temperature in the temperature range studied (4-40°C). Measurements were also carried out with whole T7 DNA and a restriction fragment containing no promoter site. A comparison of the results obtained with the various DNAs suggests that the binding of an RNA polymerase to a promoter site in the low ionic strength medium causes the disruption of a short segment of the DNA helix, of the order of ten pairs; the binding of an enzyme molecule to a promoter site appears to have a cooperative effect on the binding of enzyme molecules to adjacent non-promoter sites with concomitant disruption of DNA base pairs.

**INTRODUCTION**

In a previous paper of this series (Wang et al., 1977), we reported our results on the unwinding of the DNA helix by *Escherichia coli* RNA polymerase. As measured by the difference in the average linking numbers of double-stranded phage fd DNA samples covalently closed in the presence and absence of *E. coli* RNA polymerase, the binding of a holoenzyme at 37°C in a dilute buffer containing Mg(II) is found to unwind the DNA helix by about 240°. In arriving at this value, it has been assumed that every polymerase molecule bound to DNA is capable of unwinding the helix. Thus the measured angular alteration of the DNA helix is likely to be a lower limit. The average unwinding angle is dependent on temperature and the salt concentration of the binding buffer.

The measured unwinding of the DNA double helix by RNA polymerase lends support to the idea that the reading of the DNA sequence by the enzyme requires the unpairing of a short segment of the DNA. Alternatively, as discussed previously (Saucier and Wang, 1972), the observed unwinding could be interpreted as due to a transition of the DNA helix from one form to another, over a much longer region of the helix.

The availability of restriction enzymes makes it possible to obtain unique DNA fragments containing promoter sequences. In the first paper of this series, we reported our results on the isolation and mapping of an 1100 base pair Hinf restriction fragment of coliphage T7 DNA which contains the three early promoters for E. coli RNA polymerase and a fourth site with a high affinity for the enzyme (Hsieh and Wang, 1976). We report here our uv absorption studies on complex formation between this DNA fragment and E. coli RNA polymerase.

### MATERIALS AND METHODS

The preparation of the 1100 base pair T7 DNA fragment, (Hinf T7)<sub>1100</sub>, and E. coli RNA polymerase holoenzyme has been described (Hsieh and Wang, 1976). The concentrations of the enzyme and DNA solutions were determined spectrophotometrically. The absorptivity of RNA polymerase at 280 nm is taken as 0.66 mg<sup>-1</sup>cm<sup>2</sup> (Richardson, 1966), and the absorptivity of DNA at 260 nm is taken as 20 mg<sup>-1</sup>cm<sup>2</sup>.

### ABSORPTION DIFFERENCE SPECTROSCOPY

A Cary 14 spectrophotometer equipped with a thermostated cell holder was used in all measurements. The cell temperature was measured by a copper-constantan thermocouple and a potentiometer. A slide wire for the absorbance range between 0 and 0.1 was employed. The spectrophotometer was interfaced through digitizers (Datex Corporation) to a PDP 8/E computer, and data were recorded, normalized, and curve-smoothed by a sliding 13-point averaging algorithm (Savitzky and Golay, 1964).

A pair of optically matched quartz cells for measuring the difference spectra were purchased from Hellma Cell Inc., Germany. Each cell has two tandem compartments separated by a half-height partition, so that mixing of the solutions on the two sides of the partition can be achieved by inverting the cell. The pathlength of each compartment is 0.44 cm. In each cell, about 1.1 ml of RNA polymerase solution and DNA solution were put into the compartments separated by the half-height partition. The precise amounts of solutions were determined by weighing. The DNA concentration in these measurements was approximately 50 µg/ml. A spectrum was first taken and stored in the computer as the baseline spectrum. Then the sample cell was taken out and inverted a few times to mix the solutions on the two sides of the partition. For measurements at the lower temperatures, the mixing was performed in a cold room. After placing the sample cell in the original position in the spectrophotometer the spectrum was measured again after thermal equilibrium

had been reached. The baseline spectrum was subtracted from the sample spectrum and the results were normalized, curve-smoothed, and printed out. After the completion of the measurements, contents in the tandem compartments of the reference cell were also mixed by inverting the cell several times. The difference spectra thus obtained agreed with the baseline spectrum within experimental error.

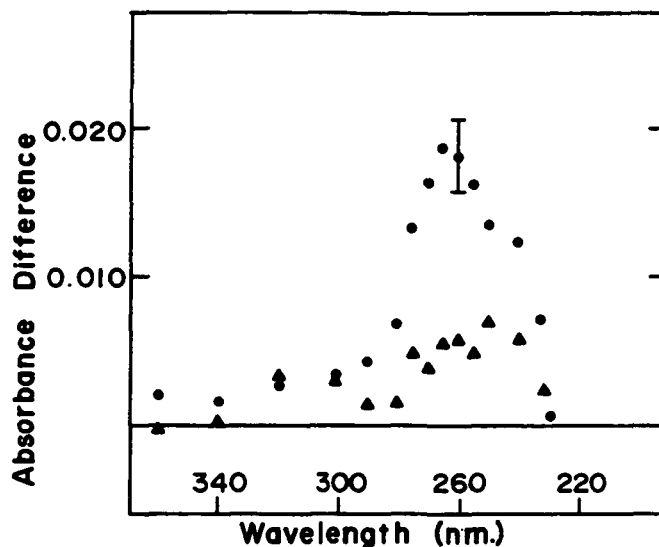
In the experiments on the spectroscopic titration of the binding of RNA polymerase to (Hinf T7)<sub>1100</sub>, the following procedure was employed. To avoid the change of ionic strength in the binding medium due to the addition of RNA polymerase in the titration procedure, RNA polymerase at a concentration of 3.4 mg/ml was first dialyzed for 8 hours at 4°C against 10 mM Tris·HCl, pH 8, 50 mM KCl, 0.1 mM Na<sub>3</sub>EDTA, 10 mM MgCl<sub>2</sub>, 1 mM 2-mercaptoethanol, and 1% (v/v) glycerol. After dialysis, the RNA polymerase concentration was measured spectrophotometrically and its enzymatic activity measured by the incorporation of radioactive <sup>3</sup>H-CTP into acid-precipitable material. The specific activity of the dialyzed sample was no less than 90% of that of the original sample. For each aliquot of the dialyzed RNA polymerase stock added to the enzyme solution compartment of the reference cell, an identical volume of the dialysis buffer was added to the DNA solution compartment of the same cell. The solution in each separate compartment was mixed by repetitive siphoning and delivering through a micropipet. The dialyzed RNA polymerase stock and the dialysis buffer solutions were also added to the sample cell compartments in a similar manner and the cell was inverted a few times to thoroughly mix the contents. Spectroscopic measurements were carried out as described in the preceding paragraph, and the results were further corrected for the dilution of the solutions during titration.

#### RESULTS

##### RNA polymerase binding to DNA causes a hyperchromic shift around 260 nm.

When solutions of RNA polymerase and the DNA restriction fragment (Hinf T7)<sub>1100</sub> were mixed at 4°C with a ratio of 6 enzyme molecules to 1 DNA molecule, a small but definitely detectable hyperchromic change in the uv region was observed (Fig. 1, triangles). Increasing the temperature increases the magnitude of the hyperchromic shift. At 37°C, the difference spectrum clearly shows a maximum around 260 nm (Fig. 1, filled circles). Although the signal to noise ratio is relatively high, the shoulders at 300 nm and 250 nm are reproducibly observed and are probably real.

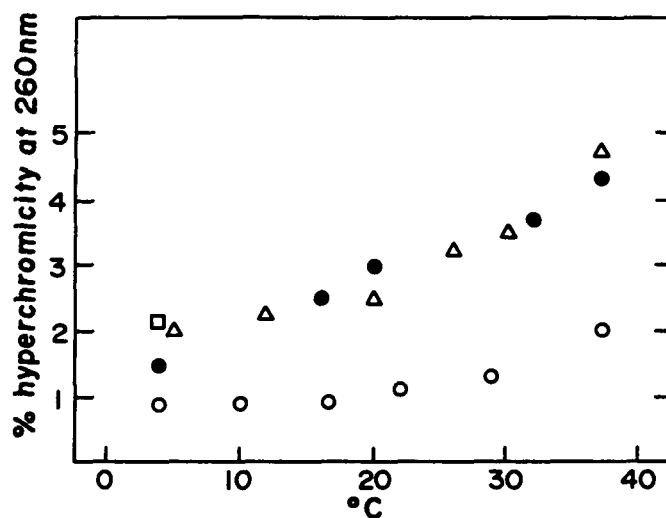
Several control experiments were carried out to see if the hyperchromic shift observed might be due to artifacts. As described in the MATERIALS AND



**Figure 1.** Difference uv absorption spectra between mixed and unmixed *E. coli* RNA polymerase and an 1100 base pair T7 DNA restriction fragment containing four promoter sites for the host enzyme. The mixing of the enzyme and DNA in the cuvette was performed at 4°C. The absorbance of the DNA solution before mixing was 0.425, corresponding to a concentration of 48.3 µg/ml. The DNA was mixed with equal volume of RNA polymerase with a concentration of 207 µg/ml before mixing. The absorbance difference measured at 4°C is given by the triangles. The filled circles give the absorbance difference measured at 37°C. The vertical bar in the figure represents the noise level of the spectroscopic measurements.

**METHODS** section, if after the recording of the difference spectrum the DNA and RNA polymerase solutions in the tandem compartments of the reference cell were also mixed and a difference spectrum was again taken, the spectrum was the same as the initial baseline spectrum. This shows that the hyperchromic shift arises from mixing of the DNA and RNA polymerase solutions and that it is reproducible. If the RNA polymerase solution in these measurements is replaced by bovine plasma albumin at a concentration of 250 µg/ml, no spectral change was observable upon mixing the contents of the sample cell. Artifacts due to the scattering of light by the contents of the cells are also unlikely to be the cause of the hyperchromic shift, as evidenced by the shape of the difference spectra.

The temperature dependence of the hyperchromic change at 260 nm is shown in Fig. 2. Since data for several DNA samples are shown in the same figure, the hyperchromic shift at 260 nm is normalized by the expected DNA absorption in the absence of the protein, and is expressed as percent hyperchromic shift.



**Figure 2.** Hyperchromic changes associated with the binding of *E. coli* RNA polymerase to various DNA samples as a function of temperature. The ratio of polymerase to DNA was approximately 6 enzyme molecules per thousand DNA base pairs, and was identical in all measurements. For each series of measurements, RNA polymerase and DNA were mixed thoroughly in the sample cuvette at 4°C. A difference spectrum was taken at 4°C and after each subsequent temperature increment. Triangles, filled circles and open circles give the results obtained with T7 DNA, (*Hinf* T7)<sub>1100</sub> and (*Hinf* T7)<sub>560</sub> respectively. The single point depicted by the open square gives the hyperchromic change when the same mixture of RNA polymerase and (*Hinf* T7)<sub>1100</sub> was first incubated at 37°C and then cooled to 4°C. The higher hyperchromic shift of this sample relative to the one without preincubation at 37°C is reproducible.

For the (*Hinf* T7)<sub>1100</sub> fragment containing the early promoters, the hyperchromicity increases with increasing temperature (filled circles) in the temperature range indicated in the figure. In these measurements, the difference spectrum was first measured at 4°C and the temperature was increased stepwise and spectral measurement was carried out after each temperature increment. After measurement at the highest temperature (37°C), the temperature was again lowered to 4°C. The last difference spectrum so obtained is hyperchromic relative to the difference spectrum initially taken at 4°C. This residual hyperchromic change persists after the cells had been kept at 4°C for 1 hr.

The dependence of the hyperchromic shift on whether the DNA fragment contains promoter sites.

In order to study whether the hyperchromic shift is dependent on the presence of promoter sites on the DNA, during the isolation of the restriction

fragment (Hinf T7)<sub>1100</sub> we also isolated a 560 base pair long fragment (Hinf T7)<sub>560</sub> from the same restriction enzyme digest. This fraction is most likely a mixture of two or three T7 DNA fragments of the same length within the resolving power of agarose gel electrophoresis. By the criteria of *in vitro* RNA polymerase binding and RNA synthesis, (Hinf T7)<sub>560</sub> contains no promoter sites.

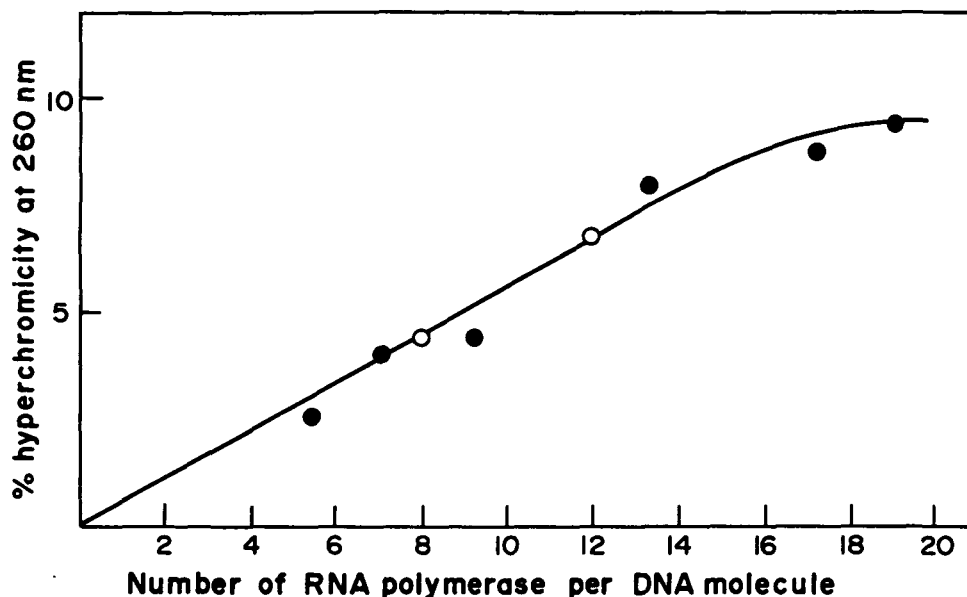
As shown in Fig. 2 (open circles), with (Hinf T7)<sub>560</sub> the hyperchromic shift observed at a given temperature is always lower than that with (Hinf T7)<sub>1100</sub>, when measurements were carried out at the same weight ratio of enzyme to DNA.

When whole T7 DNA was used, again at the same weight ratio of enzyme to DNA, the percent hyperchromic shift is identical to that observed with the promoter fragment (Hinf T7)<sub>1100</sub> (Fig. 2, triangles). As the promoter sequences constitute only a very small fraction of whole T7 DNA, one would expect that the hyperchromic shift with whole T7 DNA should be close to that with the non-promoter fragment. Clearly, the experimental result is contradictory to this expectation.

The dependence of the hyperchromic shift on the enzyme to DNA ratio.

If the hyperchromic shift results only from RNA polymerase binding to promoter sites, for a fixed DNA concentration it should increase with increasing RNA polymerase concentration and level off when the promoter sites are saturated. The results of such an experiment with the (Hinf T7)<sub>1100</sub> promoter fragment are depicted in Fig. 3 (filled circles). In the binding buffer containing 50 mM KCl and 10 mM MgCl<sub>2</sub>, the hyperchromic shift increases with increasing number of RNA polymerase per DNA molecule, but levels off at a molar ratio of enzyme to DNA of about 20. This ratio is much higher than the expected ratio of 4 for the saturation of the 4 promoters on this fragment. The trivial possibility that a large fraction of enzyme preparation is inactive is highly unlikely from results described in the first two papers of this series (Hsieh and Wang, 1976; Wang et al., 1977). The enzyme to DNA ratio at which the hyperchromic shift levels off corresponds more closely to what is needed to saturate the whole fragment completely, i.e. each enzyme molecule spans about 50 base pairs.

The same dependence of the hyperchromic shift on the RNA polymerase to DNA ratio is also observed when whole T7 DNA was used (open circles). At least at two ratios of enzyme to DNA, the percent hyperchromic shifts of whole T7 DNA upon binding RNA polymerase are the same as those of the DNA fragment (Hinf T7)<sub>1100</sub> at the corresponding weight ratios of enzyme to DNA.



**Figure 3.** Hyperchromic changes of the 1100 base pair T7 DNA restriction fragment (Hinf T7)<sub>1100</sub> upon mixing with varying amounts of RNA polymerase. A difference spectrum is taken for each sample and the percent increase in absorbance at 260 nm is plotted as a function of the molar ratio of RNA polymerase to DNA. See the text for details.

#### DISCUSSION

We have shown that the binding of *E. coli* RNA polymerase to (Hinf T7)<sub>1100</sub>, a 1100 base pair long coliphage T7 DNA fragment containing four promoter sequences for the host enzyme, causes a small but measurable hyperchromic change in the ultraviolet region. The hyperchromic change occurs in the spectral region where DNA absorbs predominantly, and is therefore likely to be largely due to a structural change of the DNA by polymerase binding.

With either the restriction fragment (Hinf T7)<sub>1100</sub> or whole T7 DNA, at a fixed ratio of RNA polymerase to DNA the magnitude of the hyperchromic change increases with increasing temperature in the range 4-40°C. At a fixed temperature, the magnitude of the hyperchromic change increases linearly with the RNA polymerase to DNA ratio in a medium containing 50 mM KCl and 10 mM MgCl<sub>2</sub>, and is not limited by the number of promoter sites available. Thus at least qualitatively the hyperchromic shift appears to correlate with the unwinding of the DNA helix by RNA polymerase (Saucier and Wang, 1972; Wang et al., 1977), in the sense that both effects show similar dependences on temperature and

polymerase to DNA ratio. The slow relaxation of the average unwinding angle to the expected value at a low temperature, following preincubation at 37°C (Wang et al., 1977), also corresponds to the residual hyperchromic effect at a low temperature after preincubation at 37°C.

The experimentally observed unwinding of the DNA helix by RNA polymerase can be interpreted as due to the disruption of a short helical segment or a transition of the DNA from one helical form to another, or a combination of these two types of changes (Saucier and Wang, 1972). The hypochromicity of the DNA double helix has been attributed to interaction between the dipoles induced in the stacked base pairs by the light (Tinoco, 1960; Rhodes, 1961). Disruption of the parallel stack of base pairs in the double helix increases the absorbance at 260 nm by about 40%. In the case of RNA polymerase binding, data shown in Fig. 3 indicate that the binding of a single RNA polymerase to the 1100 base pair long DNA fragment would cause a hyperchromic shift of approximately 0.5%. A hyperchromic shift of the same magnitude could be produced by disrupting about a dozen or so base pairs in the restriction fragment. Thus the simplest interpretation of the unwinding of the helix and the hyperchromicity is that a short DNA segment, of the order of ten base pairs, is disrupted by the binding of an RNA polymerase molecule.

There has been no evidence supporting the notion that the binding of RNA polymerase causes a gross conformational change of the DNA helix. The circular dichroism of the complex between RNA polymerase and the restriction fragment (Hinf T7)<sub>1100</sub>, in the wavelength region longer than 260 nm where the ellipticity is primarily from the contribution of DNA, is similar to that from the combination of DNA and enzyme solutions before mixing and is not significantly different from that of a DNA of the same base composition. For a mixture of (Hinf T7)<sub>1100</sub> and RNA polymerase at a molar ratio of 14 enzymes per DNA fragment, only a small increase in ellipticity (about 10% at 274 nm) is observed relative to the circular dichroism of pure DNA. The shapes of these circular dichroism spectra are also similar (Hsieh and Wang, unpublished results).

The difference in the magnitude of the hyperchromic changes observed between the non-promoter fragment (Hinf T7)<sub>560</sub> and the other promoter-containing DNAs studied presents a dilemma. On one hand, for either the restriction fragment containing the promoters or whole T7 DNA, the hyperchromic change increases linearly with increasing number of polymerase bound per DNA, until the DNA is essentially completely covered with enzyme molecules. This indicates that the hyperchromic change is not restricted to binding to promoter sites, and that binding to promoter and non-promoter sites



appear to give the same hyperchromic change. On the other hand, the magnitude of the hyperchromic change observed for the non-promoter fragment at the same number of RNA polymerase per base pair is much lower than that for the promoter containing DNA. A plausible interpretation of this apparent contradiction is that the binding of a polymerase to a promoter site exerts a cooperative effect on the binding of a polymerase to a neighboring site. This enables the polymerase to unwind a neighboring non-promoter site. This cooperative effect can persist until the DNA is essentially completely covered with polymerase. Whether such an effect might facilitate rapid transcription at a promoter site *in vivo* is not known. *In vitro* transcription experiments with the restriction fragment (Hinf T7)<sub>1100</sub> indicates that even when the DNA is fully covered with polymerase molecules, RNA synthesis mainly starts from the early promoters, and that the bound polymerase molecules apparently do not interfere with the rightward transcription from these promoters (Hsieh and Wang, unpublished results).

Finally, we note that the hyperchromic change we have discussed contradicts the much larger hypochromic change reported by Sarocchi and Darlix (1974).

#### ACKNOWLEDGEMENT

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#### REFERENCES

\*This paper is the third of a series from this laboratory, the first two being Hsieh and Wang (1976) and Wang et al. (1977).

- 1 Wang, J. C., Jacobsen, J. H. and Saucier, J.-M. (1977) *Nucleic Acids Res.* 4, 1225
- 2 Saucier, J.-M. and Wang, J. C. (1972) *Nature New Biol.* 239, 167
- 3 Hsieh, T.-S. and Wang, J. C. (1976) *Biochemistry* 15, 5776
- 4 Richardson, J. P. (1966) *Proc. Natl. Acad. Sci. USA* 55, 1616
- 5 Savitsky, A. and Golay, M. J. E. (1964) *Anal. Chem.* 36, 1627
- 6 Hsieh, T.-S. and Wang, J. C. (1975) *Biochemistry* 14, 527
- 7 Tinoco, I. Jr. (1960) *J. Amer. Chem. Soc.*, 82, 4785
- 8 Rhodes, W. (1961) *J. Amer. Chem. Soc.* 83, 3609
- 9 Sarocchi, M.-T. and Darlix, J.-L. (1974) *Eur. J. Biochem.* 46, 481

