An ω Protein from *Drosophila melanogaster*†

Walter A. Baase and James C. Wang*

ABSTRACT: A protein which converts superhelical DNA to a covalently closed form containing no superhelical turns has been partially purified from the eggs of *Drosophila melanogaster*. This activity is similar to the ω protein of *Escherichia coli* (Wang, J. C. (1971), *J. Mol. Biol. 55,* 523) in that: (1) no change except the loss of superhelical turns can be detected in the DNA; (2) the loss of superhelical turns does not follow a single-hit mechanism, and DNA intermediates with a decreasing number of superhelical turns can be obtained as the reaction proceeds; (3) no dialyzable cofactor requirement can be detected. In contrast to the *E. coli* activity, but similar to the activity from mouse embryo cells (Champoux, J. J., and Dulbecco, R. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 143), the *Drosophila* activity can relax both positive and negative superhelical DNAs. Furthermore, it does not require Mg(II) or any other divalent ion, but its activity is sharply dependent on ionic strength and temperature, with 0.2 M KCl and 28° being close to the optimal.

Recently, a protein ω from *Escherichia coli* has been shown to remove superhelical turns from a negative superhelical DNA without leaving a single chain scission in either one of the two strands (Wang, 1971, 1973). The topological constraint for a covalently closed double-stranded DNA dictates that for such a reduction in the number of superhelical turns, either there is a concomitant reduction in the number of helical turns of the DNA duplex, or a *transient* swivel is introduced during the reaction between the DNA and ω. All experimental results are consistent with the notion that ω is capable of introducing a swivel reversibly into a DNA helix. The *in vitro* function of ω is suggestive that it might have a similar role *in vivo*.

The picture is beginning to emerge that ω-type proteins are widely present in nature. Champoux and Dulbecco (1972) reported an activity from mouse cells which untwists superhelical DNA. This activity has been purified by Vosberg *et al.* (1974). A similar activity has been purified from KB cells by Keller (W. Keller, personal communication). Because of the implied *in vivo* role of an ω-type protein, and the known very high rate of DNA synthesis in developing *Drosophila* eggs (Rabinowitz, 1941; Kriegstein and Hogness, 1974), we have initiated studies on such an activity from *Drosophila*. In this paper we report the partial purification of such a protein from fertilized eggs of *Drosophila melanogaster*, and our studies on its actions on several superhelical DNA samples.

Experimental Procedure

Materials. DEAE-cellulose, 0.75 mequiv/g, was purchased from Bio-Rad. Single-stranded DNA cellulose was prepared from calf thymus DNA (Worthington) and Munktell's No. 410 cellulose (Pharmacia), according to the method of Alberts and Herrick (1971), but with an ultraviolet (uv) irradiation step as suggested by Litman (1968). The final product contained ~1 mg of DNA/ml of wet packed column material. Pancreatic DNase I, grade D, 3200 units/mg, was purchased from Worthington. *E. coli* DNA ligase was obtained from the endonuclease I deficient strain 1100 according to the procedures of Olivera and Lehman (1967). PM2 DNA was extracted from the purified phage according to the procedures of Espejo and Canelo (1968) and Espejo *et al.* (1969).

Assays. A standard assay mixture (80 μl placed in a disposable polystyrene test tube) contained 0.18 M KCl, 7.3 mM potassium phosphate (pH 6.8), 5 mM Na_3EDTA, 15–35 μg/ml of superhelical PM2 DNA, 60 μg/ml of bovine plasma albumin (Calbiochem) and a proper amount of the *Drosophila* protein. After incubating at 28° for 15 min, the reaction was stopped by the addition of 20 μl of a saturated NaCl solution containing 0.06 M Na_3EDTA. The DNA species after the reaction were examined by band sedimentation in 3 M CsCl (Harshaw optical grade)–0.01 M Na_3EDTA containing 15 μM of ethidium bromide (Calbiochem), as described previously for the assay of the *E. coli* protein (Wang, 1971). The ethidium concentration in the band sedimentation medium was chosen such that the unreacted superhelical PM2 DNA had a minimal sedimemtation coefficient. PM2 DNAs with less negative superhelical turns than the unreacted PM2 DNA became positively twisted at this dye concentration, and thus sedimented as faster moving bands relative to unreacted PM2 DNA or nicked PM2 DNA.

Purification Procedures; Crude Extract. Adult flies and larva (*D. melanogaster,* Oregon R) were maintained on media described by Lewis (1960), as modified by Kriegstein and Hogness (1974). The fly cages were kept at 26.5° and a relative humidity of 80%. A 12-hr light and 12-hr dark lighting cycle was used. Collection of the eggs was done shortly before the light to dark transition. The eggs were washed off the agar plates with water, washed briefly to remove yeast, blotted on paper towels, and then kept frozen at −15° until use. A 45 × 75 × 38 cm cage at high adult density typically yielded 10–35 g of wet eggs per day.

Twenty grams of frozen eggs were placed in a nylon strainer (with 100-μm holes), thawed at 22°, and washed extensively with distilled water. The eggs were dipped into a 2.6% sodium hypochlorite solution for 45 sec, followed by extensive washing with water, to remove the chorion. They were then washed with a 0° buffer containing 10 mM Tris (pH 8), 10 mM NaCl, and 1.5 mM $MgCl_2$, suspended in 40

† From the Chemistry Department, University of California, Berkeley, California 94720. *Received June 4, 1974.* This work was supported by a grant from the U.S. Public Health Service (GM 14621), and a professorship from the Miller Institute for Basic Research in Science.

ml of the same buffer, and broken up at 0° by 20 strokes in a Dounce homogenizer. The homogenate was spun in a Beckman Model J 21 centrifuge at 0° and 19,000*g* for 30 min. The supernatant was dialyzed against two changes of 66 mM potassium phosphate (pH 6.8)–10 mM Na_3EDTA and two changes of 66 mM potassium phosphate (pH 6.8)–1 mM Na_3EDTA. The time interval between each change of dialysate was usually 4 hr.

DNase Digestion. The above lysate was digested with pancreatic DNase I in a high salt medium as described by Alberts and Herrick (1971). Spectroscopic grade glycerol (Matheson Coleman and Bell) and stock solutions of various salts and bovine plasma albumin were added to the lysate to give a solution 55 mM in potassium phosphate (pH 6.8), 0.8 mM in Na_3EDTA, 9.5 mM in $MgCl_2$, 1.9 mM in $CaCl_2$, 425 mM in NaCl, 1 mg/ml in bovine plasma albumin, and 8% (w/v) in glycerol. Crystalline DNase I was then added to a final concentration of 0.11 mg/ml. After thorough mixing and incubation at 22° for 30 min, the solution was dialyzed against three changes of buffer A (150 mM KCl, 100 mM potassium phosphate (pH 6.8), and 1 mM Na_3EDTA) to remove Mg^{2+}. A small amount of precipitate was usually observed after dialysis. This was removed by centrifugation at 0° and 11,000*g* for 1 hr. Glycerol was then added to the supernatant to a final concentration of 10% (w/v).

DEAE- and DNA-Cellulose Chromatography. A DEAE column (1.5 cm^2 × 11 cm) and a DNA-cellulose column (1.5 cm^2 × 5 cm) were connected in series and equilibrated with buffer B (buffer A with 10% (w/v) glycerol). The supernatant was run through the columns at a flow rate of ~4 ml/hr. After loading, 10 ml of buffer B was run through both columns, and the DEAE column was then disconnected. An additional 60 ml of buffer B was run through the DNA-cellulose column. The activity was then eluted with a single step of 1.5 M KCl, 100 mM potassium phosphate (pH 6.8), 1 mM Na_3EDTA, and 10% (w/v) glycerol. The bulk of the activity was eluted at the front of the high salt eluent and was contained in ~3 ml, with an average K^+ concentration of 0.6 M. The spectrum of this fraction indicated that it contained little nucleic acids. The absorbances at 230, 260, and 280 nm were 1.19, 0.24, and 0.21, respectively. This fraction was used in most of the subsequent studies. Storage of this fraction at 0° for 2 months or in liquid N_2 for 6 months showed little loss of activity.

Comments on the Purification Procedures. Since the homogenate of the eggs was first spun at 19,000*g*, most, if not all, of the nuclei were removed. Thus, the partially purified activity is probably mainly from the cytoplasm. Trial preparations were also done from the nuclei purified by differential sedimentation. A very similar if not identical activity was obtained.

Several procedures other than the DNase treatment were also tried for the removal of nucleic acids. Streptomycin precipitation was found to be unsatisfactory since no activity was recovered. Biphasic extraction with polyethylene glycol, as described by Alberts and Herrick (1971), was partially successful in that the activity was detected after the removal of polyethylene glycol. The removal of nucleic acids, however, was not as complete as the DNase procedure.

The DNA-cellulose column can be replaced by a phosphocellulose column. In either case when step gradients were used to elute the activity, it was usually found between 0.3 and 0.9 M K^+ as a rather broad band. The broadness might be a result of heterogeneity or the association of the activity with *Drosophila* nucleic acids undigested by DNase. If the DEAE column was omitted, the uv absorption spectrum of the activity after DNA-cellulose or phosphocellulose chromatography had the appearance of a nucleic acid, with a peak at 258 nm. In several cases, storage of this material at 0°, either in 0.4 M potassium phosphate (pH 6.5)–1 mM 2-mercaptoethanol, or in 0.02 M sodium phosphate (pH 7)–1 mM 2-mercaptoethanol, led to the formation of a white precipitate and loss of activity of the supernatant. Washing the precipitate with 0.02 M sodium phosphate (pH 7)–1 mM 2-mercaptoethanol gave no activity in the wash. A suspension of the washed precipitate, however, showed activity in the standard assays. Treatment of the precipitate with sodium dodecyl sulfate followed by polyacrylamide gel electrophoresis (Shapiro *et al.*, 1967; Weber and Osborn, 1969) gave a pattern not significantly different from that of the solution prior to the formation of the precipitate. The final fraction obtained by the procedure described appears to be stable with respect to precipitate formation. Thus, the precipitation phenomenon appears to be related to the formation of a protein–nucleic acid complex.

Gel Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Shapiro *et al.*, 1967; Weber and Osborn, 1969) of the partially purified activity gave six Coomassie Blue stained bands of roughly equal intensity. The molecular weights of the bands, obtained from their mobilities with lysozyme, pepsin, trypsin, and bovine plasma albumin as standards, were 13,000, 27,000, 39,000, 47,000, 58,000, and 103,000. Urea–acid gels were also run and the gels were stained with Amido Black as described by Panyim and Chalkley (1969a,b). Six bands of roughly equal intensity were also seen. Attempts to electrophorese the activity on nondenaturing acrylamide gels to identify which band was active were unsuccessful.

Results

Evidence That the Activity Is an ω Protein. The partially purified activity is capable of converting a superhelical DNA to a covalently closed form with essentially no superhelical turns under the reaction conditions. Results of a typical set of experiments are shown in Figure 1. Superhelical PM2 DNA samples, after incubation with the activity for various time intervals, were banded in CsCl gradients containing 254 μM ethidium bromide. It has been shown that at this concentration of dye, the position of a covalently closed DNA is dependent on its superhelical density in the absence of the dye (Gray *et al.*, 1971; Wang, 1973). This is illustrated by the banding pattern of a control sample shown in Figure 1a. The control sample contained two covalently closed DNA species: the negative superhelical PM2 DNA obtained from the mature phage, and a covalently closed PM2 DNA prepared *in vitro* by treating the DNA containing a few single chain scissions (introduced by DNase I) with *E. coli* ligase. The degree of superhelicity of a superhelical DNA can be expressed in terms of ν_c, the number of bound ethidium molecules per nucleotide needed for the removal of the superhelical turns.[1] In neutral 3 M

[1] The degree of twisting of a superhelical DNA is usually expressed by the superhelical density σ, the number of superhelical turns per ten base pairs. If the unwinding angle ϕ_c (in degrees) of the DNA helix due to the binding of an ethidium is known, σ is related to ν_c by $\sigma = -\phi_c \nu_c/18$. For an ethidium–DNA complex with ν bound ethidium per

CsCl at 20°, ν_c°, with the superscript specifying the temperature and the medium, is 0.075 for PM2 DNA from the mature virus and 0.009 for the particular PM2 DNA closed *in vitro*. As shown in Figure 1a, the PM2 DNA with ν_c° = 0.009 bands at a position downfield from that of the PM2 DNA with ν_c° = 0.075. The band to the left of the ν_c° = 0.075 DNA band is linear λ c1857S7 DNA added as a marker, and the shoulder to the left of the λ DNA band is PM2 DNA containing one or more single chain scissions per molecule, which was present in these covalently closed PM2 DNA samples. As shown in Figures 1b–h, treatment of the ν_c° = 0.075 DNA with the activity in the standard assay medium at 28° progressively shifts the band downfield, indicating a continual reduction in ν_c°. In other words, treatment of the highly negatively superhelical DNA with the activity results in a gradual reduction of the number of negative superhelical turns.

As discussed in detail for the *E. coli* ω protein, in order to show that the activity from *Drosophila* eggs introduces a transient swivel into a DNA, several additional criteria must be satisfied: (1) there is no concomitant change of the number of helical turns of the DNA double helix; (2) the removal of the superhelical turns is not due to the combined action of a conventional endonuclease, which hydrolyzes a phosphodiester bond to give a hydroxyl and a phosphoryl group, and a ligase, which catalyzes the re-formation of the bond.

Two experiments indicate that there is no detectable change in the number of helical turns of the DNA. Firstly, the circular dichroism of PM2 DNA, after treatment with the activity and subsequent deproteination by phenol extraction, is virtually superimposable with that of nicked PM2 DNA. This indicates that it is highly unlikely that there is a structural transition of the double helix, which causes a reduction of the helical turns. Secondly, the buoyant density θ of the treated DNA in CsCl, measured with *Micrococcus lysodeikticus* marker DNA (θ = 1.731 g/ml), is 1.7026 g/ml. In the same run, the buoyant density of covalently closed PM2 DNA containing essentially no superhelical turns, obtained by the ligase closure of nicked PM2 DNA at 8°, is 1.7029 g/ml. The difference in θ for these samples is within experimental error. Thus, the buoyant density measurements rule out the possibility that a number of protein molecules sufficient to cause a large change in the number of superhelical turns are irreversibly bound to the DNA.

As is the case with the *E. coli* ω protein, the activity from *Drosophila* eggs requires no dialyzable cofactor for the relaxation of a superhelical DNA. This observation suggests that the *Drosophila* activity, like the *E. coli* ω protein, is not due to the combined actions of a conventional endonuclease and a conventional ligase, since the joining of a phosphoryl and a hydroxyl pair by ligase requires a cofactor. This notion is further strengthened by two types of experiments. Firstly, it was found that the *Drosophila* activity could not convert PM2 DNA containing one single-chain scission (of the 5′-phosphoryl 3′-hydroxyl type) to the covalently closed form. Secondly, if two separate enzymes are responsible for the nicking and resealing, one might hope that a certain treatment of the activity might inhibit the sealing activity more than the nicking activity. The nicking activity could then be detected. Inhibition studies with heat treatment, sodium dodecyl sulfate, and ethidium bromide were all unsuccessful in preferentially inhibiting the sealing activity. Heating at 50° or higher for 5 min inactivates the activity. Similarly, sodium dodecyl sulfate inhibits the activity at a concentration between 0.03 and 0.3 mM. In neither case was a significant extent of nicking of the DNA detected. Inhibition by ethidium will be described in more detail in a later section.

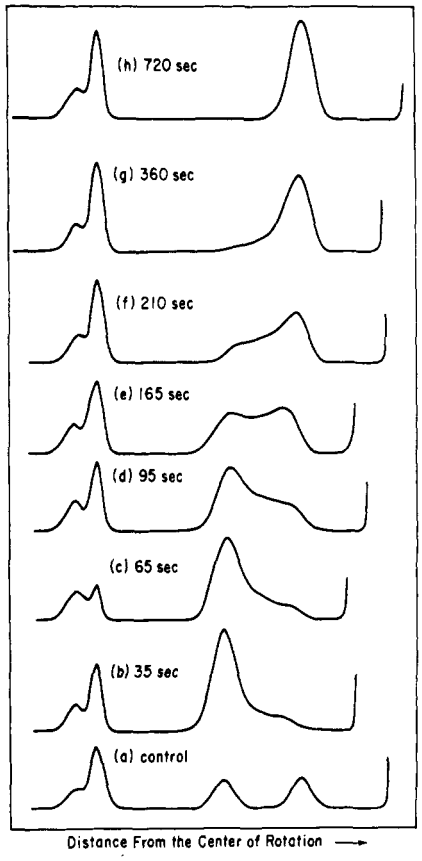

FIGURE 1: Equilibrium banding patterns in CsCl–ethidium gradients of PM2 DNA from the mature phage after incubation with the *Drosophila* activity for various times indicated in b–h. See text for a description of the control shown in a. Centrifugation was done at 39,460 rpm and 20°. Photoelectric tracings were taken at a wavelength of 340 nm after 48 hr. For a description of the technique, *cf.* Wang (1973).

Relaxation of Positively Twisted DNA. For the *E. coli* ω protein, it appears that the protein can only remove *negative* superhelical turns (Wang, 1971, 1973). On the other hand, the activity from mouse cells is capable of relaxing both negatively and positively twisted DNAs (Champoux and Dulbecco, 1972). To test whether the *Drosophila* activity has a preference for the sense of the superhelical turns, the following experiment was carried out. Ethidium bromide was added to a mixture containing roughly equal amounts of a PM2 DNA from the mature virus (ν_c° = 0.075) and a PM2 DNA covalently closed *in vitro* such that ν_c° = 0.005. To this mixture the partially purified *Drosophila* activity was added and aliquots were taken out at different times. The degrees of superhelicity of the two DNAs in each aliquot were examined by banding in CsCl–ethidium gradients. The amount of ethidium present in the reaction medium was such that if there was no topological constraint for the binding of ethidium to a covalently closed DNA, ν ethidium would be bound per nucleotide. If the *Drosophila* activity could remove *all* of the superhelical turns of a DNA irrespective of the *sense* of the twists, then

DNA molecule, $\sigma = -\phi_e(\nu_c - \nu)/18$. While ϕ_e has been generally taken as 12°, the recent unpublished results of J. C. W. indicate that ϕ_e is around 26°. Therefore, in order to avoid confusion, we express in this work the degree of superhelicity of a DNA by its ν_c value.

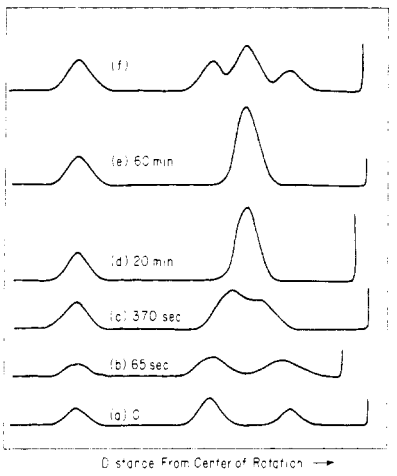

FIGURE 2: Banding patterns in CsCl–ethidium gradients of two covalently closed PM2 DNAs after treatment with the *Drosophila* activity in the presence of ethidium, as described in the text. The incubation mixture contained 15 μg/ml of PM2 DNA covalently closed *in vitro* by ligase (ν_c° = 0.009), 18 μg/ml of superhelical PM2 DNA from the mature phage (ν_c° = 0.075), and 12 μg/ml of PM2 DNA containing a few single-chain scissions per molecule. The total concentration of ethidium in the incubation mixture was 5.8 μM. Incubation was done at 28° in the standard assay medium. Aliquots were taken out at times shown in a–e of the figure, mixed with a stock solution containing CsCl and ethidium, and banded in an analytical ultracentrifuge as described in the legend of Figure 1. f is the tracing obtained for an equal volume mixture of samples after incubation with the activity for 0 sec (a) and 60 min (e).

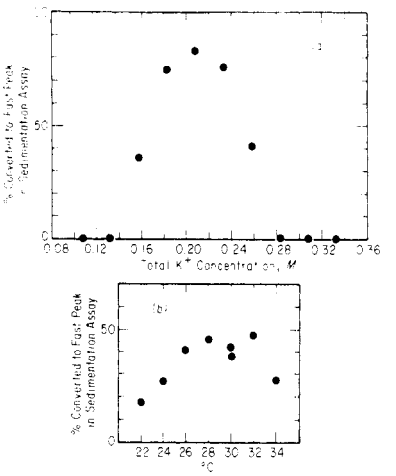

FIGURE 3: Percent of PM2 DNA (from the mature phage) converted to the fast sedimenting form at 20° in neutral 3 M CsCl containing 15 μM ethidium: (a) all incubations were done at 26° in media containing 0.022 M potassium phosphate buffer (pH 6.8) and varying amounts of KCl; (b) each assay mixture was incubated for 15 min at the temperature shown.

at the end of the reaction there would be ν bound ethidium per nucleotide for both DNAs. The ν_c° value of the product would be equal to $\nu + a$, where a is a constant to account for the difference in salt and temperature between the reaction conditions and the conditions for the measurement of ν_c° (Wang, 1969). In other words, the degrees if superhelicity of the two DNAs after treatment with the activity would be the same.

As depicted in Figure 2, this is indeed the case. The amount of ethidium in the ω reaction medium was chosen such that $\nu + a \simeq 0.04$. Since $0.005 < \nu + a < 0.075$, before the reaction the ν_c° = 0.005 DNA was positively twisted and the ν_c° = 0.075 DNA was negatively twisted. It can be seen from the tracings shown in Figure 2 that, as the reaction proceeded, both DNAs were converted to a new species with the same ν_c° value. This shows that the *Drosophila* activity can relax both negative and positive superhelical DNAs. Furthermore, within the resolution of the CsCl–ethidium gradient centrifugation technique, it appears that no residual superhelical turns were present in either DNA in the enzyme reaction mixture, since otherwise the products would have shown different degrees of superhelicity.

The last notion is confirmed, at least for a negatively superhelical DNA, by the following experiment. Covalently closed PM2 DNA from the mature virus was treated with the activity at 27°. After phenol extraction, the DNA was dialyzed back into the ω reaction medium and the sedimentation coefficient of the DNA was measured at 27° by boundary sedimentation. In the same centrifugation run, the sedimentation coefficient of PM2 DNA containing a few single-chain scissions per molecule was also measured. The sedimentation coefficients were found to be the same, indicating that under the ω reaction conditions no superhelical turns were present in the covalently closed DNA after the reaction.

Optimal Reaction Conditions. As described in the section on Experimental Procedures, for PM2 DNA from the mature phage, a reduction of the number of negative superhelical turns causes an increase in its sedimentation coefficient at 20° in neutral 3 M CsCl containing 15 μM ethidium. Figure 3a depicts the per cent of PM2 DNA converted to the faster sedimenting species, by incubating with a fixed amount of the *Drosophila* activity at 26° for 15 min, as a function of the salt concentration. Maximal amount of conversion was observed at ~0.21 M K^+. While no quantitative measurements were performed, it was found that the substitution of K^+ with Na^+ or NH_4^+ had no gross effect on the reaction. Addition of 2 mM Mg^{2+} to the 0.21 M K^+ had little effect. No activity was detectable in 0.1 M $MgCl_2$ or 0.1 M $CaCl_2$. In the medium usually used for the assay of the *E. coli* ω protein, which contains 0.01 M Tris (pH 8), 0.002 M $MgCl_2$, and 0.001 M Na_3EDTA, virtually no activity of the *Drosophila* activity is detectable. Addition of K^+ to this medium to ~0.2 M greatly increases the activity of the *Drosophila* ω. In contrast, the addition of salt to several tenths mole per liter is inhibitory for the *E. coli* ω.

As shown in Figure 3b, the temperature optimum for the *Drosophila* ω is ~30°. We have not tested the optimal pH for this activity. In the narrow pH range of 6.5–7.5, no effect on the activity was observed.

Inhibition by Ethidium. It has been suggested previously for the *E. coli* ω that the protein introduces a swivel into the DNA helix by generating a transient nick, with the protein directly attached to one of the groups at the nick. The reaction is reversible, and the dissociation of the protein from the end group is accompanied by the re-formation of the backbone bond (Wang, 1971, 1973). Past attempts to demonstrate *directly* the existence of such a transient species have been unsuccessful. Since the *Drosophila* activity, unlike the *E. coli* activity, can relax positively twisted DNA, we have attempted to use the inhibitory effect of ethidium on this activity to obtain the transient intermediate. For a positively twisted DNA in the presence of ethidium, the amount of bound ethidium ν per nucleotide is expected to increase, if a swivel is introduced into the DNA. It was

therefore hoped that in a range such that the degree of inhibition was sensitive to ν, the increase in ν for the transient species with a nick would inhibit the closure step. Reaction mixtures containing different amounts of ethidium, 20 μg/ml of a PM2 DNA covalently closed *in vitro* with a ν_c° of 0.009, and constant amounts of the *Drosophila* activity were incubated at 28° for 20 min and then examined by band sedimentation after stopping the reaction. No activity was detectable when the ethidium concentration in the ω reaction mixture was 38 μM. At an ethidium concentration of 25 μM, ~50% of the DNA–ethidium complex in the reaction mixture showed a reduction in the number of positive superhelical turns. No significant increase in the amount of nicked species was observed, however, at this ethidium concentration. Thus, we were unable to demonstrate directly the existence of a transient nicked species by ethidium inhibition.

Discussion

Since the isolation of the first ω protein from *E. coli* (Wang, 1971), a number of such activities have been detected or purified from several higher organisms, including secondary mouse cells (Champoux and Dulbecco, 1972; Vosberg *et al.*, 1974), KB cells (W. Keller, personal communication), and fertilized and unfertilized sea urchin eggs (J. C. Wang, R. Poccia, and D. Mazia, unpublished results). The partially purified activity from *Drosophila* eggs is more similar to the activities from other higher organisms than to the *E. coli* ω in two respects: (1) it causes the complete removal of both positive and negative superhelical turns; (2) it does not require Mg^{2+} or any other divalent ion, but requires ~0.2 M monovalent ion in order to function efficiently.

It has been suggested previously that the specificity of the *E. coli* ω protein for negatively twisted DNA is linked to the destabilizing effect of the double helix by such twists (Wang, 1971, 1973). For example, the binding of ω to the DNA helix preceding its catalytic function might require the disruption of several base pairs. Since a negatively twisted DNA favors the disruption of base pairs relative to the same DNA with positive superhelical turns or no turns (Bauer and Vinograd, 1970; Davidson, 1972; Botchan *et al.*, 1973; Wang, 1974), the binding of ω would be much enhanced by the presence of negative superhelical turns. An analogous case is the core RNA polymerase from *E. coli*. While this enzyme is virtually inactive on PM2 DNA containing no superhelical turns, it can synthesize RNA efficiently on PM2 DNAs containing a significant number of superhelical turns (Wang, 1974).

As the tracings depicted in Figure 2 suggest, the rate of reduction of the number of superhelical turns by the *Drosophila* activity is not grossly different for negatively and positively twisted DNAs. This in turn suggests that the binding of the *Drosophila* ω protein to the DNA double helix involves little disruption of base pairing, if the rate is dependent on the binding affinity.

The *in vivo* functions of ω proteins remain unknown. Processes such as replication, transcription, recombination, condensation, and decondensation of DNA may require, or may be promoted, by such an activity. In short, any process which involves a winding or unwinding of the double helix might be assisted by an ω protein. The significance of the differences between the *E. coli* ω and the other activities from higher organisms might emerge with the elucidation of the *in vivo* functions of such proteins.

Acknowledgment

We thank Dr. John Hearst for kindly providing us with the *Drosophila* eggs, Drs. James Allen and Carl Hanson for their instructions on the care and handling of *Drosophila*, and Mrs. Marian Malone for her assistance in the collection of the eggs.

References

Alberts, B., and Herrick, G. (1971), *Methods Enzymol. 21,* 198.

Bauer, W., and Vinograd, J. (1970), *J. Mol. Biol. 47,* 419.

Botchan, P., Wang, J. C., and Echols, H. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 3077.

Champoux, J. J., and Dulbecco, R. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 143.

Davidson, N. (1972), *J. Mol. Biol. 66,* 307.

Espejo, R. T., and Canelo, E. S. (1968), *Virology 34,* 738.

Espejo, R. T., Canelo, S., and Sinsheimer, R. L. (1969), *Proc. Nat. Acad. Sci. U. S. 63,* 1164.

Gray, H. B., Upholt, W. B., and Vinograd, J. (1971), *J. Mol. Biol. 62,* 1.

Kriegstein, H. J., and Hogness, D. S. (1974), *Proc. Nat. Acad. Sci. U. S. 71,* 135.

Lewis, E. B. (1960), *Drosophila Inform. Serv. 34,* 117.

Litman, R. M. (1968), *J. Biol. Chem. 243,* 6222.

Olivera, B. M., and Lehman, I. R. (1967), *Proc. Nat. Acad. Sci. U. S. 57,* 1426.

Panyim, S., and Chalkley, R. (1969a), *Arch. Biochem. Biophys. 130,* 337.

Panyim, S., and Chalkley, R. (1969b), *Biochemistry 8,* 3972.

Rabinowitz, M. (1941), *J. Morphol. 69,* 1.

Shapiro, A. L., Vinuela, E., and Maizel, J. V., Jr. (1967), *Biochem. Biophys. Res. Commun. 28,* 815.

Vosberg, H. P., Grossman, L. I., and Vinograd, J. (1974), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 33,* 1356, Abstr. No. 751.

Wang, J. C. (1969), *J. Mol. Biol. 43,* 25.

Wang, J. C. (1971), *J. Mol. Biol. 55,* 523.

Wang, J. C. (1973), *in* DNA Synthesis *in Vitro,* Wells, R. D., and Inman, R. B., Ed., Baltimore, Md., University Park Press, p 163.

Wang, J. C. (1974), *J. Mol. Biol.* (in press).

Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.