Purification and Characterization of the DNA Untwisting Enzyme from Rat Liver†

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ABSTRACT: The DNA untwisting enzyme has been purified approximately 300-fold from rat liver nuclei. The protein is greater than 90% pure as judged by sodium dodecyl sulfate–acrylamide gel electrophoresis. The native enzyme has a molecular weight between 64 000 and 68 000 and is composed of a single polypeptide chain. Evidence is presented that the protein can act catalytically. A trace amount of endonuclease activity associated with the most pure fraction could be a contaminant or it could be due to the action of the DNA untwisting enzyme itself.

We described an activity found in nuclear extracts of mouse cells, which has the capability of completely relaxing both negatively and positively supertwisted DNA circles (Champoux and Dulbecco, 1972). This activity was termed the DNA untwisting enzyme. A similar activity from *Escherichia coli* had been described and named the ω protein (Wang, 1971).[1] However, unlike the eucaryotic activity, the procaryotic enzyme could only partially remove negative superhelical turns and was unable to remove positive superhelical turns. Subsequently, the eucaryotic activity has been isolated and in some cases partially purified from a variety of cell types, including human KB cells (Keller and Wendel, 1974; Keller, 1975), fertilized *Drosophilia* eggs (Baase and Wang, 1974), HeLa and mouse L cells (Vosberg et al., 1975; Vosberg and Vinograd, 1975), rat liver (Champoux and Durnford, 1975), and calf thymus (Pulleyblank and Morgan, 1975).

Given the effects of these enzymes on circular DNAs, one must postulate that they act on DNA to introduce a transient nick in one strand of the duplex. During the period of time when the DNA is nicked, one strand of the helix is free to rotate relative to the other strand. The free energy associated with the superhelical turns in the closed molecule (Bauer and Vinograd, 1970) provides the driving force for the relaxation process.

In spite of the evidence that these activities must reversibly nick double-stranded DNA, there is no direct evidence for the existence of a nicked intermediate. Moreover, there is no indication as to how the enzyme stores the energy required to reseal the nick. As a prelude to our studies on the mechanism of the DNA untwisting reaction, we undertook the purification and characterization of the enzyme from rat liver nuclei.

Experimental Procedures

Materials

General. Phosphocellulose (P11) was obtained from Whatman Biochemicals, Ltd. Carboxymethyl-Sephadex (C-50) and Sephadex G-150 were purchased from Pharmacia Fine Chemicals. Poly(ethylene glycol) 6000 was obtained from J. T. Baker Co. Acrylamide, *N,N'*-methylenebisacrylamide, and sodium dodecyl sulfate were obtained from Eastman Kodak Co., and Temed[2] and ammonium persulfate were from Bio-Rad Laboratories. Dithiothreitol was purchased from Sigma Chemical Co. Type B6 nitrocellulose filters (13 mm, 0.45 μm) were purchased from Schleicher and Schuell, Inc.

Proteins. Bacterial alkaline phosphatase (BAPF) was obtained from Worthington Biochemical Corp. Bovine serum albumin and ovalbumin were obtained from Schwarz/Mann and cytochrome *c* and chymotrypsinogen from Calbiochem.

Buffers. TKM is 50 mM Tris-HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$. Column buffer is 1 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol containing variable amounts of potassium phosphate buffer (pH 7.4). The stated KPO_4 concentration refers to the concentration of PO_4^{3-}. The K^+ concentration is equal to 1.67 times the PO_4^{3-} concentration. Assay buffer is 0.39 M Na_3PO_4, 0.21 M NaH_2PO_4, 1 mM EDTA, pH 11.1.

Methods

General. The preparation of ^3H-labeled SV40 DNA and the procedure for the equilibrium centrifugation of DNAs in CsCl gradients containing propidium diiodide have been described previously (Champoux and McConaughy, 1975). Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard. The protein samples were precipitated with 5% Cl_3CCOOH to remove the dithiothreitol prior to carrying out the protein determination. The phosphate concentration in column fractions was determined by measuring the conductivity (Radiometer CDM3) of aliquots which had been diluted $1/10$ with H_2O. Protein fractions were concentrated in the cold room by pressure filtration in an Amicon stirred cell (Model 52) equipped with a PM10 membrane filter. Sodium dodecyl sulfate–acrylamide gel electrophoresis was carried out according to the procedure of Weber et al. (1972).

Assays for the DNA Untwisting Enzyme. A preliminary account of the DNA filter binding assay and the fluorometric assay has been published (Champoux and Durnford, 1975).

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[1] Other workers have since referred to the eucaryotic enzyme activity as a DNA-relaxing enzyme (Keller, 1975), a nicking-closing enzyme (Vosberg and Vinograd, 1975) and an ω protein (Baase and Wang, 1974; Pulleyblank and Morgan, 1975).

[2] Abbreviations used are: PEG, poly(ethylene glycol); TKM, 50 mM Tris-HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$; Tris,tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Temed, *N,N,N',N'*-tetramethylethylenediamine.

The diluent for the enzyme contained 20 mM KPO_4 (pH 7.4), 1 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol and 100 μg/ml bovine serum albumin.

Filter Assay. The standard reaction mixture (final volume 0.10 ml) contained 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.20 M KCl, and 2 μg/ml ^{3}H-labeled SV40 DNA. Twofold serial dilutions of the fraction to be assayed were prepared and the reactions initiated by the addition of 10 μl of the diluted enzyme. After 10 min at 37 °C, the reactions were stopped by the addition of 2 ml of assay buffer (0.39 M Na_3PO_4, 0.21 M NaH_2PO_4, 1 mM EDTA, pH 11.1). We have previously shown that superhelical SV40 DNA, presumably owing to its partial single-stranded nature, is retained by nitrocellulose filters when the filtration is carried out in the assay buffer. However, under these same conditions the relaxed closed circular product DNA is not partially single stranded and is only poorly retained by the filter (Champoux and Durnford, 1975). The filters were presoaked in H_2O and rinsed once with 2 ml of assay buffer before filtering the sample from the reaction. The filters were rinsed once more with 2 ml of assay buffer, dried, and counted in a toluene based scintillation fluid. Figure 1b shows a typical dose–response curve for the filter assay. In this experiment 100% of the substrate DNA was retained by the filter whereas only 26% of the product DNA was retained. One filter unit of enzyme is defined as the amount which results in binding midway between that of the substrate and the product.

Fluorometric Assay. We have previously shown that the DNA untwisting enzyme will remove the positive superhelical turns which are generated in a covalently closed circular DNA by the addition of excess ethidium bromide (Champoux and Dulbecco, 1972). The consequence of the removal of positive turns is that the amount of ethidium bromide bound during the course of the reaction increases. Since ethidium bromide forms a fluorescent complex when it binds to DNA (LePecq and Paoletti, 1967), the course of the reaction can be followed by observing the increase in fluorescence as a function of time (Champoux and Durnford, 1975). The enzyme reaction (final volume 0.50 ml) contained 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.20 M NaCl, 4 μg/ml SV40 DNA, and 4 μg/ml ethidium bromide. The reaction was carried out in a cuvette in an Aminco-Bowman fluorimeter equilibrated at 37 °C. The wavelength for excitation was 340 nm and the wavelength of emission was 586 nm. The time course of the reaction was monitored on a recorder attached to the fluorimeter. The rate of the reaction was determined from the time required for one-half the overall fluorescent change. Figure 1a shows that the log of the halftime for the reaction is linearly related to the log of the enzyme concentration. One fluorometric unit is arbitrarily defined as the amount of enzyme which gives a halftime of 1.0 min. The fluorometric assay, although rapid and convenient, is only suitable for assaying fractions free of DNA. For this reason, the filter assay is used to quantitate the enzyme activity through the early steps of the purification.

Isolation of Rat Liver Nuclei. Frozen rat livers were pulverized and 100-g portions were homogenized in a Waring blender in 400 ml of TKM plus 0.25 M sucrose. The homogenate was filtered through cheesecloth and the nuclei were pelleted by centrifugation at 6000*g* for 10 min. The crude nuclear pellet was washed twice with TKM plus 0.25 M sucrose and the procedure of Blobel and Potter (1966) followed for the remainder of the nuclear isolation.

Sucrose Gradient Centrifugation. The enzyme samples were layered over a 5–20% sucrose gradient containing 20 mM KPO_4 (pH 7.5), 1 mM EDTA, and 0.5 mM dithiothreitol. The gradients were centrifuged in the Beckman SW56 rotor for 14 h at 54 000 rpm and 5 °C.

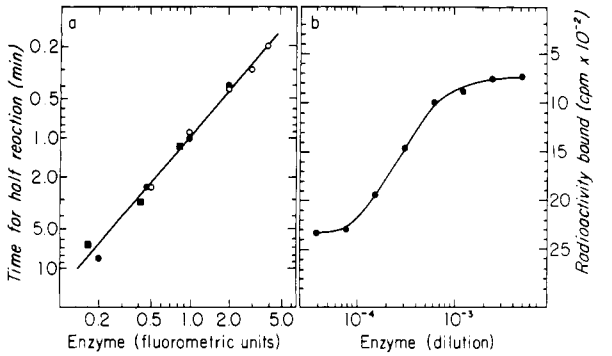

FIGURE 1: Dose–response relationships for the fluorometric and filter assays. (a) The fluorometric assay was carried out as described under Methods with the addition of varying amounts of enzyme from the pooled CM50 fraction (■), the concentrated CM50 fraction (○), and the concentrated G-150 fraction (●). The graphs were aligned by defining one fluorometric unit as the amount of enzyme which yields a halftime for the fluorescent change of 1 min. The amount of each fraction which corresponded to one fluorometric unit was 6, 1, and 5 μl for the pooled CM50, concentrated CM50, and concentrated G-150, respectively. (b) The filter assay was carried out as described under Methods (final volume 100 μl) with the addition of a constant volume (10 μl) of the pooled phosphocellulose fraction which had been twofold serially diluted as indicated. One filter unit of enzyme is defined as the amount which, under these conditions, results in the binding of the DNA to the filter at the midpoint between the amount of substrate bound and the amount of product bound. In this experiment 2290 cpm of the substrate DNA was bound by the filter, which corresponds to 100% of the input DNA.

Isolation of Single-Stranded DNA Rings. ^{3}H-Labeled SV40 DNA was allowed to accumulate breaks due to radioactive decay until approximately 40–50% of the molecules were nicked. The nicked circles were purified from the closed circles by centrifugation in CsCl–ethidium bromide gradients as described previously for the purification of SV40 DNA (Champoux and McConaughy, 1975). The nicked DNA was layered over a 5–20% alkaline sucrose gradient containing 1 mM EDTA, 0.25 M NaOH, and 0.75 M NaCl and centrifuged in the SW41 rotor at 30 000 rpm for 18 h at 18 °C. The fractions containing the 18S rings were pooled and dialyzed against 10 mM Tris-HCl (pH 7.5)–1 mM EDTA.

Nuclease Assays. Nuclease assays were carried out under two different reaction conditions employing either native SV40 DNA or single-stranded rings of SV40 DNA as substrates. Assays were performed under the standard reaction condition for the DNA untwisting enzyme [20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.20 M KCl] and under conditions more appropriate for detection of contaminating nucleases [20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 50 mM NaCl]. The extent of nicking of native SV40 DNA was monitored by centrifuging the products of the reaction in 5–20% alkaline sucrose gradients (see ring isolation) in the SW56 rotor for 75 min at 55 000 rpm and 18 °C. The nicking of single-stranded DNA as measured by the conversion of 18S rings to 16S linears or smaller fragments was monitored by alkaline sucrose gradient centrifugation under the same conditions except the time of centrifugation was extended to 2.5 h.

Results

Purification of DNA Untwisting Enzyme

In order to minimize losses of activity due to the instability of the enzyme, the entire procedure was carried out from beginning to end without interruption. All operations were carried out at 0–4 °C.

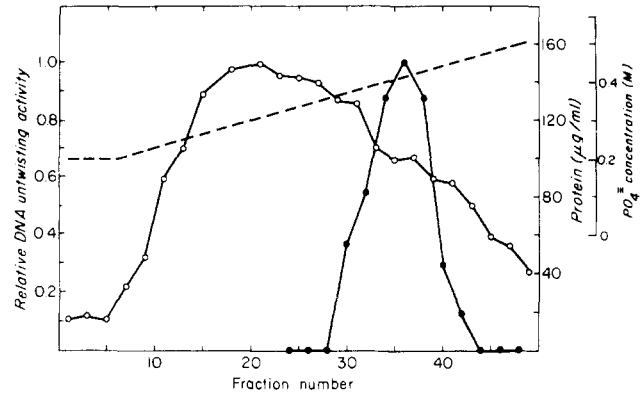

FIGURE 2: Phosphocellulose chromatography. Fraction II was chromatographed on phosphocellulose as described in the text. Each fraction contained 2.7 ml. The column was eluted with a linear gradient (shown by dashed line) from 0.20 to 0.60 M KPO_4 in column buffer. The enzyme activity (●) was determined using the fluorometric assay. Alternate fractions across the column were assayed for protein (○). The flow through plus wash fractions (not shown) contained 67% of the input protein and none of the activity.

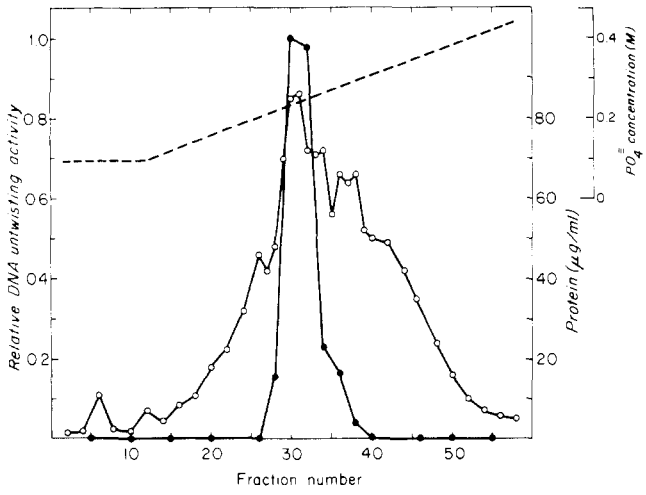

FIGURE 3: Carboxymethyl-Sephadex chromatography. Fraction III was dialyzed and chromatographed on carboxymethyl-Sephadex (C-50) as described in the text. Each fraction contained 1.3 ml. The column was eluted with a linear gradient from 0.10 to 0.60 M KPO_4 in column buffer. The enzyme activity (●) was determined using the fluorometric assay. Alternate fractions across the column were assayed for protein (○). The flow through plus wash fractions (not shown) contained 37% of the protein and none of the activity.

Crude Nuclear Extract. The isolated nuclei (see Methods) were washed in TKM and centrifuged at 400*g* for 10 min in a graduated conical centrifuge tube. The nuclei were resuspended in TKM to a volume equal to three times the volume of the nuclear pellet (final volume 20–40 ml). EDTA was added to a final concentration of 10 mM and the nuclei were lysed by the slow addition, with stirring, of an equal volume of 80 mM Tris-HCl (pH 7.5), 2 mM EDTA, 1 mM dithiothreitol, 2 M NaCl, 20% glycerol. The viscous solution was stirred gently for 5 min to yield fraction I.

Poly(ethylene glycol) Precipitation. The DNA in the nuclear extract was removed by a modification of the PEG precipitation method described by Alberts and Herrick (1971). To fraction I, 0.5 volume of 40 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.5 mM dithiothreitol, 1 M NaCl, 10% glycerol, and 18% PEG was added slowly, with stirring. The mixture was stirred for 40 min and centrifuged at 17 000*g* for 20 min. The supernatant is referred to as fraction II.

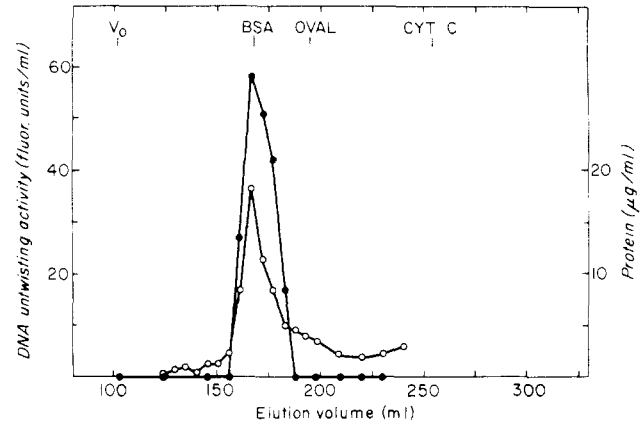

FIGURE 4: Sephadex G-150 gel filtration. Fraction IV was chromatographed on a Sephadex G-150 column as described in the text. Fractions containing 5.3 ml were collected and assayed for protein (○) and DNA untwisting activity (●). The void volume as determined with blue Dextran 2000 was 102 ml. Small molecules which are included in the gel elute at a volume of 318 ml so the internal volume (V_i) is 216 ml. The peak positions of the proteins used to calibrate the column are indicated. The molecular weights of the standard proteins, bovine serum albumin (BSA), ovalbumin (OVAL), and cytochrome *c* (CYT C), were taken from Weber et al. (1972).

Phosphocellulose Chromatography. Fraction II was dialyzed against several changes of column buffer containing 0.20 M KPO_4. The very fine precipitate which appears during dialysis was removed by centrifugation at 17 000*g* for 15 min. The supernatant was added to a phosphocellulose column (2.5 × 4 cm) which had been equilibrated with column buffer plus 0.20 M KPO_4. The column was washed with 100 ml of the same buffer and the protein eluted with a linear gradient from 0.20 to 0.60 M KPO_4 in 150 ml of column buffer. The protein concentration and enzyme activity profiles are shown in Figure 2. The fractions containing enzyme activity were pooled to give fraction III.

Carboxymethyl-Sephadex Chromatography. Fraction III was dialyzed against column buffer containing 0.10 M KPO_4 and loaded onto a carboxymethyl-Sephadex (C-50) column (1.6 × 9 cm) which had been equilibrated with the same buffer. The column was washed with 40 ml of this buffer and the protein eluted with a linear gradient from 0.10 to 0.60 M KPO_4 in 100 ml column buffer (Figure 3). The fractions containing enzyme activity were pooled and concentrated by Amicon pressure filtration (PM10 filter) in a stirred cell to give fraction IV.

Sephadex G-150 Gel Filtration. The glycerol concentration in fraction IV was increased to 25% and the sample layered over a Sephadex G-150 column (2.5 × 64 cm) which had been equilibrated with column buffer containing 70 mM KPO_4. The column was run at a flow rate of 9.5 ml/h. The protein concentration and enzyme activity profiles are shown in Figure 4. The fractions containing enzyme activity were pooled and concentrated by Amicon filtration as described above. The recovery on concentration was always quantitative. The concentrated G-150 fraction (fraction V) was stored at 0 °C for periods of several weeks or for longer periods of time in sealed tubes in liquid nitrogen.

The Sephadex G-150 chromatography removes most if not all of the extraneous nuclease activity still present in fraction IV (see below). Since the nuclease activity was found to elute from the G-150 column between 200 and 250 ml, it is advisable to exclude from the G-150 pool any of the trailing fractions containing less than about 10% of the total activity.

TABLE I: Purification Summary.

Fraction	Volume (ml)	Total Protein (mg)	Total DNA Untwisting Act. Filter Assay (Filter Units)	Fluor. Assay (Fluor. Units)	Yield (%)	Spec Act. (Fluor. Units/mg)
I. Nuclear extract[a]	100	644	3.8×10^7		100	15[b]
II. Poly(ethylene glycol) supernatant	136	134	2.6×10^7		68	49[b]
III. Pooled phosphocellulose	26.2	4.3	1.1×10^7	2748	28	641
IV. Concd carboxymethyl-Sephadex (C-50)	2.5	0.96		2528	26	2625
V. Concd Sephadex G-150	3.5	0.24		1074	11	4545

[a] The nuclear extract was prepared from 3×10^{10} nuclei which were obtained from 418 g of rat liver. [b] For ease of comparison, the specific activities of fractions I and II are expressed in fluorometric units rather than filter units. The conversion factor between the two assays is 4000.

The summary of a typical purification is presented in Table I.

Characterization of the DNA Untwisting Enzyme

Purity. The purity of the protein was assessed by electrophoresis in acrylamide gels containing sodium dodecyl sulfate (Figure 5). One major protein band was evident which migrated with a mobility very close to the bovine serum albumin standard. A second, minor band, was present in all preparations which migrated between bovine serum albumin and ovalbumin and which may correspond to the shoulder of the protein peak on the Sephadex G-150 column. If one assumes equal staining of the proteins by the dye, one can estimate their relative concentrations from the areas under the peaks in the tracing of the gel. The proportion of total protein represented by the minor band varied from 17% to the value of 4% found for our most pure preparation shown in Figure 5.

Fraction V enzyme was assayed for endonucleolytic activity on both double-stranded SV40 circles and single-stranded SV40 rings (see Methods). Under the normal enzyme assay conditions (20 mM Tris-HCl, 1 mM EDTA, 0.20 M KCl), no nuclease activity was detectable on either substrate during a 30-min incubation at 37 °C with as much as 4 μg/ml of fraction V protein. This amount of enzyme corresponds to more than 1000 times the amount required to completely untwist the substrate DNA under these same conditions. The same amount of enzyme yielded a small, but reproducible amount of nicking on native DNA when the assays were carried out in 20 mM Tris (pH 7.5), 5 mM $MgCl_2$, and 50 mM NaCl.[3] There was a 5% conversion of native closed circular SV40 DNA to the open nicked form in 30 min at 37 °C under these conditions. A similar reaction with 2 μg/ml of fraction V gave 41% nicking of single-stranded rings. This residual nuclease activity may be due to traces of a contaminant still present in fraction V or possibly due to the action of the untwisting enzyme itself (see Discussion).

Molecular Weight. The molecular weight of the native enzyme has been estimated by two methods. From the behavior of the enzyme on a Sephadex G-150 column in relation to the standard proteins (Andrews, 1964), the apparent molecular weight was found to be 64 000 ± 3000 (Figure 4). The sedimentation coefficient of the purified enzyme in sucrose (data not shown) was determined relative to bacterial alkaline phosphatase which has a sedimentation coefficient of 6.1 (Schlesinger and Barrett, 1965). The enzyme activity sedimented with an *s* value of 4.4, which was slightly slower than bovine serum albumin (4.5 S) sedimented in the same tube. Assuming the enzyme is a globular protein (Martin and Ames, 1961), and taking the molecular weight of bovine serum albumin as 68 000, the molecular weight of the enzyme was estimated to be 66 000 ± 3000.

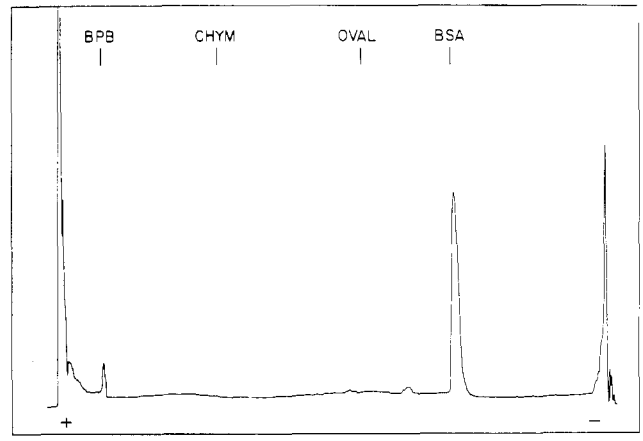

FIGURE 5: Sodium dodecyl sulfate-acrylamide gel electrophoresis of purified DNA untwisting enzyme. Fraction V (1.3 μg of protein) was run in a 10% acrylamide slab gel containing sodium dodecyl sulfate as described under Methods. Migration is from right to left with the position of the tracking dye (bromophenol blue, BPB) indicated at the left side. The gel was stained with Coomassie brilliant blue and, after destaining, scanned with a Joyce-Loebl microdensitometer. The positions of the molecular weight standards in the adjacent wells of the same gel are indicated. Chymotrypsinogen (CHYM) was used as a molecular weight standard in addition to the other standards described in Figure 4.

The polypeptide chain molecular weight was determined by electrophoresis in acrylamide gels containing sodium dodecyl sulfate (Weber et al., 1972) (Figure 5). Prior to the electrophoresis, the samples were denatured and reduced by boiling in a sodium dodecyl sulfate buffer in the presence of β-mercaptoethanol. From the average mobility of the major protein peak relative to the indicated standards run in parallel, the molecular weight of the protein was calculated to be 68 000 ± 3000. From these results we conclude that the enzyme is likely to be composed of a single subunit with a molecular weight between 64 000 and 68 000.

Stoichiometry. The total untwisting capacity of a single protein molecule was tested by carrying out the usual reaction at a high DNA concentration (230 μg/ml) for 20 h. The proportion of the molecules untwisted was measured by banding the products to equilibrium in CsCl–propidium diiodide gradients (Gray et al., 1971). At a ratio of 90 DNA molecules to one protein molecule, the DNA was completely untwisted. At higher ratios, a fraction of the DNA contained a number of superhelical turns which was intermediate between the sub-

[3] The DNA untwisting enzyme requires 0.20 M K^+ or Na^+ in the absence of Mg^{2+}, but is fully active in 50 mM NaCl in the presence of 5 mM Mg^{2+}.

strate and the completely untwisted product. In one case, the ratio of DNA molecules which were at least partially untwisted to enzyme molecules was 490. Therefore, in agreement with others (Keller, 1975; Vosberg and Vinograd, 1975), we find that the enzyme does indeed act catalytically.

Discussion

The DNA untwisting enzyme has been purified approximately 300-fold to yield a protein which is greater than 90% pure as assessed by electrophoresis in sodium dodecyl sulfate-acrylamide gels. The activity we have purified from isolated nuclei appears to represent essentially all of the cellular DNA untwisting enzyme since less than 1% was found associated with the postmitochondrial fraction of the cytoplasm (Young and Champoux, unpublished observation). The low yield (~11%) probably results from inactivation of the enzyme during the course of the purification since we observed similar losses of activity whenever the fractions at the intermediate stages of the purification were stored for a few days at 5 °C. We could find no evidence for minor forms of the enzyme which showed chromatographic properties different from the form we have purified.

If one assumes that the low yield results from inactivation during purification and that the most pure fraction contains no inactive enzyme, then from the specific activity of the crude extract one can calculate that each nucleus contains approximately 600 000 molecules of the enzyme (see Table I). This corresponds to one enzyme molecule for every 8000 base pairs of DNA in a diploid rat cell. Alternatively, if one assumes that all the inactivated protein copurifies with the active fraction, then one arrives at a minimum value of 70 000 enzyme molecules per nucleus or one enzyme for every 70 000 base pairs of DNA.

The molecular weight of the native rat liver enzyme was found to be approximately 66 000 which is close to the value of 60 000 estimated for the enzyme purified from human KB cells (Keller, 1975). The molecular weight of the native enzyme from mouse LA9 cells was estimated to be 75 000 to 80 000 by chromatography on Sephadex G-150 using bacterial alkaline phosphatase (molecular weight 86 000) as a standard (Vosberg and Vinogard, 1975). However, the apparent molecular weight of alkaline phosphatase as determined by gel filtration is considerably less than 86 000 (Andrews, 1964). In our experience, bacterial alkaline phosphatase chromatographed on G-150 with an apparent molecular weight of approximately 70 000. Taking this into consideration it would appear that the native form of the mouse cell enzyme has a molecular weight similar to the values found for the KB cell and rat liver cell enzymes. In support of this conclusion is the fact that the mouse cell enzyme, like the rat liver enzyme, sediments with an *s* value of 4.4 (Vosberg et al., 1975).

In spite of the similarities in the sizes of the native enzymes isolated from different sources, there appear to be some differences with respect to the subunit composition of the various enzymes. Whereas the enzyme from rat liver cells and KB cells (Keller, 1975) is composed of a single polypeptide chain, it has been reported that the mouse cell enzyme contains two 37 000 molecular weight subunits (Vosberg et al., 1975). The reason for this discrepancy is unknown.

The reaction carried out by the DNA untwisting enzyme must involve the introduction of a transient nick into duplex DNA. Therefore, we expect that at any given instant during the untwisting reaction at least a small fraction of the DNA substrate should exist in the form of a nicked intermediate. Under the standard reaction conditions, we have not yet been able to detect any nicking of the native substrate DNA (<3%) even at very high enzyme concentrations. In the presence of Mg^{2+} and low salt, we do detect a small amount of nicking of the substrate DNA. Under these latter conditions, single-stranded circular DNA was nicked to an even greater extent. Further experimentation will be required to determine whether the observed nicking is due to the DNA untwisting enzyme or to a small amount of a contaminating endonuclease in our most pure preparations.

Acknowledgments

We thank Patricia Bedinger for technical assistance.

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