A Deoxyribonucleic Acid Unwinding Protein Isolated from Regenerating Rat Liver

PHYSICAL AND FUNCTIONAL PROPERTIES*

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A DNA-unwinding protein has been purified from regenerating rat liver cytosol to apparent homogeneity. The protein is present in about 10⁵ copies per cell. It is a tetramer, composed of 25,000-dalton subunits which does not exhibit enzymatic activity for ATPase, DNA polymerase, or DNase. The protein is able to unwind the double helix of poly[d(A-T)], depressing the melting point of this synthetic polymer by about 40°C. It also binds to supercoiled SV40 DNA, probably by melting A-T-rich regions in the genome. The fully saturated complex of protein and SV40 DNA sediments at 30 S.

Homologous DNA polymerases-α and -β are stimulated by the protein at a different level depending on the templates used. This result argues in favor of the intervention of the unwinding protein in replication processes.

Following the work of Alberts (1), DNA-unwinding proteins have been isolated from various organisms including viruses (T, 1, 2) and M13 (3) bacteriophages, adenovirus (4), prokaryotes (5, 6) and eukaryotes, a primitive eukaryote (71, and mammals (8-13)). The main properties exhibited by these proteins are preferential binding to single-stranded DNA (which can be completely coated with protein), unwinding of the double helix (as a consequence of preferential binding), and interaction with DNA polymerases that leads to stimulation or inhibition of DNA synthesis.

Prokaryotic unwinding proteins have been extensively studied and their role in replication mechanisms appears to be essential in initiation as well as in elongation processes (6, 14).

Numerous reports of eukaryotic proteins able to bind to single- or double-stranded DNA appeared within the last 2 years, but their functional properties and the extent of analogy with prokaryotic proteins remain unknown. The most detailed investigations concerning these proteins were reported by Banks and Spanos (7) in the case of a primitive eukaryote and by Herrick and Alberts (8) in the case of mammals. Present work on a rat liver DNA-unwinding protein was undertaken in order to compare its physical and functional properties with those of similar prokaryotic and eukaryotic proteins. Moreover, the protein is useful as a tool in the study of eukaryotic DNA polymerases.

MATERIALS AND METHODS

Enzymes

Rat liver DNA polymerase-α was prepared according to the procedure of de Recondo et al. (15, 16) at a specific activity of 18,000 nmol/h (assayed at 37°C on poly[d(C)-oligo(dG)] template-primed). Homologous DNA polymerase-β was isolated on a native DNA-cellulose column in a purification procedure partly common with that of unwinding protein, its specific activity was 3,050 nmol/h.

Nucleic Acids and Nucleotides

Tritium-labeled SV40 supercoiled DNA (a gift of Dr. Michali, Institut de Recherches Scientifiques sur le Cancer) was prepared from virus-infected CV cells [10⁵ plaque-forming unit/cell according to the procedure of Hirt (17), and purified by banding to equilibrium in a CsCl-propidium diiodide gradient. Denatured SV40 DNA was prepared by heating DNA I for 10 min at 100°C followed by a rapid cooling to 0°C.

Calf thymus DNA was obtained from Cohn Laboratories, poly[d(A-T)] from Boehringer Corp., poly[d(T)] from P. L. Biochemicals Corp., poly(A) and poly(U) from Miles Corp. poly(dC)-oligo(dG) was prepared by hybridization poly(dC) from P. L. Biochemicals with [dC]₁₂⁻⁻ (from Collaborative Research). H-Labeled deoxyribonucleotides were purchased from Radiochemical Centre.

DNA cellulose was prepared from calf thymus DNA (native or denatured) and cellulose Munkell 430 by the procedure of Alberts and Herrick (18).

Sucrose Velocity Sedimentation

Protein samples (100 µl) equilibrated by dialysis against Buffer A (50 mM Tris/HCl, pH 7.6, 2 mM 2-mercaptoethanol) containing 0, 200, or 500 mM KCl are layered onto 5 ml of 5 to 20% sucrose gradients prepared in the same buffers. After a 16-h run at 45,000 rpm at 4°C in an SW 50.1 rotor, fractions of 180 µl were collected.

Gel Permeation Chromatography

Protein samples (3 to 200 µg in 50 µl of Buffer A containing 0 or 200 mM KCl) are applied to a Bio-Gel A-0.5 m agarose column (16 x 0.30 cm) packed in the same buffer, and eluted at a rate of 3 ml/h. A mixture of SV40 [³H]DNA (Form II), [³H]dGTP, alcohol dehydrogenase, and interaction with DNA polymerases that leads to stimulation or inhibition of DNA synthesis.

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ase, bovine serum albumin, chymotrypsinogen, and cytochrome c is used for the calibration of the column.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Four micromgams of each protein were denatured in the presence of 2% sodium dodecyl sulfate, 0.7 M 2-mercaptoethanol, 80 mM Tris/HCl, pH 8.0, 0.12% bromophenol blue, and 10% glycerol for 10 min at 100°C according to the procedure of Laemmli (19). Electrophoresis was performed in 15% acrylamide and 0.09% N,N'-methylenebisacrylamide in a Tris buffer containing 1% sodium dodecyl sulfate. Gels were stained with Coomassie brilliant blue R-250 and densitometry was performed on the negative photograph by a Joyce-Loebl densitometer.

Protein Determination and Storage

Protein concentrations were determined by the microtechnic of Schaffner and Weissmann (20), using bovine serum albumin as standard. Upwinding protein samples (about 200 µg/ml) were stored at -80°C in 5 mM KH2PO4/NaOH, pH 7.9, 10% glycerol buffer (Buffer A) for several months without damage.

Assay

Nitrocellulose Filter-binding Assay—The assay measures the amount of SV40 [H]DNA bound to nitrocellulose filters in the presence of unwinding protein according to the general procedure described by Jones and Berg (21). For 30 min at 37°C, 0.052 µg of SV40 DNA I (30,000 cpm/µg) was incubated with increasing amounts of protein; 0 to 1 µg in a total volume of 100 µl. Reaction mixture contained 50 mM Tris/HCl, pH 7.5, 10 mM KCl, 0.002 mM EDTA, 0.2 mM MgCl2, 5 mM 2-mercaptoethanol, and 15% glycerol (Buffer C). After dilution to 1 ml with the same buffer the mixture was filtered at a flow rate of 0.5 to 1 ml/min through a nitrocellulose filter (Schleicher and Schuell BA 85). The filter was washed with 0.6 ml of Buffer C, and dried. The radioactivity was determined in a toluene 2,5-diphenyloxazol (PPO)-1.4-bis[(5-phenyloxazolyl)]benzene (POP0) scintillation mixture. In the absence of protein, a maximum of 2% of total DNA was retained on the filter. In some experiments with denatured DNA, an alternative procedure was used according to the method of Tsai and Green (18). The supernatant was dialyzed for 22 h against 5 mM potassium phosphate, pH 7.9, 10% glycerol buffer (Buffer B) and 10 mM EDTA to a final concentration of 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol and clarified by centrifugation (20,000 x g for 30 min) (Fraction II, 210 ml, 1.500 mg).

Step 3: Differential DNA Cellulose Affinity Chromatography—Two hundred milliliters of Fraction II were loaded at 12 ml/h on two DNA-cellulose columns connected in series according to the procedure of Herrick and Alberts (8) and previously washed with Buffer D. The first column (15 x 40 mm bed) was packed with native DNA-cellulose (containing 12 mg of DNA); the second column, (15 x 80 mm bed) with denatured DNA-cellulose (containing 16 mg of DNA). After adsorption of the extract, the columns were washed with 100 ml of Buffer D, then uncoupled. In order to remove proteins which were not tightly bound to the columns, a rinse with 0.15 M NaCl in Buffer D was performed. Then, proteins were separately eluted from each column by step solution with Buffer D containing increasing amounts of NaCl. Steps were, respectively, 0.4, 0.8, 1.4 M, and 0.2, 0 M NaCl for native and denatured DNA-cellulose columns. Fractions collected (1 ml) were dialyzed overnight against 5 mM potassium phosphate, pH 7.9, 10% glycerol buffer (Buffer B), analyzed by sodium dodecyl sulfate-gel electrophoresis, and assayed for binding (1) and DNA polymerase activities (25, 26) (see "Materials and Methods"). Fractions eluted at 0.4 M NaCl from denatured DNA-cellulose column were pooled and gave Fraction III (10 ml, 1.25 mg). The main biological activities of this fraction are given in Table I.

Step 4: Phosphocellulose Chromatography—Fraction III was applied to a 1-ml bed column of phosphocellulose Whatman P11 equilibrated with Buffer R. The column was washed with 4 ml of Buffer B containing 0.3 M NaCl, then eluted with 0.5, 1.0, and 2.0 M NaCl in the same buffer. Elution was followed by absorbance at 280 nm and binding activity. Fractions eluted at 0.5 M NaCl (with a high binding activity) were pooled, concentrated to 200 to 300 µg/ml, and used in physical and functional studies (Fraction IV).

Densitometric profile of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of Fraction IV is shown in Fig. 1. Fraction IV exhibits a single protein peak (>97% of total proteins) at M, = 25,000 and possesses a high binding activity but neither detectable DNA polymerase nor ATPase, and a very slight deoxyribonuclease activity (as summarized in Table I). The recovery of unwinding protein (UP) in the previous steps cannot be estimated because binding assay and gel electrophoresis are not usable. For the same reason the amount of UP in rat liver cells can be only roughly estimated (see "Discussion") and the level of this protein cannot be quantified but seems to be approximately the same before or after hepatectomy.

Physical Properties

Fraction IV, named UP, seems to contain a single poly-

1 A binding unit is defined as the amount of protein able to retain 10^8 cpm of SV40 DNA I in the conditions of the assay described under "Materials and Methods.

2 The abbreviations used are: UP, the unwinding protein; NaDS, dextran sulfate sodium salt.

RESULTS

Purification of Unwinding Protein

All the purification steps were performed between 0 and 4°C.

Step 1: Preparation of Post-microsomal Supernatant—The cytosol from livers of 25 male WAG rats, partially hepatectomized, was prepared using a method previously described (15). Regenerating livers (137 g) removed 40 h after hepatectomy, were disrupted with a Potter homogenizer in 3 volumes of 50 mM Tris/HCl, pH 7.5, 0.25 M sucrose, 6 mM KCl, 1 mM MgCl2, 1 mM 2-mercaptoethanol, and centrifuged 2 h at 105,000 x g (Fraction I, 150 ml, 2,100 mg).

Physical Properties

Fraction IV, named UP, seems to contain a single poly-

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2 The abbreviations used are: UP, the unwinding protein; NaDS, dextran sulfate sodium salt.
Rat Liver Unwinding Protein

Retain 10 μg of SV40 DNA I in the conditions of the binding assay described under "Materials and Methods." The protein was selected in this manner and this property is also responsible for the melting effect on poly[d(A-T)]. In contrast, this kind of protein is reported to bind poorly to double-stranded DNA (7, 8, 29). We have tested the binding of the rat liver protein to a supercoiled circular double stranded DNA, the simian virus 40 DNA (Form I).

The formation of a nucleoprotein complex may be followed either by sucrose gradients sedimentation or by retention on nitrocellulose filters. In the first method, the incubation mixture of supercoiled SV40 [3H]DNA with increasing amounts of unwinding protein was sedimented in sucrose gradients as indicated in the legend of Fig. 4A. The sedimentation velocity of the complex increases from a value of 21 S for free DNA to a value of 30 S in the saturated complex, depending on the protein/DNA ratio.

In the second method, the incubation mixture is diluted in Buffer C and rapidly poured on nitrocellulose filters (see "Materials and Methods"). The binding curve obtained in plotting the amount of SV40 [3H]DNA retained on the filter against protein concentration (Fig. 4R) exhibits a pronounced sigmoidal shape. The data are independent of the extent of washing the filters and of the dilution of the sample immediately before filtration. If only 1 protein molecule bound to the

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**Table I** Biological activities detected in two last steps of UP purification

<table>
<thead>
<tr>
<th>Protein amount (μg)</th>
<th>Fraction III</th>
<th>Fraction IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,250</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Proportion of 25,000-dalton band</td>
<td>65%</td>
<td>97%</td>
</tr>
<tr>
<td>Total binding activity (unit)</td>
<td>16,000</td>
<td>9,000</td>
</tr>
<tr>
<td>DNA polymerase activity</td>
<td>Not detectable*</td>
<td>Not detectable*</td>
</tr>
<tr>
<td>ATPase activity</td>
<td>Not detectable*</td>
<td>Not detectable*</td>
</tr>
<tr>
<td>Deoxyribonuclease activity</td>
<td>Very slight</td>
<td>Very slight: 150 binding units could give only 8 nicks/100 DNA I molecules in 150 min at 37°C</td>
</tr>
</tbody>
</table>

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* A binding unit is defined as the amount of protein able to retain 10⁻² μg of SV40 DNA I in the conditions of the binding assay described under "Materials and Methods.

† Less than 2 pmol of dNTP incorporated/μg of protein/30 min.

‡ Less than 75 pmol of ADP produced/μg of protein/30 min.

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**Fig. 1.** Densitometric trace of sodium dodecyl sulfate-polyacrylamide slab gel of Fraction IV. Five micrograms of Fraction IV were subjected to a sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described under "Materials and Methods." The electrophoretic migration is from left to right. Impurities could be detected if present at a level > 2% and in this range of molecular weight, two polypeptide chains with a difference of 1000 are separable by this technique.

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peptide chain of 25,000 Mₐ, when subjected to an electrophoresis in denaturing conditions (Figs. 1 and 24).

In sucrose velocity sedimentation (Fig. 2B) at low ionic strength (μ = 0.05), binding activity is detected in two peaks, the main peak (40%) appears at 5.9 S and the second (40%) at 2.3 S. At a higher ionic strength (μ = 0.250 to 0.550) the binding activity is detected in a single peak at 2.3 S.

In order to determine the molecular weight of the protein in the various conditions tested in sedimentation, the diffusion coefficient was measured by gel filtration through Bio-Gel A-0.5m (27) in the same buffers (Fig. 2C). At low ionic strength (μ = 0.050), binding activity is eluted at a fraction corresponding to a diffusion coefficient of 5.3 ± 0.3 10⁻⁷ cm²/s. At higher ionic strength (μ = 0.250), binding activity appears at a fraction corresponding to a diffusion coefficient of 8.7 ± 0.2 10⁻⁷ cm²/s. Molecular weight was calculated from the Stokes-Einstein equation. The partial specific volume was assumed to be 0.74 ml/g; this gives a molecular weight of 104,000 ± 7,000 consistent with a tetrameric form (6) in low ionic strength conditions, and of 24,800 ± 1,500 (consistent with sodium dodecyl sulfate-polyacrylamide gel) in high ionic strength conditions.

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Melting and Renaturation of Poly[d(A-T)] in Presence of Unwinding Protein

When poly[d(A-T)] is incubated at 13.5°C with various amounts of unwinding protein, an increase in absorbance at 260 nm of the nucleic acid which corresponds to the melting of the polymer is observed (Fig. 3); at high protein/DNA ratio (15:1 to 20:1), poly[d(A-T)] was completely melted. Renaturation of the double helix could be obtained by an increase in the ionic strength or by decrease in the temperature. Salt addition has a double effect on renaturation of DNA in the presence of unwinding protein; it destabilizes the binding of the protein to DNA and promotes the formation of double helical DNA in the absence of protein. When increasing amounts of KCl are added at 13.5°C in cuvette containing poly[d(A-T)] and unwinding protein at a weight ratio of 12.5:1, half-renaturation occurs at 20 mM KCl, and double helix is completely restored at 70 mM (not shown). Consequently, the melting point of poly[d(A-T)]-protein complex is about 13.5°C at 20 mM KCl and should be compared to the melting point of free poly[d(A-T)] (about 53°C) in the same conditions.

Thermal melting experiments have been performed at various protein/DNA ratios as described in Fig. 3. In these conditions, denaturation of free poly[d(A-T)] occurs at 54°C. In the presence of protein (protein/DNA ratio from 2.4 to 10), denaturation occurs at 41°C, and is reversible with decreasing temperature.

The rat liver unwinding protein also depresses the helix melting temperature (Tm) of synthetic hybrid poly(A)-poly(dT) and double-stranded poly(A)-poly(U), indicating that both DNA and RNA are recognized by this protein.

**Binding to Simian Virus 40 DNA**

It is known that unwinding proteins bind specifically to single-stranded DNA (7, 28); moreover, the rat liver protein was selected in this manner and this property is also responsible for the melting effect on poly[d(A-T)]. In contrast, this kind of protein is reported to bind poorly to double-stranded DNA (7, 8, 29). We have tested the binding of the rat liver protein to a supercoiled circular double stranded DNA, the simian virus 40 DNA (Form I).

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The formation of a nucleoprotein complex may be followed either by sucrose gradients sedimentation or by retention on nitrocellulose filters. In the first method, the incubation mixture of supercoiled SV40 [3H]DNA with increasing amounts of unwinding protein was sedimented in sucrose gradients as indicated in the legend of Fig. 4A. The sedimentation velocity of the complex increases from a value of 21 S for free DNA to a value of 30 S in the saturated complex, depending on the protein/DNA ratio.

In the second method, the incubation mixture is diluted in Buffer C and rapidly poured on nitrocellulose filters (see "Materials and Methods"). The binding curve obtained in plotting the amount of SV40 [3H]DNA retained on the filter against protein concentration (Fig. 4R) exhibits a pronounced sigmoidal shape. The data are independent of the extent of washing the filters and of the dilution of the sample immediately before filtration. If only 1 protein molecule bound to the
Rat Liver Unwinding Protein

![Figure 2](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Physical properties of rat liver UP</th>
<th>Subunit</th>
<th>Native</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dodecyl sulfate $M_r$</td>
<td>25,000</td>
<td>104,000</td>
</tr>
<tr>
<td>$s_{20,w}$</td>
<td>2.3 S</td>
<td>5.9 S</td>
</tr>
<tr>
<td>$D_{20,w}$</td>
<td>$8.7 \times 10^{-13}$ cm$^2$/sec</td>
<td>$5.3 \times 10^{-13}$ cm$^2$/sec</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>24,800</td>
<td>104,000</td>
</tr>
<tr>
<td>Stokes radius</td>
<td>24.7 Å</td>
<td>40.5 Å</td>
</tr>
<tr>
<td>Frictional coefficient</td>
<td>1.27</td>
<td>1.29</td>
</tr>
</tbody>
</table>

The kinetics of formation of the nucleoproteic complex is relatively fast with a half-reaction time of 100 s. The reaction is inhibited by an increase in the ionic strength (50% at 40 mm NaCl), by magnesium ions (50% at 2 mm), and finally by the polyanion dextran sulfate (NaDS) (50% at 0.5 × 10⁻⁴ M). The effect of the latter could be explained by the chemical structure of NaDS, which presents some analogies with that of DNA although it cannot mimic the particular polyphosphate conformation of single-stranded DNA (8). NaDS acts as a strong competitor of the binding of the protein to DNA.

### Stimulation of Rat Liver DNA Polymerases

As recently described elsewhere (31), the rat liver unwinding protein affects the in vitro synthesis catalyzed by homologous DNA polymerases-α and -β in the presence of synthetic polymers as templates. This effect is strongly dependent on the protein/template weight ratio and also on the length of single-stranded regions of this template (31).

The 25,000 UP stimulates about 3-fold the deoxyribonucleotide incorporation catalyzed by DNA polymerase-α in the presence of a single-stranded template initiated with short oligomers, poly(dC)·(dG)$_{10}$ (31). The stimulatory effect of UP is more detectable when the initiator/template ratio is low (i.e. dG/dC ratio ≤ 1/10 in mononucleotide concentration). Maxi-
Fig. 3. A, unwinding protein-induced melting of poly[d(A-T)]. Hyperchromicity of poly[d(A-T)] at 13.5° is plotted as function of unwinding protein/poly[d(A-T)] ratio. Cuvettes contained the following: 30 μl of bidistilled water, 0.8 μg of poly[d(A-T)] in 20 μl of 5 mM Tris/HCl, pH 8.8, 1 mM EDTA, 0.1 mM dithiothreitol, 10% glycerol buffer, 0 to 15 μg of unwinding protein in Buffer B, same buffer to a final volume of 300 μl. Cuvette 1, minus poly[d(A-T)] minus protein (blank); Cuvette 2, poly[d(A-T)] (0.8 μg); Cuvette 3, poly[d(A-T)] (0.8 μg) + protein (0 to 15 μg); Cuvette 4, protein alone (0 to 15 μg). Hyperchromicity is defined as absorbance of Cuvette 3 minus the sum of Cuvette 2 and Cuvette 4 absorbances.

B, thermal denaturation of poly[d(A-T)] in the presence of rat liver unwinding protein. Final concentration of MgCl₂ was 0.5 mM; Cuvette 1 contained all components except nucleic acid and protein (blank); Cuvettes 2, 3, and 4 contained 1.0 μg of poly[d(A-T)] and, respectively, 0, 2.5, and 10 μg of unwinding protein. Temperature program was the following: linear increase from 14-64° at a rate of 0.33°/min, decrease to 20° at 1.4°/min, and final increase to 65° at 0.33°/min. The absorbance of Cuvette 1 was subtracted and the origin of Cuvettes 2, 3, 4 absorbance was shifted. In a control experiment the optical density due to protein alone was measured. An increase in absorbance indicating denaturation of the protein was never detected. This fact was confirmed by the absence of turbidity (A₅₅₀ nm) in the range of the temperatures used. The great increase in optical density above 45° probably corresponds to free poly[d(A-T)] denaturation.

maximum stimulation of DNA polymerase-α is observed at a protein/DNA weight ratio of 1.7, which is much smaller than that necessary to cover all the single-stranded regions of the template, if we assume a coverage of seven nucleotides/25,000-dalton subunit (6, 28). At a high UP/DNA weight ratio, an inhibitory effect is obtained which may correspond to blocking of DNA polymerase-α sites by unwinding protein on the template. In the presence of a double-stranded template with few 3'-OH ends, poly[d(A-T)], the DNA polymerase-β activity was never detected. This fact was confirmed by the absence of turbidity (A₅₅₀ nm) in the range of the temperatures used. The great increase in optical density above 45° probably corresponds to free poly[d(A-T)] denaturation.

Fig. 4. Binding of rat liver unwinding protein to supercoiled SV40 DNA. A, sedimentation of rat liver UP-DNA complexes. Supercoiled SV40 [³H]DNA, 0.305 μg, was incubated at 37° for 20 min without (●) or with 1.2 μg (○) or 2.5 μg (●) of rat liver unwinding protein in Buffer C in a final volume of 100 μl. Then, the three samples were loaded onto a 5 to 10% sucrose gradient in 50 mM Tris/HCl, pH 7.6, 10 mM KCl, and 5 mM 2-mercaptoethanol, which had been layered on top of a 0.1-ml cushion of 50% sucrose in the same buffer. Centrifugation was performed for 3 h at 4° and 40,000 rpm in a SW 50.1 Spinco rotor. Twenty-six equal fractions were collected from the bottom. The radioactivity in each fraction was determined by liquid scintillation counting. The arrow indicates the position of supercoiled SV40 DNA (21 S). B, retention on nitrocellulose filters. After incubation of 0.052 μg of supercoiled SV40 [³H]DNA with increasing amounts of UP, the nucleoprotein complex is filtered as indicated under "Materials and Methods" and the amount of retained DNA is plotted against UP concentration. The radioactivity remaining on the filters in the absence of protein (2% background) was subtracted.

DISCUSSION

The protein described in this communication exhibits the expected main characteristics of a DNA-unwinding protein and seems to be closely related to the 24,000-dalton calf thymus UP1 described by Herrick and Alberts (8, 22, 28). The use of dextran-sulfate 500 in the purification procedure of rat liver unwinding protein was tested but not retained: first, the protein seems to be strongly NaDS-sensitive and is partly eluted with nonspecifically bound proteins even at 0.5 mg/ml of NaDS; second, dextran-sulfate, which is scarcely eliminated from column fractions, is a strong inhibitor of DNA polymerase activities and of the binding of protein to DNA.
The purification procedure described under "Materials and Methods" has the advantage of providing a simple method to isolate DNA polymerase-α and -β besides unwinding protein in a single preparation. DNA polymerase-α stays selectively in the polyethylene glycol precipitate. DNA polymerase-β binds tightly to double-stranded DNA and is eluted from native DNA cellulose (0.8 M NaCl) at a high specific activity. DNA-unwinding protein, eluted from denatured DNA cellulose was purified to apparent homogeneity on a phosphocellulose column. The protein is free of DNA polymerase as well as deoxyribonuclease and ATPase activities. This last feature shows that the protein is not related to unwinding enzymes (DNA-dependent ATPases) described in Escherichia coli (23, 32).

In a standard preparation, the unwinding protein was extracted from the cytosol of regenerating rat liver cells and seems to be rather abundant (about 1 to 1.5 x 10^6 copies/cell). Moreover, the amount of 25,000-dalton protein in the nucleus is comparable to that of the cytosol, and the intracellular location of the protein cannot be determined accurately. On the other hand, unlike DNA polymerase-α (15), the 25,000-dalton protein level seems to be independent of the regeneration of the liver. Besides this protein, the 33,000-dalton protein described by Herrick and Alberts in calf thymus is present in very slight amount in the cytosol, but essentially appears with 25,000- and 40,000-dalton proteins in nuclear extracts (not shown).

At low ionic strength, the protein exists in a tetrameric form (about Mr = 100,000) in equilibrium with a monomeric (Mr = 25,000) form. The tetramer is dissociated at high ionic strength or under denaturing conditions. This situation seems to differ from both calf thymus 24,000-dalton and Ustilago binding proteins which had been found in a monomeric state. In contrast, phage-T4 gene 32 (1) and E. coli (β) proteins are, respectively, dimeric and tetrameric in their native forms. Cross-linking reagents could be used to investigate the state of aggregation of the protein when binding to DNA.

Like calf thymus UPI and Ustilago proteins, the 25,000-dalton rat liver protein is able to depress the melting point of poly(dA-T) by about 40°, and the effect is extremely sensitive to ionic strength. Despite its poor affinity for double-stranded DNA, the protein binds to supercoiled SV40 DNA. This binding may be facilitated by the melting of A-T-rich regions on the DNA (28, 33, 34). The hypothesis is consistent with the slight but significant increase in the absorbance at 260 nm which follows the formation of nucleoprotein complex at 37°C. The saturated complex has a sedimentation coefficient of about 30 S, indicating that many protein molecules are probably bound to the DNA. The sigmoidal shape of the nitrocellulose filters binding curve reflects either that more than 1 protein molecule is required to retain DNA on the filter, or cooperative binding of the protein to DNA. In this last case, facilitation of the binding could be mediated by direct protein-protein interactions, as already found with gene 32 protein, or may proceed via structural changes in the DNA (including melting). Sedimentation experiments of rat liver protein with single-stranded DNA and electron microscopy analysis of the complexes should resolve the problem. Using these techniques Herrick and Alberts (28) found a noncooperative binding of calf thymus UPI to fd DNA. On the other hand, Banks and Spanos (7) using nitrocellulose filters binding experiments, had reported protein-protein interactions in the binding of Ustilago unwinding protein to denatured T7 DNA.

Stimulation of rat liver DNA polymerases by an homologous DNA-unwinding protein argues in favor of the intervention of this latter in replication processes. Rat liver 25,000-dalton UP increases notably the activity of DNA polymerase-α but also that of DNA polymerase-β depending on the experimental conditions used. In the case of polymerase-α, the optimal amount of UP is much smaller than necessary to cover all the single-stranded regions of the template; this could indicate a specific interaction of the unwinding protein with polymerase-α. It is however not known if direct association of the two proteins takes place. In the case of DNA polymerase-β, stimulation might be a simple consequence of the removal of secondary structure of the DNA, without particular specific interactions between the two proteins. With calf thymus unwinding protein, Herrick and Alberts failed to observe any stimulation of DNA polymerase-β with poly(dC) oligo(dC) as template-primer. The difference with our results could be explained by the use of rabbit DNA polymerase-β, but more likely by variations in the ratio of oligo(dG) to poly(dC), i.e. in the structure of the template-primer DNA. The stimulation of polymerase-β is only observed in conditions of high oligo(dG)/poly(dC) ratio (up to 1/2) or in the presence of poly(dA-T) as a template, where the polymerase acts more as repair enzyme than as a replicase. To distinguish the effect of the rat liver unwinding protein on template from its specific interaction with homologous DNA polymerases, a comparative study with E. coli DNA polymerase I and gene 32 protein of phage T4 are in progress.

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REFERENCES
A deoxyribonucleic acid unwinding protein isolated from regenerating rat liver. Physical and functional properties.
M Duguet and A M de Recondo


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