Purification and Physical Characterization of Nucleic Acid Helix-unwinding Proteins from Calf Thymus*

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We have devised a general protein fractionation procedure which selects for eukaryotic DNA-binding proteins, some of which resemble DNA-unwinding proteins from prokaryotes. Proteins were selected which (a) pass through a native DNA-cellulose column, (b) bind to a denatured DNA-cellulose column, and (c) remain bound to the latter column during a rinse with a dilute solution of the sodium salt of the polyanion dextran sulfate. When this fractionation was applied to the soluble proteins of calf thymus, three major protein species were recovered. The predominant one has an apparent molecular weight of about 24,000 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, is isoelectric near neutrality, and elutes as a monomer from denatured DNA-cellulose at moderate NaCl concentrations. This protein, designated calf-unwinding protein 1 (UPl), has been purified to homogeneity. However, isoelectric focusing reveals four or five subspecies (apparently separated by single-charge differences) which differ appreciably in their affinities for DNA.

Two other major proteins are obtained which have apparent molecular weights in sodium dodecyl sulfate of 33,000: the first, which elutes with low salt from DNA-cellulose as a homogeneous preparation, appears to be a basic protein (although it is clearly not a histone); the other, which elutes from DNA-cellulose as the major component of a "high salt eluting fraction," is an acidic protein which co-purifies with less prominent species of higher molecular weights. Proteins similar to each of these three major calf thymus proteins have been observed by us and others in tissue culture cells of mouse, hamster, monkey, and humans, suggesting their wide occurrence among eukaryotes.

DNA-affinity chromatography provides a simple method for identifying and purifying cellular proteins which may be involved in DNA metabolism. In general, a DNA-free, soluble extract is passed through a column bed containing DNA immobilized on an inert solid support. After an extensive rinse, those proteins which have bound to the DNA on the column are eluted with high salt buffers and collected for further study. Small subclasses of such DNA-binding proteins may be specially selected, either through the use of competing polymers, or by manipulation of ionic conditions and the composition and strandedness of the affixed DNA (1).

DNA-cellulose columns have been used in several laboratories for isolation of mammalian DNA-binding proteins (1-10). In this work, we use this method to select a set of calf thymus proteins which pass through a native DNA-cellulose column, but bind to a similar column made with single-stranded DNA. To eliminate those proteins which might bind nonspecifically to any negatively charged polymer, we have chosen to study only those species which remain bound to the single-stranded DNA column during a rinse with the competing polyanion of sodium dextran sulfate.

When such a fractionation is applied to bacterial extracts, it selects for the known "DNA-unwinding proteins" from both T4 bacteriophage-infected (17) and uninfected Escherichia coli (18). These proteins, which appear to function in DNA synthesis, share two important properties. They facilitate DNA denaturation by virtue of their strong, selective binding to single-stranded DNA, forming complexes in which the otherwise folded strands are extended and completely covered by protein. In addition, they stimulate the rate of in vitro DNA synthesis by a homologous DNA polymerase by 5- to 10-fold when tested with appropriate DNA templates (see also Refs. 19 and 20). The present work with calf thymus was undertaken to determine whether there might be DNA-binding proteins in mammalian cells which resemble these DNA-unwinding proteins from prokaryotes.

Materials and Methods

Quantitation, Concentration, and Storage of Proteins—Proteins were monitored by their ultraviolet absorbance in a 1-cm path length cell, and quantities are expressed as A212 units, defined as the product of A212 nm x volume in milliliters after correction for contaminating nucleic acids (21). Extinction coefficients were determined on glyceral-free samples with the biuret reagent, comparing A212 with a standard curve generated with bovine serum albumin (E212 nm - 6.6). Samples to be saved were concentrated to at least 1 mg/ml by dialysis against solid sucrose (Schwarz/Mann Enzyme

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grade, dialyzed against 5 mM sodium phosphate, pH 7.8, plus 10% glycerol, clarified by high speed centrifugation (167,000 \( \times \) g), and quick-frozen for storage in aliquots.

Isolation of Calf Thymus DNA-binding Proteins—Thymus glands from 4- to 6-week-old calves, held on ice up to 8 hours after slaughter, were trimmed free of fat and connective tissue and cubed. Pieces were frozen at -80\(^\circ\)C, at which temperature they could be stored for at least 6 months without apparent damage to the proteins of interest. All isolation steps were performed at 0-4\(^\circ\)C, with doubly distilled water used throughout.

In a standard preparation, frozen tissue (500 g) was extracted with 1100 ml of 20 mM Tris-HCl, pH 8.8 at 20\(^\circ\)/50 mM NaCl/l mM EDTA/0.1 mM dithiothreitol/5% glycerol (v/v; Eastman Spectro grade) (Buffer A), plus an additional 0.9 mM dithiothreitol. Before the tissue thawed, it was reduced to an ice slurry in a Waring Blender run at slow speed; the slurry was then homogenized at highest speed in a blender, then run at low speed to dissolve the salt. The homogenate was centrifuged for 30 min at 15,000 \( \times \) g and the large sediment was discarded.

The supernatant (about 50 mg/ml of protein) was filtered through buffer-tissue thawed, it was reduced to an ice slurry in a Waring Blendor run at slow speed; the slurry was then homogenized at highest speed in Buffer A without glycerol. Mixing during dialysis was achieved by gentle bubbling with nitrogen; in addition, the bags were manually inverted at 2-hour intervals, except for the final 5 hours, when they were left undisturbed to permit lipids to coalesce. The impermeate is soaked cheesecloth and brought to 2 M NaCl by adding solid salt; the blender was then run at low speed to dissolve the salt. In a similar manner, the extract was made 10% (w/w) in PEG (Union Carbide Chemicals Co.) and the PEG/2 M NaCl extract was centrifuged for 30 min at 13,000 \( \times \) g to pellet any remaining DNA and particulate matter (1, 22). The clear red supernatant was subjected to a second filtration through cheesecloth and dialyzed in 24 hours.

Isoelectric Focusing in Urea-containing Polyacrylamide Gels—Protein samples, containing less than 5 \( \mu \)mol of salts, were photo-lysed into gels containing pH 3 to 10 ampholines, as described by Wrigley (29), but with 6 \( \mu \)mol urea added (Schwarz/Mann Ultrapure grade, freshly dissolved). During the initial 20 min of electrophoresis, the voltage was continuously raised from 200 V to 350 V, where it was maintained until the colored standards run in a parallel gel (cytochrome c, hemoglobin, methyl red or bromphenol blue, and xylene cyanole FF) were completely focused (1.5 to 2 hours at 20\(^\circ\)). Gels were then stained with bromphenol blue (20).

Results

In searching for possible mammalian analogues for the DNA-unwinding proteins found in bacteria, we take advantage of the fact that these DNA-binding proteins greatly destabilize regions of DNA double helix by virtue of their selective affinity for single stranded over double stranded DNA. Thus, under appropriate conditions, such proteins should pass through a native DNA-cellulose column while adhering strongly to a column containing single-stranded DNA. To analyze a hypothetical situation in quantitative terms, one estimates (from the relationship, 1/R\(_1\) = 1 + K [free DNA sites]) that a protein with an association constant (K) for native DNA \( \leq 10^{8} \) \( M^{-1} \) and an association constant for denatured DNA \( > 10^{4} \) \( M^{-1} \) would “pass through” a standard native DNA-cellulose column during the first 8 liquid column volumes of rinse, but remain “bound” to a denatured DNA-cellulose column after 70 liquid column volumes of rinse. Thus, the protocol described under “Materials and Methods” should select DNA-unwinding proteins which have as little as a 10-fold differential affinity for single-stranded over double-stranded DNA. We estimate that such a protein would depress the melting temperature of DNA by less than 1\(^\circ\). This procedure should of course also select DNA-unwinding proteins of much greater differential affinities, which are thus capable of denaturating DNA more strongly. Note that at a low enough ionic strength, a particular DNA-unwinding protein might bind to double-stranded DNA with a K \( < 10^{8} \) \( M^{-1} \) and thus be retained by such a native DNA column. However, the same

Using the notation of Martin and Synge (31), we set 1/R\(_1\) - A\(\alpha\)/A\(\alpha\) - 1/R and K\(_{eff}\) = (A\(\alpha\)/A\(\alpha\)) \(\alpha\), where 1/R\(_1\) is the number of liquid column volumes of rinse necessary to bring the protein peak to the bottom of the column and K\(_{eff}\) = [bound protein]/[free protein]. Martin and Synge derive 1/R = A\(\alpha\)/A (1 + A\(\alpha\)/A\(\alpha\)) or 1/R\(_1\) = 1 + K\(_{eff}\). As the association constant K = K\(_{eff}\) [free DNA sites], we obtain the equation in the text: 1/R\(_1\) = 1 + K [free DNA sites]. For calculations we assume DNA-cellulose with 1 mg of DNA per packed ml and a relative liquid column volume A\(\alpha\)/A = 0.9. We further assume that there are large excesses of the chromatography, and that a binding site can start at any DNA nucleotide (i.e., [free DNA sites] = [DNA nucleotides]).

For this calculation we arbitrarily choose a molar ratio of protein to DNA sites of 1 to 1, a DNA concentration of 6 \( \mu \)g/ml, a DNA site 10 nucleotides long, and an ionic strength such that the melting temperature of DNA in the absence of protein is 50\(^\circ\). The equations should be equivalent to estimate melting point depressions: for details, see Ref. 32.
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protein is likely to display exclusive affinity for a denatured DNA column at some higher ionic strength, where its general affinity for DNA is weakened.

We have chosen to screen for selective binding by mammalian proteins in a buffer containing 0.05 M NaCl, because the previously characterized Escherichia coli and T4 DNA-unwinding proteins bind selectively to denatured DNA columns at this salt concentration. We chose to fractionate the single-strand-specific proteins further on the basis of resistance to elution by a sodium dextran sulfate rinse, hoping to eliminate proteins that bind DNA nonspecifically. Hence, we began our studies by isolating the total single-strand-specific, sodium dextran sulfate-resistant DNA-binding proteins from a readily available mammalian source, calf thymus glands.

Elution profiles for a typical experiment are presented in Fig. 1. It can be seen that the denatured DNA-cellulose column bound about twice as much total protein as the native DNA-cellulose column which preceded it. Moreover, whereas sodium dextran sulfate removed essentially all of the proteins that bound to the native DNA-cellulose column (Fig. 1A), about 15% of the proteins on the denatured DNA column were not removed until a subsequent 2 M NaCl elution (Fig. 1B). Thus, an apparently single-strand-specific, sodium dextran sulfate-resistant protein fraction constitutes about 10% of the total calf DNA-binding proteins recovered (0.67 x 15%), totaling 0.60 $A_{280}$ units/g of homogenate protein.

The different protein fractions eluting from both the native and denatured DNA columns in Fig. 1 have been analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and photographs of stained gels are presented in Fig. 2. A and

![Image 1](https://www.jbc.org/)

**Fig. 1.** Fractionation of the soluble calf thymus proteins that bind to native and denatured DNA-cellulose columns linked in series. Elution profiles after uncoupling the columns are shown; for details see under “Materials and Methods.” Elution volumes shown are those from the beginning of the sodium dextran sulfate (NaDS) rinse; the 2.0 M NaCl rinse was begun where indicated. A, elution of proteins bound to the native DNA column. The total amount of protein eluted was 1.9 $A_{280}$ units/g of homogenate protein (estimated by biuret assay at 15% of the net weight of tissue). B, elution from the denatured DNA column of proteins that had passed through the native DNA column. The total amount of protein eluted was 4.1 $A_{280}$ units/g of homogenate protein. The failure of sodium dextran sulfate to elute all the proteins from this column cannot be due simply to slow equilibration, since the same fractionation can be performed with sodium dextran sulfate present in the extract, as well as in the rinse, without a major effect on the composition or yield of sodium dextran sulfate-resistant proteins (the yield of UP1 is considerably lower, however, as expected from its partial sodium dextran sulfate sensitivity; see under “Discussion”).

![Image 2](https://www.jbc.org/)

**Fig. 2.** Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of fractions of calf thymus DNA-binding proteins from Fig. 1. Electrophoresis was performed as described under “Materials and Methods;” the volume of each aliquot was chosen to give an amount of protein sufficient for readily visible bands after Coomassie blue staining, and, therefore, a comparison of band intensities between gels is not meaningful. Migration was to the right; apparent molecular weights are designated below the major bands; TD indicates the tracking dye position. A and B, samples from native (A) and denatured (B) DNA-cellulose elutions; gel numbers correspond to those fractions so marked in Fig. 1. C, band profile of single-strand-specific, sodium dextran sulfate-resistant proteins. A gel similar to that shown below (D) was scanned with a Joyce-Loebel densitometer. D, comparison of the single-strand-specific, sodium dextran sulfate-resistant proteins with calf thymus histones (Worthington Biochemical); histone bands are designated according to Ref. 33.

B. Note the very heterogeneous mixtures of proteins in the main peaks from both the native (Fig. 2A, Gels 1 to 4) and the denatured (Fig. 2B, Gels 5 to 8) columns. In striking contrast, the sodium dextran sulfate-resistant fraction from the denatured column contains only a few prominent protein species (Fig. 2B, Gels 10 to 12). In general, this fraction contains protein bands corresponding to apparent molecular weights of about 24,000, 33,000, 40,000, 11,000, 17,000, and a triplet at
about 70,000, listed in approximate order of decreasing relative abundance. A densitometer tracing of such a stained gel pattern is presented in Fig. 2C. In Fig. 2D, the migration of these proteins on sodium dodecyl sulfate-polyacrylamide gels is compared to that of calf thymus histones: as explained below, none of these proteins appear to be histones. When reapplied to native DNA-cellulose in 0.05 M NaCl, all seven of these proteins pass through the column without detectable affinity, whereas they rebind if reapplied to a denatured DNA column (see below).

As described under "Materials and Methods," one of the first steps in the preparation of the extract is a 13,000 × g centrifugation under low salt conditions, which is sufficient to remove both mitochondria (34) and chromatin (35). About 95% of the total DNA and 50% of the total protein are removed by this step. Yet this step does not lower the yield or change the composition of the single-strand-specific, sodium dextran sulfate-resistant proteins obtained, as compared with alternative procedures in which the chromatin is thoroughly disrupted (either by high salt or by pancreatic DNase I digestion, see Ref. 1). This finding suggests that these proteins are not primarily chromosomal or mitochondrial in origin. From further experiments which utilized centrifugation conditions sufficient to sediment even small particulates (2 hours at 160,000 × g), we conclude that a substantial fraction of these sodium dextran sulfate-resistant proteins are present in the soluble cell fraction (23).

Separation and Properties of Three Major Single-strand-specific, Sodium Dextran Sulfate-resistant Fractions—After rebinding the single-strand-specific, sodium dextran sulfate-resistant fraction to a second denatured DNA column, three major proteins can be distinguished by elution with increasing concentrations of NaCl. Either a series of step elutions (Fig. 3) or a salt gradient (Fig. 5) may be used. In either case, a fairly clean separation of three major protein species may be obtained. As seen from the sodium dodecyl sulfate-polyacrylamide gel patterns in Fig. 3B, a protein with an apparent molecular weight of 33,000 is eluted first, followed by the 24,000 dalton species, which elutes over a wide range of salt, a small portion being eluted only with 2 M NaCl. A second, less pure 33,000 dalton species elutes only at the high NaCl concentration, along with several less prominent proteins of higher molecular weight. These three protein fractions will henceforth be designated as the "33,000 dalton, low salting protein," the "calf-unwinding protein 1" (UP1), and the "high salting eluting proteins," respectively. In both 5% and 10% polyacrylamide sodium dodecyl sulfate gels, the two proteins with apparent molecular weights of 33,000 migrate as single bands near the position of porcine pepsin (Mr = 32,700), while UP1 migrates in both gels near bovine trypsin (Mr = 23,300).5

High Salt Eluting Proteins—These proteins can be separated from UP1 either by NaCl-gradient elution from denatured DNA-cellulose (see Fig. 5, below) or by gel permeation chromatography; the latter is shown in Fig. 4. In Fractions 4 to 8, the 33,000 dalton, high salt eluting protein appears in association with smaller amounts of the 40,000 dalton protein and other higher molecular mass species, but some also appears to chromatograph separately (e.g. in Fraction 9). There is a suggestion that the 33,000 and 40,000 dalton species may form a loose complex, since the two co-focus to pH 5.2 to 5.6 in a nondenaturing isoelectric focusing column (23) and co-elute from denatured DNA-cellulose in a salt gradient (see Fig. 5, below). After various attempts to purify the 33,000 dalton species away from the other high salt eluting proteins,
FIG. 4. Fractionation of a single-strand-specific, sodium dextran sulfate-resistant protein fraction on the basis of molecular weight. A, gel permeation chromatography (Bio-Gel A-0.5m agarose gel). The starting sample (7.5 A$\text{\text{unis}}$ units in 6 ml of Buffer A) was similar in protein composition to that used in Fig. 3, and was prepared and fractionated as described under “Materials and Methods.” B, sodium dodecyl sulfate-polyacrylamide gel analysis of fractions. Samples were prepared and gels were displayed as in Fig. 2, but with apparent native molecular weights also listed (calculated from the elution volume of the respective column fraction, assuming spherical molecules, as described under “Materials and Methods”). The bottom gel shows the composition of the sample applied to the column.

Our purest fractions contained 60 to 70% 33,000 dalton protein and had a relatively low extinction coefficient ($E_{254}$ $\approx$ 4). In further work with these proteins (32, 39), samples have been used with a composition comparable to that of Gel 16, Fig. 6A below.

The 33,000 Dalton, Low Salt Eluting Protein—This protein elutes from DNA-cellulose $>99$% pure (as judged by sodium dodecyl sulfate-polyacrylamide gels). It constitutes up to 0.04% of the total cell protein; however, in some experiments (e.g. see Fig. 5 below) we have failed to obtain significant amounts of this protein. An amino acid analysis of this 33,000 dalton, low salt eluting protein is presented in Table I. Its aromatic amino acid composition is consistent with its extinction coefficient, $E_{280}$ $\approx$ 11. The content of charged amino acids suggests that it is a basic protein. From Fig. 2D it can be seen that calf thymus histone I migrates at the same position as this protein on 10% sodium dodecyl sulfate-polyacrylamide gels (much slower than predicted from its molecular weight, see Ref. 42). However, histone I has a clearly different amino acid content: it has a ratio of basic to acidic amino acids of 4.6 (as compared with 0.67 found for this protein) and a much lower extinction coefficient (43).

UP1—This protein also elutes from DNA-cellulose as a well separated peak. It is obtained reproducibly, constituting at least 0.1% of the total cell protein. In the nondenaturing conditions of Fig. 4, it elutes from the gel permeation column with an apparent native molecular weight of 19,000 as judged by its elution relative to standard proteins (see under “Materials and Methods”). Comparing this value to the sodium dodecyl sulfate gel-derived molecular weight of 24,000, we conclude that the presumed native protein is a roughly
Amino acid analysis (Table I) of a pure UP1 sample (see Fig. 7, below) shows a paucity of tyrosine, consistent with the protein's relatively low extinction coefficient, $E_{280\text{nm}} = 4.45$. From its composition, one would predict that it should have a near neutral isoelectric point. This is the case, both in sucrose gradient-stabilized isoelectric focusing in nondenaturing conditions (pl = 7.8, Ref. 23) and in polyacrylamide gel isoelectric focusing in urea (see below).

**Identification of UP1 Heterogeneity and Purification of Subfractions**—Various initial efforts to purify UP1 by standard chromatographic techniques were frustrated by its heterogeneous behavior. For example, in the experiment shown in Fig. 5, single-strand-specific, sodium dextran sulfate-resistant proteins were fractionated by rebinding them to a small denatured DNA-cellulose column and eluting them with a linear gradient of NaCl. From sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the fractions (Fig. 6A, also see Fig. 3), it is clear that UP1 elutes from DNA over a broad range of salt. Isoelectric focusing in urea-containing, polyacrylamide gels (Fig. 6B) reveals that this broadness reflects an isoelectric point heterogeneity: UP1 appears to be composed of four or five subspecies with isoelectric points near neutrality, spaced roughly equally over a range of 0.5 to 1.0 pH unit. These subspecies elute from the column in the order of their relative basicities, the three most acidic eluting close to one another at a low NaCl concentration, and the most basic eluting considerably later.

In order to remove contaminants of different molecular weights from UP1, selected fractions from the salt gradient elution in Fig. 5 were pooled into three samples (called “Acidics,” “Mix,” and “Basics”) and subjected to gel permeation chromatography on agarose as described in Fig. 7. The peak fractions from this column were dialyzed and concentrated for storage at $-80\degree$. As judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the resulting samples were further purified by isoelectric focusing in urea-containing, polyacrylamide gels (Fig. 6B), which revealed again the presence of heterogeneity in the protein.

**Fig. 5.** Preparative fractionation of the single-strand-specific, dextran sulfate-resistant proteins by NaCl gradient elution from denatured DNA-cellulose. Such proteins, isolated from 500 g of calf thymus and dialyzed against Buffer A, were clarified by a 30-min centrifugation at 100,000 $\times$ g; 75% of the original protein was recovered in the supernatant and applied to a small denatured DNA-cellulose column at 60 ml/hour. The column was rinsed with Buffer A until absorbance reached background; gradient elution (0.05 to 1.0 M NaCl) was then initiated (Fraction 12), and at the end a 2 M NaCl step was employed (Fraction 45). The column had a packed volume of 60 ml and contained 100 mg of denatured DNA; 17-ml fractions were collected every 16 min. Sodium chloride concentrations were determined by conductivity. About 75% of the applied protein was recovered by the elution; of this, about 60% was estimated to be UP1.

**Fig. 6.** Molecular weight and charge characterization of the protein species eluted from the denatured DNA-cellulose column shown in Fig. 5. The gel numbers listed correspond to the fractions designated in Fig. 5, with the gels at the bottom representing the original material applied to the column. For each pair of gels, a sample volume corresponding to 0.045 $A_{280}$ units of protein was dialyzed against 5 mM Tris-Cl, pH 8.8/10% glycerol; it was then split, with one-third used for the sodium dodecyl sulfate-polyacrylamide gel electrophoresis and two-thirds for electrofocusing gels. A, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; apparent molecular weights are indicated. B, Isoelectric focusing of proteins in polyacrylamide gels containing urea, pH 3 to 10 gradient. Gels are displayed with the cathode (basic end) to the left; similar bands are aligned and pH 7 is marked with an arrow.
Fig. 7. Final purification of UP1 samples with different isoelectric compositions. A, gel permeation chromatography (Bio-Gel A-0.5m agarose gel). Fractions from the gradient-eluted denatured DNA column shown in Fig. 5 were pooled and named as follows: Acidics, Fractions 3 and 4; Mix, Fractions 6, 7, and 8; Basics, Fractions 9, 10, and 11. The pooled materials were concentrated and dialyzed with about 60% recovery and were applied to the same column at about 125 ml apart (see under “Materials and Methods”). Elution volumes are given from the beginning of the sample applications. Peak fractions eluted from the gel permeation column were pooled (more specifically, the top 3, 4, and 2 such fractions from the Acidics, Mix, and Basics, respectively). The resulting three samples were each concentrated, dialyzed, and stored at -80° as described under “Materials and Methods.” Final yields of UP1 ranged from 70 to 85% of the total materials applied to the column. B, sodium dodecyl sulfate-polyacrylamide gels. Aliquots containing 0.002, 0.010, and 0.025 units of each set of subspecies were applied, and gels are displayed as in Fig. 2A. The Acidics, Mix, and Basics fractions show apparent molecular weights of 23,700, 24,200, and 24,800, respectively. C, Urea polyacrylamide isoelectric focusing gels, pH 3 to 10. Aliquots representing 0.020 units were applied, and gels are displayed as in Fig. 6B.
mimic the particular polyphosphate conformations of single-stranded DNA to which these proteins bind. However, we have found (23) that UP1, but not the other two fractions, can be completely eluted from single-stranded DNA with more concentrated sodium dextran sulfate (8.75 mg/ml of sodium dextran sulfate, total [Na+] = 0.05 M). Thus, while UP1 has been isolated on the basis of its sodium dextran sulfate-resistant binding, it is only partially resistant. It is interesting to note that, like the calf UP1, both T4 bacteriophage gene 32 protein and the E. coli DNA-unwinding protein are only partially sodium dextran sulfate-resistant.

The major portion of the mammalian single-strand-specific, sodium dextran sulfate-resistant proteins appears not to be chromatin-associated, as judged by our cell fractionation studies. The possibility that the binding of these proteins to DNA is an artifact of in vitro conditions seems unlikely in view of the strong specificity of their binding (i.e. to single-stranded DNA, but not to double-stranded DNA or sodium dextran sulfate). However, while they may slightly prefer DNA, both UP1 and the high salt eluting proteins also have a substantial affinity for single-stranded RNA (32). In view of the possible excess of single-stranded RNA over single-stranded DNA in vivo, much of this protein could be bound to RNA at any instant.

If these proteins have DNA, rather than RNA, as their primary site of action, their apparent non-chromatin location might be an artifact caused by cell disruption, or the cell might contain a pool of proteins that bind to DNA only for a short period during the cell cycle, e.g. during the S period. The high molecular weight eukaryotic DNA polymerase, which presumably binds to nuclear DNA at some point and may in fact be in the nucleus in vivo, is likewise found in the non-chromatin fraction (45); in an accompanying paper we show that high molecular weight DNA polymerase is stimulated in vitro both by UP1 and by the high salt eluting proteins (39).

Calf UP1 shows a reproducible isoelectric point heterogeneity, which, from available data, could arise either genetically or from limited exopeptidase action. With regard to its possible physiological significance, it is important to note that this small charge difference between subspecies has a marked effect on DNA-affinity (Figs. 5 and 6). Thus, different subspecies could have related but different functions; alternatively, only the most basic subspecies might be functional, the less basic subspecies being inactive "turnover" products. However, the heterogeneity might also reflect heterogeneity in the thymus cell population, the state of the protein being a differentiated characteristic. None of these possibilities can be eliminated a priori, and further study of this protein, particularly in synchronized tissue culture cells, will be necessary to evaluate them adequately.

As judged by a variety of criteria (electrophoretic mobility in sodium dodecyl sulfate-polyacrylamide gels, salt elution from DNA-cellulose, insensitivity to sodium dextran sulfate elution, and specificity for single-stranded DNA), we have isolated proteins corresponding to the calf UP1 and to the calf 33,000 dalton, high salt eluting protein from extracts of cultured mouse cells (3T3 and I-10 cells; Ref. 1 and Footnote 9) and cultured monkey cells (primary African green monkey kidney and CV-1 cells). Moreover, a large scale extraction of 0.5 g of mouse I-10 cells (Leydig cell tumor; Ref. 46) yielded a single-strand-specific, sodium dextran sulfate-resistant protein fraction comparable in quantity and composition to the calf thymus preparation. Thus, these proteins which we have characterized from calf thymus may be present in all mammalian cells.

Tsai and Green (47) have identified in both mouse and human cultured fibroblasts a cytoplasmic protein, designated P8, which they purified by taking advantage of its selective affinity for single-stranded DNA. P8 is present in very large amounts, consisting of 3% of the soluble cell protein in the human cells used. This protein migrates slightly slower than our 33,000 dalton proteins on a sodium dodecyl sulfate-polyacrylamide gel. 11 Stein (14) finds a single-strand-specific DNA-binding protein, which she calls P8, in elevated levels in senescent lines of WI38 human cells; it migrates together with the P8 of Tsai and Green on sodium dodecyl sulfate electrophoresis. 12 She also sees a 25,000 dalton, single-strand-specific DNA binding protein which presumably corresponds to the calf UP1. Fox and Pardee (4) have demonstrated two prominent single-strand-specific, DNA-binding proteins in hamster cell cultures; the one that elutes from DNA-cellulose at 0.15 M NaCl has subsequently been found to co-migrate on sodium dodecyl sulfate-polyacrylamide gels with the calf thymus 33,000 dalton proteins; the other, which eluted at 0.60 M NaCl, probably corresponds in molecular weight to the calf UP1. 13 Both hamster proteins appear to be synthesized throughout the cell cycle (4). A low salt eluting, 33,000 dalton, single-stranded DNA-binding protein has been demonstrated in the cytoplasm of human cells (15). These various 33,000 dalton DNA-binding proteins may correspond to a rat liver protein (30,000 daltons, pi = 9.6) which stimulates RNA polymerase B (48).

Hotta and Stern (2, 49) have described a presumably different single-strand-specific DNA-binding protein which appears in greatly elevated amounts during meiotic prophase in germ-line tissues of both mammals and plants. Banks and Spanos (50) have isolated a single-strand-specific DNA-binding protein from the basidiomycete fungus Ustilago maydis; it behaves in many respects like the prokaryotic DNA-unwinding proteins.

In the following two articles (32, 39) we further characterize the calf single-strand-specific, sodium dextran sulfate-resistant DNA-binding proteins, with the special view of comparing them with the prokaryotic DNA-unwinding proteins.

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