# BINDING SPECIFICITY OF A HELA DNA-BINDING PROTEIN TO DNA AND HOMOPOLYMERS

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SUMMARY: A protein which has affinity for single-stranded DNA but not for double-stranded DNA has been isolated from HeLa cells by DNA-cellulose chromatography. This protein having a molecular weight of 34,000 was accounted for approximately 3% of total soluble proteins. Its binding specificity to DNA and nucleotide homopolymers has been investigated by Sephadex G-200 column chromatography. Specific binding to single-stranded DNA has been confirmed also by this method and furthermore strong binding to poly U has been found.

A number of proteins which have affinity for ss-DNA have been isolated from prokaryotic (1-5) and eukaryotic cells (6-10). The proteins which bind tightly and specifically to ss-DNA such as the gene 32 protein of phage T4 and other "unwinding proteins" of some phages and E.coli are able to unwind ds-DNA and to stimulate DNA polymerase reaction in vitro (1,2,4,5,11). From genetic studies, the gene 32 protein was shown to be required for DNA replication as well as recombination (12,13).

Recently a similar protein has been isolated from primitive eukaryotic cells, Ustilago maydis (9). As for mammalian cells, Hotta and Stern have found such a protein in meiotic cells but not in somatic cells (6). Tsai and Green have isolated P8 protein which binds specifically to ss-DNA from cultured mammalian cells(7). The P8 protein was shown to be synthesized mainly in the growing state, while its function in vivo has not been elucidated yet.

In an attempt to obtain further information on this kind of proteins, we have isolated a ss-DNA binding protein from HeLa cells and examined some of its proper-

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Abbreviations: ss-DNA, single-stranded, heat-denatured DNA
ds-DNA, double-stranded, native DNA

ties with a special reference to the binding specificity to DNA and nucleotide homopolymers.

MATERIALS and METHODS: HeLa cells were grown in Eagle's MEM containing 10% calf serum and incubated in a $CO_2$ cabinet. Cell proteins were labeled for 6 hours with [$^3$H]leucine (39 Ci/mM The Radiochemical Centre, Amersham, England) at 20 μCi/ml in fresh medium containing 0.08 mM leucine.

The cell extract was prepared as follows; cells were suspended in a solution containing 40 mM Tris-HCl (pH 8.1), 1 mM mercaptoethanol, 0.2 mM EDTA, and 10 mM $MgCl_2$ and sonicated for 45 sec. The homogenate was centrifuged at 105,000 g for 60 min. The supernatant was incubated with 20 μg/ml DNase I (Worthington) for 15 min at 25° and dialyzed against four changes of buffer containing 20 mM Tris-HCl (pH 8.1), 50 mM NaCl, 1mM mercaptoethanol, and 1 mM EDTA. The dialyzate was centrifuged at 105,000 g for 60 min to remove the precipitate and then was made 10% in glycerol.

DNA-cellulose was prepared according to Alberts and Herrick (14), using calf thymus DNA (Worthington). DNA-cellulose chromatography and SDS-polyacrylamide gel electrophoresis were performed essentially according to the procedure described by Tsai and Green (7). After electrophoresis, gels were sliced 1 mm in thickness and kept at 50°-60° overnight in 0.5 ml of Soluene 350 (Packard) to solubilize the radioactive proteins. The radioactivity was counted in 10 ml of scintillation fluid containing 10 ml of toluene, 50 mg of PPO, and 3 mg of dimethyl POPOP with a scintillation spectrometer. More than 80% of the counts applied were recovered after fractionation.

For assay of binding, Sephadex G-200 column chromatography was used. The reaction mixture (500-600 μl) containing 20 mM Tris-HCl (pH 8.1), 1 mM mercaptoethanol, 1 mM EDTA, various concentrations of NaCl in a range from 50 mM to 125 mM, 3 mg bovine serum albumin, 10% glycerol (v/v), 100 μg nucleic acid as indicated below, and 6.7 μg of DNA binding protein was allowed to stand at 4° for 60 min and chromatographed on a column of Sephadex G-200 pre-equilibrated at 4° with the buffer containing 20 mM Tris-HCl (pH 8.1), 1 mM mercaptoethanol, 1 mM EDTA, and various concentrations of NaCl. The column was eluted with the same buffer. Fractions of 1.1 ml were collected and the radioactivity of 0.5 ml aliquots were counted in 5 ml of scintillation fluid containing 3.3 ml of toluene, 1.66 ml of Triton X-100, 25 mg PPO, and 1.5 mg of dimethyl POPOP with a scintillation spectrometer.

Synthetic ribonucleotide homopolymers, poly A, poly G, and poly U were obtained from Boehringer Mannheim. Poly C was obtained from P-L Biochemicals. All nucleic acid applied to Sephadex G-200 column were sheared by passing through a syringe with a needle (0.4 x 19 mm) three times before use.

RESULTS and DISCUSSION: *Isolation of a ss-DNA binding protein by DNA-cellulose chromatography:* The proteins in exponentially growing HeLa cells were labeled with [$^3$H]leucine. The cell extracts were prepared and chromatographed on DNA-cellulose columns. Proteins eluted from DNA-cellulose column were analyzed by SDS-polyacrylamide gel electrophoresis. Fig. 1a shows the electrophoresis profile of 0.2 M NaCl eluate from ss-DNA cellulose column, in which a peak is especially prominent among several peaks detected. The main peak in Fig. 1a was not found in a SDS-polyacrylamide gel electrophoresis profile of 0.2 M NaCl eluate from ds-DNA cellulose

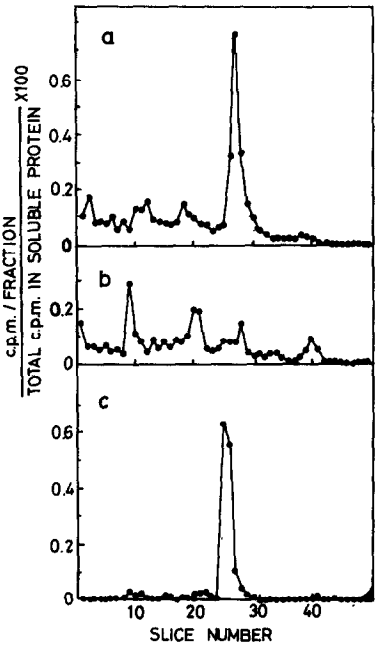
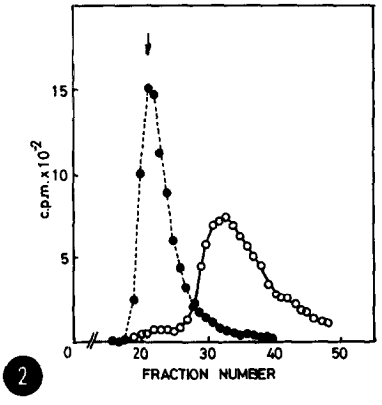



Figure 1. SDS-polyacrylamide gel electrophoresis profiles of HeLa cell proteins eluted from DNA-cellulose columns.
(a) 0.2 M NaCl eluate from ss-DNA cellulose column.
(b) 0.2 M NaCl eluate from ds-DNA cellulose column.
(c) 0.2 M NaCl eluate from ss-DNA cellulose column which passed in advance through ds-DNA cellulose column.

column (Fig.1b), indicating that the protein would have a capacity solely to bind to ss-DNA. To separate the specific ss-DNA binding protein (ss-DNA BP), we have applied the cell extract once passed through ds-DNA cellulose column to ss-DNA cellulose column and confirmed that the ss-DNA BP was almost singly eluted in 0.2 M NaCl eluate (Fig.1c). The amount of this protein was relatively high, which corresponded to about 3% of total soluble proteins. The molecular weight of the ss-DNA BP was estimated by calibration curve obtained from SDS-polyacrylamide gel electrophoresis using molecular weight standard. From this curve the molecular weight of this protein was estimated to be 34,000. On the other hand, the molecular weight estimated by Sephadex G-200 column chromatography was 110,000. This suggests that ss-DNA BP in the native form may be trimer or tetramer.

*Binding specificity:* Binding specificity was investigated by Sephadex G-200 column

chromatography as described in Materials and Methods. DNA and ss-DNA BP were mixed and chromatographed on Sephadex G-200 column. When chromatographed with ss-DNA, ss-DNA BP eluted in the excluded volume along with DNA. In the case of ds-DNA, ss-DNA BP eluted in the included volume as free protein (Fig.2). Fig.3 shows binding of ss-DNA BP to ss-DNA at various concentrations of NaCl. At 50 mM NaCl, almost all ss-DNA BP bound to ss-DNA and at 65 mM NaCl, moderate binding was observed. When NaCl concentration was increased up to 125 mM, no binding was



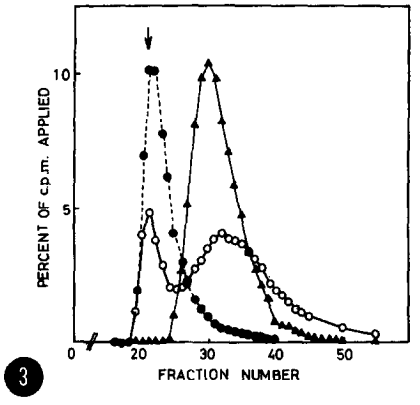




Figure 2. Binding specificity of the HeLa ss-DNA binding protein to ss-DNA in solution assayed by Sephadex G-200 column chromatography. Binding reactions and chromatography in the presence of 50 mM NaCl were carried out as described in Materials and Methods. Arrow indicats the position where DNA eluted.
(●) ss-DNA (○) ds-DNA

Figure 3. Effect of NaCl concentration on binding of the HeLa ss-DNA binding protein to ss-DNA. The protein was mixed with ss-DNA in the solution containing NaCl at concentration as indicated below. Binding reactions and chromatography on Sephadex G-200 column were carried out as described in Materials and Methods. Arrow indicats the position where DNA eluted.
(●) 50 mM NaCl (○) 65 mM NaCl (▲) 125 mM NaCl

detected. When binding assays of homopolymers were performed at 65 mM NaCl, we observed very high affinity for poly U, moderate affinity for poly A and poly C, and low affinity for poly G (Fig.4). These results suggest that major factor determining its specificity to single-stranded nucleic acid may be its affinity to base and not to phosphate group, even though the effect of tertiary structure of single-stranded form of these homopolymers can not be excluded. When HeLa cell extract was applied to ss-DNA cellulose column and eluted in linear gradient, the elution of the protein began at 140 mM NaCl. Its follows that application of this

method to investigate the binding of a protein to nucleic acid results in underestimation of binding strength but it is useful to estimate relative binding strength. Considering these results together with the fact that the binding of this protein to poly U was observed even at the concentration of NaCl over 200 mM by centrifugation method (not shown), it can be concluded that ss-DNA BP indeed has high affinity to poly U. Although the affinity of the protein to deoxyribonucleic acid homopolymers remains to be examined, this apparent high affinity for poly U seems to be very interesting as the transcriptional control RNA is reported

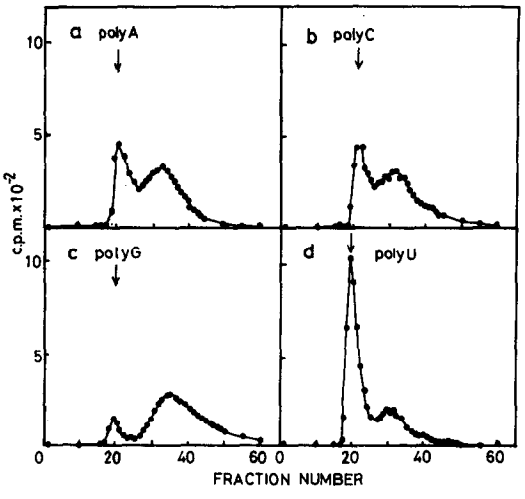



Figure 4. Binding pattern of the HeLa ss-DNA binding protein to nucleotide homopolymers. The protein was mixed with nucleotide homopolymer as indicated in the reaction mixture containing 65 mM NaCl. Binding reactions and chromatography were carried out as described in Materials and Methods. Arrow indicats the position where nucleotide homopolymer eluted.
(a) poly A (b) poly C (c) poly G (d) poly U

to contain poly U sequences (15). Work is now in progress to clarify the in vivo function of this protein.

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