Nucleic Acids Research

Single-strand DNA binding protein from rat liver: interactions with supercoiled DNA

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ABSTRACT

As shown by competition experiments, the single-strand DNA binding protein from normal rat liver (S25) interacts preferentially with supercoiled DNA compared to relaxed DNA duplexes. When followed both by sedimentation analysis and by nitrocellulose filter assay, the binding of S25 to SV40 supercoiled DNA (FI) appears to be non-cooperative. Saturation is reached at a protein to DNA weight ratio of about 2. The S25-DNA complexes prefixed with glutaraldehyde appear as beaded structures having an average of 14 to 16 beads per SV40 DNA molecules. Cross-linking of S25 bound to SV40 DNA by dimethyl suberimidate allows to detect oligomeric structures containing a maximum of twenty monomers of S25. When complexes are treated by glutaraldehyde, 10 % of the genome become resistant against micrococcal nuclease. Moreover, S25 affects the DNA helical structure. Superhelical forms are generated by the association of S25 with SV40 DNA, in the presence of nicking-closing enzyme.

INTRODUCTION

In previous works (1,2,3), we have described the purification and some properties of a single-strand specific DNA binding protein from rat liver.The protein isolated from regenerating liver, called HD25, was able both to lower the melting point of poly d(A-T) (1) and to stimulate rat liver DNA polymerases (4). These results supported the idea that it could be involved in DNA replication. In contrast the protein from normal rat liver, called S25, although it is a single-strand binding protein, was not found to be a melting protein, and in some conditions, inhibits homologous DNA polymerases (2). At this time, the only difference found between these proteins is the ability of HD25 to oligomerize, a property that is not shared by S25. Otherwise HD25 and S25 proteins are undistinguishable, even by the pattern of proteolytic degradation (2,3).

In search for a possible function of S25 in non dividing cells, we decided to look at the way it binds to DNA. In this paper we described its differential affinity for various DNAs and its specific interaction with supercoiled DNA.

MATERIALS AND METHODS

S25 protein, enzymes and nucleic acids.S25 protein was purified from normal
rat liver as previously described (1). The protein was 97% pure as judged
by SDS polyacrylamide gel electrophoresis. It was free of endo-or exonuclease
activity. Micrococcal nuclease was from Worthington. Rat liver nicking-clo-
sing enzyme was purified according to Champoux and Mac Conaughy (5) from
nuclei, through phosphocellulose pool (Fraction III). This fraction still
contained a residual nuclease activity, which was not detectable under the
conditions used in the present study (i.e. in the absence of magnesium).
Single-stranded M13 [^{32}P] DNA was a gift from Dr. E. Richet. Tritium labeled
SV40 supercoiled DNA, prepared according to Hirt method (6) was from Dr.M.
Mechali,unlabeled SV40 DNA was from Dr. J. Feunteun. Relaxed, covalently
closed SV40 DNA was prepared by 20 min incubation with nicking-closing enzyme
in the conditions described by Champoux and Mac Conaughy (5).
After deproteinization, the DNA was recovered by ethanol precipitation.
Value of 3.3 10^6 was used for the molecular weight of SV40 DNA. Calf thymus
DNA was obtained from Choay Laboratories. Denatured calf thymus DNA was
prepared by heating DNA for 15 min at 100°C followed by a rapid coiling to
0°C. DNA concentrations were determined by using E $\frac{260}{mg/ml}$ = 20.
Nitrocellulose filter-binding assay. The assay, used for competition experi-
ments, measured the amount of M13 [^{32}P] DNA bound to nitrocellulose filters
in the presence of protein and was essentially that developed by Tsai and
Green (7). Single-stranded M13 [^{32}P] DNA (0.032 µg) first mixed with an
unlabeled DNA, was incubated with S25 protein in 20 mM Tris-HCl (pH 8.0),
1 mM EDTA, 2 mM 2-mercaptoethanol, 30 mM NaCl and 5% glycerol (Buffer A) in
a total volume of 100 µl, at 37°C for 30 min. 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 prewashed with 10 ml of buffer A containing
1% dimethylsulfoxide. The filter was washed 3 times with 3 ml buffer A, 1%
dimethylsulfoxide, dried and the radioactivity determined in a toluene
2,5-diphenyloxazole (PPO)-1,4-bis [2-(5 phenyloxazolyl)] benzene (POPOP)
scintillation mixture. The nitrocellulose filters (Schleicher and Schüll BA85)
were boiled in water and soaked overnight in buffer A, 1% dimethylsulfoxide,
before use. In the absence of S25 protein, about 1% of the total DNA in the
assay was retained by the filter.

     In experiments with SV40 supercoiled DNA, an alternative procedure
was used according to the method of Jones and Berg (8). SV40 [^{3}H]DNA I
(0.048 µg) was incubated with increasing amounts of protein (0 to 1 µg) in

a total volume of 100 µl for 30 min at 37°C. Reaction mixture contained
50 mM Tris-HCl (pH 7.5), 10 mM KCl, 2.10^{-3} mM EDTA, 0.2 mM $MgCl_2$, 5 mM
2-mercaptoethanol, 15% glycerol (Buffer B). The amount of labeled DNA retai-
ned to the filter was determined as described above.For these experiments,
nitrocellulose filters were incubated in 10 ml buffer B for 30 min before
use.

Electron microscopy. Samples of DNA or protein-DNA complexes were fixed for
10 min at 37°C with 1% glutaraldehyde ; diluted 10 fold in distilled water
and adsorbed on grids according to Dubochet et al. (9). Preparations were
observed with Siemens Elmiskop A electron microscope.

Reaction of dimethyl suberimidate with S25 protein-SV40 DNA I complexes.
Intramolecular cross-linking with dimethyl suberimidate was performed as
described by Davies and Stark (10). Dimethyl suberimidate (25 mg/ml) was
dissolved immediately before use in 0.2 M triethanolamine-HCl, pH 8.5.Cross-
linking was carried out at 20°C in the same buffer. S25 protein-SV40 DNA I
complexes, where S25 protein concentration was 0.2 m/ml, were mixed with
dimethyl suberimidate at a final concentration of 2.5 mg/ml. The reaction
mixture was left at 20°C for 2 h. Cross-linking reagent was again added
to give a final concentration of 4.5 mg/ml and the mixture was left for
12 h at room temperature. DNAase I was then added for 2 h at 37°C prior
addition of 1.5% sodium dodecyl sulfate. The resulting cross-linking pro-
ducts were then analyzed by sodium dodecyl sulfate polyacrylamide gel elec-
trophoresis.(Under these conditions, S25 protein alone was not cross-linked,
whereas cross-linking of oligomeric proteins as *E. coli* RNA polymerase
occured).

Micrococcal nuclease digestion of unfixed or fixed S25-SV40 DNA complexes.
Complexes between SV40 DNA I and S25 were formed by mixing protein and
DNA in a ratio of 2 : 1, for 30 min at 37°C. An aliquot was treated with 1%
glutaraldehyde for 10 min at 37°C to covalently link the protein to the DNA
with which it was associated. The glutaraldehyde was removed by dialysis for
72 h against 10 mM phosphate buffer, pH 7.9, 20% glycerol.

The protein-DNA complex, the SV40 DNA and the protein-DNA complex
covalently bound by glutaraldehyde treatment were digested by micrococcal
nuclease (0.15 units/µg DNA) using 0.2 mM $CaCl_2$. Incubations were carried
out at 37°C for the indicated times when aliquots were removed and precipita-
ted with 0.6 ml trichloracetic acid 5% onto Whatman GF/C glass fibre filters.
Filters were washed and dried before to be counted.

Assay for the generation of superhelical turns. The standard incubation mix-

ture contained, in a total volume of 30 µl, 10 mM Tris-HCl, pH 8.0, 1 mM

EDTA, 30 mM NaCl, 1.2 µg DNA, 1 µl nicking-closing enzyme (Fraction III) and

various amounts of S25 protein. After a time of incubation indicated in the

legend of figure , the reaction was stopped by 1% SDS (final concentration)

for 5 min at 37°C, and the DNA was analyzed by agarose gel electrophoresis.

Electrophoresis were performed in 1% agarose, using horizontal slab gels pre-

pared and run in 36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA, pH 7.8. Gels were

run at 2 v/cm for 26 h at room temperature and stained with ethidium bro-

mide. Photographs of the gels were taken under short wavelenght UV light

with Ilford HP4 film. Densitometric traces were made using a Joyce Loebl

microdensitometer.

RESULTS

Differential affinity of S25 protein for various DNAs

      S25 protein was isolated by its preferential binding to single-

stranded DNA. Indeed, applied to double-stranded DNA and single-stranded DNA

columns connected in series, S25 protein failed to bind to double-stranded

DNA column at 0.05 M NaCl.It was dissociated with 0.4 M NaCl from the single-

stranded DNA column (1-3). Nitrocellulose filter binding assay was used to

mesure the relative ability of S25 protein to bind various nucleic acids.

Single-stranded [^{32}P] M13 DNA was first mixed with an unlabeled DNA, then

incubated with S25 protein and the solution was filtered.

      Unlabeled DNA mixed with single-stranded [^{32}P] M13 DNA reduced

the amount of labeled DNA bound by S25 to the filter as a function of the

affinity of the protein for competing DNA. The results are given in Fig.1.

Denatured calf thymus DNA was found to strongly compete with [^{32}P] M13 DNA,

providing that S25 protein forms complex with single-stranded DNA, regardless

of base sequence. As expected, duplex DNA does not compete with M13 DNA, even

when present in large excess. From the above data, it can be estimated that

the apparent affinity of S25 is more than 3 orders of magnitude greater for

single-standed M13 DNA than for double-stranded DNA. In this assay, S25 pro-

tein was found to have some affinity for supercoiled DNA, which is only 20 to

40 fold weaker than for M13 single-stranded DNA. When the supercoiled DNA of

SV40 was converted to the relaxed circular form, its competition with single

-stranded DNA was completely abolished. The efficiency of complex formation

with S25 protein thus depends on the superhelical conformation of the DNA.

Interactions of S25 protein with supercoiled DNA.

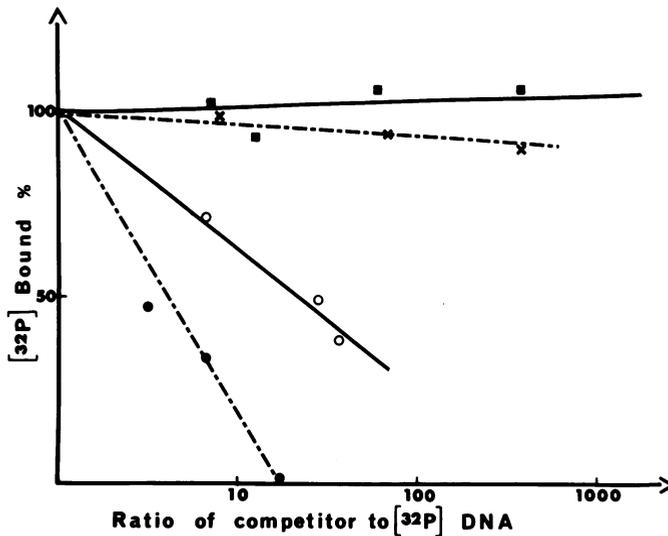   From the above data, S25 protein was found to have a special abi-

Fig. 1 : Competition between M13 single-stranded DNA and various DNAs for the binding to S25 protein .[^{32}P] labeled M13 DNA (0.4 µg/ml) was mixed with different unlabeled DNAs in different ratios, followed by addition of S25 protein(2.8 µg/ml). Binding assays were performed at 37°C and the solution was filtered through nitrocellulose filters as described in Methods. Without competing DNA, 45% of the input [^{32}P] M13 DNA was bound to the filter. This value was set at 100% to calculate the level of competition. Competing DNA : (●-●), denatured calf thymus DNA ; (O—O), SV40 DNA I ; (×-×), native calf thymus DNA ; (■-■), relaxed SV40 DNA I .

lity to interact with supercoiled DNA. The binding of S25 protein to SV40 supercoiled DNA was first studied using nitrocellulose filters. Fig. 2 shows the binding curve obtained by plotting the amount of SV40 [^{3}H] DNA retained on the filter as a function of protein concentration. The data were independent of the extent of washing the filters and of the dilution of the sample immediately before filtration, and indicated that S25 protein saturated the DNA at a weight ratio of about 2-2.5 : 1. The binding curve exhibited a sigmoidal shape that could reflect either that more than one protein molecule was required to retain DNA on the filter, or the cooperative binding of the protein to DNA.

An increase in the salt concentration resulted in a large reduction of the binding (Fig.3). The salt sensitivity of the complex appeared independent of protein/DNA ratio. This data argues for a non-cooperative binding of the protein to DNA. Once formed, the complex appeared to be rather unstable. In order to study the dissociation of the protein from
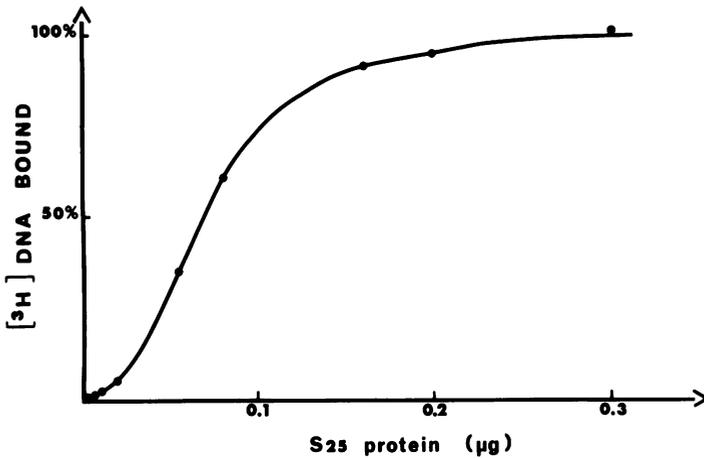
Fig. 2 : Complex formation between S25 protein and SV40 DNA I. After incuba-
tion of 0.05 µg of supercoiled SV40 [³H] DNA with increasing amounts of S25
protein for 30 min at 37°C, nucleoprotein complex was filtered as indicated
in Methods, and the amount of retained DNA was plotted against S25 concentra-
tion. The radioactivity remaining on the filters in the absence of protein
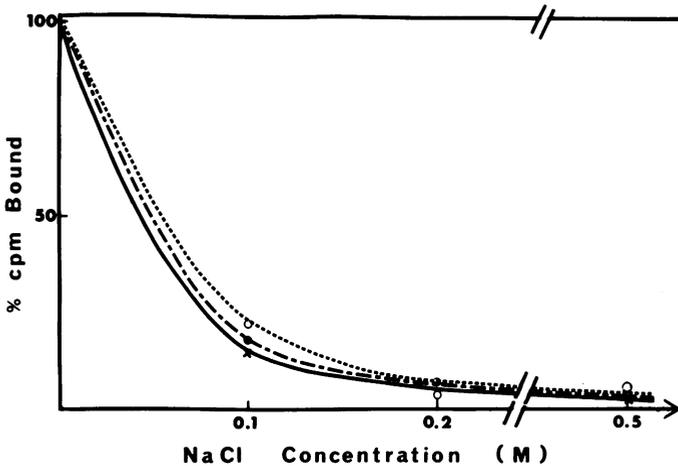(2% background) was subtracted.

Fig. 3 : Stability of the S25-SV40 DNA I complexes in the presence of salt.
At each protein concentration, the radioactivity of DNA retained to the fil-
ters in absence of salt was set at 100%. SV40 DNA I (0.05 µg) was incubated
with S25 protein for 30 min at 37°C in 0.1 ml buffer B, then 0.9 ml of buf-
fer B containing the NaCl concentration indicated was added. After 5 min at
37°C the solution was filtered and the filters rinsed with the buffer contai-
ning salt. Protein to DNA ratio : (O-O), 0.8 ; (●-●), 1.4 ; (✗—✗), 4.

supercoiled DNA, an excess of unlabeled DNA was added to a preformed complex containing [³H] labeled DNA. The mixture was incubated at 37°C and samples were filtered at various times. The rate of release of the protein was measured by the decrease of labeled DNA retained by the filter as a function of time. As shown in Fig. 4, release of the protein proceeds rapidly, with a half time of about 10 min at 37°C and the redistribution was completed within 45 min.

The stoichiometry of S25 protein–SV40 DNA complexes was investigated in more details by sedimentation studies. Complexes obtained with a fixed quantity of labeled DNA and increasing amounts of S25 protein were sedimented through linear sucrose gradients, as indicated in the legend of Fig. 5. Peak displacement in the sedimentation profiles was a linear function of the protein added and reached a plateau when the complexes were saturated. By this criterion, saturation was reached at a protein to DNA weight ratio of about 2:1. This value, when compared to the ratio of 14:1 obtained with single–stranded DNA,(manuscript in preparation) suggests that the SV40 would be only partially covered by the protein.Sedimentation profiles were in in favor of a non-cooperative binding of the protein to DNA.

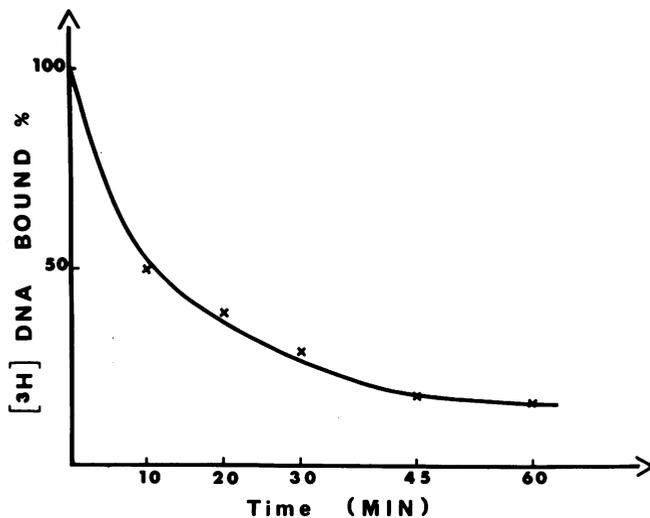S25 – SV40 DNA complexes were visualized as described by Dubochet

Fig. 4 : Dissociation of the complex between S25 protein and SV40 DNA I. Complex between [³H] labeled SV40 DNA I and S25 was formed by mixing protein and DNA in a ratio of 2 : 1. After 30 min at 37°C, a 10 fold excess of unlabeled SV40 DNA I was added and the mixture was incubated at 37°C. Samples were filtered at various times.
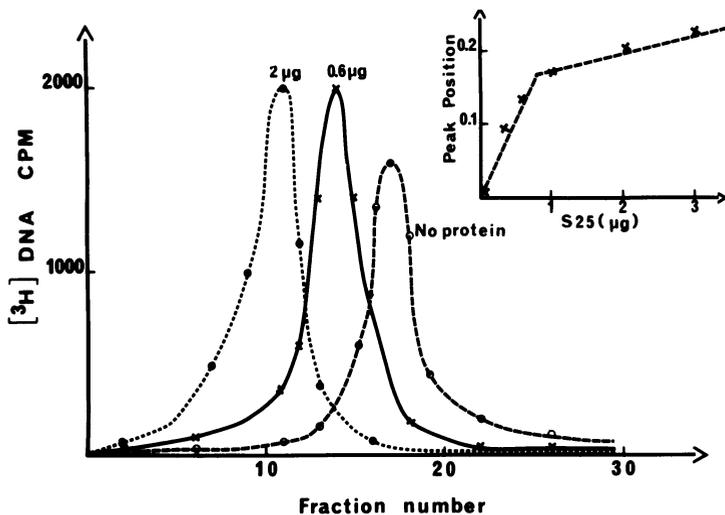
Fig. 5 : Sedimentation of supercoiled SV40 DNA complexed with S25 protein. Supercoiled SV40 [³H] DNA(0.440 µg) was incubated at 37°C for 30 min without (O--O) or with 0.6 µg (×—×) or 2 µg (●-●) of S25 protein in buffer B, 2.5 % glycerol, in a final volume of 100 µl. Then, the samples were loaded onto a 5 to 20% 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°C and 40.000 rpm in a SW50.1 Spinco rotor. Twenty-eight equal fractions were collected from the bottom. The radioactivity in each fraction was determined by liquid scintillation counting. Inset : the peak position in each gradient is plotted against the amount of protein added.

et al. (9). The complexes prefixed with glutaraldehyde, appeared as entangled and beaded structures (Fig. 6). The average number of beads, at protein saturation, was 14 to 15 per SV40 DNA molecule, depending on the preparation. At subsaturating amounts of protein, an intermediate number of beads per SV40 DNA molecule was observed (not shown). In the absence of fixation the beaded structure was lost during spreading on the activated carbon grids, providing the relative unstability of these complexes. These structures consisting of a chain of beads, resemble the nucleosome structures obtained by the assembly of SV40 DNA I with the four core histones (11) or with *E.coli* HU protein (12). The most disturbing difference is the fact we failed to observe a significant compaction of DNA complexed with S25 protein. This suggests that DNA would not be wrapped around S25 protein.

The state of aggregation of the protein bound to SV40 DNA was analyzed by cross-linking with dimethyl suberimidate. Alone, the protein
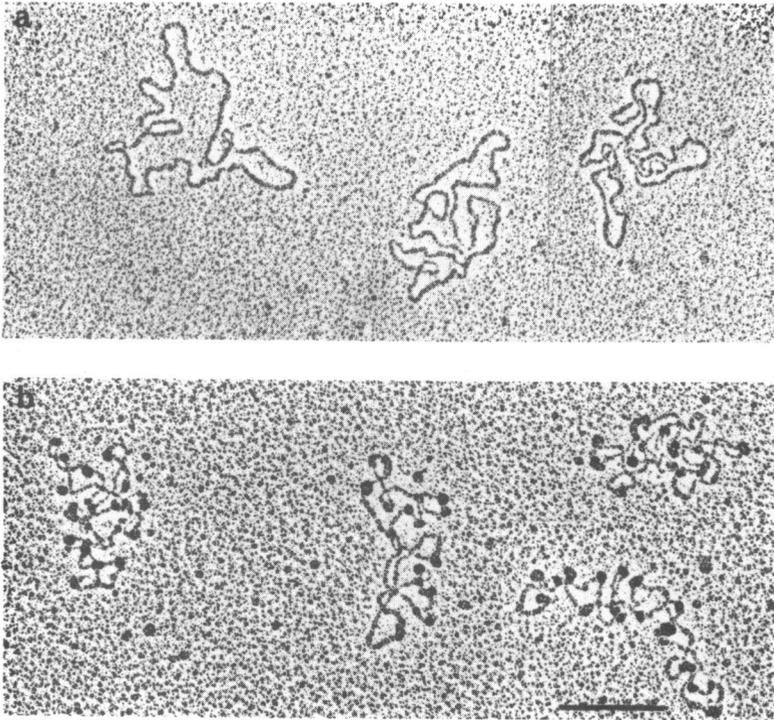
Fig. 6 : Electron microscopy of S25-SV40 DNA complexes. SV40 DNA I and S25 were incubated in 10 mM potassium phosphate pH 7.0, for 30 min at 37°C and processed as described in Materials and Methods. (a) SV40 DNA I control; (b) S25-SV40 DNA complexes at protein saturation. The bar represents 0.2 μm.

was not cross-linked by the reagent, providing that the native protein contains a single polypeptide chain.SDS polyacrylamide gel of S25 protein cross-linked when bound to DNA at a weight ratio of 1:1 is shown in Fig. 7. Densitometer tracing reveals several bands corresponding to cross-linked oligomers containing two, three, four up to a maximum of approximately twenty monomers . Similar patterns were also obtained at different protein to DNA ratios (0.5 : 1 and 1.5 : 1). These results suggest that DNA binding induces oligomerization of the protein. An oligomeric structure of about twenty monomers (500 000 daltons) might correspond to the size of the beaded structures observed by electron microscopy.

Micrococcal nuclease digestion was also used to investigate the structure of S25 - SV40 DNA complexes. Incubation of protein-DNA complexes was performed under the conditions described in Materials and Methods and
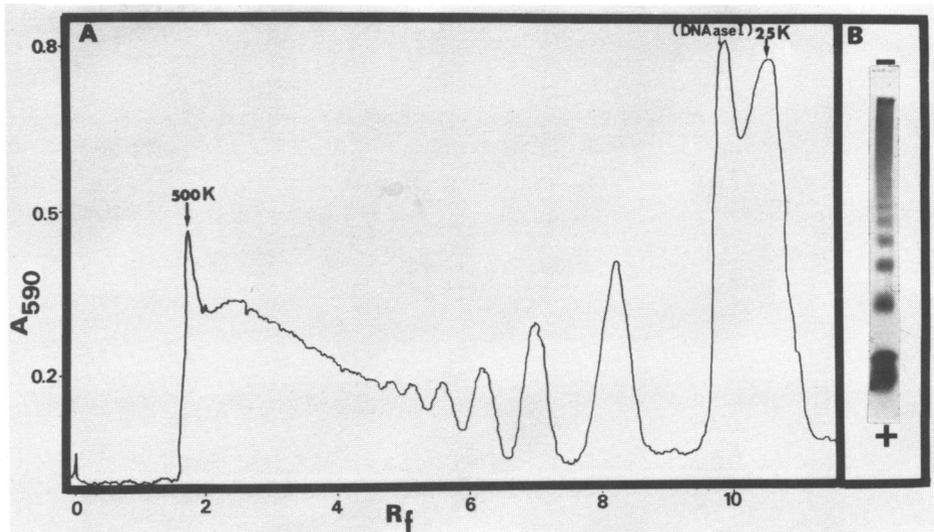
Fig. 7 : Sodium dodecyl sulfate gel analysis of S25 bound to SV40 DNA I and
cross-linked with dimethyl suberimidate. S25 (5 µg) complexed with SV40 DNA I
was cross-linked with dimethyl suberimidate as indicated under Methods.
DNA was digested with DNase I (molecular weight : 38 K), prior addition of
SDS and analysis by SDS polyacrylamide gel electrophoresis. The weight ratio
of S25 protein to DNA was 1 : 1. Electrophoresis was performed on a SDS 2.5-
15 % polyacrylamide gradient gel. (A) densitometer scan, (B) stained gel .

the extent of digestion determined by precipitation with trichloracetic acid.
The rate of hydrolysis of SV40 was slightly reduced in the complex with S25
protein, proportionnaly to the amount of protein added, to be minimum at
protein saturation. But, at all concentration of S25 protein, total diges-
tion of the genome still occurs. As shown in Fig. 8, incubation of the satu-
rated complex with micrococcal nuclease resulted in digestion of almost all
the DNA within 2 min, whereas free SV40 DNA was digested within less than
1 min. When the result of such an incubation was analyzed on polyacryla-
mide gel, it was impossible to detect any protected DNA fragment. This re-
sult is consistent with the unstability of the complexes observed both in
the electron microscope and in filter binding experiments.It was then deci-
ded to link the protein to DNA within the complex by glutaraldehyde treat-
ment. Then, when subjected to nuclease digestion, two differences were ob-
served as compared to non-treated complex (Fig. 8) : The rate of hydrolysis
was once more reduced, and moreover 10% of the genome remained undigested
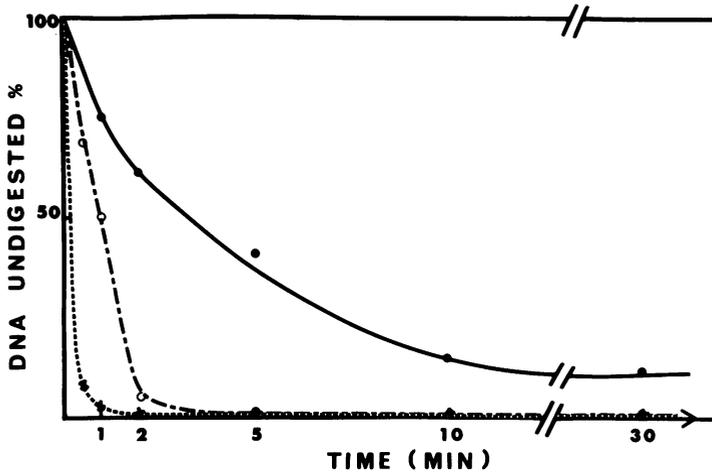after 30 min of incubation.

Fig. 8 : Time course of hydrolysis of SV40 DNA I by micrococcal nuclease in absence or presence of S25 protein, after fixation by glutaraldehyde or not. SV40 DNA I and S25-SV40 DNA complexes were digested with micrococcal nuclease as described in Methods. Samples were removed at intervals, trichloracetic acid precipitated onto filters and counted. (x--x) SV40 DNA I , (O-O) S25-DNA complex,(●—●)glutaraldehyde-treated S25-DNA complex.

The aspect of the S25-SV40 DNA complexes observed by electron microscopy lead us to study the effect of S25 on the structure of closed circular DNA. We used for this approach the procedure described by Germond et al (11) to study the *in vitro* formation of nucleosomes on SV40 DNA. In the presence of rat liver nicking-closing enzyme (5), S25 could introduce superhelical turns into SV40 DNA, as indicated by migration of deproteinized DNA in agarose gels. As shown in Fig. 9, the number of superhelical turns increased with S25 protein added to reach a plateau of 15 to 16 turns, for protein to DNA ratios of 1.7 to 2.6 (w/w). This number could be correlated with the average number of beads visualized by electron microscopy per SV40 DNA molecule. In other experiments, performed with another supercoiled DNA, i.e. PM2 DNA, S25 protein again generated superhelical turns (Duguet et al., submitted to publication).

DISCUSSION

This study shows that the single-strand DNA binding protein isolated from the cytosol of normal rat liver (S25) binds to SV40 DNA I in a characteristic manner. Its interactions with double-stranded SV40 DNA
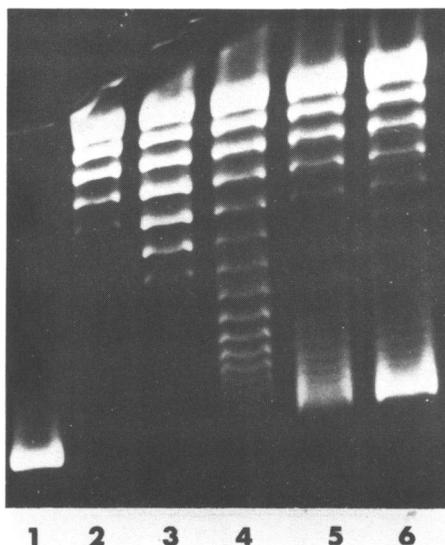
Fig. 9 : Generation of superhelical turns in SV40 DNA. SV40 DNA I was incu-
bated with increasing amounts of S25 at 30 mM NaCl in the presence of nicking
-closing enzyme for 45 min at 37°C, deproteinized and electrophoresed as
described in Materials and Methods. (1), DNA I ;(2 to 6), complexes with S25
at protein to DNA ratio of : (2), 0.0 ; (3), 0.22 ; (4), 0.88 ; (5), 1.76 ;
(6) , 2.65.

depends on the superhelical state of the molecule. Our studies on the stoi-
chiometry of the complex show that saturation occurs at a protein to DNA
weight ratio of about 2, whereas a weight ratio of 14 was found for single-
stranded DNA, suggesting that the DNA would be only partially covered by
the protein. This assumption was confirmed by electron microscopy studies
and nuclease digestion . S25-SV40 DNA complexes fixed with glutaraldehyde
appear as beaded structures that resemble minichromosomes. The average
number of beads at protein saturation was 14 to 15 per genome. These struc-
tures appeared rather unstable, and in the absence of fixation the beaded
structure is lost during spreading on the activated carbon grids. In the
same way, competition experiments revealed a redistribution of protein mo-
lecules on the DNA.

As demonstrated by cross-linking experiments, S25 protein formed
large aggregates on the DNA, whereas it was not cross-linked in the absence
of DNA. The maximum number of monomers by oligomeric structure could be
roughly estimated to twenty. Keeping in mind the number of beads per SV40

DNA molecules observed in electron microscopy, this value is in good correlation with the weight ratio reported above for the saturated complex.

        Although the binding was found non cooperative by sedimentation
or filtration studies,the formation of oligomeric structures may involve
direct interactions between the monomers. Because of the unability of S25
to self associate, one would assume that DNA binding induces a conformationnal change in the protein, that allows its aggregation. At intermediate protein to DNA ratios, an intermediate number of beads was observed without
change in the limit molecular weight obtained after cross-linking. No permanent protection of SV40 DNA complexed with the S25 protein against micrococcal nuclease was noted, unless the protein and DNA were first cross-linked
by glutaraldehyde.After such treatment, 10% of the DNA (about 500 base pairs)
became resistant to nuclease. If this proportion of the genome protected
(10%) reflects the total length of the binding sites of S25, it is possible
to estimate that each oligomeric structure would occupy a site of 35 to 40
base pairs long in the saturated complex.

        Furthermore, S25 affects the DNA helical structure. Superhelical
forms are generated after incubation of S25 with SV40 DNA in the presence of
nicking-closing enzyme and deproteinization. Moreover, the number of negative superhelical turns corresponds to the number of beaded structures. Conformationnal changes in DNA imposed by protein binding were already demonstrated with histones (11), HMG 1 and HMG 2 proteins (13) and *E. coli* HU protein (12). As reported here, no reduction in the length of the DNA was found
upon S25 binding, providing that DNA would not be wrapped around the protein
as in the case of histones and HU protein ; then the binding of S25 may
induce either a change in DNA helix rotation, or a local separation of DNA
strands by preferential binding of S25 to single-stranded DNA, possibly
within the beaded structures. An unwinding of the double helix was also proposed to explain the DNA helical change introduced by the binding of HMG
proteins (14).

    In view of its strong specificity of binding to single-stranded DNA,
its effects on the helix parameters, and its relative abundance in the cell,
S25 may serve as structural element in chromatin. As it was previously
described, S25 does not exhibit the properties of a helix destabilizing protein (HD protein) (i.e. lowering the Tm of DNA, and stimulating homologous
DNA polymerases). Electron microscopy studies also confirm this difference.
In fact, it could share some properties with non histones proteins HMG 1 and
HMG 2 which also change the helical structure of DNA. As these non histone

proteins, S25 is present either in cytosol and in nuclear extracts.
Serological techniques may be useful in studies its cellular distribution
as its possible relationship with non histone proteins.

REFERENCES

1 Duguet, M. and De Recondo, A.M. (1978) J. Biol. Chem. 253, 1660-1665
2 Bonne, C., Duguet, M. and De Recondo, A.M. (1979) FEBS Letters 106,
   292-296.
3 De Recondo, A.M., Bonne, C. and Duguet, M. (1980) in Mechanistic studies
   of DNA replication and genetic recombination, ICN-UCLA Symposia on Mole-
   cular and Cellular Biology, Vol. XIX, eds.Bruce Alberts and C. Fred Fox
   Academic Press (in press).
4 Duguet, M., Soussi, T., Rossignol, J.M., Mechali, M. and De Recondo,A.M.
   (1977) FEBS letters 79, 160-164.
5 Champoux, J.J. and Mac Conaughy, B.L. (1976) Biochemistry 15, 4638-4642.
6 Hirt, B. (1967) J. Mol. Biol. 26, 365-369
7 Tsai, R.L. and Green, H. (1973) J. Mol. Biol. 73, 307-316.
8 Jones, O.W. and Berg, P. (1966) J. Mol. Biol. 22, 99-209.
9 Dubochet, J., Ducommun, M., Zollinger, M. and Kellenberger, E. (1971)
   J. Ultrastructure Res. 35, 147-167.
10 Davies, G.E., and Stark, G.R. (1970), Proc. Natl. Acad. Sci. USA 66,
   651-656.
11 Germond, J.E., Hirt, B., Oudet, P., Gross-bellard, M. and Chambon, P.
   (1975) Proc. Natl. Acad. Sci. USA 72,1843-1847.
12 Rouviere-Yaniv, J., Yaniv, M. and Germond, J.E. (1979) Cell 17, 265-274.
13 Javaherian, K., Liu, L.F. and Wang, J.C. (1978) Science 199, 1345-1346.
14 Javaherian, K., Sadeghi, M., and Liu, L.F. (1979) Nucleic Acids Res. 6,
   3569-3580.