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Escherichia coli single-strand binding protein organizes single-stranded DNA in nucleosome-like units

(electron microscopy/nuclease digestion/nucleosomal structure)

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ABSTRACT **Electron microscopy shows that complexes of the single-strand DNA binding protein (SSB) of *Escherichia coli* and phage fd DNA appear as beaded fiber loops containing an average of 38 beads, 1 per 170 bases of DNA. Extensive digestion of native unfixed SSB–fd DNA complexes with micrococcal nuclease reveals a protected DNA fragment of 145 bases, while shorter digestion periods result in a sequence of fragments in multiples of 160 ± 25 bases. Digestion of these complexes with DNase I produces a repeating pattern of bands, multiples of approximately 15 bases with strong bands at 60, 105, 118, 130, 145, 150, and 210 bases. Isopycnic banding in CsCl solution yields densities of 1.272 and 1.700 g/ml, respectively, for SSB alone and for fd DNA and, after fixation, of 1.388 g/ml for fd DNA–SSB beaded fibers and 1.373 g/ml for the individual protein–DNA beads. Based on these data and the molecular weights of SSB and fd DNA, we suggest that the nucleoprotein chain consists of eight molecules of SSB bound to 145 bases of DNA, with these units linked by roughly 30 bases of protein-free DNA. The excellent concord between results obtained by enzyme digestion of unfixed native samples and, after fixation, by electron microscopy and density banding supports the conclusion that SSB organizes single-stranded DNA in a manner similar to the organization of duplex DNA by histones.**

DNA binding proteins perform vital roles in all cells. A single-stranded DNA (SS DNA) binding protein present in *Escherichia coli* (SSB) was first identified and purified by Sigal *et al.* (1). Isolation of mutants of this protein and *in vivo* studies have shown that it is required in replication of the chromosomes of *E. coli* and several small DNA phages (2–5) and involved in genetic recombination and SOS repair (6–9). One must conclude that SSB interacts in a precise manner with enzymes in the replication and recombination pathways and that changes in these interactions are highly detrimental to the cell. Despite the central role of this protein, little is known about the three-dimensional structure of the SSB–DNA complex. This represents a serious gap in our knowledge because it is likely that the structure of the SSB–DNA complex is a key element in its interaction with other proteins, in particular with RecA, another SS DNA binding protein, also present in substantial amounts in the normal cell.

In previous studies, electron microscopy (EM) has been used to visualize SSB–SS DNA complexes (10, 11) and a regular beaded substructure has been noted (12). Although it has been shown that the fixation methods used in those studies retain the native features of nucleoprotein structures (13, 14), in any morphological study by microscopy, the structural features of apparent interest may reflect the preparative procedure and not the native form. The only secure argument against such artifact is to establish a strong accord between results obtained by EM and by other methods not dependent on fixation or drying. Here, we have used a variety of techniques including EM, nuclease digestion, and equilibrium density banding. We present direct evidence for a repeating structural unit of 145–170 bases from two very different approaches: enzyme digestion of native unfixed samples and EM visualization after fixation. This agreement supports our use of the fixation method in measuring the number of SSB molecules per unit length in the complexes. The value we obtained by this method suggests that an octamer of SSB forms the core of each "SSB nucleosome." The striking similarity of the organization of SS DNA by SSB to that of duplex DNA by histones (15, 16) must reflect important functional features.

MATERIALS AND METHODS

SSB and DNA. The SSB protein used in these studies was (*i*) the gift of Arthur Kornberg (Stanford University) [three different preparations prepared by the method of Weiner *et al.* (17)], (*ii*) the gift of Claus Christiansen and Robert Baldwin (Stanford University) (prepared as above), and (*iii*) purified in this laboratory (three preparations) from an overproducing strain by the method of Chase *et al.* (18). Isolation of phage fd SS DNA labeled with ^{32}P (ICN) was as described by Makino *et al.* (19). SSB–fd SS DNA complexes were formed at a protein/DNA ratio of 10:1 unless otherwise stated.

Enzymes and Reagents. DNase I (Sigma) was further purified by affinity chromatography on Agarose-UMP, as described by Brison and Chambon (20). Micrococcal nuclease was from Worthington.

Gel Electrophoresis. Products of DNase I digestions were analyzed on 10% polyacrylamide gels as described by Peacock and Dingman (21). Electrophoresis of micrococcal nuclease digestion products was on 4–10% polyacrylamide gels with an acrylamide/bis-acrylamide ratio of 30:1.2. The gels and running buffer were 0.02 M Tris base/0.04 M NaOAc/1 mM EDTA. All gels were dried and analyzed by autoradiography with Kodak X-Omat AR film.

RESULTS

Electron Microscopy of SSB–fd DNA Complexes. Direct visualization of SSB–SS DNA complexes by EM revealed a repeating beaded substructure. In a typical preparation, SSB and fd SS DNA were mixed at protein/DNA mass ratios of 1:1 to 40:1 in 0.01 M Tris base, pH 7.5/1 mM EDTA, allowed to remain 10 min at 4°C, and then fixed with sequential addition of formaldehyde and glutaraldehyde (14). The unbound protein was removed by chromatography on Sepharose 4B, and the samples were prepared for EM by adsorbing them onto thin

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Abbreviations: EM, electron microscopy; SS DNA, single-stranded DNA; SSB, *E. coli* SS DNA binding protein.

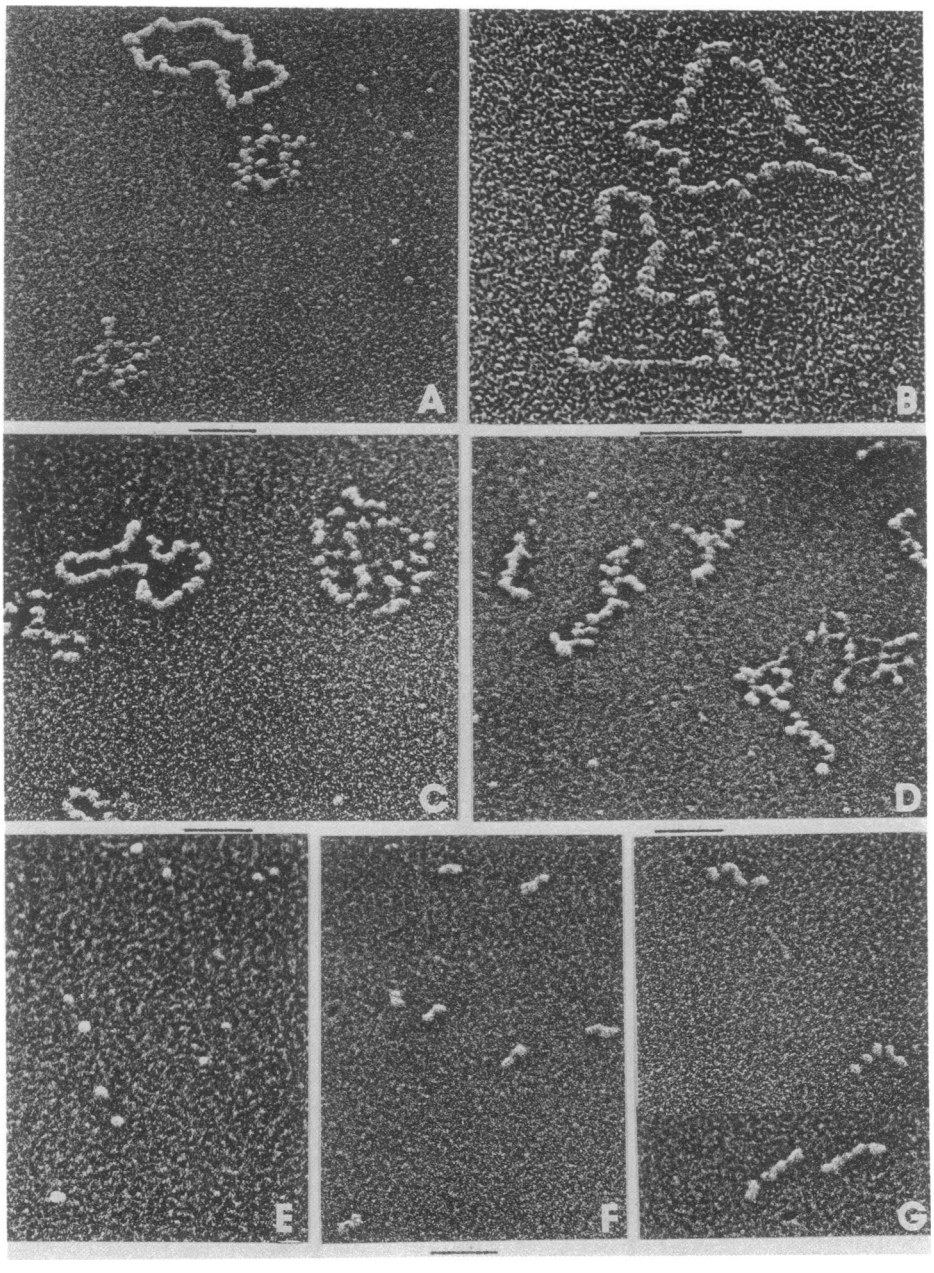

FIG. 1. Visualization of SSB bound to fd SS DNA. Complexes of SSB and intact fd circles were formed at protein/DNA ratios of 3:1 (*A*) or 10:1 (*B*) in buffer alone (10 mM Tris base, pH 7.5/1 mM EDTA) or at a protein/DNA ratio of 9:1 in buffer containing 0.1 M (*C*) or 0.3 M (*D*) NaCl. Addition of SSB to SS fd DNA fragments of 158 bases (*E*), 309+311 bases (*F*), and 849 bases (*G*) in buffer containing 0.1 M NaCl (protein/DNA ratio, 9:1) yielded units of one, two, or five beads. The complexes were fixed with 1% formaldehyde and 0.6% glutaraldehyde. Samples were prepared for EM by directly mounting aliquots onto thin carbon films, washing, drying, and shadow casting with tungsten (14). Reverse-contrast results are shown. Bars = 0.1 μm, but the micrographs were taken at a 45° tilt in the EM to enhance contrast and these magnifications therefore are only approximate.

carbon films, washing, dehydrating in ethanol, and light shadow casting with tungsten (14). The cooperative nature of SSB binding to fd SS DNA was apparent. At low protein levels, most of the DNA remained as collapsed single-strand tangles but a few molecules were extended into open nucleoprotein fiber loops (Fig. 1*A*). At mass ratios of 10:1 or greater (Fig. 1*B*), these nucleoprotein fiber loops were the only species present, in agreement with previous estimates of the amount of SSB needed to drive the DNA into a fully complexed form (22, 23) (note that this does not measure the amount of protein in the complexes themselves). The fd SS DNA–SSB complexes had a contour length of 0.54 μm ± 10% (50 measurements) or 30% the length of protein-free fd duplex DNA (1.9 μm). The nucleoprotein fiber was 12 nm wide (after correction for shadowing) and appeared to be formed from closely packed discrete spherical beads 12 nm in diameter. The beaded nature was most apparent when the complexes were formed in solutions containing 0.1 M NaCl (Fig. 1*C*). At salt concentrations of 0.3–0.5 M, the DNA appeared to remain mostly collapsed, with small regions extended into beaded loops (Fig. 1*D*). Unlike the binding of RecA protein to fd SS DNA, which appears to be cold sensitive (24), no difference in structure was observed by EM if the complexes were prepared and fixed at 4°C, 20°C, or 37°C. In all preparations, some of the complexes had a more smooth contoured appearance (Fig. 1*A*), suggesting that the beads had become so closely packed as to obscure their resolution by shadow casting. However, in many instances, it was possible to count the number of beads per complex, and values of 32–42 (average, 38) were obtained. Because each fd SS DNA circle contains 6,400 nucleotides (25), this corresponded to 170 bases of DNA per repeating unit, a number similar to the eukaryotic nucleosomal repeat number.

Micrococcal Nuclease Treatment. Micrococcal nuclease has provided a powerful probe into the native structure of chromatin in solution (26), and this enzyme readily cleaves SS DNA. Complexes of fd SS [^{32}P]DNA and SSB were formed in 0.01 M Tris base, pH 7.5/5 mM $CaCl_2$/2–200 mM NaCl and the fragmentation pattern of the DNA was examined after addition of the enzyme. After digestion for 7.5–15 min [conditions under which 90–95% of the DNA remained acid insoluble (Fig. 2*A*)],

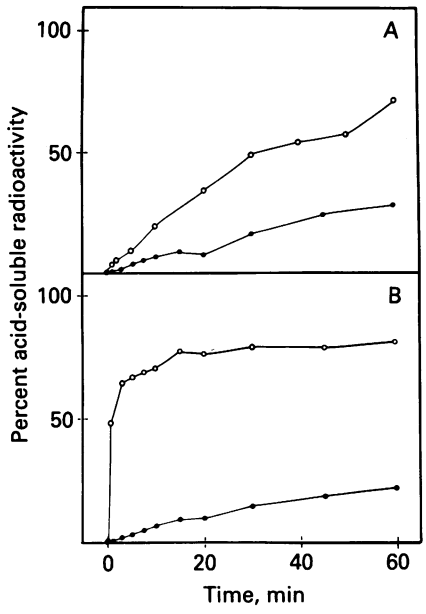

FIG. 2. Protection of fd DNA by SSB from nuclease digestion. SSB–fd [^{32}P]DNA complexes (●) and free fd [^{32}P]DNA (○) were incubated with micrococcal nuclease (1 μg/μg of DNA) in 10 mM Tris base, pH 7.5/0.1 M NaCl/5 mM $CaCl_2$ (*A*) or with DNase I (1 μg/μg of DNA) in 5 mM $MgCl_2$ (*B*) at 37°C. The fraction of acid-soluble DNA was determined by assaying the amount of ^{32}P label remaining after addition of trichloroacetic acid to 10% for 10 min on ice and removal of the precipitate by centrifugation.

the reaction products were deproteinized with 1% $NaDodSO_4$ and analyzed by polyacrylamide gel electrophoresis and autoradiography. A discrete band was observed corresponding to 145 bases (Fig. 3*A*). When the digestion was stopped after only 1–4 min, a ladder of bands, multiples of 160 ± 25 bases was produced (Fig. 3 *B* and *C*). With longer times (up to 45 min), only the 145-base fragment was seen, with no shorter discrete fragments observed (Fig. 3*C*). We estimate that, after a 15-min digestion, more than 75% of the DNA from the original complexes was in the form of 145-base fragments.

Visual inspection of the digestion products by EM (fixed after digestion) revealed progressively shorter fragments of the beaded nucleoprotein fiber loops with increased digestion times. At 20 min, only fields of single particles similar to those in Fig. 1*E* were seen.

To further confirm the correspondence between the repeat units revealed by micrococcal nuclease digestion and the beads seen by EM, complexes were formed using purified fd DNA *Hae* III restriction fragments of 158, 309+311, and 849 base pairs. From the nuclease digestion data, these fragment lengths correspond closely to repeats of one (145), two (320), and five (845) units, respectively. By EM, the complexes formed with heat-denatured 158-base fragments yielded only single beads (Fig. 1*E*) while the (309+311)- and 849-base fragments yielded complexes of two and five beads (Fig. 1 *F* and *G*).

DNase I Digestion. DNase I also cleaves SS DNA at physiological pH and salt concentrations and has been used to probe the substructure of the nucleosome (27). When fd SS DNA-SSB complexes were treated with DNase I, we observed strong protection of the DNA as contrasted to protein-free DNA (Fig. 2*B*). Autoradiographic visualization of the digestion products from reaction times of 30 and 45 min showed a repeating pattern of bands starting at 25 bases and then spaced roughly at intervals of 15 bases. The pattern was modulated, with particularly strong bands at 60, 105, 118, 130, and 145 bases. Strong bands were also seen at 150 and 210 bases (Fig. 4). Thus, DNase I digestion of native unfixed complexes confirms the presence of a nucleosome-like repeating substructure and suggests that the DNA is wrapped about a protein core.

Measurement of the Number of SSB Molecules per Unit Length by Equilibrium Density Banding. The agreement between the results from nuclease digestion of native unfixed samples and the EM results using a sequential fixation with formaldehyde and glutaraldehyde encouraged us to use fixation to aid in measuring the DNA/protein mass ratio of the complexes. This approach has been used successfully in studies of chromatin structure (28). Direct measurement of this value is important because only a fraction of the SSB molecules in solution enter the complex. Here, SSB was added to ^{32}P-labeled fd SS DNA at supersaturating levels and the samples were fixed with sequential addition of formaldehyde and glutaraldehyde (as for EM) and banded to equilibrium in CsCl solutions. The complexes formed a sharp peak at a density of 1.388 g/ml (Fig. 5) and, by EM, were beaded loops as in Fig. 1. In parallel gradients, the densities of SSB alone (unfixed) and fd DNA alone were 1.272 and 1.700 g/ml, respectively. Based on simple ratios

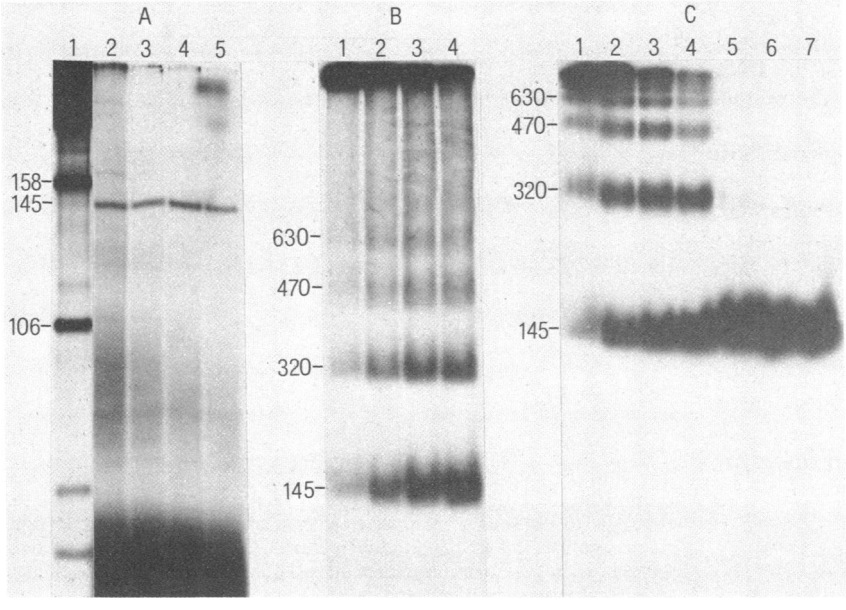

FIG. 3. Repeating pattern of DNA fragments from SSB–fd DNA complexes revealed by micrococcal nuclease digestion. SSB–fd [^{32}P]DNA complexes in 10 mM Tris base, pH 7.5/5 mM $CaCl_2$/0.02 M NaCl (*A*), 0.05 M NaCl (*B*), or 0.17 M NaCl (*C*) were incubated with micrococcal nuclease (1 μg/μg of DNA) at 37°C for various times. The reactions were stopped with 10 mM EDTA and the complexes were denatured with 1% $NaDodSO_4$, electrophoresed on 10% (*A*), 4% (*B*), or 7% (*C*) polyacrylamide gels, and analyzed by autoradiography. (*A*) Lanes: 1, *Hae* III single-stranded fragments of fd (markers); 2–5, complexes digested for 7.5, 10, 12.5, and 15 min, respectively. (*B*) Lanes: 1–4, complexes digested for 1, 2, 3, and 4 min, respectively. (*C*) Lanes: 1–7, complexes digested for 1, 2, 3, 4, 20, 30, and 45 min, respectively. Numbers to the left of the gels indicate numbers of bases.

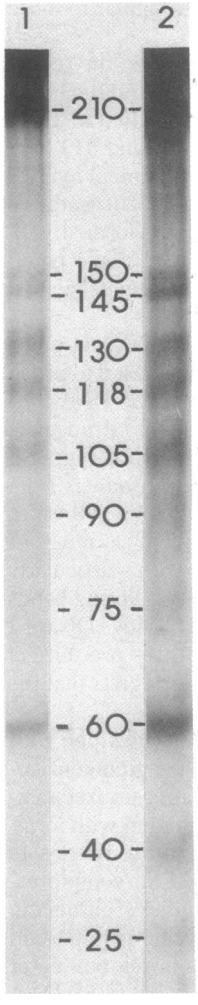

FIG. 4. Repeating pattern of DNA fragments from SSB–fd DNA complexes revealed by DNase I digestion. SSB–fd [^{32}P]DNA complexes were incubated in 10 mM Tris base, pH 7.5/0.17 M NaCl/5 mM $MgCl_2$ with DNase I (1 μg/μg of DNA) at 37°C for 30 min (lane 1) and 45 min (lane 2). The reaction was stopped with 10 mM EDTA, and the complexes were denatured with 1% $NaDodSO_4$, electrophoresed on a 10% polyacrylamide gel, and analyzed by autoradiography. Sizes (bases) were determined from *Hae* III and *Hpa* II restriction endonuclease digests of fd DNA run in parallel.

of the two components, material banding at 1.388 g/ml would have a protein/DNA ratio of 2.7:1. From the known molecular weights of SSB (18) and fd DNA (25), we calculate that this is equivalent to 88 bases of DNA per SSB tetramer or 176 bases per octamer.

As a test of the cooperativity of SSB binding, complexes were formed at protein/DNA mass ratios of 1:1, 10:1, and 40:1, fixed, and banded in CsCl solutions. Independent of the amount of protein available in solution, the complexes banded at the same density (data not shown). At subsaturation levels, the peaks were somewhat broader, suggesting that the DNA molecules may contain protein-free tracts or that the protein molecules are less compactly arranged.

To measure the protein/DNA ratio in the individual "SSB monosomes," complexes were digested with micrococcal nuclease for 20 min as above and fixed, and the monosomes were isolated by gel filtration. After banding in CsCl, the monosomes were found in a peak at a density of 1.373 g/ml (Fig. 6), corresponding to a protein/DNA ratio of 3.3:1 or 144 bases of DNA per SSB octamer. By EM, the peak material appeared to be particles as in Fig. 1*E*, while the fractions with a higher density contained mostly small pieces of free DNA and some short fragments of nucleoprotein fibers. Thus, we conclude that SSB–SS DNA complexes consist of "core" particles, each with a SSB octamer bound to 145 bases of DNA and linked by stretches of protein-free DNA roughly 30 bases long.

DISCUSSION

We report that a protein from *E. coli*, SSB, can organize SS DNA in a manner similar to the way in which the histones com-

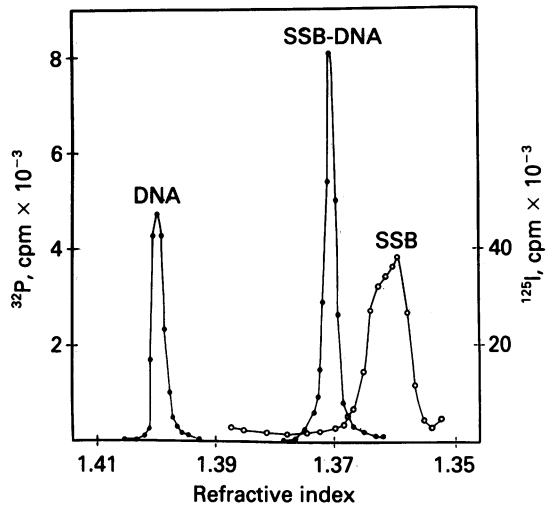

FIG. 5. Equilibrium density banding of SSB–fd DNA complexes, free SSB, and free fd DNA in CsCl solution. SSB–fd [^{32}P]DNA complexes were formed in 10 mM Tris base, pH 7.5/0.1 M NaCl, fixed as in Fig. 1, and banded in a CsCl solution. Free fd [^{32}P]DNA and ^{125}I-labeled SSB were banded in parallel gradients and are shown here superimposed by refractive index measured at 21°C. Sedimentation was for 24 hr at 47,000 rpm in a Sorvall TV-865 rotor.

pact double-stranded DNA in chromatin. The central feature of the SSB complex is a repeating chain of spherical particles. By EM, SSB binds to and extends SS DNA in solutions containing up to 0.2 M NaCl while, at salt concentrations of 0.3–0.5 M, it appeared to bind to, but was incapable of disrupting, the DNA secondary structure stabilized by the higher salt concentrations. Equilibrium density banding and nuclease digestion experiments were carried out at NaCl concentrations from 1 to 220 mM. No variations were seen in the results, confirming the EM observation of repeating chains of beads under this range of salt conditions.

The excellent agreement between the EM and nuclease digestion data reinforces our confidence in the presence of a repeating beaded substructure. This structure may also exist *in vivo*, as suggested by the beaded appearance of SSB assembled onto nascent SS ϕX174 DNA by the complex replication machinery of the *E. coli* cell (10).

Measurements using fixation and banding in CsCl solutions suggest that each bead consists of an octamer of SSB bound to

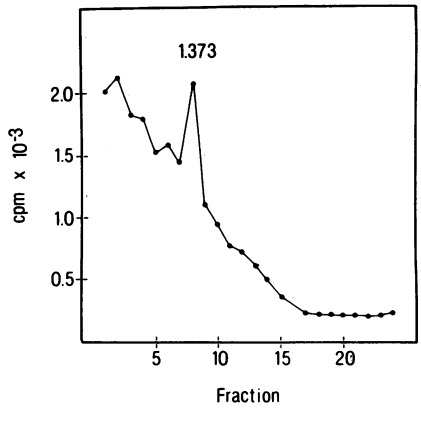

FIG. 6. Equilibrium density banding of individual SSB–DNA units in CsCl solution. SSB–fd [^{32}P]DNA formed in 10 mM Tris base, pH 7.5/0.1 M NaCl were digested with micrococcal nuclease for 20 min as described in Fig. 3. Individual protein–DNA monosomes were fixed, isolated by gel filtration on Sephadex G-150, and sedimented as in Fig. 5. Peak density is given in g/ml.

145 bases of DNA. This method has been used in studies of chromatin, where the density of fixed chromatin complexes correlated with the known mass per unit length as determined by other means (28). In applying this method, however, it is important that the protein binds DNA tightly (as with histone and SSB) or that supersaturating amounts of protein be used (also used here) because it has been reported that fixation of materials can lead to the net release of protein (29). The presence of an octamer of SSB is further supported by protein crosslinking studies (not shown here) in which we observed SSB octamers following crosslinking of SSB–DNA complexes but not with crosslinked SSB alone. Finally, the presence of a ladder of nine bands produced by DNase I digestion of the 145-base core particle could be explained on the basis of one cut on either side of eight protein subunits about which the DNA is presumably wrapped.

Our estimate of eight molecules of SSB associating with 145 bases of DNA would appear in conflict with earlier estimates that each SSB tetramer binds 30–36 bases (1, 17, 22, 23). However, it is important not to confuse the amount of protein stably bound in the complex (mass per unit length) with the total amount added in solution. Several methods have been used to measure the maximal stimulation of a SSB-dependent reaction as a function of the amount of SSB added to the DNA (17, 23, 30). In these experiments, as in our EM studies, saturation is reached at 9 μg of SSB per μg of SS DNA. We would argue that, because excess protein is needed to drive such complexes to complete formation, only a fraction of the protein will be present in the stable complex at any one time. This is analogous to a similar situation in *in vitro* histone reconstitutions, in which a histone/DNA input ratio of 2.5:1 is needed to form nucleosomal chains in which the actual protein/DNA ratio is somewhat less than 1:1 (histone H1 being absent) (11, 16).

In solution, SSB exists as a stable tetramer (17), and our studies reveal that its organization of short oligonucleotides may differ significantly from that of large natural DNAs. Thus, binding site sizes determined with short homopolymers (22, 30) to which only tetramers could bind may not reflect the binding of SSB to large DNAs. Finally, variations in SSB preparations may exist. We observed that SSB does not withstand repeated freezing and thawing. After such treatment, the SSB appeared to bind as strings of tetramers (the protein beads were smaller, their number was greater, the fd loops were longer) and, when such SSB was used for equilibrium density banding experiments, the binding was less cooperative and varied with the protein/DNA input ratio.

We have presented evidence that SS DNA is organized by a prokaryotic protein, SSB, in much the same manner that duplex DNA is arranged by the histones. SSB is not the only protein in *E. coli* that binds cooperatively to SS DNA. The RecA protein is also abundant and arranges fd SS DNA into open nucleoprotein loops as visualized by EM (24, 31). Because both these proteins are involved in genetic recombination, their structural interactions are of great interest. In our EM studies of RecA–DNA filaments (24), RecA binding appeared even more cooperative than SSB binding and the filament contour was smooth and nonbeaded. We propose that the bead-and-linker arrangement of SSB–DNA complexes provides a mechanism for extending the DNA while allowing other proteins access to a portion of the DNA. If another protein were to bind in a highly cooperative manner, beginning in the linker region, this might lead to release of the SSB. This hypothesis can be tested through the use of EM and mutants in RecA and SSB (6, 32). Such studies could elucidate our understanding of SSB–RecA interactions and possibly some histone–nonhistone associations as well.

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1. Sigal, N., Delius, H., Kornberg, T., Gefter, M. L. & Alberts, B. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3537–3541.
2. Wickner, S. & Hurwitz, J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4120–4124.
3. Meyer, R. R., Glassberg, J. & Kornberg, A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1702–1705.
4. Scott, J. F., Eisenberg, S., Bertsch, L. L. & Kornberg, A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 193–197.
5. Fuller, R. S., Kaguni, J. M. & Kornberg, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7370–7374.
6. Johnson, B. F. (1977) *Mol. Gen. Genet.* **157**, 91–97.
7. Meyer, R. R., Glassberg, J., Scott, J. V. & Kornberg, A. (1980) *J. Biol. Chem.* **256**, 2897–2901.
8. Glassberg, J., Meyer, R. R. & Kornberg, A. (1979) *J. Bacteriol.* **140**, 14–19.
9. McEntee, K., Weinstock, G. & Lehman, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 857–861.
10. Eisenberg, S., Griffith, J. & Kornberg, A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3198–3202.
11. Dunn, K. & Griffith, J. (1980) *Nucleic Acids Res.* **8**, 555–566.
12. Chrysogelos, S., Dunn, K., Griffith, J., Manning, M. & Moore, C. (1981) *Annu. Proc. Electron Microsc. Soc. Am.* **39**, 448–451.
13. Christiansen, G. & Griffith, J. D. (1977) *Nucleic Acids Res.* **4**, 1837–1851.
14. Griffith, J. & Christiansen, G. (1978) *Annu. Rev. Biophys. Bioeng.* **7**, 19–37.
15. Griffith, J. D. (1975) *Science* **187**, 1202–1203.
16. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1843–1847.
17. Weiner, J. H., Bertsch, L. L. & Kornberg, A. (1975) *J. Biol. Chem.* **250**, 1972–1980.
18. Chase, J. W., Whittier, R. F., Auerbach, J., Sancar, A. & Rupp, D. W. (1980) *Nucleic Acids Res.* **8**, 3215–3227.
19. Makino, S., Woolford, J. L., Tanford, C. & Webster, R. E. (1975) *J. Biol. Chem.* **250**, 4327–4332.
20. Brison, O. & Chambon, P. (1976) *Anal. Biochem.* **75**, 402–409.
21. Peacock, A. C. & Dingman, C. W. (1967) *Biochemistry* **6**, 1818–1827.
22. Ruyechan, W. T. & Wetmur, J. G. (1975) *Biochemistry* **14**, 5529–5534.
23. Molineux, I. J., Pauli, A. & Gefter, M. (1975) *Nucleic Acids Res.* **2**, 1821–1837.
24. Dunn, K., Chrysogelos, S. & Griffith, J. (1982) *Cell* **28**, 757–765.
25. Schaller, H. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 401–408.
26. Noll, M. (1974) *Nature (London)* **251**, 249–251.
27. Prunel, A., Kornberg, R. D., Lutter, L., Klug, A., Levitt, M. & Crick, F. H. C. (1979) *Science* **240**, 855–858.
28. Jackson, V. & Chalkley, R. (1974) *Biochemistry* **13**, 3952–3956.
29. Noll, M. & Noll, H. (1976) *J. Mol. Biol.* **105**, 111–130.
30. Krauss, G., Sindermann, H., Schomburg, U. & Maass, G. (1981) *Biochemistry* **20**, 5346–5352.
31. Flory, J. & Radding, C. (1982) *Cell* **28**, 747–756.
32. Gudas, L. S. & Mount, D. W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5280–5284.