

Conformations of the Single-Stranded DNA of Bacteriophage M13*

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Communicated by Norman Davidson, August 28, 1970

Abstract. At least two conformations of M13 single-stranded DNA have been demonstrated by measuring differences in sedimentation coefficient and by direct visualization in the electron microscope. Which form is obtained from infected cells and/or intact phage depends on the pH, ionic strength, and temperature. The slower-sedimenting form can be converted to the faster-sedimenting, single-stranded form by low ionic strength, alkali treatment, formamide, or formaldehyde, but not by exposure to 100°C in 1.0 M NaCl. The ability to assume either conformation appears to be a function of the nucleic acid alone. Whether or not these different conformations are of biological significance is still unknown.

M13 is a small fibrous bacteriophage with a genome consisting of a circular, single-stranded DNA molecule of molecular weight 2×10^6 . The base composition is inconsistent with Watson-Crick pairing and the optical density at 260 nm increases gradually with increasing temperature in contrast to the sharp melting of double-stranded DNA.^{1,2} The absorbance at 260 nm and the sedimentation coefficient vary greatly with ionic strength, suggesting a flexible conformation with significant base stacking in aqueous solution.^{3,4} We have isolated from M13-infected cells, and from intact phage, a circular, single-stranded DNA form which sediments in 1.0 M NaCl at a rate 1.16 times as fast as that of RF I, in contrast to the form usually observed which sediments at a rate 1.31-1.35 times that of RF I. The isolation and characterization of the slower-sedimenting single-stranded DNA (SS*), and the interconversions between the slow- and fast-sedimenting (SS) forms, are described below.

Materials and Methods. Sources of materials, media, bacterial growth, phage infection and assay, lysis of cells, centrifugation techniques, and radioactivity assays have all been described (ref. 5, and manuscript in preparation).

Buffers used are: (1) Tris-EDTA, 10 mM Tris (pH 8.0)-1 mM EDTA; (2) Tris-EDTA-NaCl is Tris-EDTA with the indicated molarity of NaCl. Radioactive M13 single-stranded DNA, used as a sedimentation marker, was prepared as follows: labeled phage were incubated with 100 μ g/ml pronase and 0.1% sodium dodecyl sulfate for 20 min at 60°C in Tris-EDTA or Tris-EDTA-1 M NaCl, then were deproteinized by adding one-half volume of 3 M NaClO₄ and one volume of chloroform-octanol (9:1) and vortex mixing. The aqueous phase was then dialyzed against Tris-EDTA.

Material was prepared for electron microscopy by the method described by Davis, Simon, and Davidson.⁶ The spreading solution contained 0.5 M NH₄C₂H₃O₂-1 mM EDTA-10 mM Tris, pH 7.5, and the hypophase contained 0.25 M NH₄C₂H₃O₂-0.5 mM

EDTA-5 mM Tris, pH 7.4. The preparations were shadowed at low angle with platinum-palladium and examined in a Phillips model 200 electron microscope. (We are indebted to Dr. John Fessler, Mrs. June Baumer, and Dr. Frederick Eisinger for help and advice with electron microscopy.)

Results. Isolation and characterization of SS* from infected cells: When M13-infected *Escherichia coli* are lysed with lysozyme-EDTA and sarcosyl detergent in Tris-EDTA-0.1 M NaCl, the intracellular, single-stranded, DNA cosediments with a viral SS marker at 1.31-1.35 times the rate of RF I in a sucrose gradient that contains 1.0 M NaCl. In contrast, if infected cells are lysed with lysozyme-EDTA (in Tris-EDTA-0.1 M NaCl) without sarcosyl, the single-stranded DNA quantitatively appears as the slower-sedimenting SS* form, which sediments 1.16 times as fast as RF I (Fig. 1). SS* can be ob-

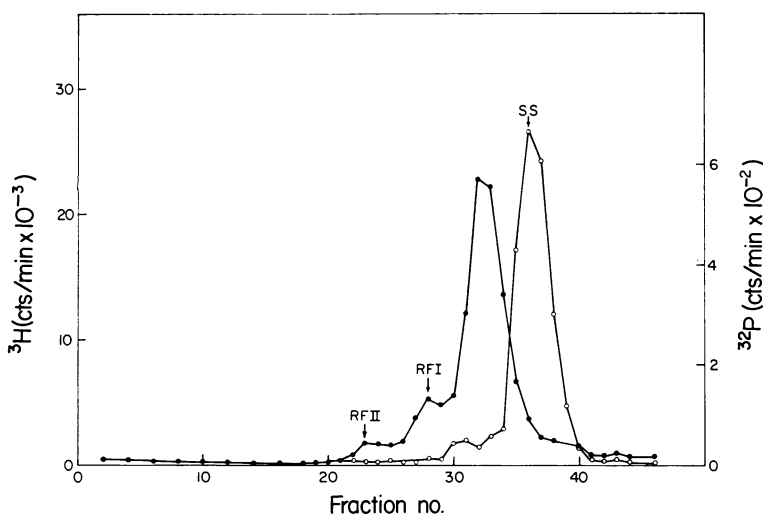


FIG. 1. Isolation of intracellular SS*. A culture of *E. coli* K12 5274 was grown to 1×10^8 cells/ml and infected with M13 at a phage to cell ratio of 40. The cells were labeled from 3 to 60 min after infection with $20 \mu\text{Ci/ml}$ [^3H]deoxythymidine. At the end of the labeling period, the cells were washed, lysed with lysozyme-EDTA, mixed with a [^{32}P]SS marker, and analyzed by velocity sedimentation through a 5-20% linear sucrose gradient in Tris-EDTA-1 M NaCl for 16 hr at 24,000 rpm and 5°C in a SW27 rotor. Sedimentation is from left to right. ●—●, ^3H cpm; ○—○, ^{32}P cpm.

tained after lysozyme-EDTA and sarcosyl lysis, however, if the NaCl concentration is 1.0 M during lysis. SS*, under these lysis conditions, is more stable at high ionic strength.

SS* purified by sucrose gradient sedimentation is stable at 37°C in 1.0 M NaCl, but not in 0.1 M NaCl. The SS* form is stable in 0.1 M NaCl at 37°C only in the presence of the crude lysate. The conversion of SS* to SS, in sarcosyl-treated lysates in 0.1 M NaCl, may possibly be due to denaturation of proteins or disruption of the lipoprotein cell membrane, either of which may be responsible for the stabilization of the structure under these conditions.

In general, agents or conditions which disrupt hydrogen bonds result in conversion of SS* to SS. At salt concentrations of 0.01 M or lower, SS* is converted

to SS even at 5°C. Alkali (0.1 N NaOH), formamide (40%), and formaldehyde (6%) act similarly, although exposure to 100°C for 10 min in Tris-EDTA-1.0 M NaCl does not convert SS* to SS. In Tris-EDTA-0.5 to 1.0 M NaCl, SS* is unaffected by pronase, sodium dodecyl sulfate, sarcosyl detergent, ether or chloroform extraction, deproteinization with perchlorate and chloroform-octanol, or deproteinization with phenol and/or ethanol precipitation.

Isolation of SS* from infectious M13 bacteriophage particles: The slower-sedimenting single-stranded DNA can be obtained from M13 by heating the phage in Tris-EDTA-1.0 M NaCl at 100°C for 10 min, or by deproteinization of the phage with NaClO₄ and chloroform-octanol (9:1) followed by dialysis at 5°C against Tris-EDTA that contains from 0.5 to 1.0 M NaCl (Fig. 2). The deproteinizing procedure for obtaining SS* requires phage particles as the starting material. If SS DNA is deproteinized with NaClO₄ and chloroform-octanol (9:1) and dialysed against Tris-EDTA-1.0 M NaCl at 5°C or below, it remains

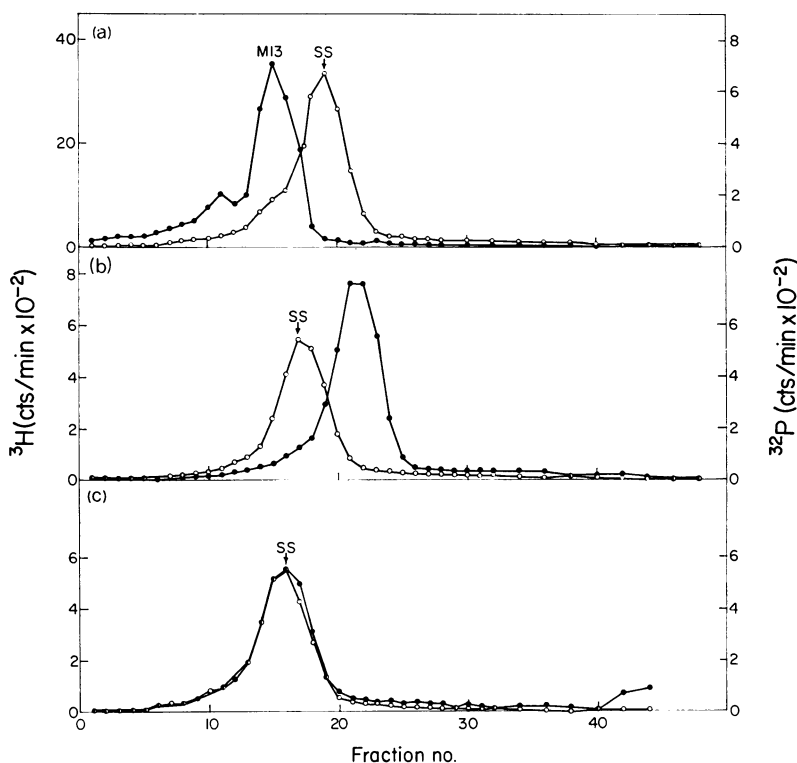


Fig. 2. Isolation of SS* from intact M13 phage particles. A sample of [^3H]deoxythymidine-labeled M13 was deproteinized with sodium perchlorate and chloroform-octanol and divided into two portions. One part was dialyzed against Tris-EDTA-1.0 M NaCl and the other against Tris-EDTA. Each sample was mixed with a [^{32}P]SS marker and analyzed by velocity sedimentation through a linear 5–20% sucrose gradient in Tris-EDTA-1.0 M NaCl for 2.75 hr at 60,000 rpm and 5°C in a SW65 rotor. Sedimentation is from right to left. (a) [^3H]-M13 phage (b) [^3H]SS* (deproteinized [^3H]M13 dialyzed against Tris-EDTA-1.0 M NaCl), (c) [^3H]SS (deproteinized [^3H]M13 dialyzed against Tris-EDTA). ●—●, ^3H cpm; ○—○, ^{32}P cpm.

as SS. Deproteinization of the phage, followed by dialysis against Tris-EDTA, produces the fast-sedimenting SS form (Fig. 2c). Sedimentation of the phage prior to deproteinization is shown in Fig. 2a. (The small, fast-sedimenting peak of phage material in Fig. 2a is thought to be an aggregation artifact in our phage preparations.) The properties of SS* obtained from phage are identical to those of the SS* isolated from infected cells.

Both the slow-sedimenting SS* and the fast-sedimenting SS have identical ultraviolet absorption spectra in the range from 220 nm to 320 nm and show A_{260}/A_{280} ratios, in Tris-EDTA-1.0 M NaCl, of 1.67. These data, and the resistance of SS* to proteolytic enzymes and to the protein denaturing conditions described above, suggested that this was not a protein-mediated conversion. Failure to label SS* with [^3H]leu or [^{35}S]sulfate supported this hypothesis. The radioactive phage used contained label such that 0.5-1% of the phage proteins could have been detected in association with the DNA.

Heating and cooling of SS DNA: The above data suggested that SS* and SS are two different conformations of the viral nucleic acid *per se*. To further test this hypothesis, we attempted to convert SS back to the slower sedimenting SS*. At the same time, a similar single-stranded DNA from ϕX174 (provided by R. Schekman) was examined. A ^{32}P -labeled sample of DNA from M13 and a ^{32}P -labeled sample of DNA from ϕX174 were separately heated in Tris-EDTA-1.0 M NaCl to 80°C, allowed to cool slowly over a period of 6 hr, and analyzed by velocity sedimentation through a neutral sucrose gradient, in 1.0 M NaCl, in the presence of the appropriate ^3H -labeled SS-marker DNA. [^3H]RF markers were also added in the case of M13. The results are shown in Fig. 3.

For M13 DNA, the unheated ^{32}P -labeled sample cosedimented with the marker of [^3H]M13 SS DNA, at a rate 1.31 times that of RF I, whereas the heated and cooled sample sedimented at 1.22 times the rate of RF I (Fig. 3a and b). For ϕX174 , a similar result was obtained, although the difference between the sedimentation coefficients was not as great (Figs. 3c and d). If the DNA was quick-cooled on ice, similar results were observed but with more forward skewing of the DNA.

If SS in Tris-EDTA is dialyzed overnight against Tris-EDTA-1.0 M NaCl at 23 or 37°C, a result very similar to that obtained by heating and cooling is observed. At 23°C, SS is converted to a form sedimenting at 1.22 times the rate of RF I, while at 37°C SS appears as a form sedimenting at 1.25 times the rate of RF I in 1.0 M NaCl. Under the conditions used, SS sedimented at 1.32 times the rate of RF I. Dialysis of SS against Tris-EDTA-1.0 M NaCl at 5°C does not alter its sedimentation rate.

Electron microscopy: The fast-sedimenting SS and the slower-sedimenting SS* were also examined by electron microscopy as shown in Fig. 4. The fast-sedimenting SS appears as a single puddle of collapsed DNA or as a dumbbell-shaped object, i.e., two puddles. The slow-sedimenting SS* has a much more extended structure, with an average length of 0.3 μm , and appears as a chain having an average of 6 single-stranded puddles, possibly separated by double-stranded regions. The nature of the regions between the single-strand puddles, i.e., whether they represent regions of extensive homology, or some peculiar

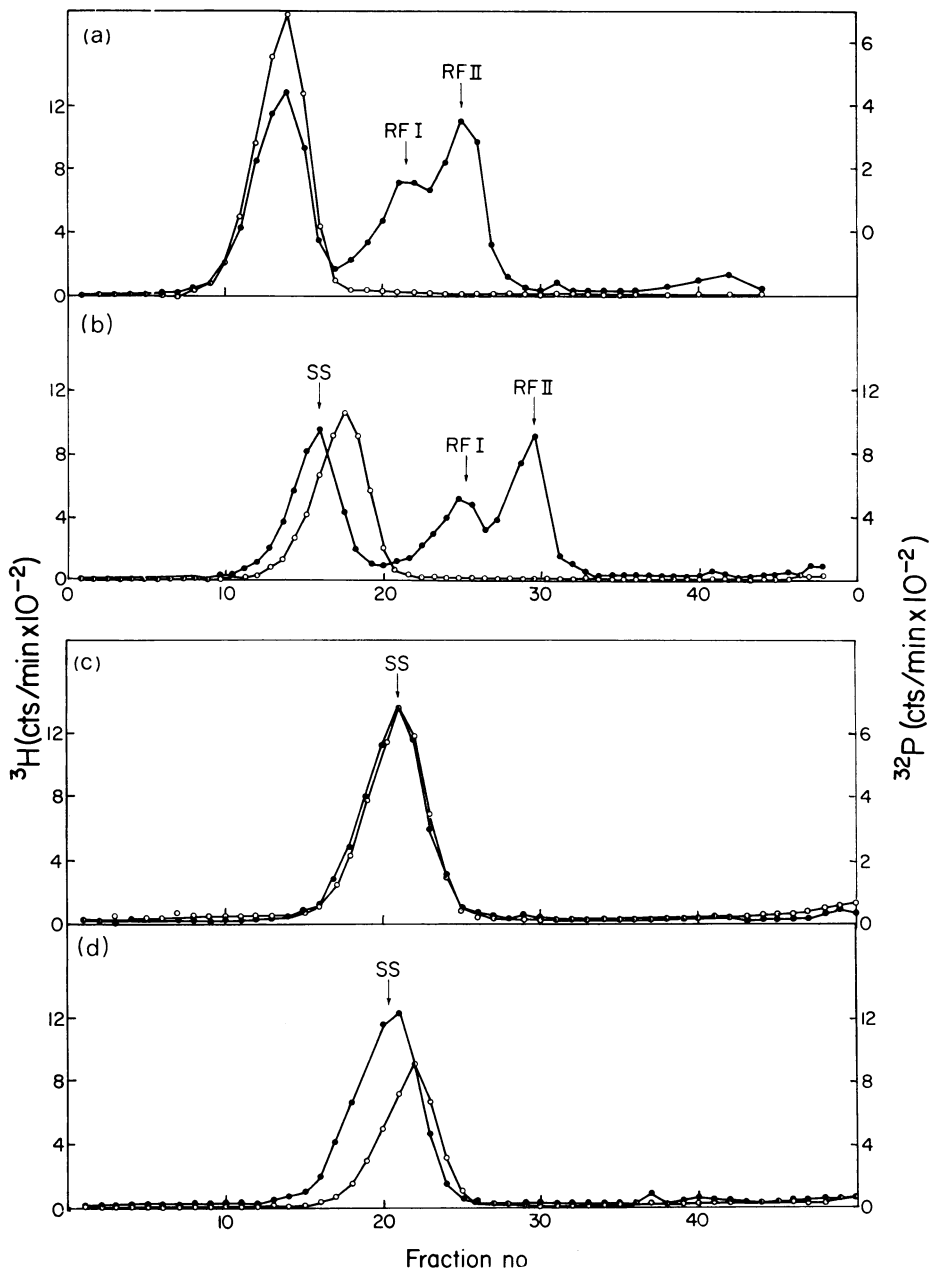


FIG. 3. Heating and cooling of SS. ^3H -labeled- and ^{32}P -labeled-M13 SS was prepared as described. ^3H -labeled- and ^{32}P -labeled- ϕX174 SS was prepared by phenol treatment of the phage, alcohol precipitation of the aqueous phase, and resuspension in Tris-EDTA. The ^3H -labeled SS from the appropriate phase was used as a marker in each case. A sample of each [^{32}P]SS preparation was sedimented (untreated) as a control, and a second sample was heated to 80°C for 30 min in Tris-EDTA-1.0 M NaCl and allowed to cool slowly to room temperature for 6 hr. ^3H -labeled-M13 RF markers were added to the M13 samples. Velocity sedimentation was as described in Fig. 2. Sedimentation is from right to left. (a) ^{32}P -labeled-M13 SS, unheated; (b) ^{32}P -labeled-M13 SS, heated to 80°C and cooled; (c) ^{32}P -labeled- ϕX174 SS, unheated; (d) ^{32}P -labeled- ϕX174 SS, heated to 80°C and cooled. ●—●, ^3H cpm, ○—○, ^{32}P cpm.

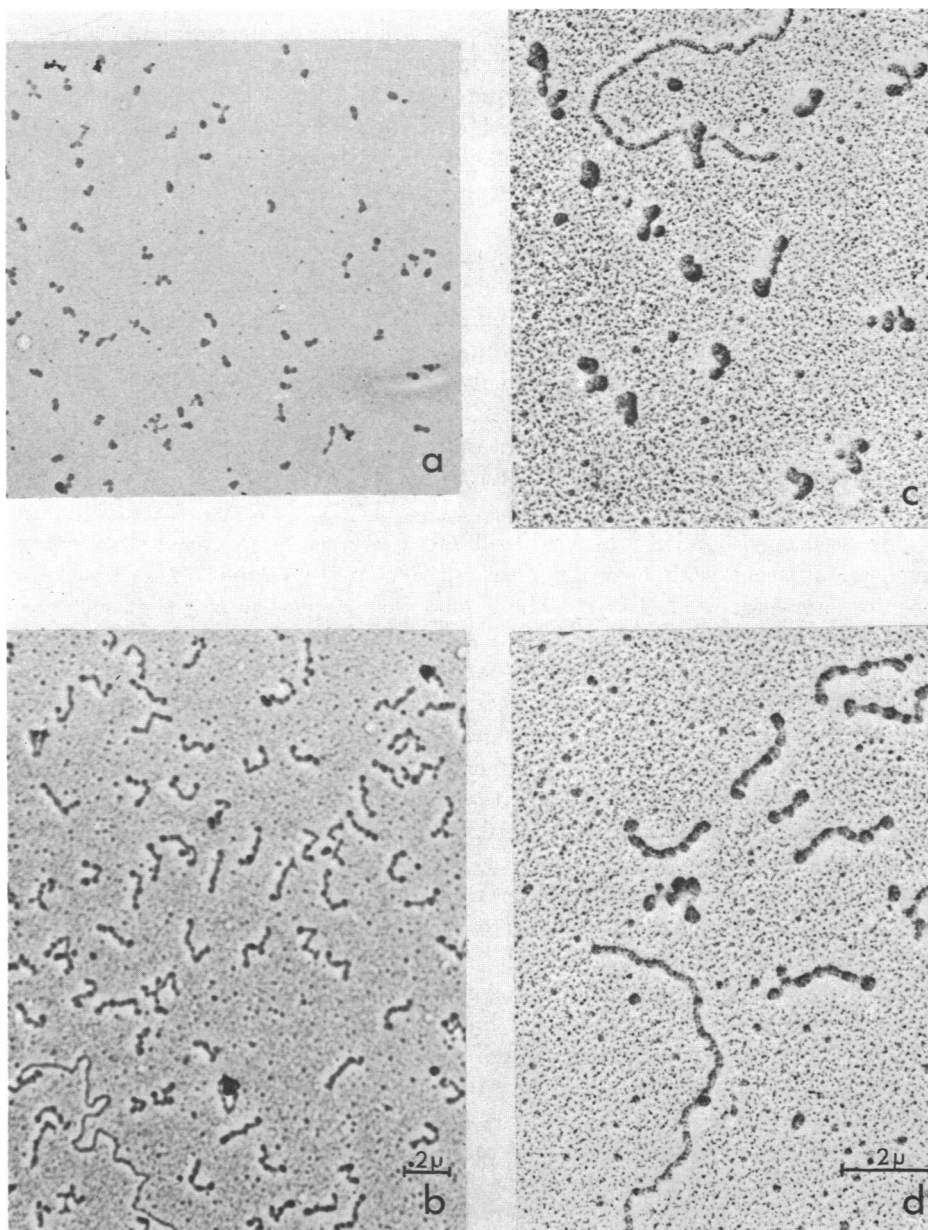


FIG. 4. Electron micrographs of the two forms of M13 single-stranded DNA. (a) and (c), SS at two different magnifications; (b) and (d) SS* at the same magnifications.

twisting of one part of the strand around another, is unknown. (The long, thread-like structure is part of a double-stranded λ DNA molecule used as a marker.) We have been unable to utilize the formamide technique of Westmoreland, *et al.*⁷ to spread the single-stranded regions because the method depends on very low ionic strength, a condition under which SS* is unstable.

Discussion. We have demonstrated, by differences in sedimentation rate and by visualization in the electron microscope, that there are at least two stable conformations of the M13 single-stranded DNA. Either conformation can be obtained from phage or from infected cells, depending on the ionic strength, pH, and temperature. It is perhaps significant that the conversion of SS* to SS occurs at conditions close to physiological conditions (0.1–0.2 M NaCl and 37°C).

DNA is obtained in the fast-sedimenting SS form at low ionic strength, and in the slow-sedimenting SS* form at high ionic strength. Conversion of SS* to SS, by dialysis against buffer containing 0.01 M NaCl or less, is quantitative. Conditions for quantitative conversion of SS to SS* have not yet been found. Heating and slow-cooling of SS, or dialysis of SS against 1.0 M NaCl at 23 or 37°C, produces a form sedimenting 1.22 or 1.25 times as fast as RF I in 1.0 M NaCl. These forms sediment at rates intermediate between those of SS (1.31–1.35 times the rate of RF I) and SS* (1.16 times the rate of RF I). It is possible, therefore, that M13 DNA, and perhaps ϕ X174 DNA, can exist in several metastable, hydrogen-bonded states with different degrees of extension and, consequently, different sedimentation rates. Discrete metastable states have also been observed for MS2 RNA.⁸ The results with ϕ X174 suggest that such conformations could be general for all small single-stranded DNAs.

The electron micrographs suggest that the fast-sedimenting SS is a very compact structure under these spreading conditions. On the other hand, the slower-sedimenting structure appears more rigid and elongated, and suggests a chain of single-stranded bushes separated by double-stranded regions. The extended conformation would have a much greater frictional coefficient than the more compact one and would be expected to sediment more slowly, as observed. Careful measurements of length and of the distances between single-strand puddles may provide meaningful data on whether this slow-sedimenting conformation is a unique ordered arrangement, facilitated by specific base pairing along the length of the molecules, or whether it is the result of random association of strands stabilized by a few base pairs. The techniques used here, however, do not adequately resolve all the single-strand bushes from the assumed double-stranded regions; finer resolution is being sought. Some of the regions between single-strand puddles appear to be thicker than the double-stranded DNA used as marker.

Two other models may be considered. One is that the slower-sedimenting form is a result of aggregation of the fast-sedimenting SS. This model is inconsistent with the dimensions observed in the electron microscope, i.e., the single-strand bushes of the fast-sedimenting form are much larger than the bushes of the elongated SS*. Furthermore, the aggregation reaction would probably be dependent upon concentration whereas formation of SS* is independent of concentration over several orders of magnitude. Another explanation is that in aqueous solution, the slower-sedimenting form is simply a coiled structure with a larger radius of gyration than the fast-sedimenting form, which might have certain additional, specific, internal hydrogen-bonded regions permitting it to contract. On the basis of our data, we favor the interpretation suggested by

the electron micrographs that the SS* form has single-strand "bushes," separated by double-stranded regions, whereas the SS form is a compact structure with many random hydrogen bonds.

The suggestion that there are specific double-stranded regions in the single-stranded molecule is attractive because such double-stranded regions might serve as enzyme recognition sites for the initial events in replication of the phage DNA. In addition, formation of a double-stranded region might provide a means for circularization of a newly-synthesized, linear, single strand by polynucleotide ligase.⁹ The extended form might also be the necessary DNA precursor for assembling the phage, the single-stranded regions being stretched out, in the phage particle by interaction with the coat protein. The biological significance of either conformation, however, is still unknown. Whatever the biological roles, a knowledge of the conformations of the single-stranded DNA of M13 is an important first step to understanding and elucidating these roles.

Abbreviations: RF, replicative form; RF I, the covalently closed, double-stranded, ring form; SS, fast-sedimenting single-stranded DNA; SS*, slow-sedimenting single-stranded DNA.

* Supported by NSF grant GB 18074, University of California Cancer Research Funds, and an NSF predoctoral traineeship to A.B.F.

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