Studies on the Cooperative Binding of the *Escherichia coli* DNA Unwinding Protein to Single-Stranded DNA†

William T. Ruyechan and James G. Wetmur*

ABSTRACT: The cooperative binding of the *Escherichia coli* DNA unwinding protein to single-stranded DNA has been studied by electron microscopy. Analysis of the electron microscopic data by means of a simple statistical mechanical model has yielded a value of 3.8–7.6 × 10^{10} l./mol for the cooperative binding constant in 0.15 *M* NaCl. Studies under elevated salt conditions have shown that the average DNA protein complex length is 50% of the length found at 0.04 or 0.15 *M* NaCl.

Alberts and Frey (1970) isolated the product of T4 gene 32 by taking advantage of this protein's strong and preferential binding to single-stranded DNA. Characterization of the protein and its interaction with DNA was of particular interest since there was direct genetic evidence implicating gene 32 product in both replication and recombination. Studies with amber (Kozinski and Felgenhauer, 1967) and temperature sensitive (Alberts et al., 1968) gene 32 mutants showed that the gene product was required throughout the infectious cycle of T4. Gene dosage experiments were also done (Sinha and Snustad, 1971) and indicated that gene 32 product was required in stoichiometric rather than catalytic amounts during replication. Evidence for the role of gene 32 product in recombination came from the work of Tomizawa et al. (1966) with amber gene 32 mutants which failed to form hydrogen-bonded, joint DNA molecules believed to be the initial products of the recombination process.

In their initial study, Alberts and Frey (1970) found that T4 gene 32 product bound to single-stranded DNA in a cooperative manner and was able to accelerate DNA denaturation and renaturation under certain conditions. Further characterization of the protein (Huberman et al., 1971; Carrol et al., 1972, 1975; Delius et al., 1972) has shown that it (a) stimulates the activity of T4 DNA polymerase but not *Escherichia coli* DNA polymerases I, II, or III, (b) forms a weak complex with T4 DNA polymerase in the absence of DNA, (c) self-aggregates at concentrations above 0.1 mg/ml, and (d) holds single-stranded DNA in an extended configuration even in the presence of spermine. Owing to the last property mentioned, gene 32 product was dubbed a DNA "unwinding" protein, and models for replication which involve unwinding of the DNA double-helix by gene 32 product were proposed (Alberts and Frey, 1970; Alberts, 1973).

Since the initial isolation of T4 gene 32 product, a number of single-stranded DNA binding proteins have been obtained from prokaryotic and eukaryotic sources. The prokaryotic proteins include those from the bacteriophages T7 (Reuben and Gefter, 1973, 1974; Scherzinger et al., 1973) and fd (Alberts et al., 1972; Oey and Knippers, 1972) and the bacterium *Escherichia coli* (Sigal et al., 1972). Eukaryotic binding proteins have been found in calf thymus tissue (Herrick and Alberts, 1973), meiotic cells of *Lilium* and mammalian spermatocytes (Hotta and Stern, 1971a,b), adenovirus types 2 and 5 (Van der Vliet and Levine, 1973; Yamashita and Green; 1974, Shanmugam et al., 1975), mitotic cells of the basidiomycete fungus *Ustilago maydis* (Banks and Spanos, 1975), and leukemic lymphocytes (Huang et al., 1975).

Although genetic evidence for the involvement of these proteins in replication and recombination is lacking, most of them do show some properties, beyond preferential binding to single-stranded DNA, which are qualitatively similar to those exhibited by T4 gene 32 product. However, only the fd (Alberts et al., 1972), *E. coli* (Sigal et al., 1972), and calf thymus (Herrick and Alberts, 1973) DNA binding proteins have been reported to hold single-stranded DNA in an extended conformation and only the *E. coli* protein exhibits properties analogous to all those catalogued for the T4 gene 32 product (Sigal et al., 1972); Molineux and Gefter, 1974; Molineux et al., 1974).

To date, with the exception of the fd unwinding protein, whose complexes with DNA have been extensively studied by circular dichroism (Day, 1973; Anderson et al., 1975), and determinations of dissociation rate constants for the T7 (Reuben and Gefter, 1974) and *E. coli* (Weiner et al., 1975) proteins, very little quantitative data concerning the interaction of these proteins with DNA have been presented. It would be of particular interest to obtain information concerning their cooperative binding since it is this process which has been postulated (Alberts, 1973) to unwind the DNA helix at the replication fork and hold the resulting single-strand template in a configuration favorable to polymerase action.

In the work presented here, we have quantitated the cooperative interaction of the *E. coli* unwinding protein with single-stranded DNA. The binding constant obtained is of the same order of magnitude as that estimated by Alberts and Frey (1970) for the binding of T4 gene 32 product to fd DNA.

Materials and Methods

Isolation of λ DNA. A stock solution of λ vir phage and a plate of *Escherichia coli* C600 were the kind gifts of Dr. A. Garro. Phage were prepared by infection of a logarithmically growing *E. coli* culture in tryptone broth plus 0.002

† From the Department of Microbiology, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029. *Received June 20, 1975.* This work was supported by Grant No. USPH GM 22029 from the National Institutes of Health.

M $MgCl_2$. After lysis solid NaCl was added to produce a concentration of 2 M. The phage were purified by differential centrifugation and banding in CsCl (density 1.50 g/ml). The phage band was removed with a syringe and dialyzed against 0.01 M Tris (pH 7.8), 0.0025 M $MgCl_2$, and 0.015 M NaCl.

λ vir DNA was prepared by the phenol extraction procedure of Mandell and Hershey (1960) followed by two chloroform extractions. The extracted DNA was dialyzed against 0.2 M NaCl, 0.01 M Tris (pH 7.8), and 0.001 M EDTA and stored at 4°C.

Preparation of Single-Stranded λ DNA. Single-stranded λ DNA was prepared by alkali denaturation using either of the following procedures: (1) the DNA solution (A_{260} = 0.5) was dialyzed overnight in the cold against 0.04 M NaCl, 0.001 M EDTA, and 0.5 M NaOH, or (2) the DNA solution was mixed 1:1 with a 1 M NaOH–0.16 M EDTA solution and incubated for 15 min at 0°C. The denatured DNA was then dialyzed against 0.01 M Tris (pH 7.6), 0.001 M EDTA, plus either 0.04 M or 0.2 M NaCl and stored at 4°C until use.

All manipulations were carried out at 0–4°C and all buffers were prechilled to 4°C to prevent renaturation of the DNA.

Preparation of the E. coli Unwinding Protein. Single-stranded DNA cellulose was prepared according to the method of Alberts and Herrick (1971) using calf thymus DNA (Worthington) and cellulose powder (Whatman) having a particle size which would not pass through a 200 mesh sieve. Frozen *E. coli* K12 cell paste was purchased in 1-lb lots from Grain Processing Corp., Muscatine, Iowa.

The isolation procedure of Sigal et al. (1972), scaled up for processing 450 g of cell paste, was followed without modification through elution of the unwinding protein and two contaminants from a single-stranded DNA-cellulose column with 2 M NaCl. The 2 M eluate was dialyzed against 0.01 M NaCl, 0.001 M EDTA, 0.001 M β-mercaptoethanol, 0.02 M Tris (pH 7.6), and 10% (v/v) glycerol (buffer B of the Sigal et al. (1972) procedure), and adsorbed to a DEAE-cellulose column (Bio-Rad, cellex-D) which had previously been washed to optical purity with high (0.6 M) and low (0.01 M) salt buffer B solutions. Following adsorption of the proteins, the column was eluted in a stepwise gradient with NaCl concentrations of 0.01, 0.06, 0.16, 0.26, and 0.51 M. Protein eluting from the column was monitored by absorbance readings at 280 nm. The unwinding protein was found in the 0.16 M fraction and was free of contaminants as judged by the sodium dodecyl sulfate gel electrophoresis method of Weber and Osborn (1964). Coelectrophoresis with ribonuclease A (Sigma), cytochrome c (Sigma), trypsin (Pentex), ovalbumin (Mann), and bovine serum albumin (Sigma) indicated that the molecular weight of the protein was about 20000, in good agreement with the molecular weight of 22000 reported by Sigal et al. (1972).

The purified protein was concentrated by dialysis against dry Sephadex G-150 (Pharmacia), then dialyzed against 0.2 M NaCl, 0.01 M Tris (pH 7.8), and 0.001 M EDTA and stored at 4°C until use. In some cases the protein was dialyzed against 0.05 M NaCl, 0.001 M EDTA, 0.001 M β-mercaptoethanol, 0.02 M Tris, and 10% (v/v) glycerol and stored frozen at −80°C. This second method of storage was avoided due to loss of protein through aggregation.

Quantitation of the Protein. The unwinding protein was quantitated with the micro-biuret technique developed by Goa (1953) using chymotrypsin (Sigma) as a standard. The molar absorptivity coefficient of the protein at 280 nm was determined to be 3.3×10^4 based on a molecular weight of 22000.

Preparation and Electron Microscopy of Single-Stranded λ DNA. Unwinding Protein Complexes. Single-stranded λ DNA ($3.7–4.4 \times 10^{-6}$ M phosphate) in 0.01 M Tris (pH 7.8), 0.001 M EDTA, and either 0.04 or 0.2 M NaCl was mixed at 0°C with varying amounts of unwinding protein in the corresponding buffer. Cold distilled water was added to the 0.2 M mixtures to give a final Na^+ concentration of 0.15 M. The mixtures were then incubated at 4°C for times ranging from 10 min to 20 hr. After incubation, an aliquot of 5% (v/v) glutaraldehyde solution was added (1.25 μg/100 μl mixture) and the protein–DNA complexes were fixed by heating for 10 min at 50°C.

Preparation of the complexes for visualization by electron microscopy was done using the modified Kleinschmidt technique described by Davis et al. (1971). The glutaraldehyde-treated solutions were made 0.1 mg/ml in cytochrome c and 0.4 M in ammonium acetate, then spread onto an aqueous hypophase of 0.4 M ammonium acetate. The cytochrome film was picked up on parlodian-coated copper grids and stained with uranyl acetate for 20–30 sec. The grids were then rinsed for 10 sec in isopentane and air dried.

Photographs were taken with an AEI EM801 at magnifications of 6300× and 10000×. Photographic negatives were displayed on a blackboard with a lantern-slide projector and the lengths of the DNA–protein complexes were traced with a map-measuring device (Keufel and Esser).

Theoretical

If the *E. coli* unwinding proteins binds single-stranded DNA in a cooperative manner without specificity for the nucleotide sequence and only two types of binding sites are allowed, the system can be described using the one-dimensional Ising model (Ising, 1925) discussed by Davidson (1962) and Schneider et al. (1968). In this model, the two types of binding interaction which can occur are (a) binding to an isolated site: an initiation interaction with a binding constant K_1 or (b) binding next to an already filled site: a propagation interaction with a binding constant K_2. The binding sites are assumed to be nonoverlapping.

An alternative characterization for this type of system has been described by McGhee and Von Hippel (1974) using a conditional probability approach. The authors examined in detail the effects of potential binding site overlaps which arise from the fact that each protein binding site corresponds to an array of several adjacent nucleotides. Their calculations show that with noncooperative binding this condition leads to smaller than binding site-sized gaps between bound protein molecules while with cooperative binding the molecules will bind in unbroken complexes the length of which is dependent on the value of a cooperativity constant ω. This constant is equivalent to the ratio of the initiation and propagation binding constants, K_2/K_1, in our treatment. Under the conditions of nonoverlap and large ω the authors' equation defining the average complex length is analogous to the one derived below.

In the Ising model formulation the partition function for binding for a molecule of length N in terms of binding sites will be:

$$Q = [1,0]\begin{bmatrix} 1 & \frac{K_1}{K_2}K_2(U) \\ 1 & K_2(U) \end{bmatrix}^N \begin{bmatrix} 1 \\ 1 \end{bmatrix} \quad (1)$$

where (U) ≡ concentration of free protein.

The average number of protein molecules bound to the DNA ($\bar{\nu}$) is then given by:

$$\bar{\nu} = [\partial \ln Q / \partial \ln (K_2(U))]_{(K_1/K_2)} \quad (2)$$

and the average number of initiation complexes on the DNA ($\bar{m}$) is given by:

$$\bar{m} = [\partial \ln Q / \partial \ln (K_1/K_2)]_{(K_2(U))} \quad (3)$$

In order to obtain workable expressions for $\bar{\nu}$ and $\bar{m}$ the following determinantal equation must be solved:

$$\begin{vmatrix} 1-\lambda & K_1(U) \\ 1 & K_2(U)-\lambda \end{vmatrix} = 0 \quad (4)$$

The roots λ_0 and λ_1 are obtained in the form:

$$\lambda_{0,1} = (1 + K_2[U] \pm [(1 - K_2(U))^2 + 4K_1(U)]^{1/2})/2 \quad (5)$$

and the partition function can now be written as:

$$Q = [\lambda_0{}^N(1 - \lambda_1) + \lambda_1{}^N(\lambda_0 - 1)]/(\lambda_0 - \lambda_1) \quad (6)$$

If we are in the region where cooperative binding is occurring, i.e., $K_2(U) \approx 1$, and the length N of the DNA molecules is such that $N(K_1/K_2)^{1/2} \gg 1$ the expression for Q can be approximated as:

$$Q \approx \frac{\lambda_0{}^N(1 - \lambda_1)}{\lambda_0 - \lambda_1} \text{ or } \ln Q/N \approx \ln \lambda_0 \quad (7)$$

The expressions for $\bar{\nu}$ and $\bar{m}$ then become:

$$\bar{\nu} \approx \frac{NK_2(U)}{\lambda_0}\left(\frac{\partial\lambda_0}{\partial(K_2(U))}\right)_{K_1/K_2} \quad (8)$$

and

$$\bar{m} \approx \frac{N(K_1/K_2)}{\lambda_0}\left(\frac{\partial\lambda_0}{\partial(K_1/K_2)}\right)_{K_2[U]} \quad (9)$$

Performing the indicated differentiations in eq 8 and 9 and combining the results yields the following expression for $\bar{l}$, the average length of an uninterrupted DNA–protein complex:

$$\bar{l} = \frac{K_2}{2K_1}\left\{[(1 - K_2(U))^2 + 4K_1(U)]^{1/2} + K_2(U) - 1 + \frac{2K_1}{K_2}\right\} \quad (10)$$

It can be shown that the cooperative transition

$$\text{protein} + \text{DNA} \rightarrow \text{protein:DNA} \quad (11)$$

occurs within the limits:

$$1 - 2(K_1/K_2)^{1/2} < K_2(U) < 1 + 2(K_1/K_2)^{1/2} \quad (12)$$

A number of special cases within these limits have been calculated with regard to $\bar{l}$ and the fractional number of sites occupied: $\bar{\nu}/N$. The results of these calculations are listed in Table I.

Line five of Table I indicates that when 50% of the available sites are occupied, the average length of the DNA–protein complexes in terms of sites will be the square root of the ratio of the two binding constants. Thus, if the average complex length, $\bar{l}$, and K_1, the initiation, or more conventionally, the intrinsic, binding constant, were known the value of the propagation or cooperative binding constant could be determined.

A value for K_1 has been obtained by equilibrium dialysis of the protein against radioactively labeled oligomers. Similar experiments have also shown no DNA sequence dependence in the binding reaction, thus justifying one of the initial assumptions of this model (W. T. Ruyechan and J. G. Wetmur, results to be published). Values of $\bar{l}$ have been obtained from electron photomicrographs in this study. The resulting value for K_2 showed that the condition $N(K_2/K_1) \gg 1$ did not hold for the DNA molecules measured. As a result a computer program was written using the matrix form of the partition function in eq 1. Values of Q for a series of values of $K_2(U)$, N, and K_2/K_1 were calculated. The results were then compared with those obtained experimentally (see Results and Discussion).

Table I: Calculation of $\bar{\nu}/N$ and $\bar{l}$ at Specific Values of $K_2(U)$.

$K_2(U)$	$\bar{\nu}/N$	$\bar{l}$
$1 - 2(K_1/K_2)^{1/2}$	0.146	$0.414(K_2/K_1)^{1/2}$
$1 - (K_1/K_2)^{1/2}$	0.276	$0.681(K_2/K_1)^{1/2}$
$1 - 0.5(K_1/K_2)^{1/2}$	0.378	$0.780(K_2/K_1)^{1/2}$
$1 - 0.25(K_1/K_2)^{1/2}$	0.437	$0.883(K_2/K_1)^{1/2}$
1	0.500	$(K_2/K_1)^{1/2}$
$1 + 0.25(K_1/K_2)^{1/2}$	0.562	$1.13(K_2/K_1)^{1/2}$
$1 + 0.5(K_1/K_2)^{1/2}$	0.621	$1.28(K_2/K_1)^{1/2}$
$1 + (K_1/K_2)^{1/2}$	0.723	$1.62(K_2/K_1)^{1/2}$
$1 + 2(K_1/K_2)^{1/2}$	0.854	$2.41(K_2/K_1)^{1/2}$

Results and Discussion

Estimation of K_1. Sigal et al. (1972) found that the *E. coli* unwinding protein bound to single-stranded DNA in a ratio of eight nucleotides per 22000 dalton monomer. In the same study they presented electron microscopic evidence that the protein bound in an oligomeric form. It has since been shown (Molineux et al., 1974; Weiner et al., 1975) that the *E. coli* unwinding protein exists as a tetramer under nondenaturing conditions, and Weiner et al. (1975) have determined the binding stoichiometry to be 30–36 nucleotides per tetramer.

We have approached the question as to whether the monomer or tetramer is responsible for the cooperative binding phenomenon by means of equilibrium dialysis experiments. The protein was dialyzed against a series of radioactively labeled, single-stranded DNA oligomers containing 4, 6, 8, and 12–18 nucleotides. The increased binding seen with increasing oligomer length is consistent with a model in which the internal sites of a tetramer are being filled in noncooperative manner as opposed to a model in which the 22000 dalton monomer is the cooperatively binding species (W. T. Ruyechan and J. G. Wetmur, results to be published).

This study has allowed us to estimate a value of 1.4–2.8 $\times 10^5$ l/M for the binding constant of a tetramer binding to 32 nucleotides. This binding constant corresponds to K_1, the initiation interaction constant previously discussed. The spread of a factor of two in this value is negligible when compared to the five orders of magnitude separating K_1 and K_2 (see below).

Equilibrium Considerations. Weiner et al. (1975) have found that in 0.003 M Na^+ the rate of release of unwinding protein from DNA protein complexes has a half-time of about 120 min at 37°C. In the light of this fact the following control experiments were done to determine whether or not we could obtain equilibrium lengths of the DNA protein complexes with higher salt, short time incubations at 4°C.

Using the weight ratio of eight nucleotides per protein

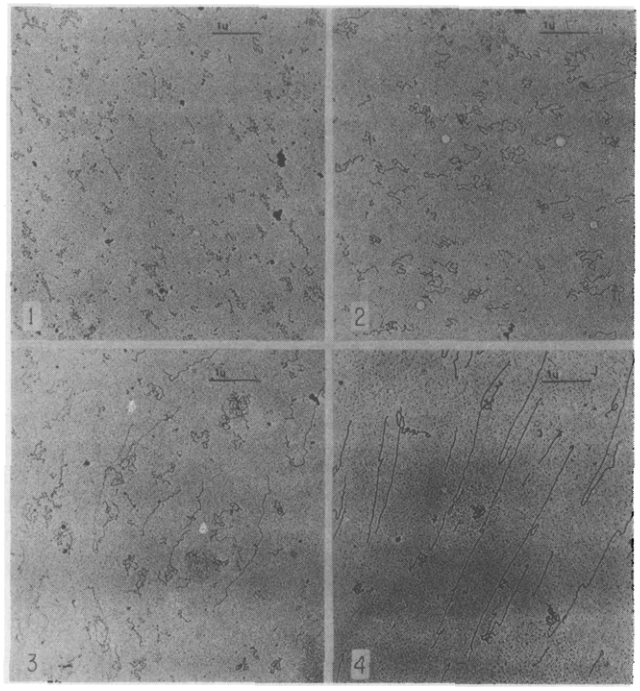

PLATE 1: Electron photomicrographs of DNA–unwinding protein complexes at varying protein/DNA ratios in 0.15 *M* NaCl. The photographs correspond to (1) 11%, (2) 23%, (3) 38%, and (4) 47% coverage of the available DNA binding sites.

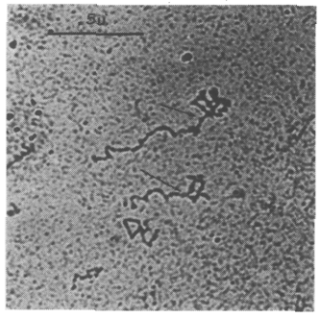

PLATE 2: Electron photomicrographs of DNA–unwinding protein complexes at 38% coverage. The arrows indicate junctions between the extended, protein bound regions and the collapsed, irregular protein free areas.

monomer determined by Sigal et al. (1972), single-stranded DNA unwinding protein complexes corresponding to 15 and 30% coverage of the DNA sites were prepared in 0.04 *M* NaCl as described in Materials and Methods. Each mixture was then divided into two parts, one of which was incubated for 10 min at 4°C before glutaraldehyde fixation while the other was incubated for 20 hr prior to fixation. Subsequent measurements of electron photomicrographs of the complexes showed that, within experimental error, there was no difference in complex length between the 10-min and 20-hr incubations in either the 15 or 30% coverage experiments.

A second type of control was done in which DNA–protein complexes at 50% site coverage were prepared in 0.04 *M* and 0.54 *M* NaCl. Following a 10-min incubation, 4 *M* NaCl was added to the low salt mixture to give a final NaCl concentration of 0.54 *M*. Both mixtures were then incubated for an additional 10 min before glutaraldehyde fixation. Length measurements of the complexes obtained showed no significant difference between the two preparations. In comparison, DNA–protein complexes prepared in 0.04 *M* NaCl at 50% coverage are twice the length of those prepared at 0.54 *M* NaCl at 50% coverage (see below).

Table II: Results of Length Measurements in 0.15 *M* NaCl at Varying Site Coverage.

% Sites Covered	$\bar{l}$ (μ)	$\bar{l}$ (sites)
11	0.39	68
19	0.65	114
23	0.80	140
38	0.93	163
47	1.25	219
94	1.44	252

These results indicate that under the conditions used in this study a stable equilibrium between DNA unwinding protein complexes and free protein and DNA is achieved in a few minutes. All subsequent preparations were incubated for 10–15 min before fixation.

Determination of K_1/K_2 and K_2. DNA unwinding protein complexes were prepared in 0.15 *M* NaCl at protein/DNA ratios corresponding to 11, 19, 23, 38, 47, and 94% coverage of the available DNA sites. Following fixation and preparation for electron microscopy, random fields of molecules from each mixture were photographed at a magnification of 10000×.

Photographs from control experiments where no protein had been added showed the characteristic bushlike structures which result when single-stranded DNA is mounted under aqueous conditions. Some double-stranded material was also seen due to the small but finite renaturation rate of single-stranded DNA under our experimental conditions. These molecules were not a source of error since the staining characteristics of the complexes and double-stranded DNA are markedly different; the latter being less distinct and having a smoother contour than the former. Plate 1 illustrates that the complexes increase in length with increasing amounts of protein and are easily distinguished from the single-stranded bushes which have no protein bound to them. Plate 2 illustrates the junctions between protein bound and protein free regions on individual molecules.

The number average lengths of the molecules measured at each protein/DNA ratio are presented in Table II. The number average lengths are used since l as calculated above is also a number average quantity. The lengths are reported both in microns and in number of sites. The length in terms of number of sites occupied was computed assuming a tetrameric binding species which bound 32 nucleotides (Weiner et al., 1975) and a spacing of 1.8 Å/base in the DNA–protein complex (Sigal et al., 1972). Each length was obtained from measurements on 125–200 molecules.

Line six of Table II indicates that the average length of the complexes at nearly saturating protein concentration is 1.44 μ. A full length single-stranded λ genome would be expected to give a value of ~7 μ under the same conditions. The fact that our molecules were 20% of this value on the average is probably the result of breakage during handling and some preexisting single-stranded nicks.

Comparison of line five with line six in Table II indicates that average complex length near 50% coverage is about equal to the average length of the single-stranded DNA molecules in our preparations. Thus N, the number of sites at 100% coverage, is probably not large enough to justify the assumption made in order to simplify the expression for the partition function in eq 7. The value of K_2/K_1 obtained,

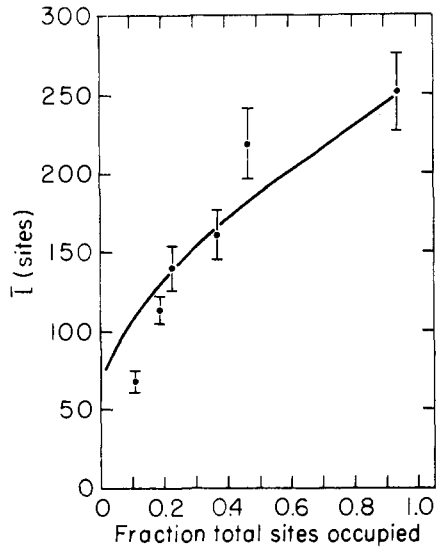

FIGURE 1: The number average lengths of DNA-unwinding protein complexes, given in terms of sites, are plotted as a function of the fraction of total available DNA binding sites occupied. The complexes were prepared in 0.15 *M* NaCl. The continuous curve shows the results of the best fit calculation described in the text.

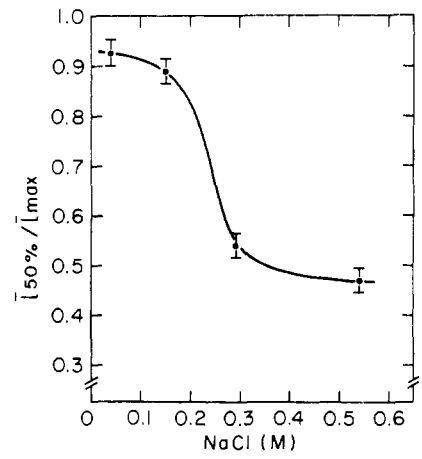

FIGURE 2: The ionic strength dependence of the average length of DNA-unwinding protein complexes at 50% site coverage. The ratio $\bar{l}_{50\%}/\bar{l}_{max}$, where $\bar{l}_{max}$ is the coverage at 100% in low salt, is plotted against NaCl concentration.

at 50% coverage, is 4.8×10^4 which makes $N(K_1/K_2)^{1/2}$ about 1 rather than much greater than 1.

In order to obtain the correct value for K_2/K_1 and, therefore, K_2 a computer program was written which calculated the logarithms of the partition functions for the distribution of molecular lengths in the 94% coverage experiment. The partition functions were calculated using the matrix formulation in eq 1; hence no simplifying assumptions were made. $\bar{\nu}$, $\bar{m}$, and $\bar{l}$ were then evaluated for a series of values of K_1/K_2 at various percentages of site coverage. The value of K_1/K_2 which gave the best fit to all the data was 3.7×10^{-6}. The data and the theoretical curve are presented in Figure 1.

From these results, K_2/K_1 is 2.7×10^5 yielding a range of values for K_2 of $3.8\text{–}7.6 \times 10^{10}$ for K_1 equal to $1.4\text{–}2.8 \times 10^5$. This value is in agreement with the upper limit of $<10^{-9}$ for the dissociation constant of gene 32 product from single-stranded fd DNA determined by Alberts and Frey (1970).

Ionic Strength Dependence of K_2/K_1. The DNA binding capacity of the *E. coli* unwinding protein is diminished at elevated salt concentrations (Sigal et al., 1972) with a drop of approximately 50% going from 0.003 to 0.5 *M* NaCl (Weiner et al., 1975). Equilibrium dialysis experiments done in our laboratory have shown that K_1 decreases rapidly at NaCl concentrations above 0.2 *M* (W. T. Ruyechan and J. G. Wetmur, results to be published).

In order to determine whether or not K_2 also changes as a result of variation in ionic strength, DNA–protein complexes at ~50% coverage were prepared in 0.04, 0.29, and 0.54 *M* NaCl using a fresh preparation of single-stranded DNA. The 0.04 *M* mixtures were set up as described in Materials and Methods. The average length of the complexes at 98% coverage in 0.04 *M* NaCl for this second DNA preparation was 1.57 μ.

For the higher salt mixtures, 4 *M* NaCl was added to 0.04 *M* preparations in order to obtain the desired ionic strength. The samples were incubated 10 min following addition of the concentrated NaCl solution. The values of $\bar{l}$ obtained at the various salt concentrations are presented in Table III. The 0.15 *M* value is that obtained from experiments described in the preceding section. It can be seen that $\bar{l}$ decreases with increasing NaCl concentration implying that K_2/K_1 and K_2 are also decreasing with K_2 falling more rapidly than K_1. A plot of $\bar{l}_{50\%\text{ coverage}}/\bar{l}_{max}$ vs. [NaCl], where $\bar{l}_{max}$ is the length obtained at ~100% site coverage in 0.04 or 0.15 *M* NaCl is shown in Figure 2. A sharp drop occurs in $\bar{l}/\bar{l}_{max}$ in the vicinity of 0.2 *M* NaCl and is followed by a more gradual decline.

Table III: Results of Length Measurements at 50% Site Coverage with Varying NaCl Concentration.

NaCl (*M*)	$\bar{l}$ (sites)	NaCl (*M*)	$\bar{l}$ (sites)
0.04	254	0.29	145
0.15	219	0.54	128

The results presented here indicate that both the initiation and propagation interactions are diminished at NaCl concentrations above 0.2 *M*. Studies designed to determine the nature of this destabilization are planned.

Summary

We have shown that the cooperative binding of *E. coli* DNA unwinding protein to single-stranded DNA can be analyzed using a simple statistical mechanical model. At physiological salt concentrations, the cooperative binding constant is found to have a value of $3.8\text{–}7.6 \times 10^{10}$ l./mol. Finally both K_2 and the ratio of the cooperative and intrinsic binding constants K_2/K_1 decrease with increasing salt concentration.

A detailed study of the intrinsic binding constant K_1 and the parameters affecting it will be presented in a forthcoming paper.

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