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Interaction of bacteriophage T4 and T7 single-stranded DNA binding proteins with DNA

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Abstract

Bacteriophage T4 and T7 are well studied model replication systems, which have allowed researchers to determine the roles of many proteins central to DNA replication, recombination, and repair. Here we discuss the results from two recently developed single molecule methods to determine the salt-dependent DNA binding kinetics and thermodynamics of the single-stranded DNA (ssDNA) binding proteins (SSBs) from these systems. We use these methods to characterize both the equilibrium double-stranded DNA (dsDNA) and ssDNA binding of the SSBs T4 gene 32 protein (gp32) and T7 gene 2.5 protein (gp2.5). Despite the overall two order of magnitude weaker binding of gp2.5 to both forms of DNA, we find that both proteins exhibit four orders of magnitude preferential binding to ssDNA relative to dsDNA. This strong preferential ssDNA binding as well as the weak dsDNA binding are essential for the ability of both proteins to search dsDNA in one dimension to find available ssDNA binding sites at the replication fork.

Keywords

single molecule; DNA melting; SSB; DNA binding; DNA replication

Introduction

The last decade has seen rapid development in single molecule force spectroscopy. This emerging technique has provided new information about the biophysical properties of single biological macromolecules, such as nucleic acids and proteins [1–9]. In order to obtain a better understanding of biological processes involving DNA, it is important to study the properties of single DNA molecules and molecules that interact with them under different conditions presented *in vivo*. By isolating a single molecule, we can simplify the system we are studying, in the absence of interactions between molecules, and obtain fine details which otherwise could have been lost due to ensemble averaging in a bulk experiment. Also, studies can be extended to solution conditions not available to bulk studies, allowing us to gain new insights into specific DNA-ligand interactions in biological systems. Various nanomanipulation techniques, including atomic force microscopy (AFM), optical tweezers, and magnetic tweezers exploit different methods to determine forces on the molecular scale [8,6,7,10]. In these experiments both ends of a single biomolecule such as DNA is strongly tethered between a surface and a

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force detector. When the molecule is subjected to tension, forces required to produce given molecular extensions can be measured and the response of an individual biomolecule to these applied forces can be studied. We can then quantify how this behavior is altered in the presence of DNA binding ligands to characterize DNA interactions, as discussed below.

Genomic replication is a complex process with numerous interconnected steps, each of which exhibits a high degree of complexity and involves many proteins properly assembled at the DNA replication fork. The replication system of bacteriophage T7 and T4 are similar to those of higher organisms. They have been used extensively in molecular biology research and provide excellent model systems for analyzing DNA replication. Bacteriophage T7 and T4 encode most of their own replication proteins, therefore bypassing the host replication machinery. Decades of research on the molecular machinery responsible for the coordination of events at the replication fork of bacteriophages T7 and T4, have made considerable progress in understanding the elaborate process of genomic replication [11]. However, many questions concerning the central roles of individual proteins in catalyzing the reactions at the replication fork still remain to be uncovered.

In the work described here, we will study the DNA binding properties the single-stranded DNA binding proteins (SSBs) from T7 and T4, the T7 gene 2.5 protein (gp2.5) and the T4 gene 32 protein (gp32), as well as their C-terminal truncated mutants. gp2.5 and gp32 are key components of the machinery for DNA replication, recombination, and repair in bacteriophages T7 and T4, respectively [11]. In addition to having a strong ssDNA-binding preference that is believed to eliminate secondary structure in long ssDNA formed on the lagging strand of the replication fork, gp2.5 and gp32 are also believed to be key components in coordinating other reactions at replication forks. Both proteins share a conserved DNA-binding fold and an acidic C-terminus that, in the absence of DNA, can contact the DNA-binding domain [12–18]. In order to better understand the function of these proteins in the cell, we have developed new single molecule methods to quantify the thermodynamics and kinetics of the protein-protein and protein-nucleic acid interactions. Using these methods, we will compare the DNA-binding properties of both proteins and show how each protein is able to rapidly search dsDNA to find ssDNA binding sites in a very similar manner despite having several very different biophysical mechanisms of DNA interaction.

Determining the binding of T7 gp2.5 and T4 gp32 to DNA from DNA stretching

Force-induced melting of single DNA molecules

Experiments discussed in this paper use dual beam optical tweezers [18]. In this setup an optical trap (~1 μm diameter) is formed by focusing two counter-propagating diode lasers (~200 mW, near-infrared) within a flow cell, using water immersion microscope objectives. Two streptavidin coated polystyrene beads (~5 μm diameter) are held in the trap and on the end of a glass micropipette where a captured phage lambda DNA (~48,500 base pairs, biotin labeled on each 3' terminus) is tethered between the two beads. The glass micropipette, mounted on a piezoelectric stage, is moved, causing the single DNA molecule to be stretched. The resulting force on the DNA is determined by the displacement of the trapping lasers.

DNA stretching has been used as a probe to study the kinetics and thermodynamics of DNA-ligand interactions. When single DNA molecules are stretched beyond their B-form contour length, an abrupt phase transition, referred to as DNA overstretching, occurs in which the extension of the DNA increases to almost twice its B-form contour length over a very small force range. A quantitative model has been proposed by Rouzina and Bloomfield [19,20] in which this transition is described as force-induced melting, in which double-stranded DNA (dsDNA) is converted to ssDNA, analogous to thermal separation of the strands. This model can be used to quantitatively predict the dependence of the overstretching force on changes in

solution conditions and the presence of DNA-binding ligands [21,22,9]. The overall structure of DNA during the transition has been recently investigated by studying DNA overstretching in the presence of glyoxal, a chemical that forms a stable DNA adduct with solution-exposed guanine residues [23]. The results demonstrate that a majority of the DNA base pairs are exposed to solution when the DNA is overstretched under a wide range of solution conditions including physiological ionic strength and pH. This result provides quantitative evidence that DNA overstretching is accompanied by a disruption of the DNA helical structure, including exposure of the DNA base pairs to solution, and has further demonstrated the validity of the force-induced melting model without relying on the thermodynamic conclusions.

Properties of T7 gp2.5 and T4 gp32

The T7 SSB, gp2.5 [24], contains 232 residues and appears to exist as a stable homodimer in the absence of DNA [25,26]. It physically interacts with both T7 DNA polymerase and T7 helicase-primase [27,28] and plays multiple roles in T7 DNA replication and recombination [27–29]. gp2.5 facilitates the annealing of complementary strands of DNA more efficiently than T4 phage gp32 and *E. coli* SSB [30–33]. The crystal structure of a gp2.5 lacking the C-terminal 26 residues [25] revealed a core that consists of a conserved oligosaccharide/oligonucleotide-binding fold (OB-fold). This domain consists of aromatic residues surrounded by positively charged amino acids and is well adapted for interactions with ssDNA. gp2.5 has a highly acidic C-terminus like other prokaryotic and mitochondrial ssDNA-binding proteins, which is required for dimer formation and for interactions with T7 DNA polymerase and the helicase-primase [27,28]. The C-terminus ends with an aromatic residue, Phenylalanine (F), which is essential for the function of gp2.5 and can add another negative charge due to its free carboxyl group [12]. In previous proposed models [25,17], the CTT interacts with the DNA-binding core of the protein. It functions as a biological switch, effectively protecting the positively charged DNA-binding cleft from binding to random negatively charged surfaces, and coordinating multiple reactions occurring at the replication fork. It has been shown that the T7 helicase-primase also has an acidic C-terminus [34] that can compete with the C-terminus of gp2.5 for binding to the same site [13].

Bacteriophage T4 gene 32 protein (gp32) is another classical paradigm of SSB proteins that is analogous in function to those of T7 and *E. coli*. It destabilizes DNA secondary structure, binds selectively and cooperatively to ssDNA, and is involved in replication, recombination, and repair [32,35–37]. The full length gp32 is comprised of 301 residues, contains three domains: C-terminal domain (CTD), N-terminal domain (NTD), and core. The acidic CTD (residues 254–301) is the site of heterotypic protein-protein interactions. The NTD (residues 1–21) is responsible for gp32-gp32 interactions resulting in highly cooperative ssDNA binding, and the central core (residues 22–253) contains a ssDNA binding site consisting of a zinc-stabilized OB fold [38–40]. gp32 has been studied extensively in bulk studies [32,41,42]. However, these studies were not able to explicate the details of the protein's interaction with DNA. On a thermodynamic basis, it was previously predicted that gp32 should lower the thermal melting temperature (T_m) of dsDNA by ~50°C based on its preferential binding to ssDNA [43]. However, thermal melting studies were not able to observe any change in the melting temperature. In contrast, *I, a C-terminal truncated form of gp32 which appeared to bind 2–3 times more strongly to ssDNA than gp32, can lower T_m by the suggested amount [44]. This result led to the proposal of a 'kinetic block' that prevents DNA destabilization by T4 gp32 [43,45]. In the work discussed here [14,16], stretching studies have been performed in the presence of intact gp32 as well as its proteolytically defined truncated form, *I, and this work resolves the apparent contradiction between DNA binding and thermal melting studies.

Quantifying T7 gp2.5 and T4 gp32 binding to ssDNA

Although gp32 and gp2.5 have several important similarities and the same single molecule method is used to study their effects on the DNA stability, each protein has different properties that affect the results. gp2.5-Δ26C and *I lack the C-terminal tail and bind ssDNA more tightly than do the full-length proteins. The results of typical force-induced melting experiments in the presence of gp2.5 and gp2.5-Δ26C or gp32 and its C-terminal truncate *I are shown in figure 1a and c, respectively. In the presence of all proteins studied, the melting force is lowered relative to that observed in the absence of protein and depends on the rate of DNA stretching. Although it seems that gp32 is incapable of destabilizing DNA under these conditions by having no effect on the DNA overstretching force, *I significantly lowers this force at the pulling rate of 100 nm/s. These results reflect a kinetic barrier to DNA melting as well as other important effects on DNA binding involving the C-terminus, to be discussed below [16].

Unlike the relaxation curve for bare DNA, the presence of an SSB protein results in a remarkable degree of hysteresis in which the relaxation curve does not overlap the stretching curve. These results indicate that protein dissociation from exposed ssDNA regions and subsequent DNA reannealing upon relaxation is slower than the time scale of a typical stretching/relaxation cycle. Although the same strong hysteresis is observed in the presence of gp2.5-Δ26C or *I, the melting force is significantly smaller, indicating that the truncated protein has a higher affinity for ssDNA than the wild type protein. The observed hysteresis upon relaxation, as well as the dependence of the overstretching force on the pulling rate, reveals the nonequilibrium nature of these experiments. This implies that the observed melting force (F_k) does not represent an equilibrium melting force (F_m). In addition, the character of the hysteresis in the presence of gp2.5 and gp2.5-Δ26C is completely different compared to that of gp32 and *I. For gp32 and *I, the relaxation curve resembles ssDNA to very low forces, with a very small slope even at 5–10 pN. In contrast, for gp2.5 and gp2.5-Δ26C at forces of 5–10 pN, the DNA is almost completely reannealed and the slope of the curve is very high, similar to that expected for dsDNA. This observation shows that gp2.5 dissociates from ssDNA faster than gp32.

As we pull DNA slower in the presence of these SSB proteins, the observed melting force F_k decreases, and is expected to converge to its equilibrium value F_m . However, for the proteins studied here, this limit is not achieved even with the slowest pulling rates of our instrument. Therefore, in order to determine F_m , a series of time-dependent force measurement experiments have been performed [17,15,16] as shown in figure 1b and d. In these experiments, the DNA-protein complex is rapidly stretched to a fixed position at the midpoint of the plateau, relative to the center of the optical trap and force is monitored as a function of time. Over a time scale of several minutes, the observed force exponentially converges to an equilibrium value (F_m), representing additional DNA melting due to protein binding to the transiently exposed single-stranded regions in the middle of DNA molecule. The equilibrium nature of this force is confirmed by the fact that both denaturation and renaturation forces converge to the same value at long times.

Analogous to thermal melting studies [46,47], by measuring the change in the equilibrium melting force (F_m) as a function of protein concentration (C), (shown in Figure 2), the equilibrium binding constants of these proteins to ssDNA (K_{ss}) can be determined by the following relation [17]:

$$F_m = F_m^0 - \frac{2k_B T}{n_{ss} \Delta x} \ln(1 + K_{ss} C). \quad (1)$$

Here, F_m^0 , n_{ss} , and Δx are the melting force in the absence of protein, protein binding site size on ssDNA in nucleotides, and the increment in length of protein-bound ssDNA relative to dsDNA, respectively. We have neglected the contribution of dsDNA binding to change in melting force, as it is always negligible for the SSBs studied here. Fits to equation (1) for gp2.5 and its C-terminal truncation mutant are shown as lines in Figure 2a and b. Each fit determines the equilibrium association constant to ssDNA for a given salt concentration, and the results will be presented below. The same approach [18, 17] was also used to determine the equilibrium binding constants of gp32 and *I to ssDNA and dsDNA [14–16], and the data and fits to equation (1) are shown in Figure 2c and d.

Quantifying T7 gp2.5 and T4 gp32 binding to dsDNA

DNA stretching curves in the presence of gp2.5 (Figure 1a) and gp32 (Figure 1c) exhibit a rate-dependent stretching force that can be used to study the kinetics of the protein-DNA binding [14, 15, 18]. The typical pulling rate dependence of the non-equilibrium melting force (F_k) for DNA stretching in the presence of gp2.5-Δ26C and *I are shown in figures 3a and c, respectively. In these experiments, DNA-protein complexes are stretched and relaxed at a constant rate, where the pulling rate is the change in position as a function of time. Analogous to dsDNA thermal melting studies, this rate dependence can be used to study protein binding kinetics. While the DNA melting force depends weakly on the pulling rate (v) in the absence of protein, it varies linearly with the logarithm of the pulling rate in the presence of gp2.5-Δ26C and *I [14, 15, 18]. Similar results have been obtained for gp2.5 and gp32 (data not shown), and all of the results follow the predictions of a simple model for rate-dependent force-induced melting. In this model, proteins bind ssDNA with binding site size n_{ss} as thermal fluctuations cause the DNA to open transiently at the ends of the DNA molecule, and the melting transition occurs at force $F_k(v)$ when the DNA pulling rate v is equal to the rate of increase in DNA length due to protein binding $n_{ss}\Delta x$:

$$F_k(v) = \frac{k_B T}{n_{ss} \Delta x} \ln \left(\frac{v}{2n_{ss} \Delta x k_a} \right) + F_m^0, \quad (2)$$

where F_m^0 is the equilibrium DNA melting force in the absence of protein. The rate-dependent kinetics of DNA melting can be used to obtain measurements of n_{ss} as well as k_a , the rate of these SSBs finding the transiently formed ssDNA binding sites at the ends of the DNA molecule [14].

The concentration dependence of k_a is shown in Figure 3b and d, along with the concentration dependence that would be expected in the theoretical 3D diffusion limit [48] given by $k_{diff} = 4\pi DR = 2k_B T / 3\eta \approx 10^9 \text{ M}^{-1} \text{ s}^{-1}$. Here, D is the diffusion coefficient, η is the solution viscosity, and $R \approx 1 \text{ nm}$ is the approximate protein radius [14, 15]. Under many conditions, the 3D diffusion limit is exceeded by the proteins studied here. In addition, the measured protein binding rate appears to depend nonlinearly on protein concentration. These two unusual types of behavior can be explained by recognizing that both proteins can bind dsDNA weakly and noncooperatively. Therefore, the enhanced binding kinetics can be explained by a model in which the proteins bind noncooperatively to dsDNA and diffuse in one dimension until colliding with one of the ssDNA binding sites at the force-frayed ends of the dsDNA molecule [14, 18]. If this model holds, then the measured ssDNA binding rate (k_a) can be expressed as

$$k_a = (2\Theta / n_{ds})^2 k_s. \quad (3)$$

Here, n_{ds} is the protein binding site size on dsDNA in nucleotides, $k_s \sim 10^7 \text{ s}^{-1}$ is the conventional sliding rate on dsDNA, and Θ is the binding site occupancy on dsDNA that varies between 0 and 1 and can be determined from McGhee and von Hippel protein-DNA binding isotherm [49]

$$\Theta = K_{ds} n_{ds} C \frac{(1 - \Theta)^{n_{ds}}}{(1 - \Theta + \Theta/n_{ds})^{n_{ds}-1}} \quad (4)$$

where K_{ds} is the equilibrium binding constants of these proteins to dsDNA. Fitting the dependence of k_a on protein concentration allows for determination of K_{ds} [14,15,18].

Salt-dependent binding of T7 gp2.5 and T4 gp32 to dsDNA and ssDNA

Figure 4a and b show the salt-dependent equilibrium association constants to both dsDNA and ssDNA for all four proteins discussed here. Comparing the binding of gp2.5 and its C-terminal truncation mutant to the two forms of DNA, we see that the salt-dependence of binding by gp2.5 to dsDNA is similar to its binding to ssDNA, and the same trend holds for gp2.5-Δ26C binding. This suggests that these proteins bind both forms of DNA via the same or similar cationic binding site. In addition, the binding affinities of gp2.5 and gp2.5-Δ26C for ssDNA binding exceed those for dsDNA binding by four orders of magnitude. This difference can be attributed to nonelectrostatic interactions, such as stacking of the aromatic residues of the OB site with unpaired DNA bases.

Comparing the binding of gp2.5 to its C-terminal truncation mutant, we see that gp2.5-Δ26C binding to both forms of DNA is stronger and more salt-dependent relative to that of gp2.5. The most likely explanation for this reduction in gp2.5's binding affinity is that the binding of the negatively charged C-terminal tail to the cationic DNA-binding cleft [13] occludes DNA binding by wild type gp2.5. However, it is known that the wild type protein forms dimers in solution [26]. Considering that the C-terminal deletion mutants do not dimerize [25], we conclude that the C-terminal tail stabilizes the dimer form by binding to the DNA-binding site of the other monomer (Figure 5b). The free energy of this dimerization, ΔG_{dimer} (or the negative of this number, the dimer binding free energy), can be obtained as [18]

$$\Delta G_{\text{dimer}} = k_B T \cdot \ln \left[\left(\frac{K_{gp2.5-\Delta 26C}}{K_{gp2.5}} \right)^2 - 1 \right]. \quad (5)$$

The dimerization free energy is strongly salt-dependent, as expected given the fact that the dimerization interaction involves the highly acidic C-terminus. Figure 5b and c demonstrate a model for regulation of gp2.5-DNA binding by a dimerization interaction involving the C-terminus [17].

As was observed for gp2.5, in the case of gp32 the salt dependence of wild type protein-DNA binding is minor, while the C-terminal truncate, *I, shows strong salt dependence. Given that the only difference between these two proteins is that *I lacks the acidic C-terminal domain, it is clear that the C-terminus interferes with DNA binding in a salt-dependent manner. Although gp32 does not dimerize, regulation of DNA binding by the C-terminus can be explained by a model similar to that shown for gp2.5 in Figure 5. In the case of gp32, however, the C-terminus likely binds directly to the DNA binding site of the same protein monomer, thus inhibiting DNA binding, as shown in Figure 5d, e, and f. Here the DNA binding affinity is reduced by the probability of fluctuational opening of the C-terminal flap that is bound to

the protein core, which in turn allows us to calculate the free energy of interaction between the C-terminus and the protein core:

$$\Delta G_{C\text{-terminus}} = -k_B T \ln(K_{*I} / K_{gp32} - 1). \quad (6)$$

Here, K_{*I} and K_{gp32} are the equilibrium DNA-binding constants of $*I$ and gp32, respectively. The C-terminus-protein core interaction $\Delta G_{C\text{-terminus}}$ is strongly salt-dependent, resulting in the strong difference in salt-dependent binding between gp32 and its C-terminal truncation mutant $*I$ [15].

Comparing DNA-binding properties of T7 gp2.5 and T4 gp32

The salt-dependant binding constants of gp2.5, gp32, and their truncated mutants to single- and double-stranded DNA are compared in Figure 4a and b [14, 15, 18, 17]. Both proteins bind to ssDNA $\sim 10^4$ -fold stronger than dsDNA. They also both have an acidic C-terminus that lowers the DNA-binding affinity and must be removed prior to single- or double-stranded DNA binding (Figure 5). They have similar binding site sizes (~ 7 nt), and the same 1D sliding mechanism on dsDNA regulates their ssDNA binding.

Several differences between the T4 and T7 SSBs are also observed. First, gp2.5 binds ssDNA with insignificant cooperativity [26] consistent with its faster dissociation from ssDNA [17, 18] compared to gp32 [50–52, 14–16] (Figure 1a and c), a property that likely helps gp2.5 facilitate the annealing of complementary strands of DNA more efficiently than T4 gp32 and *E. coli* SSB [32, 33, 31]. Because gp2.5 does not bind ssDNA cooperatively, its 10^4 -fold salt-independent preferential ssDNA binding comes from stacking of aromatic residues or other nonelectrostatic interactions with unpaired DNA bases. In contrast, in the case of gp32, with a cooperativity parameter of $\sim 10^3$ for ssDNA (but not dsDNA) binding these direct nonelectrostatic interactions of the protein with ssDNA are weaker, generating only an additional factor of ten in preferential ssDNA binding beyond what is generated by interactions with the bound protein neighbours. Second, $*I$ exhibits a stronger salt dependence to its ssDNA and dsDNA binding compared to gp2.5- $\Delta 26C$. This difference can be attributed to the higher cationic charge of the gp32 DNA-binding site that results in a stronger binding of this protein to ssDNA and dsDNA at low and moderate salt concentrations. Finally, while gp2.5 forms a dimer in solution, gp32 is a monomer in solution. The C-terminal segments stabilize the dimer form of gp2.5 by a domain swapping interaction [53] across the dimer interface [25, 54] (Figure 5b). The free energy of C-terminal interactions with the protein is weaker for gp32 than for gp2.5, resulting in a stronger DNA-binding affinity for the former protein.

Conclusions

Prokaryotic ssDNA-binding proteins, or SSBs, share a common domain structure, regardless of their lack of sequence homology [55, 25, 56, 57, 38, 58, 59]. They have conserved oligosaccharide/oligonucleotide binding (OB) domains composed of aromatic residues surrounded by positively charged amino acids. These aromatic residues stack with the DNA bases, while the positively charged amino acids contact the phosphate backbone. In addition, they all have an acidic C-terminal tail or domain that is essential for phage growth and DNA replication. The replication machinery of phage T7 is less complex compared to that of T4. gp2.5 is one of the four proteins that comprise T7 replisome, and has a key role in coordinating protein-protein interactions, as well as sophisticated DNA-binding properties. Previous studies show that gp2.5 binding to ssDNA is weaker and almost noncooperative relative to gp32 [18]. Here we directly compare the properties of gp2.5 and gp32 in order to understand the biophysical basis for their roles in their respective replication systems.

The ssDNA association rates of gp2.5 and gp32 as well as their C-terminal truncation mutants, gp2.5-Δ26C and *I, have been determined using a method that mimics the growth of protein clusters at the DNA replication fork *in vivo*. We found that these association rates are enhanced by one-dimensional sliding of proteins along dsDNA, prior to their strong binding to ssDNA. This means that these SSB proteins have two distinct binding modes. First, they can establish weak nonelectrostatic interactions with the backbone of the dsDNA. Second, they can bind strongly to exposed ssDNA regions through electrostatic and nonelectrostatic interactions. Both of these properties are likely essential for the capabilities of these proteins to act effectively as part of the DNA replication process.

By comparing the binding of T7 gp2.5 and T4 gp32 to both ssDNA and dsDNA, we can answer important questions concerning the nature of the DNA interactions of these proteins. First, it has never been clear how T7 gp2.5 can function as well as T4 gp32 in bacteriophage replication, given that its equilibrium binding affinity to ssDNA is two orders of magnitude weaker. Our study clearly shows that, while gp2.5's binding to both ssDNA and dsDNA is weaker than that of gp32, its preferential binding to ssDNA relative to dsDNA is almost exactly the same. Thus, it is the preferential ssDNA binding that allows both proteins to strongly stabilize ssDNA that is created at the replication fork. Another aspect of SSB-DNA interactions that is not understood is how two SSBs such as T7 gp2.5 and T4 gp32 can have similar replication functions, and yet one protein binds highly cooperatively, while the other is not believed to bind with any significant cooperativity. The studies described here demonstrate that cooperative binding is not needed for an SSB such as gp2.5 to achieve strong preferential ssDNA binding. Thus, T4 gp32 makes up for a lack of direct preferential ssDNA binding with strong additional cooperative protein-protein interactions, but both gp2.5 and gp32 achieve essentially the same result in terms of their ability to stabilize ssDNA. Finally, much weaker ssDNA binding by gp2.5 gives it much more rapid ssDNA dissociation kinetics, as demonstrated by the lower hysteresis observed upon DNA relaxation relative to that observed in the presence of T4 gp32 (Figure 1a and c). This rapid dissociation distinguishes T7 gp2.5 from other SSBs, including gp32 and *E. coli* SSB [60–63], and is most likely responsible for gp2.5 being most efficient among other SSBs in mediating homologous DNA base pairing [26, 31] and strand annealing [31]. These SSB functions are specific manifestations of their general nucleic acid chaperone activity, which is known to strongly depend on rapid protein-nucleic acid interaction kinetics [64–66]

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Figure 1.

(a) Rate-dependent stretching (solid line) and relaxation (dashed line) curves for λ -DNA in the absence of protein (black) at the pulling rate of 250 nm/s and in the presence of 30 μ M gp2.5 at pulling rates of 250 nm/s (red) and 25 nm/s (orange), 300 nM gp2.5- Δ 26C at pulling rates of 250 nm/s (blue) and 25 nm/s (light blue). Data are taken in 10 mM Hepes pH 7.5, 50 mM $[\text{Na}^+]$. (b) Time-dependence of DNA stretching force at constant position in the absence of protein (black) and in the presence of 10 μ M gp2.5 (dark red) and 300 nM gp2.5- Δ 26C (dark blue) as well as the time-dependence of DNA renaturation in which the DNA molecule has been overstretched and relaxed back to the same position in the presence of 10 μ M gp2.5 (red) and 300 nM gp2.5- Δ 26C (blue). Data are taken in 10 mM Hepes pH 7.5, 25 mM $[\text{Na}^+]$. (c) Stretching (solid line) and relaxation (dashed line) curves for λ -DNA at a pulling rate of 100 nm/s in the absence of protein (black) and in the presence of 200 nM gp32 (red) and 25 nm/s (orange), 200 nM *I (blue) Data are taken in 10 mM Hepes pH 7.5, 100 mM $[\text{Na}^+]$. (d) Time-dependence of DNA stretching force at constant position in the absence of protein (black) and in the presence of 200 nM gp32 (red) and 200 nM *I (green) as well as the time-dependence of DNA renaturation in which the DNA molecule has been overstretched and relaxed back to the same position in the presence of 200 nM gp32 (blue) and 200 nM *I (gold).

Figure 2.

(a) The measured (symbol) DNA equilibrium melting force as a function of protein concentration for T7 gp2.5. Measurements are shown in 5 mM Na^+ (diamond), 25 mM Na^+ (square), 50 mM Na^+ (circle). (b) The measured (symbol) DNA equilibrium melting force as a function of protein concentration for T7 gp2.5- Δ 26C. Measurements are shown in 25 mM Na^+ (circle), 50 mM Na^+ (diamond), 75 mM Na^+ (triangle), and 100 mM Na^+ (square). (c) The measured (symbol) DNA equilibrium melting force as a function of protein concentration for T4 gp32. Measurements are shown in 50 mM Na^+ (square), 75 mM Na^+ (triangle), 100 mM Na^+ (circle), 150 mM Na^+ (diamond), and 200 mM Na^+ (open square). (d) The measured (symbol) DNA equilibrium melting force as a function of protein concentration for the T4 gp32 C-terminal truncate *I. Measurements are shown in 50 mM Na^+ (square), 75 mM Na^+ (triangle), 100 mM Na^+ (circle), 150 mM Na^+ (diamond), and 200 mM Na^+ (open square). For all parts, lines were fitted to data using equation (1) and a χ^2 analysis.

Figure 3.

(a) Measured nonequilibrium DNA melting force F_k as a function of the rate of pulling v . Data are shown in the absence of protein (closed square), in the presence of 10 μ M gp2.5 (closed circle), 20 μ M gp2.5 (closed triangle), 30 μ M gp2.5 (closed diamond), 230 nM gp2.5- Δ 26C (open circle), 300 nM gp2.5- Δ 26C (open triangle), 460 nM gp2.5- Δ 26C (open circle). Linear fits are shown as continuous lines. Data is taken in 10 mM Hepes pH 7.5, 50 mM Na^+ . (b) Protein binding rate (k_a) as a function of protein concentration for gp2.5 in 5 mM Na^+ (close circle), 25 mM Na^+ (close triangle), 50 mM Na^+ (close diamond). (c) Protein binding rate (k_a) as a function of protein concentration for gp2.5 in 25 mM Na^+ (close circle), 50 mM Na^+ (close triangle), 100 mM Na^+ (close diamond). In both part (b) and (c), lines are fit to the data using equation (4), and dashed lines show the theoretical 3D diffusion limit.

Figure 4.

(a) Measured (symbols) equilibrium association constants for T4 gp32 (red) and T7 gp2.5 (black) to ssDNA (closed symbols) and dsDNA (open symbols). (b) Measured (symbols) equilibrium association constants for T4 gp32 C-terminal truncate *I (red) and T7 gp2.5-Δ26C (black) to ssDNA (closed symbols) and dsDNA (open symbols).

Figure 5.

Models for regulation of DNA binding by the C-terminal regions of T4 and T7 single-stranded DNA binding proteins. (a) Model for T7 gp2.5-Δ26C, which contains a cationic DNA binding site and dimer interface. (b) Model for T7 gp2.5 dimerization, in which the C-terminus of each dimer partner binds to the DNA binding site, thereby preventing DNA binding. (c) Model for DNA binding by T7 gp2.5, in which the dimer must be broken and the C-terminus exposed to solution in order for DNA binding to occur. (d) Model for the T4 gp32 C-terminal truncate *I, which contains a cationic DNA binding site available for DNA binding. (e) Model for T4 gp32 in low salt. Under these conditions the C-terminus binds to the DNA binding site, thereby preventing DNA binding by the protein. (f) Model for DNA-bound T4 gp32, in which the C-terminal domain must be exposed to solution in order for the protein to bind DNA.