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E. coli SSB tetramer binds the first and second molecules of $(dT)_{35}$ with heat capacities of opposite sign

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ABSTRACT

We have previously shown that formation of a 1:1 fully wrapped complex of *Escherichia coli* SSB tetramer with $(dT)_{70}$ displays a temperature-dependent sign reversal of the binding heat capacity (ΔC_P). Here we examine SSB binding to shorter oligodeoxynucleotides ($(dX)_{35}$) to probe whether this effect requires binding of one or two $(dX)_{35}$ molecules per SSB tetramer. We find that the ΔC_P for the first molecule of $(dX)_{35}$ is always negative. However, a sign reversal of ΔC_P from negative to positive occurs with increasing temperature for binding of the second $(dX)_{35}$. This striking behavior of ΔC_P for the second $(dX)_{35}$ appears linked to conformational changes within the ssDNA–SSB complex that are required to form a fully wrapped $(SSB)_{65}$ binding mode. These results also underscore that binding heat capacities of macromolecular interactions have multiple origins that cannot be understood simply on the basis of examining static structures.

1. Introduction

Single stranded (ss)DNA binding proteins (SSBs) play essential roles in DNA replication, recombination and repair [1]. These proteins bind selectively to ssDNA with high affinity and little sequence specificity, functioning to stabilize and protect ssDNA intermediates that form during DNA metabolism. SSB proteins also bind directly to an array of enzymes and proteins in order to bring those proteins to their sites of function on DNA [2]. The *Escherichia coli* ssDNA binding protein (*Eco*SSB) is the most studied representative of the large class of SSB proteins that are typically found in bacteria [3,4]. Most bacterial SSB proteins are tetrameric as is the case for *Eco*SSB and contain one oligonucleotide/oligosaccharide binding fold (OB-fold) per subunit, where each OB-fold contains an ssDNA binding site [4–6].

Due to the presence of its four potential ssDNA binding sites, the *Eco*SSB tetramer can bind to long, polymeric ssDNA in multiple binding modes that differ by the amount of ssDNA that is wrapped around the tetramer. Two of the major binding modes, denoted $(SSB)_{35}$ and $(SSB)_{65}$ differ by the number of subunits, either two or four, respectively, that interact with ssDNA with corresponding occluded site sizes of ~35 and 65 nucleotides [7]. The relative stabilities of these binding modes are influenced by salt concentration type and valence and protein binding density [7–12]. At moderate to high salt concentrations (≥0.2 M NaCl) *Eco*SSB forms exclusively the $(SSB)_{65}$ binding mode, whereas at low salt concentrations (≤20 mM NaCl) and high ratios of protein to DNA the $(SSB)_{35}$ binding mode is favored. In the $(SSB)_{35}$ binding mode, tetramers can bind to ssDNA with high "unlimited" inter-tetramer cooperativity [7,8,10,12–15]. In the $(SSB)_{65}$ mode, tetramers bind with little cooperativity or at most can form dimers of tetramers (octamers) [9,10].

Fig. 1 shows models for how ssDNA wraps around the SSB tetramer in both the $(SSB)_{65}$ and $(SSB)_{35}$ binding mode suggested based on x-ray crystal structures of two molecules of $(dC)_{35}$ bound to a C-terminal truncation of SSB, SSBc, in which the last 42 amino acids of each subunit are missing [6]. The tetrameric ssDNA binding core is comprised of four subunits (N-terminal residues 1–112), containing the four OB-folds. In the proposed $(SSB)_{65}$ structure (Fig. 1A), 65 nucleotides of ssDNA wrap around all four subunits using a topology similar to the stitching on a baseball, such that the 5′ and 3′ ends of the ssDNA enter and exit the tetramer in close proximity. The proposed ssDNA wrapping in the $(SSB)_{35}$ binding mode (Fig. 1B) is more speculative and is based on the observation that in several crystal structures, adjacent SSB tetramers form an interface through the so-called L_{45} loops and the assumption that this interface is involved in the inter-tetramer cooperativity on ssDNA [5,6,16]. In this mode 35 nucleotides of ssDNA interact with an average of 2 subunits and the ends of ssDNA enter and exit an SSB tetramer on opposite sides near the L_{45} loops.

The four C-termini of the SSB tetramer (residues 112–177) are unstructured even when SSB is bound to ssDNA [16]. The last 8–10 amino acids in these C-termini serve as the primary sites to which more than a dozen other proteins bind to SSB [2,17]. These C-terminal tails can also interact with the SSB ss-DNA binding core and inhibit

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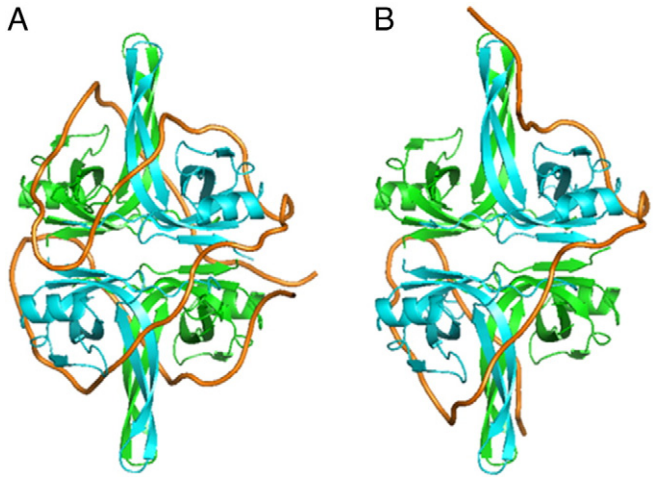


Fig. 1. The topologies of ssDNA wrapping around SSB tetrameric core in $(SSB)_{65}$ (A) and $(SSB)_{35}$ (B) binding modes suggested based on the crystal structure of two molecules of $(dC)_{35}$ bound to SSB [6]. The 65 and 35 nucleotides long ssDNA is shown in orange.

ssDNA binding [18]. Even though SSB tetramers bind with very high affinity to ssDNA, individual SSB tetramers can diffuse along ssDNA while in the $(SSB)_{65}$ binding mode [19].

Equilibrium studies of *Eco*SSB binding to both ss polydeoxynucleotides and oligodeoxynucleotides have been extensive (reviewed in Refs. [4,14]). The DNA binding sites on an SSB tetramer can be saturated with one molecule of $(dX)_{70}$ (X = T, C or A) or two molecules of $(dX)_{35}$. As such, an SSB-$(dT)_{70}$ complex has been used as a model for the $(SSB)_{65}$ binding mode whereas a 1:1 SSB-$(dT)_{35}$ complex has been used as a model for the $(SSB)_{35}$ mode [12,17,20–27]. The binding of two $(dX)_{35}$ molecules to SSB occurs with intra-tetramer negative cooperativity, which is strongly dependent on salt concentration and nucleotide base type [20–24]. The thermodynamics of forming an SSB tetramer bound to two molecules of $(dX)_{35}$ are very similar to the thermodynamics of forming a 1:1 SSB-$(dT)_{70}$ complex [23]. Hence, current evidence suggests that both complexes serve as useful models for a fully wrapped $(SSB)_{65}$ complex. Although at moderate and high salt concentrations (>0.2 M) SSB always binds in the $(SSB)_{65}$ mode (1:1 complex with $(dX)_{70}$) independent of protein to DNA ratio, at lower salt concentrations two SSB tetramers can bind to $(dX)_{70}$ at high protein to DNA ratios, presumably in an $(SSB)_{35}$-like mode [15].

Isothermal Titration Calorimetry (ITC) has been used to study the energetics of SSB binding to $(dX)_{70}$ and $(dX)_{35}$ as a function of solution conditions and temperature [23–25,27,28]. The binding thermodynamics are complex with contributions from multiple linked equilibria that accompany SSB–DNA binding. Among these are the linkage of cation and anion binding [23,27,28], protonation [25], and conformational transitions within DNA [24] and protein [27].

In previous studies we investigated the thermodynamics of forming 1:1 SSB:$(dT)_{70}$ complexes as a model for the $(SSB)_{65}$ binding mode. We observed very large effects of salt concentration and type on both the binding enthalpy (ΔH_{obs}) and heat capacity ($\Delta C_{p,obs}$). We also observed a dramatic and rarely observed effect of temperature on $\Delta C_{p,obs}$ such that it changed sign as a function of temperature ($\Delta C_{p,obs}<0$ at T<35 °C and $\Delta C_{p,obs}>0$ at T>35 °C). To explain this behavior we suggested that the SSB protein and SSB–DNA complex undergo temperature dependent conformational transitions modulated by weak binding of anions to the free SSB, which are released upon binding ssDNA. As mentioned above, a 1:1 fully wrapped SSB–$(dT)_{70}$ complex is formed exclusively at monovalent salt concentrations ≥0.2 M [12,21–23,26,27]. Hence, one is limited to studying this system at these higher salt concentrations. Here we have examined the binding of $(dX)_{35}$ (X = T or C) to an SSB tetramer, which enables us to extend those studies to lower salt concentration. This also allows us to examine the thermodynamics of SSB tetramer binding to one vs. two molecules of $(dX)_{35}$. Our results show that the observed reversal in sign of the binding heat capacity occurs only for binding of the second molecule of $(dX)_{35}$ to an (SSB–$(dX)_{35}$ complex and is consistent with a conformational change that is required to form a fully wrapped $(SSB)_{65}$ complex. Along with our previous studies [24,25,27] these results indicate that there can be multiple origins for heat capacity changes and that these cannot be understood simply by calculating changes in accessible surface area from static structures.

2. Materials and methods

2.1. Reagents and buffers

All solutions were prepared with reagent grade chemicals and glass distilled water that was subsequently treated with a Milli Q (Millipore, Bedford, MA) water purification system. Buffer T is Tris (tris(hydroxymethyl) aminomethane), buffer H is Hepes (4-(hydroxyethyl)-1-piperazineethanesulfonic acid). All buffer concentrations were 10 mM and contained 0.1 mM Na_3EDTA (ethylenediaminetetraacetic acid). The concentrations of NaF, NaCl and NaBr in each buffer are specified in the text. The pHs of the buffers were adjusted to 8.1 at 25 °C by titrating with 5 M NaOH (Hepes) or 5 M HCl (Tris). Since the pK_a and ΔH_{ion} of the buffers are dependent on temperature, the final pH of the solution and ΔH_{ion} of the buffers were calculated for each particular experimental temperature, T, as described [25,29] using the following reference values (25 °C): $pK_a = 7.45$; $\Delta H_{ion} = 5.02$ kcal/mol and $\Delta C_{p,ion} = 12$ cal/mol K (Hepes) [29] and $pK_a = 8.06$; $\Delta H_{ion} = 11.34$ kcal/mol and $\Delta C_{p,ion} = -17$ cal/mol K (Tris) [30]. For experiments performed at different temperatures (5–40 °C), the resulting pH of the buffers varied over this temperature range from 8.7 to 7.8 for Tris and 8.4 to 8.0 for Hepes, respectively. The error in calculating the pH for a particular experiment is within ±0.1 pH unit as estimated based on direct measurements of the pH of the buffer at several temperatures.

2.2. E. coli SSB protein and nucleic acids

SSB protein was purified as described [31] with the addition of a double stranded DNA cellulose column to remove a minor exonuclease contaminant [32]. SSB protein concentration was determined spectrophotometrically in buffer T in the presence of 0.2 M NaCl using the extinction coefficient, $\in_{280} = 1.13\times10^5\ M^{-1}$(tetramer) cm^{-1} [7]. The SSB tetramer is stable at all protein concentrations, NaCl, NaBr and NaF concentrations [15,23,33] and studied temperatures [27]. The oligodeoxynucleotides, $(dT)_{35}$ and $(dC)_{35}$ were synthesized and purified as described [15] and were ≥98% pure as judged by denaturing gel electrophoresis and autoradiography of a samples that were 5′ end-labeled with ^{32}P using polynucleotide kinase. Concentrations of $(dT)_{35}$ and $(dC)_{35}$ were determined spectrophotometrically in buffer T (pH 8.1), 0.1 M NaCl using the extinction coefficients, $\in_{260} = 8.1\times10^3\ M^{-1}$ (nucleotide) cm^{-1} [34] and $\in_{269} = 7.6\times10^3\ M^{-1}$(nucleotide) cm^{-1} [35,36], respectively.

2.3. Isothermal titration calorimetry (ITC)

ITC experiments were performed using both an OMEGA titration microcalorimeter and VP-ITC titration microcalorimeter (MicroCal Inc., Northhampton, MA) [37]. Generally, experiments were carried out by titrating SSB solutions (0.3–1.4 μM tetramer) with oligodeoxynucleotide (generally stock concentrations ranging from 5 to 30 μM). The heats of dilution were usually obtained from a reference titration in which DNA in the syringe is titrated into the cell containing only buffer solution. All SSB and oligodeoxynucleotides samples were dialyzed

extensively against the corresponding buffer at the indicated salt concentration that was used in each ITC experiment.

An SSB tetramer binds two molecules of $(dX)_{35}$ [20,21]. As in the previous studies [23–25] for the analysis of equilibrium ITC titration data we used a 2 site sequential binding model characterized by two microscopic binding constants ($k_{1,obs}$ and $k_{2,obs}$) and two binding enthalpies ($\Delta H_{1,obs}$ and $\Delta H_{2,obs}$) as described by Eq. (1),

$$Q_i^{tot} = V_0 \cdot M_{tot} \cdot \left(\frac{2k_{1,obs}X \cdot \Delta H_{1,obs} + k_{1,obs}k_{2,obs}X^2\left(\Delta H_{1,obs} + \Delta H_{2,obs}\right)}{1 + 2k_{1,obs}X + k_{1,obs}k_{2,obs}X^2} \right) \quad (1)$$

where the concentration of free ligand (X = DNA) was obtained by solving Eq. (1a).

$$X_{tot} = X + X_{bound} = X + \frac{2k_{1,obs}X + 2k_{1,obs}k_{2,obs}X^2}{1 + 2k_1X + k_{1,obs}k_{2,obs}X^2} M_{tot} \quad (1a)$$

In Eq. (1) $-X_{tot}$ and M_{tot} are the total concentrations of the ssDNA and SSB (macromolecule), respectively, in the calorimetric cell after the *i*-th injection. Non-linear least squares fitting of the data was performed using the "ITC Data Analysis in Origin" software provided by the manufacturer. The conversion of integral heats (Q_i^{tot}) to differential heats (heats per injection observed in the experiment) and the fitting routine including corrections for heat displacement effects and ligand and macromolecule dilutions in the calorimetric cell were performed as described [23] (see also the *ITC Data Analysis in Origin Tutorial Guide* (Microcal Inc.)).

For the two step reaction including binding and conformational transition shown in Scheme 1 the observed equilibrium constant and corresponding observed enthalpy change can be expressed as in Eqs. (2) and (3):

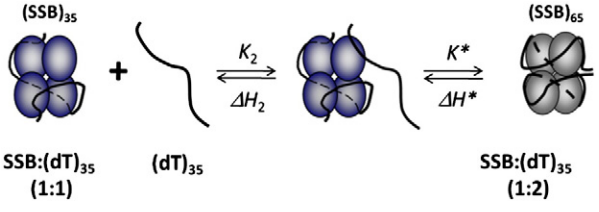
$$ln\, k_{2,35,obs} = ln\, K_2 + ln(1 + K^*) \quad (2)$$

$$\Delta H_{2,35,obs} = \Delta H_2 + \frac{K^* \cdot \Delta H^*}{1 + K^* \cdot \Delta H^*} \quad (3)$$

Where K_2, ΔH_2 and K^*, ΔH^* are the binding parameters corresponding to the first and the second step in Scheme 1. Data shown in Fig. 7A and B were globally fit to Eqs. (2) and (3), assuming no dependence of ΔH_2 and ΔH^* on temperature ($\Delta Cp_2 = \Delta Cp^* = 0$), so the temperature dependences of K_2 and K^* can be expressed as in Eq. (4):

$$K_i = K_{i,ref}\ exp\left(\frac{\Delta H_i}{R} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \quad (4)$$

Where *R* is the gas constant and $K_{i,\ ref}$ is either $K_{2,ref}$ or K^*_{ref} at reference temperature T_{ref}. These and all additional fittings and simulations were performed using the nonlinear regression package in Scientist (MicroMath Scientist Software, St. Louis, MO). All uncertainties reported are at the 68% confidence level (one standard deviation).



Scheme 1. Kozlov and Lohman.

3. Results

3.1. The binding heat capacity ($\Delta C_{P,obs}$) for SSB–$(dT)_{35}$ binding at low salt concentration shows a sign change for the binding of the first vs. the second DNA molecule

One SSB tetramer can bind two molecules of $(dX)_{35}$ (where X = T,A or C), although with negative cooperativity that decreases with increasing monovalent salt concentration and is dependent on the salt type and the nature of the base [20–22]. Using isothermal titration calorimetry (ITC) we examined the binding of two $(dT)_{35}$ molecules to SSB at low concentrations of NaF, NaCl and NaBr from ~7 to ~50 °C. Fig. 2 shows typical isotherms obtained in buffer H (0.1 mM EDTA, 20 mM NaCl) at 12 °C, 25 °C and 37 °C. In each isotherm, the first molecule of $(dT)_{35}$ binds to the SSB tetramer with very high affinity (stoichiometrically), such that only $\Delta H_{1,35,obs}$ can be estimated from the flat portion of the isotherm at $[(dT)_{35}]_{tot}/[SSB]_{tot} \leq 1$; no information on the equilibrium constant is obtainable. The binding of the second molecule of $(dT)_{35}$ is much weaker at all temperatures, as reflected by the more slanting character of the isotherm at $[(dT)_{35}]_{tot}/[SSB]_{tot} \geq 1$. These titrations also show that the affinity of SSB for the second $(dT)_{35}$ decreases as the temperature increases. At 37 °C binding of the second $(dT)_{35}$ is not detectable under these conditions.

Titrations at different temperatures were performed in Na^+ salts with different anions (NaCl, NaBr and NaF) to obtain $k_{1,35,obs}$ $\Delta H_{1,35,obs}$ and $k_{2,35,obs}$, $\Delta H_{2,35,obs}$, the microscopic binding constants and enthalpy changes for the binding of the first and the second molecules of $(dT)_{35}$, respectively. Each titration was fit to a two sequential sites binding model (Eq. (1) in Materials and methods), although we note that in all cases $k_{1,35,obs}$ is too high ($>10^9\ M^{-1}$) to be determined with reliability. The estimates of $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$, as well as $lnk_{2,35,obs}$ at 20 mM NaF, NaCl and NaBr are plotted in Fig. 3A and C, respectively. At low temperatures binding of the first $(dT)_{35}$ shows a large and negative $\Delta H_{1,35,obs}$, which decreases gradually (almost linearly) with increasing T ($\Delta C_{P,1} < 0$) (Fig. 3A). Binding of the second $(dT)_{35}$ shows a much larger negative binding enthalpy ~−(15–20) kcal/mol more favorable at T<15 °C. However, as T increases above 20 °C, $\Delta H_{2,35,obs}$ increases nonlinearly ($\Delta C_{P,2} > 0$) intersecting with $\Delta H_{1,35,obs}$, at T~28 °C. Determination of $\Delta H_{2,35,obs}$ at T>32 °C in these conditions becomes more difficult because

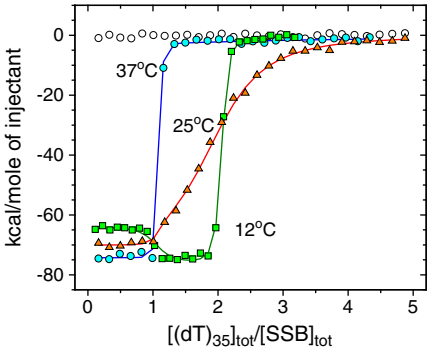


Fig. 2. Typical titrations of SSB (~1.4 μM) with $(dT)_{35}$ performed in buffer H (20 mM NaCl) at different temperatures: 12 °C (green squares), 25 °C (orange triangles), 37 °C (cyan circles). Reference titrations of $(dT)_{35}$ into the buffer at different temperatures (shown in open circles) are comparable and provide very low heat responses. Binding isotherms demonstrate biphasic behavior indicative of sequential binding of two $(dT)_{35}$ with negative cooperativity, with the first $(dT)_{35}$ molecule binding stoichiometrically, for which the affinity cannot be estimated and the second one binding with lower affinity, decreasing with increasing temperature, such that at 37 °C its binding is not detectable.

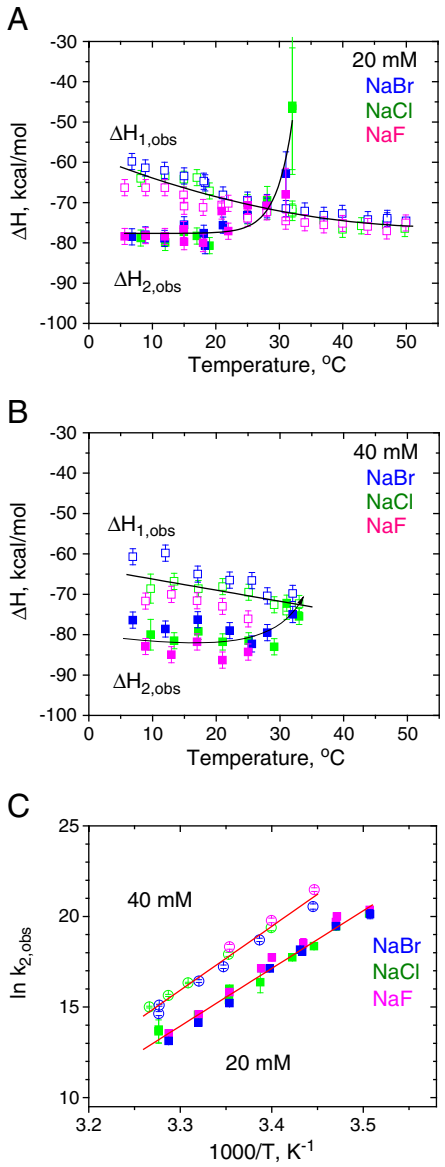


Fig. 3. The effect of temperature and type of the salt (low concentrations) on $(dT)_{35}$ binding parameters (buffer H). (A) — Temperature dependences of $\Delta H_{1,35,obs}$ (open squares) and $\Delta H_{2,35,obs}$ (closed squares) obtained in the presence of 20 mM NaF (magenta), NaCl (green) and NaBr (blue). (B) — Temperature dependences of $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ obtained in the presence of 40 mM NaF, NaCl and NaBr (all designations are the same as in panel (A)). (C) — Van't Hoff plots of the dependences of $k_{2,35,obs}$ on temperature in the presence of 20 mM (closed squares) and 40 mM (open circles) of NaF (magenta), NaCl (green) and NaBr (blue).

of the very steep decrease in $k_{2,obs}$ with increasing T (Fig. 3C). It appears that there is no effect of anion type on $\Delta H_{1,35,obs}$, $\Delta H_{2,35,obs}$ (Fig. 3A) or $k_{2,35,obs}$ (Fig. 3C). This agrees with our previous studies of SSB binding to $(dT)_{70}$ [23,27], showing that the effects of anion concentration and type on the binding enthalpy become pronounced only at [salt]>0.1 M and follow the Hofmeister series, $Br^- > Cl^- >> F^-$ (with little effect observed in NaF). We also note that the temperature dependence of $\Delta H_{tot,35,obs}$ ($=\Delta H_{1,35,obs}+\Delta H_{2,35,obs}$) below 20 °C (see Supplementary Fig. S2) is quite similar to that observed for $\Delta H_{70,obs}$ for SSB binding to $(dT)_{70}$ in 0.2 M NaF over the same temperature range [27].

Binding of $(dT)_{35}$ to SSB is accompanied by a net protonation of the SSB [24,25,27]. We have previously studied these linked protonation reactions for SSB binding to $(dT)_{35}$ at constant temperature by varying pH and salt concentration [25]. In the current study we observe that uptake of protons occurs upon binding both molecules of $(dT)_{35}$ as indicated by the decrease in the magnitudes of $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ for experiments performed in Tris vs. Hepes buffer (Supplementary Fig. S1). Corrections for these protonation effects have been applied as described in Supplementary materials and show that these protonation effects cannot explain the very different trends in the temperature dependences of $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ (see Discussion).

Upon increasing the salt concentration to 40 mM (Fig. 3B) the influence of anion type becomes noticeable ($\Delta C_{P,1,NaBr} < \Delta C_{P,1,NaCl} < \Delta C_{P,1,NaF}$), although there is still only a slight effect of anion type on $\Delta H_{2,35,obs}$. The dependences of $\Delta H_{2,35,obs}$ on T at 40 mM are similar to those observed at 20 mM, although the temperature at which $\Delta H_{2,35,obs}$ starts to increase (decrease in magnitude) is higher (>30 °C). It also appears that at 40 mM salt concentration the binding of the second $(dT)_{35}$ may be slightly more (~5–10 kcal/mol) favored enthalpically. This is consistent with a previous observation of a increase in the magnitude of $\Delta H_{2,35,obs}$ of ~10 kcal/mol upon increasing the NaCl concentration from 10 to 50 mM at constant temperature (25 °C) [23].

At both 20 and 40 mM salt concentrations the van't Hoff plots of $\ln k_{2,35,obs}$ are essentially linear with little effect of anion type (Fig. 3C), although the values of $k_{2,35,obs}$ are shifted upward by ~10-fold at 40 mM. This is due to the decrease in negative cooperativity for binding of the second $(dT)_{35}$ at the higher salt concentration [20,21,23].

3.2. Binding of $(dC)_{35}$ to SSB at low salt concentrations is similar to that observed for $(dT)_{35}$

To examine the generality of the temperature effects on the binding of SSB to $(dT)_{35}$ at low salt we also examined binding to $(dC)_{35}$ under similar conditions (buffer H, 20 mM NaCl). Two molecules of $(dC)_{35}$ can also bind to the SSB tetramer with a salt-dependent negative cooperativity, although the extent of this negative cooperativity is lower for $(dC)_{35}$ than for either $(dT)_{35}$ or $(dA)_{35}$ [22,24].

As for $(dT)_{35}$, the temperature dependences of $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ for the binding of $(dC)_{35}$ show opposite trends increasing and decreasing in magnitude with increasing temperature (Fig. 4A). The heat capacity for binding the first $(dC)_{35}$ remains negative ($\Delta C_{P,1}<0$), although it deviates from linearity at T>35 °C. However, $\Delta H_{2,35,obs}$ remains relatively constant up to ~25 °C ($\Delta C_{P,2}\leq 0$), but then increases sharply with increasing temperature ($\Delta C_{P,2}>0$). Both $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ are approximately 10 kcal/mol more negative than the corresponding values for $(dT)_{35}$ binding (see Fig. 3A) reflecting a preference for SSB binding to the deoxycytosines as shown previously [22,24]. As in the case of $(dT)_{35}$, experiments performed in Tris buffer (data not shown) indicate that binding of both molecules of $(dC)_{35}$ is accompanied by the uptake of protons.

The van't Hoff plots of $k_{2,35,obs}$ shown in Fig. 4B are practically linear over the entire temperature range. In fact, the sharp increase in $\Delta H_{2,35,obs}$ observed in the data in Fig. 4A shows up only as a very slight deviation in the linearity of the van't Hoff plot at the highest temperatures. This emphasizes the fact that it is often difficult to detect even large changes in binding heat capacity from measurements of a binding constant; one needs direct calorimetric measurements of ΔH. Due to the lower negative cooperativity associated with binding of the second molecule of $(dC)_{35}$, the values of $k_{2,35,obs}$ are ~10 fold higher than the corresponding values for $(dT)_{35}$ and therefore can be determined (as well as values of $\Delta H_{2,35,obs}$) over a broader temperature range (up to 38 °C). These results indicate that the binding of $(dT)_{35}$ and $(dC)_{35}$ to SSB displays similar thermodynamics, supporting a general mechanism for SSB interactions with these short ssDNA (see Discussion section).

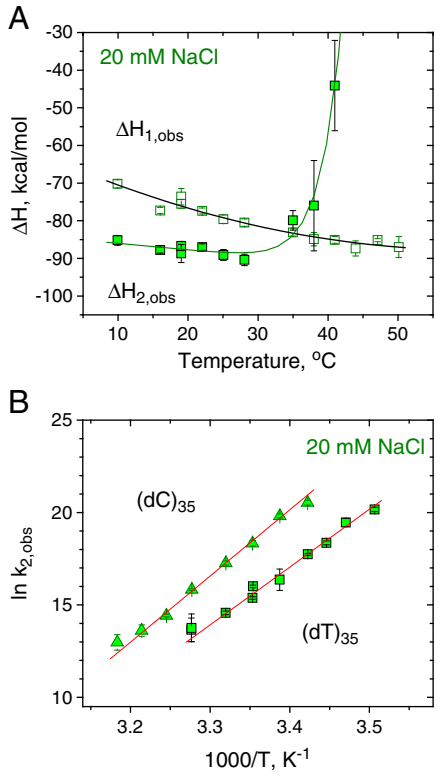


Fig. 4. The effect of temperature on $(dC)_{35}$ binding parameters (buffer H, 20 mM NaCl). (A) — Temperature dependences of $\Delta H_{1,35,obs}$ (open squares) and $\Delta H_{2,35,obs}$ (closed squares) (B) — van't Hoff plot of the dependence of $k_{2,35,obs}$ on temperature (triangles). The dependence for $(dT)_{35}$ from Fig. 3C is shown for comparison (squares).

3.3. At higher salt concentrations (>0.2 M) binding of the first and the second molecule of $(dT)_{35}$ shows a similar behavior but displays significant effects of salt concentration and type

Our previous thermodynamic studies of the binding of SSB to $(dT)_{70}$ showing a temperature-dependent sign reversal for the binding heat capacity were performed at monovalent salt concentrations ≥ 0.2 M [27]. Hence, for comparison, we next examined the binding of $(dT)_{35}$ to SSB at these higher salt concentrations from 6 to 38 °C at 0.2 and 1.0 M NaCl and NaBr. The binding isotherms were fit to a two site binding model and the resulting binding parameters ($\Delta H_{1,35,obs}$, $\Delta H_{2,35,obs}$ and $k_{2,35,obs}$) are shown in Fig. 5. Again, we note that $k_{1,35,obs}$ could not be determined reliably for most of the titrations due to the high binding affinity of the first $(dT)_{35}$.

Binding of the first and second $(dT)_{35}$ molecules to SSB in 0.2 M NaCl (see Fig. 5A) is accompanied by large, negative binding enthalpies differing in magnitude $|\Delta H_{1,35,obs}| < |\Delta H_{2,35,obs}|$, but showing similar linear temperature dependences below 25 °C ($\Delta C_{P,1,35,obs} \approx \Delta C_{P,2,35,obs}$, see Table 1). Similar behavior is observed in 0.2 M NaBr (Fig. 5B and Table 1), although the binding enthalpies are shifted by ~8–10 kcal/mol reflecting the previously observed effects of Br^- vs. Cl^- [23,27]. Importantly, for both salts, $\Delta H_{2,35,obs}$ starts to increase at temperatures above 30 °C indicating that $\Delta C_{P,obs}$ becomes positive indicating a sign reversal, although this change is less dramatic than at lower salt conditions.

Increasing the salt concentration to 1 M (see Fig. 5A and B) results in a significant increase in both $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ at all temperatures with larger effects for NaBr as shown previously at 25 °C [23,27]. However there is little difference in ΔC_P at the different salt concentrations (see Table 1). One interesting difference is that at either 1 M NaCl or 1 M NaBr, binding of the first $(dT)_{35}$ molecule shows a slightly more favorable enthalpy than for the second molecule $|\Delta H_{1,35,obs}| > |\Delta H_{2,35,obs}|$.

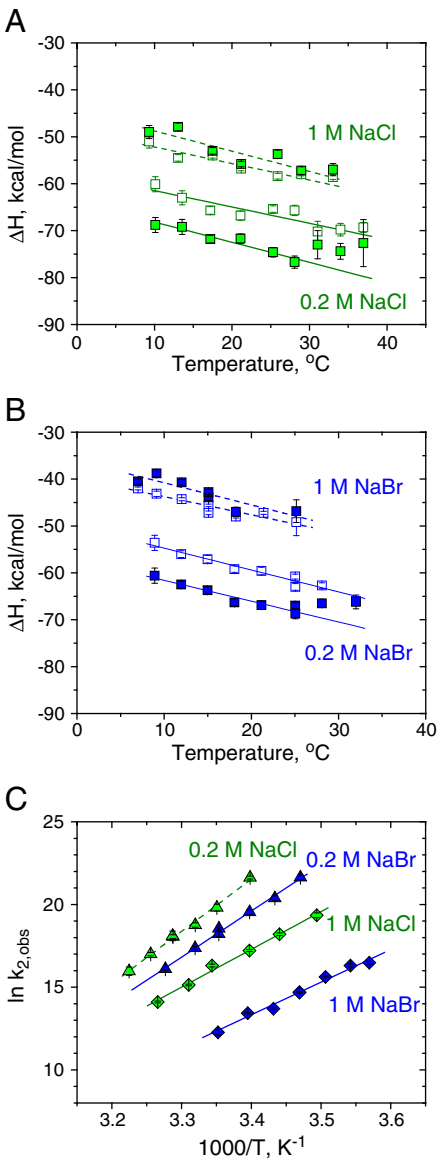


Fig. 5. The effect of temperature and type of the salt (moderate and high concentrations) on $(dT)_{35}$ binding parameters (buffer H). (A) — Temperature dependences of $\Delta H_{1,35,obs}$ (open squares) and $\Delta H_{2,35,obs}$ (closed squares) obtained in the presence of 0.2 M NaCl and 1 M NaCl. (B) — The dependences obtained in the presence of 0.2 M and 1 M NaBr. (C) — van't Hoff plots of the dependences of $k_{2,35,obs}$ on temperature in the presence of 0.2 M of NaCl and NaBr (green and blue triangles, respectively) and 1 M of NaCl and NaBr (green and blue diamonds, respectively).

3.4. Estimates of ion uptake/release upon binding the second molecule of $(dT)_{35}$ to SSB

The data in Figs. 3C and 5C allow us to examine the dependencies of $\log k_{2,35,obs}$ on log[salt] at 15 20, 25 and 30 °C (see Fig. 6A and B). Within this temperature range all $k_{2,35,obs}$ show linear van't Hoff plots at each salt concentration (0.02 M; 0.04 M; 0.2 M and 1.0 M). Fig. 6A and B shows that at each temperature the affinity first increases and then decreases with increasing salt concentration, indicative of a net uptake of ions ($SK_{obs} \equiv (\partial \log k / \partial \log[salt])_T > 0$) at low salt concentrations and a net release of ions ($SK_{obs} \equiv (\partial \log k / \partial \log[salt])_T < 0$) at higher salt concentrations. The salt dependences obtained previously in NaCl and NaBr at 25 °C [23] are also included for comparison.

It is evident that the slopes (SK_{obs}) in Fig. 6 are temperature dependent reflecting an effect of temperature on ion uptake/release.

Table 1
Heat capacity changes for SSB binding to $(dT)_{35}$ obtained from linear-least squares analysis of data at low temperature (5–25 °C) at moderate (0.2 M) and high (1.0 M) concentrations of NaCl and NaBr (buffer H).

Salt concentration	$\Delta Cp_{1,35}$ kcal mol^{-1} K^{-1}	$\Delta Cp_{2,35}$ kcal mol^{-1} K^{-1}	$\sum \Delta Cp_{35}$ kcal mol^{-1} K^{-1}	ΔCp_{70}[a] kcal mol^{-1} K^{-1}
0.2 M NaCl	−0.35 ± 0.07	−0.43 ± 0.06	−0.78 ± 0.09	−0.94 ± 0.05
0.2 M NaBr	−0.47 ± 0.04	−0.44 ± 0.05	−0.91 ± 0.07	−1.14 ± 0.11
1 M NaCl	−0.35 ± 0.06	−0.43 ± 0.11	−0.79 ± 0.13	−1.00 ± 0.15
1 M NaBr	−0.39 ± 0.06	−0.47 ± 0.11	−0.86 ± 0.12	−1.10 ± 0.03

[a] From reference [27].

The estimates of SK_{obs} based on only two salt concentrations at both low (0.02 and 0.04 M) and high (0.2 M and 1 M) salt are plotted as a function of temperature in Fig. 6C and D, respectively. In the high salt region (Fig. 6D) the net release of Na^+ plus Br^- is higher than for Na^+ plus Cl^-, although both slopes decrease as the temperature increases. In the low salt region (Fig. 6C) there is no detectable effect of anions on $k_{2,35,obs}$, whereas the net uptake of ions (most likely Na^+) decreases with increasing temperature. Since SK_{obs} is temperature dependent it follows from Eq. (5) [28] that ΔH_{obs} must be dependent on [salt].

$$\left(\frac{\partial SK_{obs}}{\partial T^{-1}}\right)_{[salt]} = -\frac{1}{2.3R}\left(\frac{\partial \Delta H_{obs}}{\partial\, log\ _{[salt]}}\right)_T \quad (5)$$

Applying Eq. (5) in the high salt range, where NaCl or NaBr concentrations are changing from 0.2 to 1 M, we estimate that the expected shift in the magnitude of $\Delta H_{2,obs}$, associated with the release of Cl^- or Br^- ions should be ~17–18 kcal/mol, which is in good agreement with the observed shift of ~20 kcal/mol (see Fig. 5A or B). In the low salt range (from 0.02 to 0.04 M), where the enthalpy change is associated with the uptake of cations, Eq. (5) predicts an ~7 kcal/mol shift in the magnitude of $\Delta H_{2,35,obs}$, which is consistent with the fact that the observed enthalpy becomes 5–10 kcal/mol more negative at 40 mM [salt] (see Fig. 3; see also Fig. 7 in reference [23]).

It is important to note that in the analysis presented above we have not corrected either $k_{2,35,obs}$ or $\Delta H_{2,35,obs}$ for the slight contribution due to the change in pH with temperature or for the contribution due to protonation of SSB upon binding $(dT)_{35}$. These effects appear to be temperature dependent only [25,27] and should contribute equally at each particular salt concentration. Consequently, when k_{obs}, and ΔH_{obs} are analyzed in terms of derivatives relative to [salt] (either SK_{obs} or $\partial\Delta H_{obs}/\partial \log[salt]$, for example) these pH and protonation effects should cancel.

4. Discussion

The molecular basis for heat capacity changes in macromolecular interactions has been the subject of much interest [24,25,27,38–46] and

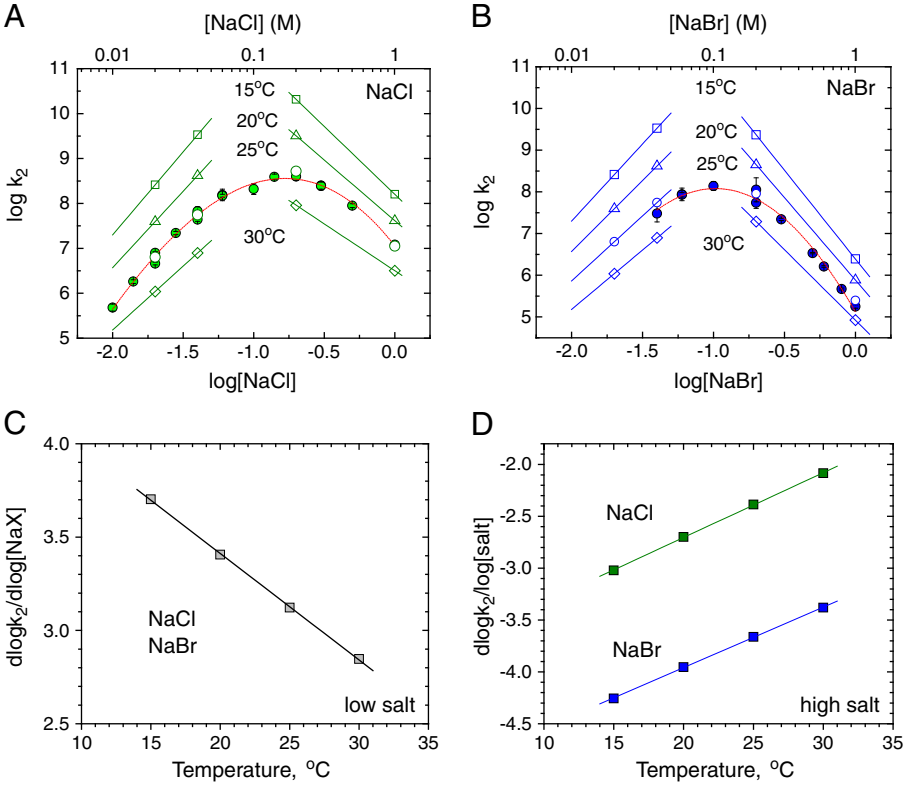


Fig. 6. Estimates of the net uptake/release of ions upon binding of the second molecule of $(dT)_{35}$ to SSB tetramer at different temperatures and salt conditions. Log$k_{2,35,obs}$-log[salt] dependences in NaCl (A) and NaBr (B) at four different temperatures (15 °C, 20 °C, 25 °C and 30 °C) obtained based on the analysis of the data presented in Figs. 3C and 5C (see text for details). Data shown in closed circles for 25 °C are from reference [23]. Net uptake (C) and release (D) of ions as a function of temperature obtained from the slopes of log$k_{2,35,obs}$-log[salt] dependences shown in panels (A) and (B) for low (20–40 mM) and high (0.2–1.0 M) salt concentrations, respectively.

several origins have been proposed: (1) net burial of nonpolar surface area upon complexation (hydrophobic effect) [38,40–42] (2) changes in the vibrational modes of the macromolecules and participating water [38,43,46], and (3) thermodynamic coupling of one or more binding or conformational equilibria to the main binding reaction of interest [24,25,27,38,39,47,48]. We have observed that multiple temperature-dependent linked equilibria, including protonation, cation and anion binding, as well as protein and DNA conformational changes contribute significantly to the observed binding heat capacity of SSB–ssDNA interactions [24,25,27]. Observations such as these indicate that the origins of these heat capacity changes are complex and cannot be understood simply from structural information (i.e., changes in accessible surface area).

Our previous studies of SSB binding to $(dT)_{70}$ performed at moderate and high salt concentrations showed a dramatic temperature dependent change in the sign of the binding heat capacity. We observed that ΔH_{obs} decreased with temperature from 5° to 35 °C ($\Delta C_{p,obs}<0$), but then increased at higher temperatures up to 60 °C ($\Delta C_{p,obs}>0$) and that both salt concentration and anion type have large effects on ΔH_{obs} and $\Delta C_{p,obs}$. To explain these observations we suggested a model in which SSB protein can undergo a temperature dependent conformational transition (below 35 °C), the midpoint of which shifts to higher temperature (above 35 °C) for the SSB–ssDNA complex and that this transition is modulated by salt concentration and anion type [27]. Here, we have examined the heat capacities for binding of SSB to one vs. two molecules of $(dT)_{35}$ or $(dC)_{35}$ and show that the change in the sign of the heat capacity occurs as well, but only for the binding of the second DNA molecule. This suggests that this temperature-dependent sign reversal of the heat capacity is a property that results from the formation of a fully wrapped SSB–ssDNA structure. Furthermore, a temperature-dependent conformational change, or rearrangement of the SSB–ssDNA complex is the likely origin of this sign reversal of the binding heat capacity. At a minimum this indicates that the number of thermally accessible states increases upon binding a second molecule of $(dT)_{35}$ to form a fully wrapped SSB–DNA complex.

The previous studies of $(dT)_{70}$ binding to SSB had to be restricted to high salt concentrations (≥0.2 M) to ensure that only the fully wrapped $(SSB)_{65}$ binding mode is formed. The use of $(dT)_{35}$ or $(dC)_{35}$ allowed us to extend our studies to lower salt concentrations since one can readily differentiate SSB complexes formed with one vs. two molecules of $(dX)_{35}$. SSB tetramers can bind two molecules of $(dX)_{35}$ at any salt concentration, although with negative cooperativity [21,23]. Complexes of SSB bound to one vs. two molecules of $(dX)_{35}$ have been used as models for the $(SSB)_{35}$ vs. $(SSB)_{65}$ mode, thus these studies also may provide information on the thermodynamics of the transitions between SSB binding modes.

4.1. Opposite heat capacity trends are observed for the binding of the first vs. the second molecule of $(dX)_{35}$ to an SSB tetramer

Previous studies [22,24] show that binding of either $(dT)_{35}$ or $(dC)_{35}$ occurs with negative cooperativity ($k_{1,35,obs}>>k_{2,35,obs}$). Over a broad temperature range binding of both the first and the second molecules of DNA is enthalpically driven with $\Delta H_{2,35,obs}<\Delta H_{1,35,obs}$. We show here that the heat capacity for binding the first molecule of $(dX)_{35}$ is always negative. However, for binding of the second molecule of $(dX)_{35}$, the binding heat capacity changes sign from negative to positive with increasing temperature. This suggests that the previously observed temperature-dependent reversal of sign of the heat capacity for binding of SSB to $(dT)_{70}$ is a property of forming a fully wrapped SSB–ssDNA complex, such as what is observed in the $(SSB)_{65}$ binding mode.

What might be the molecular basis for this behavior? It has been shown that preferential binding of anions to SSB and their concomitant release upon formation of the SSB–ssDNA complex results in salt dependent ΔH_{obs} and ΔCp, although these effects diminish as the salt concentration decreases [27]. Another large contribution to ΔH_{obs} and ΔCp is linked protonation reactions that accompany the formation of SSB–ssDNA complexes [24,25]. These protonation reactions are temperature dependent and thus can potentially contribute differentially to ΔH_{obs} at different temperatures as well as to K_{obs}. Since the protonation sites on SSB have been identified and quantified previously [24,25,27] we can correct our current data (both ΔH_{obs} and observed association constant $k_{2,35,obs}$) for these contributions (see Supplementary materials). The corrected data from Fig. 3A and C obtained in Hepes buffer are combined with the data obtained in Tris buffer (20 mM NaCl) in Fig. 7B and A, respectively. These data clearly indicate that the opposite behaviors of

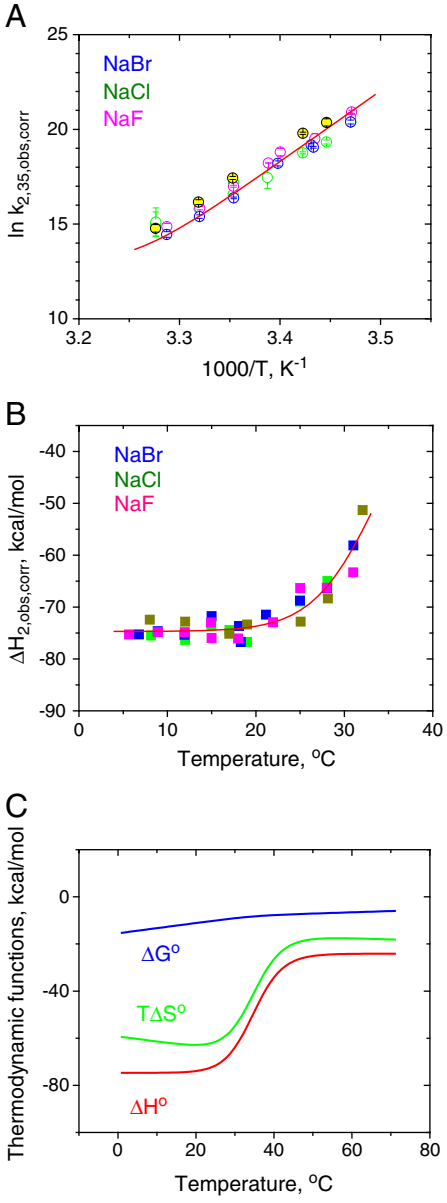


Fig. 7. Predicted effects of temperature on the observed association constant ($k_{2,35,obs}$) and enthalpy change ($\Delta H_{2,35,obs}$) for the interaction of the second $(dT)_{35}$ molecule to SSB–$(dT)_{35}$ (1:1) complex based on the model in Scheme 1. (A) — Temperature dependences of $k_{2,35,obs}$ in the form of van't Hoff plots obtained in 20 mM NaCl (green), NaBr (blue) and NaF (magenta) in Hepes buffer and in 20 mM NaCl in Tris buffer (yellow) after correction for protonation. (B) — $\Delta H_{2,35,obs}$ dependences on temperature obtained under the same conditions (panel A) after corrections for buffer ionization and protonation. Solid curves through the data points in both panels represent the simulation based on the parameters shown in Table 2 (see Discussion). (C) — thermodynamic profile for the data in panels (A) and (B) predicted over the broader temperature range from 0 up to 70 °C.

the heat capacities for binding the first vs. the second molecule of $(dT)_{35}$ with SSB remain. The overall $\Delta H_{tot,35,obs}$ ($\Delta H_{1,35,obs} + \Delta H_{2,35,obs}$) shows a decrease followed by an increase with temperature above 28 °C (see Supplementary Fig. S2). The change in the sign of $\Delta C_{P,obs}$ observed previously in experiments performed with $(dT)_{70}$ at higher salt concentrations (≥0.2 M) occurred at higher temperatures (35–40 °C) [27]. There are only a few examples of a positive heat capacity change for a protein–DNA association reaction. One has been reported for the binding of a mutant Sac7d protein to poly(dGdC) [48] and was ascribed to conformational changes within the protein–DNA complex. The other example is our previous observation of a positive heat capacity change for the binding of SSB to $(dT)_{70}$ at high temperatures [27].

4.2. Large enthalpy driven rearrangements of the SSB–ssDNA complex may explain the positive heat capacity for binding the second DNA molecule

We suggest Scheme 1 to explain the sign reversal in the heat capacity change for binding of the second $(dX)_{35}$ molecule. In this scheme, "loose" binding of the second $(dX)_{35}$ to an SSB–$(dX)_{35}$ complex is followed by a rearrangement of both DNA molecules within the complex. In the initial 1:1 complex the first molecule of $(dX)_{35}$ interacts with an average of two SSB subunits (possibly as shown in Fig. 1B) leaving two DNA binding sites (OB folds) partially available for the binding of the second $(dX)_{35}$. The second DNA can bind to one of these sites, although it may also readily dissociate due to constraints (both configurational and electrostatic) imposed by the first bound DNA molecule. It has been shown that SSB can diffuse along ssDNA possibly using a "rolling mechanism" that incorporates unwrapping/rewrapping of the ends of bound ssDNA molecule [19,49,50]. A similar mechanisms could be used by the second $(dX)_{35}$ to obtain the optimal configuration for both molecules to form a complex similar to the $(SSB)_{65}$ binding mode (Fig. 1A). This rearrangement should be entropically unfavorable and therefore would need to be accompanied by a large favorable binding enthalpy.

The data for $(dT)_{35}$ binding shown in Fig. 7A and B were globally fit to the model in Scheme 1 (Eqs. (2)–(4) (see Materials and methods)). The first two parameters of this Scheme, K_2 (at T_{ref}) and ΔH_2, describe the loose binding of $(dT)_{35}$ to the preformed 1:1 SSB–$(dT)_{35}$ complex, while the second two parameters, T_{ref} and ΔH^*, reflect the conformational rearrangement within the 1:2 SSB–$(dT)_{35}$ complex, (T_{ref} is the temperature for the middle of the transition ($K^*(T_{ref}) = 1$)). Fig. 7 (see Table 2 for parameters estimates) shows that this model provides a good description of the dependences of both $k_{2,35,obs}$ and $\Delta H_{2,35,obs}$ on temperature. We note that this analysis has assumed that both ΔH_2 and ΔH^* are independent of temperature in order to reduce the number of parameters in the model. The resulting parameters indicate that the first step for binding the second $(dT)_{35}$ occurs with weak affinity ($K_2 \approx 5\times10^5\ M^{-1}$ at $T_{ref} \approx 33$ °C or $K_2 \approx 2\times10^7\ M^{-1}$ at T = 5 °C) and a moderate binding enthalpy ($\Delta H_2 \approx -24 \pm 5$ kcal/mol), whereas the subsequent conformational rearrangements within the complex are accompanied by a very large enthalpy change ($\Delta H_2 \approx -51 \pm 4$ kcal/mol). The latter yields a very sharp change of K^* with temperature (from $K^* = 1$ at $T_{ref} \approx 33$ °C to $K^* = 2.5\times10^3$ at T = 5 °C).

Table 2
Parameters for the binding of the second $(dX)_{35}$ molecule to SSB:$(dX)_{35}$ = 1:1 complex obtained globally fitting the data at 20 mM salt using the model shown in Scheme 1 and described by Eqs. (2)–(4).

Parameters	$(dT)_{35}$	$(dC)_{35}$
$K_{2,ref}$, M^{-1}	$(4.2 \pm 1.0)\times10^5$	$(1.4 \pm 0.4)\times10^6$
ΔH_2, kcal/mol	-24.1 ± 5.2	-36.2 ± 9.5
T_{ref}, °C	33.5 ± 0.2	37.6 ± 2.4
ΔH^*, kcal/mol	-50.6 ± 3.8	-50.3 ± 8.7

The same model can fit the data for $(dC)_{35}$ (Fig. 4), after correction for protonation effects, yielding parameters with similar trends (see Table 2). However, for $(dC)_{35}$ the transition temperature, T^*, is shifted to a higher value (~38 °C) indicating that formation of the fully wrapped $(dC)_{35}$:SSB (2:1) complex is more favorable. Larger values for K_2, and the overall $\Delta H_{2,35obs}$ ($= \Delta H_2 + \Delta H^*$) reflect the preference of SSB for binding deoxycytosines over deoxythymidines [22,24].

Using the parameters in Table 2 from the fits to Scheme 1, we can calculate the thermodynamic profiles (Fig. 7C) for the binding of the second molecule of $(dT)_{35}$ from 0 °C to 70 °C (the temperature at which SSB starts to dissociate and unfold [27]). We see that at temperatures below 30 °C formation of the SSB–$(dT)_{35}$ (1:2) complex is more favorable energetically and is driven by a large enthalpy change needed to overcome the unfavorable entropic contribution accompanying the DNA rearrangement within the complex. On the other hand as the temperature increases formation of the SSB–$(dT)_{35}$ (1:2) complex becomes much less favorable and the equilibrium shifts to favor a (1:1) complex. Thus, this model predicts that increasing temperature should increase the observed negative cooperativity for binding a second molecule of $(dX)_{35}$. The effects of temperature on the negative cooperativity parameter have been examined for $(dT)_{16}$ binding to SSB, but only over a limited low temperature range (8 °C to 25 °C) [21]. These show a slight trend in the predicted direction, although a much wider temperature range needs to be examined to provide a rigorous test of this prediction. However, the current thermodynamic profiles shown in Fig. 7C suggest that the fully wrapped $(SSB)_{65}$ mode would be preferred at lower temperatures, whereas the $(SSB)_{35}$ binding mode would be favored at higher temperatures.

4.3. The thermodynamics of forming a fully wrapped SSB complex using either one molecule of $(dT)_{70}$ or two molecules of $(dT)_{35}$ are very similar at moderate and high salt concentrations

Anions have a negligible effect on $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ and the apparent affinity ($k_{2,35,obs}$) at low salt concentrations over the temperature range that we have examined (see Fig. 3). This indicates that these anions bind weakly to the SSB tetramer and thus do not contribute much to the observed parameters at low salt [23,27]. However, above 0.2 M significant anion effects are observed (see Fig. 5).

In the high salt range, the magnitudes of both $\Delta H_{1,35,obs}$ and $\Delta H_{2,35,obs}$ decrease significantly as the salt concentration increases and this effect is much more pronounced in NaBr. In addition, the temperature dependences of $\Delta H_{2,35,obs}$ change its sign ($\Delta Cp_2 < 0$) and can be approximated as linear over the temperature range from 5 to 25 °C. Both $\Delta Cp_{1,35,obs}$ and $\Delta Cp_{2,obs}$ appear to be similar within experimental uncertainties and vary within −0.35 to −0.47 kcal/mol deg (see Table 1). However, there appears to be a trend toward a somewhat larger magnitude of ΔCp determined in NaBr. These observations are consistent with those obtained for $(dT)_{70}$ binding to SSB tetramer under the same conditions [27].

Indeed, the temperature dependences of the total enthalpy for the binding of two $(dT)_{35}$ molecules ($\Delta H_{tot,35,obs} = \Delta H_{1,35,obs} + \Delta H_{2,35,obs}$) are in a good agreement with those for $(dT)_{70}$ binding (see Supplementary Fig. S2 and Table 1 for comparison of $\Delta Cp_{35,tot}$ and ΔCp_{70}). Therefore, formation of the fully wrapped SSB–$(dT)_{35}$ (1:2) complex and the fully wrapped SSB–$(dT)_{70}$ (1:1) complex is affected by salt similarly [23,27]. These results also suggest that the final complexes of an SSB tetramer bound with one molecule of $(dT)_{70}$ or two molecules of $(dT)_{35}$ at moderate and higher salt conditions are identical in terms of ssDNA wrapping and resemble the $(SSB)_{65}$ binding mode.

It is remarkable to note that the dependences of $\Delta H_{70,obs}$ on temperature obtained previously at 0.20 M monovalent salt concentrations [27] show a reversal of the sign of ΔCp_{70} at 38–40 °C. Under the same conditions our data clearly show the same trend for $\Delta H_{35,tot}$

at temperatures above 30 °C (see Supplementary Fig. S2), primarily due to the contribution from $\Delta H_{2,35,obs}$, (see Fig. 5A and B). Since we ascribe the positive $\Delta Cp_{,35,obs} > 0$ to temperature dependent rearrangements of $(dT)_{35}$ molecules within the complex, we suggest that the interaction of $(dT)_{70}$ with SSB undergoes similar conformational changes. In this case, $(dT)_{70}$ first forms a partially bound complex (possibly resembling the $(SSB)_{35}$ binding mode), but must then rearrange to form the fully wrapped $(SSB)_{65}$ binding mode. The formation of the latter is favored at increased salt concentrations and decreased temperatures, whereas formation of the $(SSB)_{35}$ mode is favored at low salt and increased temperatures. Salt concentration, ion type, temperature and SSB concentration are all important factors in determining the preferences for forming the different modes of ssDNA binding to SSB, which, in turn, may function to regulate the variety of roles that SSB plays in DNA metabolic processes [2].

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bpc.2011.05.005.

References

[1] J.W. Chase, K.R. Williams, Single-stranded DNA binding proteins required for DNA replication, Annu. Rev. Biochem. 55 (1986) 103–136.
[2] R.D. Shereda, A.G. Kozlov, T.M. Lohman, M.M. Cox, J.L. Keck, SSB as an organizer/mobilizer of genome maintenance complexes, Crit. Rev. Biochem. Mol. Biol. 43 (2008) 289–318.
[3] R.R. Meyer, P.S. Laine, The single-stranded DNA-binding protein of *Escherichia coli*, Microbiol. Rev. 54 (1990) 342–380.
[4] T.M. Lohman, M.E. Ferrari, *Escherichia coli* single-stranded DNA-binding protein: multiple DNA-binding modes and cooperativities, Annu. Rev. Biochem. 63 (1994) 527–570.
[5] S. Raghunathan, C.S. Ricard, T.M. Lohman, G. Waksman, Crystal structure of the homo-tetrameric DNA binding domain of *Escherichia coli* single-stranded DNA-binding protein determined by multiwavelength x-ray diffraction on the selenomethionyl protein at 2.9-A resolution, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 6652–6657.
[6] S. Raghunathan, A.G. Kozlov, T.M. Lohman, G. Waksman, Structure of the DNA binding domain of E-coli SSB bound to ssDNA, Nat. Struct. Biol. 7 (2000) 648–652.
[7] T.M. Lohman, L.B. Overman, Two binding modes in *Escherichia coli* single strand binding protein-single stranded DNA complexes. Modulation by NaCl concentration, J. Biol. Chem. 260 (1985) 3594–3603.
[8] W. Bujalowski, T.M. Lohman, *Escherichia coli* single-strand binding protein forms multiple, distinct complexes with single-stranded DNA, Biochemistry 25 (1986) 7799–7802.
[9] S. Chrysogelos, J. Griffith, *Escherichia coli* single-strand binding protein organizes single-stranded DNA in nucleosome-like units, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 5803–5807.
[10] J.D. Griffith, L.D. Harris, J. Register III, Visualization of SSB–ssDNA complexes active in the assembly of stable RecA-DNA filaments, Cold Spring Harb. Symp. Quant. Biol. 49 (1984) 553–559.
[11] W. Bujalowski, L.B. Overman, T.M. Lohman, Binding mode transitions of *Escherichia coli* single strand binding protein-single-stranded DNA complexes. Cation, anion, pH, and binding density effects, J. Biol. Chem. 263 (1988) 4629–4640.
[12] R. Roy, A.G. Kozlov, T.M. Lohman, T. Ha, Dynamic structural rearrangements between DNA binding modes of *E. coli* SSB protein, J. Mol. Biol. 369 (2007) 1244–1257.
[13] T.M. Lohman, L.B. Overman, S. Datta, Salt-dependent changes in the DNA binding co-operativity of *Escherichia coli* single strand binding protein, J. Mol. Biol. 187 (1986) 603–615.
[14] T.M. Lohman, W. Bujalowski, *E. coli* SSB protein: multiple binding modes and cooperativities, in: A. Revzin (Ed.), The Biology of Nonspecific DNA–Protein Interactions, CRC Press, 1990, pp. 131–170.
[15] M.E. Ferrari, W. Bujalowski, T.M. Lohman, Co-operative binding of *Escherichia coli* SSB tetramers to single-stranded DNA in the (SSB)35 binding mode, J. Mol. Biol. 236 (1994) 106–123.
[16] S.N. Savvides, S. Raghunathan, K. Futterer, A.G. Kozlov, T.M. Lohman, G. Waksman, The C-terminal domain of full-length E-coli SSB is disordered even when bound to DNA, Protein Sci. 13 (2004) 1942–1947.
[17] A.G. Kozlov, M.J. Jezewska, W. Bujalowski, T.M. Lohman, Binding specificity of *Escherichia coli* single-stranded DNA binding protein for the chi subunit of DNA pol III holoenzyme and PriA helicase, Biochemistry 49 (2010) 3555–3566.
[18] A.G. Kozlov, M.M. Cox, T.M. Lohman, Regulation of single-stranded DNA binding by the C termini of *Escherichia coli* single-stranded DNA-binding (SSB) protein, J. Biol. Chem. 285 (2010) 17246–17252.
[19] R. Roy, A.G. Kozlov, T.M. Lohman, T. Ha, SSB protein diffusion on single-stranded DNA stimulates RecA filament formation, Nature 461 (2009) 1092–1097.
[20] W. Bujalowski, T.M. Lohman, Negative co-operativity in *Escherichia coli* single strand binding protein–oligonucleotide interactions. I. Evidence and a quantitative model, J. Mol. Biol. 207 (1989) 249–268.
[21] W. Bujalowski, T.M. Lohman, Negative co-operativity in *Escherichia coli* single strand binding protein–oligonucleotide interactions. II. Salt, temperature and oligonucleotide length effects, J. Mol. Biol. 207 (1989) 269–288.
[22] T.M. Lohman, W. Bujalowski, Effects of base composition on the negative cooperativity and binding mode transitions of *Escherichia coli* SSB–single-stranded DNA complexes, Biochemistry 33 (1994) 6167–6176.
[23] A.G. Kozlov, T.M. Lohman, Calorimetric studies of E-coli SSB protein single-stranded DNA interactions. Effects of monovalent salts on binding enthalpy, J. Mol. Biol. 278 (1998) 999–1014.
[24] A.G. Kozlov, T.M. Lohman, Adenine base unstacking dominates the observed enthalpy and heat capacity changes for the *Escherichia coli* SSB tetramer binding to single-stranded oligoadenylates, Biochemistry 38 (1999) 7388–7397.
[25] A.G. Kozlov, T.M. Lohman, Large contributions of coupled protonation equilibria to the observed enthalpy and heat capacity changes for ssDNA binding to *Escherichia coli* SSB protein, Proteins (Suppl. 4) (2000) 8–22.
[26] A.G. Kozlov, T.M. Lohman, Stopped-flow studies of the kinetics of single-stranded DNA binding and wrapping around the *Escherichia coli* SSB tetramer, Biochemistry 41 (2002) 6032–6044.
[27] A.G. Kozlov, T.M. Lohman, Effects of monovalent anions on a temperature-dependent heat capacity change for *Escherichia coli* SSB tetramer binding to single-stranded DNA, Biochemistry 45 (2006) 5190–5205.
[28] T.M. Lohman, L.B. Overman, M.E. Ferrari, A.G. Kozlov, A highly salt-dependent enthalpy change for *Escherichia coli* SSB protein–nucleic acid binding due to ion–protein interactions, Biochemistry 35 (1996) 5272–5279.
[29] H. Fukada, K. Takahashi, Enthalpy and heat capacity changes for the proton dissociation of various buffer components in 0.1 M potassium chloride, Proteins 33 (1998) 159–166.
[30] J.J. Christensen, L.D. Hansen, R.M. Izatt, Handbook of Proton Ionization Heats, John Wiley & Sons, New York, 1976.
[31] T.M. Lohman, J.M. Green, R.S. Beyer, Large-scale overproduction and rapid purification of the *Escherichia coli* ssb gene product. Expression of the ssb gene under lambda PL control, Biochemistry 25 (1986) 21–25.
[32] W. Bujalowski, T.M. Lohman, Monomer–tetramer equilibrium of the *Escherichia coli* ssb-1 mutant single strand binding protein, J. Biol. Chem. 266 (1991) 1616–1626.
[33] L.B. Overman, W. Bujalowski, T.M. Lohman, Equilibrium binding of *Escherichia coli* single-strand binding protein to single-stranded nucleic acids in the (SSB)65 binding mode. Cation and anion effects and polynucleotide specificity, Biochemistry 27 (1988) 456–471.
[34] S.C. Kowalczykowski, N. Lonberg, J.W. Newport, P.H. von Hippel, Interactions of bacteriophage T4-coded gene 32 protein with nucleic acids. I. Characterization of the binding interactions, J. Mol. Biol. 145 (1981) 75–104.
[35] R.B. Inman, Transitions of DNA homopolymers, J. Mol. Biol. 9 (1964) 624–637.
[36] B.A. Adler, L. Grossman, G.D. Fasman, Single-stranded oligomers and polymers of cytidylic and 2′-deoxycytidylic acids: comparative optical rotatory studies, Proc. Natl. Acad. Sci. U. S. A. 57 (1967) 423–430.
[37] T. Wiseman, S. Williston, J.F. Brandts, L.N. Lin, Rapid measurement of binding constants and heats of binding using a new titration calorimeter, Anal. Biochem. 179 (1989) 131–137.
[38] J.M. Sturtevant, Heat capacity and entropy changes in processes involving proteins, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 2236–2240.
[39] M.R. Eftink, A.C. Anusiem, R.L. Biltonen, Enthalpy–entropy compensation and heat capacity changes for protein–ligand interactions: general thermodynamic models and data for the binding of nucleotides to ribonuclease A, Biochemistry 22 (1983) 3884–3896.
[40] J.H. Ha, R.S. Spolar, M.T. Record Jr., Role of the hydrophobic effect in stability of site-specific protein–DNA complexes, J. Mol. Biol. 209 (1989) 801–816.
[41] K.P. Murphy, E. Freire, Thermodynamics of structural stability and cooperative folding behavior in proteins, Adv. Protein Chem. 43 (1992) 313–361.
[42] R.S. Spolar, M.T. Record Jr., Coupling of local folding to site-specific binding of proteins to DNA, Science 263 (1994) 777–784.
[43] C.J. Morton, J.E. Ladbury, Water-mediated protein–DNA interactions: the relationship of thermodynamics to structural detail, Protein Sci. 5 (1996) 2115–2118.
[44] L. Jen-Jacobson, L.E. Engler, J.T. Ames, M.R. Kurpiewski, A. Grigorescu, Thermodynamic parameters of specific and nonspecific protein–DNA binding, Supramol. Chem. 12 (2000) 143–160.

[45] J.A. Holbrook, O.V. Tsodikov, R.M. Saecker, M.T. Record Jr., Specific and non-specific interactions of integration host factor with DNA: thermodynamic evidence for disruption of multiple IHF surface salt-bridges coupled to DNA binding, J. Mol. Biol. 310 (2001) 379–401.
[46] S. Bergqvist, M.A. Williams, R. O'Brien, J.E. Ladbury, Heat capacity effects of water molecules and ions at a protein–DNA interface, J. Mol. Biol. 336 (2004) 829–842.
[47] M.E. Ferrari, T.M. Lohman, Apparent heat capacity change accompanying a nonspecific protein–DNA interaction. *Escherichia coli* SSB tetramer binding to oligodeoxyadenylates, Biochemistry 33 (1994) 12896–12910.
[48] W.B. Peters, S.P. Edmondson, J.W. Shriver, Thermodynamics of DNA binding and distortion by the hyperthermophile chromatin protein Sac7d, J. Mol. Biol. 343 (2004) 339–360.
[49] R. Romer, U. Schomburg, G. Krauss, G. Maass, *Escherichia coli* single-stranded DNA binding protein is mobile on DNA: 1H NMR study of its interaction with oligo- and polynucleotides, Biochemistry 23 (1984) 6132–6137.
[50] S.V. Kuznetsov, A.G. Kozlov, T.M. Lohman, A. Ansari, Microsecond dynamics of protein–DNA interactions: direct observation of the wrapping/unwrapping kinetics of single-stranded DNA around the *E. coli* SSB tetramer, J. Mol. Biol. 359 (2006) 55–65.