Adenine Base Unstacking Dominates the Observed Enthalpy and Heat Capacity Changes for the *Escherichia coli* SSB Tetramer Binding to Single-Stranded Oligoadenylates†

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ABSTRACT: Isothermal titration calorimetry (ITC) was used to test the hypothesis that the relatively small enthalpy change (ΔH_{obs}) and large negative heat capacity change ($\Delta C_{p,obs}$) observed for the binding of the *Escherichia coli* SSB protein to single-stranded (ss) oligodeoxyadenylates result from the temperature-dependent adenine base unstacking equilibrium that is thermodynamically coupled to binding. We have determined $\Delta H_{1,obs}$ for the binding of 1 mole of each of $dT(pT)_{34}$, $dC(pC)_{34}$, and $dA(pA)_{34}$ to the SSB tetramer (20 mM NaCl at pH 8.1). For $dT(pT)_{34}$ and $dC(pC)_{34}$, we found large, negative values for $\Delta H_{1,obs}$ of -75 ± 1 and -85 ± 2 kcal/mol at 25 °C, with $\Delta C_{p,obs}$ values of -540 ± 20 and -570 ± 30 cal mol^{-1} K^{-1} (7−50 °C), respectively. However, for SSB−$dA(pA)_{34}$ binding, $\Delta H_{1,obs}$ is considerably less negative (-14 ± 1 kcal/mol at 25 °C), even becoming positive at temperatures below 13 °C, and $\Delta C_{p,obs}$ is nearly twice as large in magnitude (-1180 ± 40 cal mol^{-1} K^{-1}). These very different thermodynamic properties for SSB−$dA(pA)_{34}$ binding appear to result from the fact that the bases in $dA(pA)_{34}$ are more stacked at any temperature than are the bases in $dC(pC)_{34}$ or $dT(pT)_{34}$ and that the bases become unstacked within the SSB−ssDNA complexes. Therefore, the $\Delta C_{p,obs}$ for SSB−ssDNA binding has multiple contributions, a major one being the coupling to binding of a temperature-dependent conformational change in the ssDNA, although SSB binding to unstacked ssDNA still has an "intrinsic" negative $\Delta C_{p,0}$. In general, such temperature-dependent changes in the conformational "end states" of interacting macromolecules can contribute significantly to both $\Delta C_{p,obs}$ and ΔH_{obs}.

An understanding of the stabilities and specificities of protein−nucleic acid interactions requires thermodynamic information. Furthermore, with the increasing availability of high-resolution structural information for many proteins, nucleic acids, and their complexes, efforts have been made to provide structural interpretations of the thermodynamic profiles observed for these interactions. Such approaches can provide some useful connections between structure and energetics. Over the past several years, there has been increasing interest in obtaining a molecular interpretation of the changes in heat capacity, $\Delta C_{p,obs}$, associated with the formation of bio-macromolecular complexes, such as protein−ligand, protein−protein, and protein−nucleic acid interactions (*1*, *2*). This is a difficult task because of the numerous possible contributions to observed heat capacity changes for macromolecular processes, which can include (1) the hydrophobic effect with the associated release of constrained water associated with the burial of nonpolar surfaces (*1*, *3*, *4*), (2) restriction of "soft" vibrational modes of polar groups and bound water molecules mediating the interaction (*3*, *5*), and (3) the coupling or linkage to binding of any temperature-dependent "hidden" or "ignored" equilibria, such as protonation or conformational changes within the interacting macromolecules (*6*−*9*). Adding to this complexity is the fact that caution must be exercised in structurally interpreting thermodynamic profiles since the energetics of macromolecular interactions are extremely sensitive to solution conditions (temperature, pH, salt concentration, and type), whereas most crystallographic structures can be determined only under a limited range of solution conditions that often differ from the conditions under which the thermodynamic profiles were determined.

For many protein binding processes, correlations have been noted between the magnitude of the experimental heat capacity change and changes in the solvent accessible surface area that occur upon formation of the protein−ligand complex (*2*, *10*, *11*). However, in some cases, the heat capacity changes for a variety of protein interactions are found to be generally larger in magnitude than those calculated according to these empirical relationships and the available crystallographic data (*1*, *5*, *12*−*15*). In these cases, it has been suggested (*1*) that the additional contributions to ΔC_p result from some additional folding of an unstructured region of the protein that is coupled to binding. Although this hypothesis can explain the excess in ΔC_p for many processes (*1*, *16*), deviations are still apparent for a number of systems (*5*, *13*−*15*).

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Ferrari and Lohman (*7*) previously reported the effects of temperature on the equilibrium association constant, K_{obs}, for binding of the homotetrameric *Escherichia coli* single-stranded binding (SSB) protein to a series of single-stranded (ss) oligodeoxyribonucleotides: $dA(pA)_N$, $dT(pT)_N$, and $dC(pC)_N$ ($N = 34$, 55, or 69). Although linear van't Hoff plots were obtained for SSB binding to $dT(pT)_N$ and $dC(pC)_N$, SSB binding to $dA(pA)_N$ displayed van't Hoff plots that were distinctly nonlinear with K_{obs} maxima near 25 °C, indicating a large negative heat capacity change. It was suggested that the nonlinear van't Hoff plots resulted from the coupling or linkage of two temperature-dependent processes: (1) the unstacking of $dA(pA)_N$ bases (occurring with $\Delta H° > 0$ and $\Delta C_p° = 0$) and (2) the binding of SSB to the unstacked DNA (occurring with a $\Delta H°$ of <0). Thus, the large negative $\Delta C_{p,obs}°$ was proposed to be due mainly to the coupling of SSB binding to the temperature-dependent adenine base unstacking reaction, rather than to the net burial of nonpolar residues and the resulting hydrophobic effect.

To further test this hypothesis, we have performed isothermal titration calorimetry (ITC) studies of SSB tetramer binding to $dT(pT)_{34}$, $dC(pC)_{34}$, and $dA(pA)_{34}$, to obtain ΔH_{obs} and its dependence on temperature, from which we estimate $\Delta C_{p,obs}$ for these interactions. For the binding of all three oligodeoxynucleotides, we find negative values of $\Delta C_{p,obs}$. However, whereas the same value of $\Delta C_{p,obs}$ is obtained for SSB binding to $dT(pT)_{34}$ and $dC(pC)_{34}$, we find a value of $\Delta C_{p,obs}$ that is ∼2-fold larger in magnitude, although still negative, for SSB binding to $dA(pA)_{34}$. This additional contribution to $\Delta C_{p,obs}$ for the SSB−$dA(pA)_{34}$ interaction appears to be due to the pre-existing temperature-dependent adenine base unstacking equilibrium within $dA(pA)_{34}$ that is coupled to the SSB binding process (*7*).

MATERIALS AND METHODS

Reagents and Buffers. All chemicals were reagent grade, and all solutions were made with distilled water that was subsequently treated with a Milli Q (Millipore, Bedford, MA) water purification system. Titrations were performed in either buffer H or buffer T. Buffer H is 10 mM Hepes [4-(hydroxyethyl)-1-piperazineethanesulfonic acid] and 0.1 mM Na_3EDTA (pH 8.1). Buffer T is 10 mM Tris [tris(hydroxymethyl)aminomethane] and 0.1 mM Na_3EDTA (ethylenediaminetetraacetic acid) (pH 8.1). Buffers were prepared by adjusting the pH at 25 °C of a series of stocks of 10 mM Hepes-acid or 10 mM Tris-HCl solutions with 5 M NaOH so that the resulting pH of each buffer was kept at 8.1 ± 0.1 at all temperatures, based on the dependence of the pK_a of each buffer on temperature [for Hepes buffer, $pK_a(25\ °C) = 7.48$ and $dpK_a/dT = -0.014$; for Tris buffer, $pK_a(25\ °C) = 8.06$ and $dpK_a/dT = -0.028$ (*17*)]. As a result, the total Na^+ concentration contributed by the NaOH increased from 4 mM for the experiments at 7 °C to 9 mM at 50 °C.

E. coli SSB Protein and Nucleic Acids. SSB protein was purified as described previously (*18*) with the addition of a double-stranded DNA cellulose column for removing a minor exonuclease contaminant (*19*). The SSB protein concentration was determined spectrophotometrically in buffer T and 0.20 M NaCl using an extinction coefficient ϵ_{280} of 1.13×10^5 M^{-1} (tetramer) cm^{-1} (*20*). The oligodeoxynucleotides, $dT(pT)_{34}$, $dA(pA)_{34}$, and $dC(pC)_{34}$, were synthesized and purified as described previously (*21*) and were ≥98% pure as judged by denaturing gel electrophoresis and autoradiography of a sample that was 5′ end-labeled with ^{32}P using polynucleotide kinase. DNA concentrations were determined spectrophotometrically in buffer T (pH 8.1) and 0.1 M NaCl using the following extinction coefficients: for $dT(pT)_{34}$, $\epsilon_{260} = 8.1 \times 10^3$ M^{-1} (nucleotide) cm^{-1} (*22*, *23*); for $dA(pA)_{34}$, $\epsilon_{260} = 9.65 \times 10^3$ M^{-1} (nucleotide) cm^{-1} (*22*, *24*); and for $dC(pC)_{34}$, $\epsilon_{269} = 7.6 \times 10^3$ M^{-1} (nucleotide) cm^{-1} (*25*, *26*). DNA and SSB samples were dialyzed extensively versus buffer H or T at the indicated salt concentration for use in ITC experiments.

Isothermal Titration Calorimetry (ITC). ITC experiments were performed using an OMEGA titration microcalorimeter (MicroCal Inc., Northhampton, MA) (*27*) as described previously (*28*). Generally, experiments were carried out by titrating SSB solutions (ranging from 0.9 to 1.5 μM tetramer) with DNA [stock concentrations ranging from 30 to 50 μM for $dT(pT)_{34}$ and $dC(pC)_{34}$]. However, higher concentrations of the SSB protein (2.5−4.5 μM tetramer) were required in the experiments with $dA(pA)_{34}$ (stocks ranging from 0.9 to 1.7 mM) due to the lower magnitude of the enthalpy change for these reactions. The heats of dilution obtained for reference titrations of DNA into buffer were independent of DNA concentration at all temperatures. All corrections for heats of dilution were applied as described previously (*28*).

The tetrameric form of SSB has been shown to be stable over a wide range of protein concentrations (0.3−4.9 μM tetramer) and in different salts (*28*, *29*) and at temperatures from 5 to 37 °C (*7*). In some of the ITC experiments performed in this study, we used temperatures as high as 50 °C, since preliminary differential scanning calorimetry (DSC) experiments performed with the SSB protein [9−14 μM tetramer (pH 8.1) and 10−300 mM NaCl] showed no dissociation or unfolding transitions until 71 °C, where a sharp irreversible transition occurred which was independent of salt concentration (A. G. Kozlov, unpublished data). Although the SSB concentrations used in the ITC experiments were lower than those used in the DSC experiments, the ITC results gave no indication of tetramer dissociation or unfolding at this higher temperature.

Analysis of ITC Isotherms. At saturation, one SSB tetramer can bind two molecules of $dX(pX)_{34}$ (X being A, C, or T), although the second DNA molecule binds with a salt-dependent negative cooperativity (*28*, *30*−*33*). The degree of negative cooperativity is also strongly dependent on base composition, increasing in the following order: $dC(pC)_{34} < dT(pT)_{34} < dA(pA)_{34}$ (*7*, *33*). The partition function (P) in eq 1, which specifies two sites for $dX(pX)_{34}$ (X being T, C, or A) binding per SSB tetramer, was used to analyze the ITC isotherms

$$P = 1 + 2k_1X + k_1k_2X^2 \qquad (1)$$

where k_1 and k_2 are the microscopic equilibrium association "binding" constants for $dX(pX)_{34}$ binding to the first and second sites on the SSB tetramer, respectively, and X is the free $dX(pX)_{34}$ concentration.

The data were fit to this model using the "ITC Data Analysis in Origin" software provided by the manufacturer. For this model, the total heat after the ith injection, Q_i^{tot}, is given by eq 2

$$Q_i^{tot} = \frac{M_i^{tot} V_o}{P}[\Delta H_1\, 2k_1X_i + (\Delta H_1 + \Delta H_2)k_1k_2X_i^2] \quad (2)$$

where X_i, the free $dX(pX)_{34}$ concentration after the ith injection, is obtained by solving eq 3

$$X_i^{tot} = X_i + \nu M_i^{tot} = X_i + M_i^{tot}\left(\frac{2k_1X_i + 2k_1k_2X_i^2}{P}\right) \quad (3)$$

and ΔQ_i (the heat for the ith injection) is given by eq 4

$$\Delta Q_i = Q_i^{tot} - Q_{i-1}^{tot} + \frac{dV_i}{2V_o}(Q_i^{tot} + Q_{i-1}^{tot}) \quad (4)$$

In eqs *2*−*4*, X_i^{tot} and M_i^{tot} are the total concentrations of $dX(pX)_{34}$ and SSB tetramer, respectively, V_o is the volume of the calorimetric cell (1.34 mL), and ΔH_1 and ΔH_2 are the observed enthalpy changes for the binding of the first and second molecules of $dX(pX)_{34}$, respectively. Since the binding of the first molecule of $dX(pX)_{34}$ is stoichiometric under the conditions used in our study, the reported values of ΔH_1 were calculated as an average of normalized heats corrected for the heat of dilution when $[dX(pX)_{34}]/[SSB] < 1$.

Corrections for Contributions to ΔH_{obs} Due to Proton Release from the Buffer. Values of $\Delta H'_{obs}$ determined by ITC can have contributions from the heat of ionization of the buffer if there is any net protonation or deprotonation of the interacting species upon formation of the protein−DNA complex (*34*, *35*). We use a prime to designate experimental values of the ΔH_{obs} determined in a particular buffer that may include contributions from the heat of ionization of the buffer. Values of ΔH_{obs} without a prime have been corrected for the contribution due to the heat of ionization of the buffer using eq 5

$$\Delta H'_{obs} = \Delta H_{obs} + \Delta n_{H^+}\Delta H_{ion} \quad (5)$$

where Δn_{H^+} is the number of protons released (positive value) or absorbed (negative value) by the buffer as a result of binding or release, respectively, of protons upon SSB−$dX(pX)_{34}$ complexation (*34*, *35*). We note, however, that even after this correction, ΔH_{obs} still includes any contributions from the heat of protonation of the ionizable group (or groups) on the protein or DNA; $\Delta H_{obs} = \Delta H_{intr} + \Delta n_{H^+}\Delta H_{prot}$, where ΔH_{prot} is the enthalpy of protonation of protein or DNA residues and ΔH_{intr} is the intrinsic enthalpy change for SSB−DNA binding. The values of ΔH_{obs} and Δn_{H^+} were determined at each temperature by performing two measurements of $\Delta H'_{obs}$ in Tris and Hepes buffers at each temperature and solving eqs 6a and 6b.

$$\Delta H'_{obs,Hepes} = \Delta H_{obs} + \Delta n_{H^+}\Delta H_{ion,Hepes} \quad (6a)$$

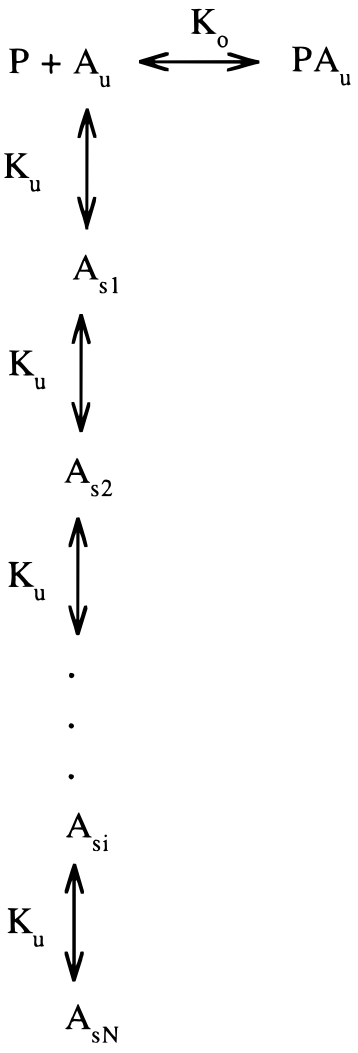
$$\Delta H'_{obs,Tris} = \Delta H_{obs} + \Delta n_{H^+}\Delta H_{ion,Tris} \quad (6b)$$

The values of ΔH_{ion} at 25 °C and $\Delta C_{p,ion}$ used to calculate ΔH_{ion} at each temperature were 5.01 kcal/mol and 12 cal mol^{-1} K^{-1}, respectively, for Hepes and 11.34 kcal mol^{-1} and −17 cal mol^{-1} K^{-1}, respectively, for Tris (*36*).

THEORETICAL BACKGROUND

Analysis of Enthalpy and Heat Capacity Changes for the Binding of dA(pA)$_{34}$ to SSB Tetramers. To interpret the effects of temperature on ΔH_{obs} for SSB binding to $dA(pA)_{34}$, we used the noncooperative base unstacking model described for the analysis of the nonlinear van't Hoff plots observed for SSB−ss-oligodeoxyadenylate binding (*7*). This model (see Scheme 1) accounts for the fact that the bases within oligo- and polydeoxyadenylates exist in equilibrium among a continuum of stacked states A_{si}, each containing i stacked bases. The base unstacking equilibrium is noncooperative (*7*, *37*) and described by equilibrium constant $K_u = [U]/[S]$, where [S] and [U] are the concentrations of stacked and unstacked bases, respectively. The enthalpy for unstacking is ΔH_u = 3.0−3.4 kcal/mol (per stack) (*38*, *39*) so that stacking is less populated at higher temperatures (*37*, *40*−*44*). We further assume that the ssDNA bases are completely unstacked (state A_u) when bound by SSB (P) (*45*) and that binding of SSB to A_u is described by equilibrium constant $K_0 = [PA_u]/[P][A_u]$, and the intrinsic enthalpy change ΔH_0. On the basis of Scheme 1, the observed binding constant for SSB binding to $dA(pA)_{34}$, independent of its degree of base stacking, K_{obs}, is given by eq 7.

Scheme 1



$$K_{obs} = K_0[K_u/(1 + K_u)]^N \quad (7)$$

The expression for the observed enthalpy change, ΔH_{obs}, for

SSB−oligo(dA) binding, obtained by differentiation of eq 7 with respect to T^{-1}, is given in eq 8.

$$\Delta H_{obs} = \Delta H_0 + N\Delta H_u(1 + K_u)^{-1} \quad (8)$$

The expression for the change in heat capacity, $\Delta C_{p,obs}$, obtained by differentiating eq 8 with respect to T, is given in eq 9

$$\Delta C_{p,obs} = \Delta C_{p,0} + N\Delta C_{p,u}(1 + K_u)^{-1} - N(\Delta H_u^2/RT^2)[K_u/(1 + K_u)^2] \quad (9)$$

where $\Delta C_{p,0} = (\partial \Delta H_0/\partial T)_P$ and $\Delta C_{p,u} = (\partial \Delta H_u/\partial T)_P$. Thus, the observed heat capacity change, $\Delta C_{p,obs}$, consists of three terms. The first term, $\Delta C_{p,0}$, reflects the contribution from binding of SSB to fully unstacked DNA; the second is the contribution associated with the base unstacking equlibrium, and the last term results from the coupling of the two processes (*6*, *46*, *47*), binding and base unstacking, and thus represents an *apparent* heat capacity change. This last term is always negative; thus, even if $\Delta C_{p,0} = 0$ and $\Delta C_{p,u} = 0$, a negative heat capacity change will be observed due to the coupling of the base unstacking to the binding (if $\Delta H_u \neq 0$) (*7*). Calorimetric studies suggest that $\Delta C_{p,u} = 0$ for base unstacking in poly(A) (*39*).

For the analysis of our ITC data, we have modified eq 8, assuming that there is no heat capacity change associated with adenine base unstacking, i.e., $\Delta C_{p,u} = 0$ (*39*). We also assume, as appears to be the case for SSB binding to $dT(pT)_{34}$ and $dC(pC)_{34}$, that the enthalpy change for the interaction of SSB with unstacked $dA(pA)_{34}$ is a linear function of temperature as in eq 10

$$\Delta H_0 = \Delta H_{T_m} + \Delta C_{p,0}(T - T_m) \quad (10)$$

where T_m is the temperature at the midpoint of the oligodeoxyadenylate unstacking transition [$T_m = 55$ °C (*7*, *37*)] and ΔH_{T_m} is the enthalpy change for SSB binding to fully unstacked $dA(pA)_{34}$ at T_m. Insertion of eq 10 and $K_u = \exp[(\Delta H_u/R)(1/T_m - 1/T)]$ into eq 8 yields eq 11.

$$\Delta H_{obs} = \Delta H_{T_m} + \Delta C_{p,0}(T - T_m) + N\Delta H_u\{1 + \exp[(\Delta H_u/R)(1/T_m - 1/T)]\}^{-1} \quad (11)$$

Our ITC measurements were analyzed using eq 11 and the nonlinear least-squares regression analysis package in Scientist (MicroMath Scientific Software, Salt Lake City, UT).

RESULTS

Temperature Dependence of $\Delta H_{1,obs}$ for SSB Binding to $dA(pA)_{34}$, $dT(pT)_{34}$, and $dC(pC)_{34}$ at 0.02 M NaCl. Earlier fluorescence (*7*, *33*) and ITC (*28*) studies showed that two molecules of $dX(pX)_{34}$ (X being A, T, or C) can bind per SSB tetramer, although the second molecule of $dX(pX)_{34}$ binds with a salt-dependent negative cooperativity (*28*, *32*). We chose a salt concentration of 20 mM NaCl to perform the ITC experiments reported here for the following reasons. First of all, in this range of NaCl concentrations, the effect of NaCl concentration on the enthalpy change for SSB−ssDNA binding is negligible (*28*). Second, the binding of $dX(pX)_{34}$ to the first site on the SSB tetramer is stoichiometric at this salt concentration (*33*) for all the oligodeoxynucleotides that were studied, which allows us to determine $\Delta H_{1,obs}$ with great precision. In addition, due to the large negative cooperativity, $\Delta H_{1,obs}$ is identical to the intrinsic ΔH for $dX(pX)_{34}$ binding to the first site.

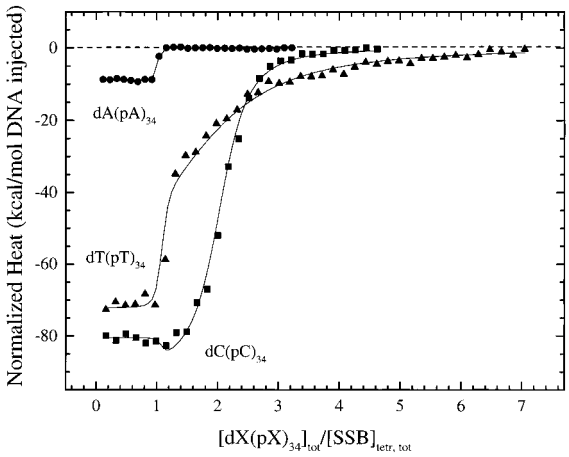


FIGURE 1: Isothermal titration calorimetric studies of *E. coli* SSB tetramer binding to $dA(pA)_{34}$, $dT(pT)_{34}$, and $dC(pC)_{34}$. Titrations were performed at 27−28 °C in 10 mM Hepes (pH 8.1) and 20 mM NaCl as described in Materials and Methods. (●) SSB protein (4.6 μM tetramer) was titrated with $dA(pA)_{34}$ (141 μM) at 27.1 °C. (▲) SSB protein (1.4 μM tetramer) was titrated with $dT(pT)_{34}$ (44 μM) at 28.1 °C. (■) SSB protein (1.1 μM tetramer) was titrated with $dC(pC)_{34}$ (50 μM) at 28.1 °C. Data are the integrated heat response per injection, normalized to the amount of injected ligand after correction for the contribution due to the heats of dilution, and are plotted vs $[dX(pX)_{34}]_{tot}/[SSB\ tetramer]_{tot}$. The smooth curves represent the best fits to a model for two interacting DNA sites per SSB tetramer (see eqs 2−4).

A series of calorimetric titrations of the SSB protein with $dA(pA)_{34}$, $dT(pT)_{34}$, and $dC(pC)_{34}$ was performed over the temperature range of 7−50 °C [20 mM NaCl and buffer H (pH 8.1)]. Typical results are shown in Figure 1 for ITC titrations of the SSB tetramer with $dA(pA)_{34}$ (27.1 °C), $dT(pT)_{34}$ (28.1 °C), and $dC(pC)_{34}$ (28.1 °C) in buffer H (pH 8.1) and 20 mM NaCl. The constant value of the normalized heats observed when $[dX(pX)_{34}]/[SSB] < 1$ for each type of DNA indicates that the binding of the first oligodeoxynucleotide to the SSB tetramer is stoichiometric. The plateau values of the normalized heats in this region of the titration provide an estimate of the binding enthalpy, $\Delta H'_{1,obs}$, per mole of $dX(pX)_{34}$. A prime is used to designate binding enthalpies that have not been corrected for any potential contributions from the heat of ionization of the buffer due to any exchange of protons between the buffer and the interacting macromolecules (*34*), whereas values of ΔH_{obs} without a prime have been corrected for the contribution due to the heat of ionization of the buffer (see Materials and Methods).

As seen in Figure 1 (see also Table 1), although $\Delta H'_{1,obs}$ is negative for all three ssDNAs, it is substantially smaller in magnitude for $dA(pA)_{34}$ (−8.8 ± 0.3 kcal/mol) than for $dT(pT)_{34}$ (−71.1 ± 1.7 kcal/mol) or $dC(pC)_{34}$ (−80.7 ± 0.9 kcal/mol). In all three titrations, it is clear that the second molecule of $dX(pX)_{34}$ (data at $[dX(pX)_{34}]/[SSB] > 1$) binds weaker than the first, reflecting the substantial negative cooperativity. In fact, the change in the values of the normalized heats with increasing $[dX(pX)_{34}]/[SSB]$ is sharper for $dC(pC)_{34}$ than for $dT(pT)_{34}$, whereas no binding is

Table 1: Temperature Dependence of $\Delta H'_{1,obs}$ (Kilocalories per Mole) for $dX(pX)_{34}$ Binding to the SSB Tetramer[a]

$dC(pC)_{35}$			$dT(pT)_{35}$			$dA(pA)_{35}$		
T (°C)	$\Delta H'_{1,Hepes}$	$\Delta H'_{1,Tris}$	T (°C)	$\Delta H'_{1,Hepes}$	$\Delta H'_{1,Tris}$	T (°C)	$\Delta H'_{1,Hepes}$	$\Delta H'_{1,Tris}$
7.0	−69.0(±1.4)		7.5	−63.9(±1.0)				
				−62.4(±1.0)				
9.9	−72.1(±1.1)		8.1	−64.8(±1.3)	−58.5(±1.0)	8.6	5.62(±0.20)	8.82(±0.68)
			12.0	−64.3(±1.1)	−60.4(±1.0)	12.0	3.73(±0.24)	8.12(±0.24)
							4.36(±0.27)	
			15.0	−66.3(±1.6)		15.0	0.87(±0.13)	
				−62.7(±1.1)				
16.0	−78.0(±1.1)							
			17.0	−63.6(±1.1)	−61.8(±1.4)			
19.0	−75.3(±1.4)		19.0	−66.2(±0.8)	−62.8(±0.9)			
	−76.6(±1.1)							
22.0	−78.0(±1.2)		22.0	−70.0(±1.1)				
25.1	−79.7(±1.4)	−73.4(±0.7)	25.0	−68.0(±1.9)	−64.3(±0.8)			
			25.1	−69.9(±1.0)				
28.1	−80.7(±1.1)	−73.4(±1.3)	28.1	−71.1(±1.7)	−64.3(±0.8)	27.1	−9.18(±0.32)	
							−8.78(±0.33)	
32.0	−80.7(±0.9)		32.1	−71.6(±3.0)	−64.5(±1.1)	32.0	−11.46(±0.6)	
35.0	−82.8(±1.3)	−74.5(±1.0)						
38.0	−84.3(±1.2)	−73.8(±12)	37.0	−73.8(±1.2)	−64.6(±1.0)	37.0	−20.2(±0.7)	
41.0	−84.1(±1.1)		40.0	−76.1(±1.5)		42.0	−26.7(±0.4)	−16.5(±0.3)
43.9	−86.9(±2.0)		42.9	−75.3(±1.9)				
47.0	−84.8(±1.4)	−76.7(±1.6)	46.9	−76.1(±1.1)	−65.3(±1.4)	47.0	−34.1(±0.4)	−21.7(±0.4)
50.2	−87.0 (±2.8)	−75.4(±1.7)	49.9	−76.4(±1.1)	−67.3(±1.2)	51.9	−35.7(±1.2)	

[a] Conditions: 10 mM Hepes or 10 mM Tris (pH 8.1) and 20 mM NaCl.

observed for a second molecule of $dA(pA)_{34}$. This indicates that the affinity of $dX(pX)_{34}$ for the second site decreases in the following order: $dC(pC)_{34}$ > $dT(pT)_{34}$ > $dA(pA)_{34}$, consistent with previous fluorescence studies of SSB binding (*33*). The values of $\Delta H'_{2,obs}$ and k_2 obtained by fitting the data in Figure 1 to eqs *2*−*4* are −69.5 ± 3.5 kcal/mol and (2.1 ± 0.2) × 10^6 M^{-1} for $dT(pT)_{34}$ and −90.4 ± 1.6 kcal/mol and (3.2 ± 0.4) × 10^7 M^{-1} for $dC(pC)_{34}$, respectively.

The dependencies of $\Delta H'_{1,obs}$ on temperature (7−52 °C) for $dA(pA)_{34}$, $dT(pT)_{34}$, and $dC(pC)_{34}$ binding to SSB are given in Table 1. For $dT(pT)_{34}$ and $dC(pC)_{34}$, the values of $\Delta H'_{1,obs}$ are large and negative, but decrease with increasing temperature from −63.9 ± 1.0 to −76.4 ± 1.1 kcal/mol and from −69.0 ± 1.4 to −87.0 ± 2.8 kcal/mol for $dT(pT)_{34}$ and $dC(pC)_{34}$, respectively. The heat capacity changes, $\Delta C'_{p,1,obs} = (\partial \Delta H'_{1,obs}/\partial T)_P$, calculated from the slopes of the linear least-squares fits of the data in Table 1, are −350 ± 20 and −380 ± 30 cal mol^{-1} K^{-1} for $dT(pT)_{34}$ and $dC(pC)_{34}$, respectively, which are the same within our experimental uncertainties. In contrast, the values of $\Delta H'_{1,obs}$ for $dA(pA)_{34}$ binding are much less negative, even becoming positive at temperatures below 16 °C. In addition, the dependence of $\Delta H'_{1,obs}$ on temperature is also negative for $dA(pA)_{34}$ binding, with the slope indicating a heat capacity change $\Delta C'_{p,1,obs}$ = −990 ± 40 cal mol^{-1} K^{-1}, which is nearly 3-fold larger in magnitude than that for SSB binding to $dT(pT)_{34}$ or $dC(pC)_{34}$.

Corrections for Contributions to $\Delta H_{1,obs}$ and $\Delta C_{p,obs}$ Due to the Heat of Ionization of the Buffer. It has previously been shown that there is some linkage of pH and salt effects on the binding of SSB to poly(U), such that 3 ± 1 protons are bound per tetramer upon formation of the SSB−poly(U) complex in its $(SSB)_{65}$ mode, where all four subunits of the tetramer interact with poly(U) (*48*). We therefore expect some protonation to accompany the binding of SSB to the ss-oligodeoxynucleotides studied here. Any such protonation of the SSB−DNA complex will result in some deprotonation of the buffer, which will then contribute to the observed enthalpy due to the heat of ionization of the buffer (*34*). We examined this by performing a series of ITC titrations in both Hepes and Tris buffers, which differ significantly in ionization enthalpy [11.34 kcal/mol for Tris and 5.01 kcal/mol for Hepes at 25 °C (*36*)]. Titrations were performed in each buffer as a function of temperature (at a constant pH of 8.1 ± 0.1) for all three oligodeoxyribonucleotides. As seen from Table 1, the absolute value of $\Delta H'_{1,obs}$ obtained in Tris is lower than that obtained in Hepes, with the difference in $\Delta H'_{1,obs}$ increasing with increasing temperature from 3−5 to 9−12 kcal/mol at the extremes. This clearly indicates that protonation occurs upon SSB−oligodeoxynucleotide binding, and that the extent of protonation is temperature-dependent.

We analyzed the data in Table 1 (obtained in both Tris and Hepes buffers) using eqs 6a and 6b as described in Materials and Methods to estimate Δn_{H^+}, the number of protons released by the buffer as a result of binding of protons upon SSB−$dX(pX)_{34}$ complexation, as well as $\Delta H_{1,obs}$, the value of the binding enthalpy after correction for the heat of ionization of the buffer. It should be remembered, though, that $\Delta H_{1,obs}$ itself still includes any contributions from the protonation of an ionizable group (or groups) on the protein or DNA. The resulting values of Δn_{H^+} are shown in Figure 2 and Table 2 (column 2). All values of Δn_{H^+} are positive, indicating that protons are absorbed upon formation of the SSB−$dX(pX)_{34}$ complex, and thus, there is net proton release from the buffer. Furthermore, the number of protons released by the buffer increases from approximately 0.4 at 7 °C to 2 at 50 °C. The number of protons released is independent of the nature of the oligodeoxyribonucleotide, suggesting that protonation involves residues on the SSB protein. The values of Δn_{H^+} showed an approximately linear dependence on temperature, where $\Delta n_{H^+,appr} = 0.147(\pm 0.132) + 0.035(\pm 0.004)T$ (Figure 2). The values of $\Delta n_{H^+,appr}$ calculated according to this linear approximation are listed in Table 2 (column 3) and were used

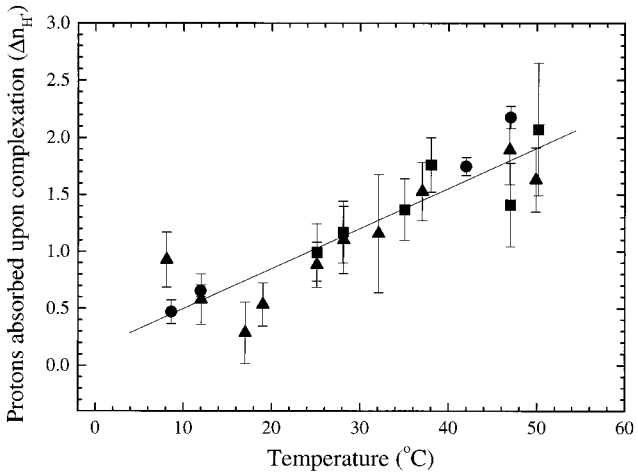


FIGURE 2: Temperature dependence of the number of protons (Δn_{H^+}) absorbed upon formation of SSB−dX(pX)$_{34}$ complexes. Δn_{H^+} was determined from data obtained in Tris and Hepes buffers using eqs 6a and 6b as described in Materials and Methods for dA(pA)$_{34}$ (●), dT(pT)$_{34}$ (▲), and dC(pC)$_{34}$ (■) (see Table 1). The linear least-squares line describing the data is $\Delta n_{H^+,appr} = 0.15(\pm 0.13) + 0.035(\pm 0.004)T$ (see Table 2).

to calculate $\Delta H_{1,obs}$ according to eq 6a using representative values of $\Delta H'_{1,obs}$ obtained in buffer H for all three oligonucleotides.

The resulting values of $\Delta H_{1,obs}$ are listed in Table 2 and plotted as a function of temperature in Figure 3. For the binding of dT(pT)$_{34}$ and dC(pC)$_{34}$ to SSB, $\Delta H_{1,obs}$ decreases with increasing temperature from -65.8 ± 1.2 to -86.5 ± 1.7 kcal/mol and from -70.9 ± 1.5 to -97.1 ± 3.1 kcal/mol, respectively. The resulting values of $\Delta C_{p,1,obs}$, obtained from the slopes of the linear least-squares fits to the data in Figure 3, are -540 ± 20 cal mol^{-1} K^{-1} for dT(pT)$_{34}$ and -570 ± 30 cal mol^{-1} K^{-1} for dC(pC)$_{34}$. These are larger in magnitude than the uncorrected values obtained in buffer H. For the binding of dA(pA)$_{34}$ to SSB, $\Delta H_{1,obs}$ also decreases with increasing temperature from 3.44 ± 0.68 to -46.2 ± 1.4 kcal/mol (Table 2), but with a much larger value for $\Delta C_{p,1,obs}$ of -1180 ± 40 cal mol^{-1} K^{-1} (Table 3).

DISCUSSION

Equilibrium studies of SSB binding to a series of oligodeoxyadenylates [dA(pA)$_N$ (N = 34, 55, and 69)], monitored by tryptophan fluorescence quenching (*7*), showed that van't Hoff plots of the temperature dependence of the equilibrium association constant, K_{obs}, were distinctly nonlinear, with K_{obs} having an optimum value near 25 °C. In contrast, similar van't Hoff plots of SSB binding to oligodeoxypyrimidines [dC(pC)$_N$ and dT(pT)$_N$] appeared to be linear over the temperature range that was investigated (5−37 °C). Therefore, SSB binding to the dA(pA)$_N$ appeared to display a significant negative heat capacity change, whereas no heat capacity change could be determined for SSB binding to either dC(pC)$_N$ or dT(pT)$_N$. It was suggested that the nonlinear van't Hoff plots for SSB−dA(pA)$_N$ binding were the result of a coupling to binding of a temperature-dependent unstacking of adenine bases within the oligodeoxyadenylates. That is, the conformational "end state" of at least one of the interacting species, the free ssDNA, changes with temperature. Furthermore, the "noncooperative base unstacking model" shown in Scheme 1 provided a semiquantitative explanation of the apparent heat capacity change for SSB binding to oligodeoxyadenylates (*7*).

Table 2: Temperature Dependence of the Binding Enthalpy $\Delta H_{1,obs}$ for dX(pX)$_{34}$ Binding to the SSB Tetramer after Correction for Contributions from Proton Release (Δn_{H^+}) from the Buffer[a]

T (°C)	Δn_{H^+}[b]	$\Delta n_{H^+,appr}$[c]	$\Delta H_{1,obs}$ (kcal/mol)
		dC(pC)$_{35}$	
7.0		0.39	−70.9(±1.5)
9.9		0.50	−74.5(±1.3)
16.0		0.71	−81.5(±1.3)
19.0		0.82	−79.4(±1.6)
22.0		0.92	−82.6(±1.4)
25.1	0.99(±0.25)	1.03	−84.8(±1.6)
28.1	1.17(±0.27)	1.14	−86.5(±1.4)
32.0		1.27	−87.9(±1.3)
35.0	1.37(±0.27)	1.38	−89.9(±1.6)
38.0	1.76(±0.24)	1.48	−92.0(±1.5)
41.0		1.59	−92.4(±1.5)
43.9		1.69	−95.8(±2.3)
47.0	1.41(±0.37)	1.80	−94.3(±1.8)
50.2	2.07(±0.58)	1.91	−97.1(±3.1)
		dT(pT)$_{35}$	
7.5		0.41	−65.8(±1.2)
			−64.4(±1.2)
8.1	0.93(±0.24)	0.43	−66.9(±1.5)
12.0	0.58(±0.22)	0.57	−67.0(±1.3)
15.0		0.68	−69.6(±1.7)
			−66.0(±1.3)
17.0	0.29(±0.27)	0.75	−67.3(±1.3)
19.0	0.54(±0.19)	0.82	−70.2(±1.1)
22.0		0.92	−74.6(±1.3)
25.0	0.88(±0.20)	1.03	−75.1(±1.3)
			−73.2(±2.0)
28.1	1.10(±0.30)	1.14	−76.9(±1.9)
32.1	1.16(±0.52)	1.28	−78.1(±3.1)
			−77.3(±1.5)
37.0	1.53(±0.26)	1.45	−81.2(±1.5)
40.0		1.55	−84.2(±1.9)
42.9		1.66	−84.0(±2.2)
46.9	1.89(±0.30)	1.80	−85.6(±1.6)
49.9	1.63(±0.28)	1.90	−86.5(±1.7)
		dA(pA)$_{35}$	
8.6	0.47(±0.10)	0.45	3.44(±0.68)
9.12		0.47	2.83(±0.91)
12.0	0.65(±0.05)	0.57	1.61(±0.73)
			0.97(±0.72)
15.0		0.68	−2.43(±0.72)
27.1		1.10	−14.7(±0.9)
			−14.3(±0.9)
32.0		1.27	−18.0(±1.1)
37.0		1.45	−27.6(±1.2)
42.0	1.74(±0.08)	1.62	−35.2(±1.2)
47.0	2.18(±0.10)	1.80	−43.6(±1.3)
51.9		1.97	−46.2(±1.4)

[a] Conditions: pH 8.1 and 20 mM NaCl. [b] Calculated according to eq 6. [c] Calculated on the basis of $\Delta n_{H^+,appr} = 0.15(\pm 0.13) + 0.035(\pm 0.004)T$.

Although the absence of observable curvature in the van't Hoff plots for SSB binding to the oligodeoxypyrimidines (*7*) indicated a clear difference between those energetics and SSB binding to the oligoadenylates, it was not possible to estimate a value of $\Delta C_{p,obs}$ for the SSB−oligodeoxypyrimidine [dT(pT)$_N$ or dC(pC)$_N$] interactions. Furthermore, due to the much higher values of K_{obs} for SSB binding to dT(pT)$_N$ and dC(pC)$_N$ versus binding to dA(pA)$_N$, it was not possible to compare the van't Hoff plots for all three binding interactions under identical solution conditions. Therefore, to pursue these studies further, we have made direct measurements of ΔH_{obs} as a function of temperature using

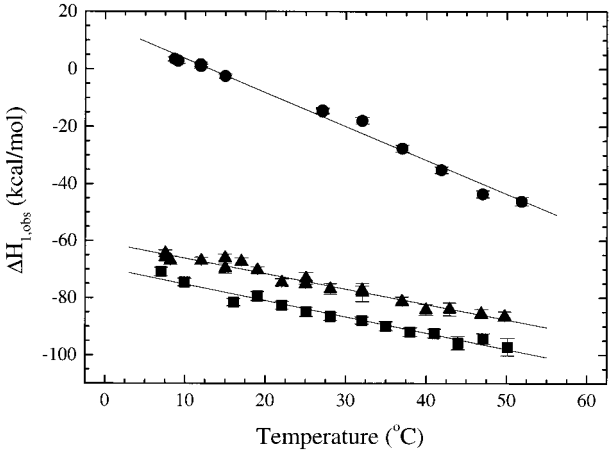


FIGURE 3: Temperature dependences of the enthalpy changes for the binding of 1 mol of SSB tetramer to 1 mol of $dA(pA)_{34}$, $dT(pT)_{34}$, and $dC(pC)_{34}$. Values of $\Delta H_{1,obs}$ (pH 8.1) after correction for the heats of ionization due to proton release by the buffer (see eq 6a and Table 2) for $dA(pA)_{34}$ (●), $dT(pT)_{34}$ (▲), and $dC(pC)_{34}$ (■).

Table 3: Heat Capacity Changes for $dX(pX)_{34}$ (X Being A, T, or C) Binding to the SSB Tetramer after Correction for Buffer Ionization[a,b]

$dC(pC)_{34}$	$dT(pT)_{34}$	$dA(pA)_{34}$	
$\Delta C_{p,1,obs}$ (kcal mol^{-1} K^{-1})	$\Delta C_{p,1,obs}$ (kcal mol^{-1} K^{-1})	$\Delta C_{p,1,obs}$ (kcal mol^{-1} K^{-1})	$\Delta C_{p,0}$[c] (kcal mol^{-1} K^{-1})
−0.57(±0.03)	−0.54(±0.02)	−1.18(±0.04)	−0.67(±0.05)

[a] Conditions: pH 8.1 and 20 mM NaCl. [b] Uncertainties represent standard errors of linear least-squares fitting. [c] From fitting to eq 11.

isothermal titration calorimetry (ITC) for SSB binding to $dA(pA)_{34}$, $dT(pT)_{34}$, and $dC(pC)_{34}$ under identical solution conditions (20 mM NaCl at pH 8.1), over a wide range of temperatures (7−50 °C), from which we have estimated values of $\Delta C_{p,obs}$. The results demonstrate that SSB binding to $dA(pA)_{34}$ does indeed exhibit a significantly larger negative heat capacity change (−1180 ± 40 cal mol^{-1} K^{-1}) than is observed for binding to either $dT(pT)_{34}$ (−540 ± 20 cal mol^{-1} K^{-1}) or $dC(pC)_{34}$ (−570 ± 30 cal mol^{-1} K^{-1}). Another major difference is the fact that the ΔH_{obs} for SSB binding to the oligodeoxypyrimidines is very large and negative, whereas it is significantly less negative for binding to $dA(pA)_{34}$, even becoming positive when T is less than ∼13 °C.

The More Negative $\Delta C_{p,obs}$ for SSB Binding to $dA(pA)_{34}$ Can Be Explained by the Coupling to Binding of a Temperature-Dependent Adenine Base Unstacking Equilibrium. As discussed previously (*7*), oligo- and polydeoxyadenylates undergo a temperature-dependent base stacking transition (*37*, *40*−*44*), whereas the bases within the oligo- and polydeoxypyrimidines have a much lower tendency to stack (*37*). Furthermore, the bases within SSB−ssDNA complexes have been shown to be less stacked than in the free nucleic acid (*45*). We have therefore interpreted the thermodynamics of SSB binding to $dA(pA)_{34}$ determined from our ITC experiments using Scheme 1 and the resulting eqs 9 and *11*. For SSB binding to $dA(pA)_{34}$, we consider that the observed heat capacity change, $\Delta C_{p,obs}$, has contributions from only the first and third terms in eq 9. The first term, $\Delta C_{p,0}$, is the "intrinsic" heat capacity change that would result from SSB binding to a fully base-unstacked $dA(pA)_{34}$ [or for binding to $dC(pC)_{34}$ or $dT(pT)_{34}$], whereas the third term is due to the coupling of the temperature-dependent adenine base unstacking equilibrium. The second term, $\Delta C_{p,u}$, is assumed to be zero, on the basis of the results of Filimonov and Privalov (*39*), who showed that the adenine base unstacking enthalpy is not temperature-dependent. In contrast, the values of $\Delta C_{p,obs}$ for SSB binding to $dT(pT)_{34}$ or $dC(pC)_{34}$ are assumed to be due to only $\Delta C_{p,0}$, the first term in eq 9.

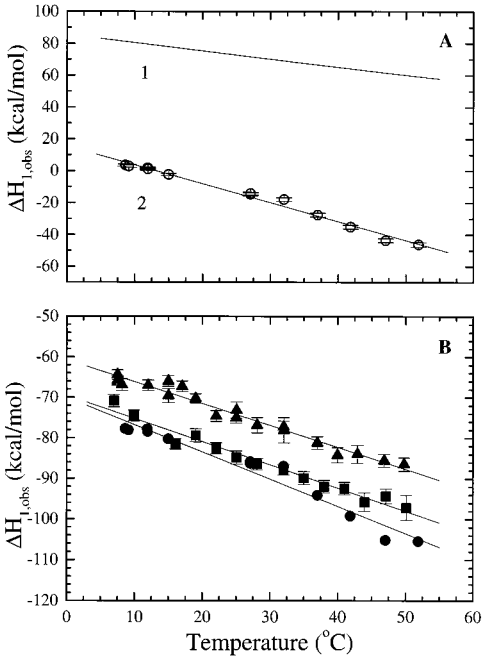


FIGURE 4: Temperature dependence of the contribution of the enthalpy change due to adenine base unstacking to $\Delta H_{1,obs}$ for the SSB−$dA(pA)_{34}$ binding interaction. (A) Curve 1 depicts the contribution to the observed $\Delta H_{1,obs}$ due to the coupling of $dA(pA)_{34}$ unstacking (third term in eq 11) calculated assuming $\Delta H_u = 3.4$ kcal/mol and $T_m = 55$ °C. Curve 2 depicts the observed temperature dependence of $\Delta H_{1,obs}$ for $dA(pA)_{34}$ binding to SSB. (B) (●) Predicted values of $\Delta H_{1,obs}$ as a function of temperature for SSB binding to fully unstacked $dA(pA)_{34}$ determined by subtraction of curve 1 from curve 2 in panel A. $\Delta H_{1,obs}$ for SSB binding to $dT(pT)_{34}$ (▲) and $dC(pC)_{34}$ (■). The values of $\Delta C_{p,obs}$, evaluated from the linear least-squares slopes of these dependencies, are given in Table 3.

In our analysis of the temperature dependence of $\Delta H_{1,obs}$ for SSB−$dA(pA)_{34}$ binding based on Scheme 1 and eqs 9 and 11, we constrained the values of T_m and ΔH_u for the adenine unstacking transition to reduce the number of parameters. As in our previous analysis, we use a T_m of 55 °C (*7*, *37*) and a ΔH_u of 3.4 kcal/mol (per stack), which was determined calorimetrically for the oligoribonucleotide A_7 (*38*). This value is close to the value of 3.0 kcal/mol reported for poly(A) unstacking (*39*), and we have assumed that it does not differ for the deoxy analogues. Using these values of T_m and ΔH_u, and assuming that all of the adenine bases become fully unstacked upon binding SSB (i.e., $N = 34$), we can calculate the expected contributions to $\Delta C_{p,obs}$ and ΔH_{obs} due to the last terms in eqs 9 and 11, respectively. The last term of eq 11, representing the enthalpy change due to adenine base unstacking, is plotted as a function of temperature in Figure 4A (curve 1). This term is highly endothermic and decreases from approximately 83 to 60 kcal/mol over the temperature range of 5−50 °C. The last term in eq 9, reflecting the apparent heat capacity change due to the coupling of base unstacking to binding, which we call

$\Delta C_{p,app}$, varies from −523 to −473 cal mol^{-1} K^{-1}. To simplify the following calculations, we have approximated this term as a constant, using an average value for $\Delta C_{p,app}$ of −510 ± 20 cal mol^{-1} K^{-1}.

Subtraction of the contribution to the enthalpy change due to adenine base unstacking (line 1 in Figure 4A) from the experimental values of $\Delta H_{1,obs}$ (corrected for buffer ionization enthalpy) (line 2 in Figure 4A) provides a prediction of the dependence of $\Delta H_{1,obs}$ on temperature for SSB binding to fully unstacked dA(pA)$_{34}$. This dependence is shown in Figure 4B (●). Direct fitting of the dependence of $\Delta H_{1,obs}$ on temperature (line 2 in Figure 4A) to eq 11 [with fixed parameters T_m = 55 °C, ΔH_u = 3.4 kcal/mol (per stack), and N = 34] yields a ΔH_{T_m} of −106.9 ± 1.4 kcal/mol and a $\Delta C_{p,0}$ of −670 ± 50 cal mol^{-1} K^{-1} (see Table 3), the latter being the predicted contribution to $\Delta C_{p,obs}$ for SSB binding to fully unstacked dA(pA)$_{34}$.

For comparison, Figure 4B also shows the values of $\Delta H_{1,obs}$ determined for SSB binding to dC(pC)$_{34}$ and dT(pT)$_{34}$ (see also Table 3). After correction for the contribution due to adenine base unstacking, the predicted values of $\Delta H_{1,obs}$ and $\Delta C_{p,0}$ for SSB binding to a fully unstacked dA(pA)$_{34}$ are very close to those determined for SSB binding to dT(pT)$_{34}$ and dC(pC)$_{34}$. This suggests that the major differences in the energetics of SSB binding to oligodeoxyadenylates versus oligodeoxypyrimidines may result from the coupling of adenine base unstacking equilibrium to the SSB binding equilibrium. However, we emphasize that the quantitative results of this calculation are based on the assumption that all of the adenine bases become fully unstacked upon binding SSB. If not all of the bases become unstacked, then the predicted values of ΔH_1 for SSB binding to "unstacked" dA(pA)$_{34}$ will be larger (less negative) than for SSB binding to dT(pT)$_{34}$ or dC(pC)$_{34}$.

Other Contributions to the Heat Capacity Change for SSB−ssDNA Binding. Our ITC studies show that SSB binding to both dC(pC)$_{34}$ and dT(pT)$_{34}$ is accompanied by a relatively large negative heat capacity change (−540 to −570 cal mol^{-1} K^{-1}), even though the level of base stacking should be relatively small for these oligodeoxynucleotides. Although our previous fluorescence studies of SSB binding to dT(pT)$_N$ and dC(pC)$_N$ showed no detectable curvature in van't Hoff plots over the temperature range of 5−37 °C (*7*), this result is consistent with our ITC studies. The maximum curvature in a van't Hoff plot will occur in the temperature range near T_H, the temperature at which ΔH_{obs} = 0 (*4*). From eq 12, which describes the temperature dependence of ΔH_{obs}

$$\Delta H_{obs} = \Delta H_{ref} + \Delta C_p(T - T_{ref}) \quad (12)$$

we obtain an expression for T_H given in eq 13.

$$T_H = T_{ref} - \Delta H_{ref}/\Delta C_p \quad (13)$$

For SSB binding to dT(pT)$_{34}$ in 20 mM NaCl (pH 8.1), for which $\Delta H_{1,obs}$(25 °C) = −73.2 kcal/mol and $\Delta C_{p,obs}$ = −540 cal mol^{-1} K^{-1} (Tables 2 and 3), eq 13 predicts that T_H = −111 °C. As previously discussed (*49*), since this value of T_H is obviously very far from the experimental temperature range used in our previous SSB−dT(pT)$_N$ studies (*7*), it indicates that no detectable curvature would be expected in van't Hoff plots obtained near 25 °C, even though $\Delta C_{p,obs}$ is significant. This serves to emphasize the point that, although one can estimate a value of $\Delta C_{p,obs}$ from a van't Hoff plot when significant curvature is observed, the absence of curvature does not necessarily indicate that $\Delta C_{p,obs}$ = 0.

Previous studies have suggested that $\Delta C_{p,obs}$ is expected to be near zero for nonspecific binding of proteins to duplex DNA (*1*, *50*, *51*), since such interactions are not expected to involve major conformational changes or burial of significant amounts of nonpolar surface. However, a recent ITC study reports a significant negative heat capacity change ($\Delta C_{p,obs}$ = −250 ± 10 cal mol^{-1} K^{-1}) for nonspecific binding to duplex DNA of the Sso7d protein from *Sulfolobus solfataricus* (*52*). The interactions of SSB with dT(pT)$_{34}$ and dC(pC)$_{34}$ reported here provide another example of nonsequence specific DNA binding interactions that exhibit even larger negative heat capacity changes, presumably in the absence of significant base unstacking equilibria. Other factors that might contribute to this large, negative $\Delta C_{p,0}$ are (1) the coupling to binding of other temperature-dependent equilibria, such as protonation or protein conformational changes, (2) significant burial of nonpolar surface area (*1*, *3*), and (3) the restriction of "soft" vibrational modes of polar groups and bound water molecules mediating the interaction (*3*, *5*, *53*). Although we cannot evaluate most of these contributions quantitatively, we can provide an estimate of the likely contribution due to a coupled protonation.

Our ITC experiments show that SSB−oligodeoxynucleotide binding is accompanied by a net uptake of protons, with the number of protons absorbed varying from 0.4 to 1.9 as the temperature is increased from 7 to 50 °C (see Figure 2 and Table 2). Therefore, this linkage of the protonation of the complex will make a temperature-dependent contribution to the overall $\Delta H_{1,obs}$ and thus will also contribute to the observed heat capacity change, $\Delta C_{p,obs}$. Since Δn_{H^+} exhibits the same dependence on temperature for all three oligodeoxynucleotides (Figure 3), the protonation likely occurs on protein residues. Overman and Lohman (*48*) showed that the interaction of poly(U) with SSB in its SSB$_{65}$ binding mode is accompanied by a net uptake of protons. Although a strong linkage of some of the protonation to salt concentration (mainly anion concentration) was observed, a required uptake of protons was also observed that is not linked to ion release. In fact, 3 ± 0.9 protons, with an average pK_a of ∼8.3, are required to bind per tetramer at 25 °C (*48*). This is consistent with our estimate of an average of two protons bound per dX(pX)$_{34}$ at 25 °C (see Table 2), since dX(pX)$_{34}$ interacts with only two SSB subunits instead of all four subunits when poly(U) binds in the SSB$_{65}$ binding mode. Assuming one ionizable group per SSB subunit (ΔH_{prot} = −6.3 kcal/mol, pK_a = 8.3), simulations predict a contribution to the heat capacity change of approximately −100 cal mol^{-1} K^{-1}, leaving approximately −400 cal mol^{-1} K^{-1} to be explained.

Our previous fluorescence studies (*7*) and ITC studies (*28*) of SSB−oligopyrimidine binding performed at higher salt concentrations where $\Delta G°$, ΔH, and $\Delta S°$ can all be determined show that both $T\Delta S°$ and ΔH are negative and decrease (becoming more negative) with decreasing NaBr concentrations. Although the large salt dependence of ΔH_{obs} observed mainly at high salt concentrations and which is primarily an anion effect (*28*, *54*) is still not fully understood, it is clear that SSB binding to both dT(pT)$_N$ and dC(pC)$_N$ is

enthalpically driven and accompanied by a large, negative ΔC_p.

We have discussed previously the possible sources of the unusually large and negative ΔH_{obs} for SSB−ssDNA binding (*28*, *54*). Major contributions most likely result from interactions of aromatic amino acids (Trp-54, Trp-88, and Phe-60) involved in stacking interactions with nucleic acid bases, with smaller contributions from positively charged amino acids (Lys-73 and Arg-3, -21, -56, -84, and -86) interacting with phosphates. All of these residues are clustered within a region that likely forms part of the DNA binding site within each SSB subunit (*55*). However, simple estimates (assuming additivity) based on thermodynamic data for oligopeptide−ss-polynucleotide binding (*56*−*58*) predict a value for ΔH_1 that, although large, does not exceed −40 kcal/mol per two SSB subunits, which is still ∼2-fold lower in magnitude than the $\Delta H_{1,obs}$. This suggests that the missing contributions to $\Delta H_{1,obs}$ may result from conformational changes within the SSB protein and/or the ssDNA. Any such conformational changes in the protein may contribute significantly to $\Delta C_{p,0}$ as well. Experimental estimates of the other possible contributions to $\Delta C_{p,0}$ must await further studies.

In summary, the dominant contribution to $\Delta C_{p,obs}$ for SSB binding to oligodeoxyadenylates is due to the coupling to binding of a temperature-dependent conformational change in the DNA (adenine base unstacking). However, large, negative heat capacity changes are still observed for SSB binding to ss-oligodeoxypyrimidines, although these are smaller than those observed for oligodeoxyadenylate binding. As discussed previously (*6*−*8*), it is likely that similar temperature-dependent conformational changes which are coupled to macromolecular binding are at least partially responsible for large negative heat capacities observed for other systems as well. Such contributions need to be considered more generally, especially if attempts are made to invoke structural interpretations of the origins of observed heat capacity changes in macromolecular interactions.

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