

# Thermodynamics of the binding of *Thermus aquaticus* DNA polymerase to primed-template DNA

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## ABSTRACT

**DNA binding of the Type 1 DNA polymerase from *Thermus aquaticus* (Taq polymerase) and its KlenTaq large fragment domain have been studied as a function of temperature. Equilibrium binding assays were performed from 5 to 70°C using a fluorescence anisotropy assay and from 10 to 60°C using isothermal titration calorimetry. In contrast to the usual behavior of thermophilic proteins at low temperatures, Taq and KlenTaq bind DNA with high affinity at temperatures down to 5°C. The affinity is maximal at 40–50°C. The  $\Delta H$  and  $\Delta S$  of binding are highly temperature dependent, and the  $\Delta C_p$  of binding is  $-0.7$  to  $-0.8$  kcal/mol K, for both Taq and KlenTaq, with good agreement between van't Hoff and calorimetric values. Such a thermodynamic profile, however, is generally associated with sequence-specific DNA binding and not non-specific binding. Circular dichroism spectra show conformational rearrangements of both the DNA and the protein upon binding. The high  $\Delta C_p$  of Taq/KlenTaq DNA binding may be correlated with structure-specific binding in analogy to sequence-specific binding, or may be a general characteristic of proteins that primarily bind non-specifically to DNA. The low temperature DNA binding of Taq/KlenTaq is suggested to be a general characteristic of thermophilic DNA binding proteins.**

## INTRODUCTION

Taq DNA polymerase (Taq), the Type 1 polymerase from the thermophilic eubacterium *Thermus aquaticus*, has proven to be an immensely important biotechnological reagent due to its widespread use in the polymerase chain reaction (PCR). The enzyme belongs to the same family as *Escherichia coli* DNA polymerase 1 (Pol 1), and is a single polypeptide comprised of a C-terminal polymerase domain, a 3' exonuclease domain which is inactive, and an N-terminal 5' nuclease domain (1–6). Removal of the 5' nuclease domain produces the KlenTaq 'large fragment' of the polymerase, by analogy with the Klenow fragment of *E.coli* Pol 1 (7). The three-dimensional structures of KlenTaq and Klenow polymerases are almost identical (8–10) and the proteins have ~49% sequence

identity. The optimal physiological growth temperatures of *T.aquaticus* and *E.coli* differ by ~40°C [ $\sim 37^\circ\text{C}$  for *E.coli* and  $70\text{--}75^\circ\text{C}$  for *T.aquaticus* (11)].

We have recently examined the differences and similarities in the salt dependence of DNA binding by Taq and *E.coli* DNA polymerases (12). In this study, we have examined the temperature dependence of DNA binding by Taq polymerase and its KlenTaq large fragment domain in order to begin to understand some of the thermodynamic driving forces and non-covalent interactions involved in the functioning of Taq polymerase. The energetic forces that drive a macromolecular interaction are defining characteristics of that particular interaction. Understanding thermodynamic profiles for whole classes of interactions, such as DNA–protein interactions, allows one to begin to understand the energetic constraints or requirements for evolution and/or *de novo* design of such interactions. In this study, we have performed equilibrium DNA binding experiments with Taq polymerase using both fluorescence anisotropy and isothermal titration calorimetry and determined, as a function of temperature, the core thermodynamic quantities for this interaction ( $\Delta G$ ,  $\Delta H$ ,  $\Delta S$ ,  $\Delta C_p$ ).

We find that DNA binding for Taq and KlenTaq occur with sub-micromolar affinity across a broad temperature range, with maximal affinities near 40–50°C. Although it has been shown that Taq polymerase has little or no catalytic activity at room temperature (4,6), surprisingly, we find that Taq and KlenTaq polymerases bind DNA quite well down to at least 5°C. We also find that the DNA binding of Taq/KlenTaq is associated with an unusually large heat capacity change for a non-sequence-specific binding protein (13–17). Because of this, as found for most sequence-specific DNA binding proteins (17), the driving force for DNA binding of Taq/KlenTaq shifts from entropy driven to enthalpy driven as the temperature is increased. At its physiological temperature, DNA binding is enthalpy driven for Taq/KlenTaq polymerase.

## MATERIALS AND METHODS

### Materials

The proteins examined are full-length Taq DNA polymerase and the KlenTaq large fragment of the polymerase. Preparation of the proteins has been described in detail previously (12). No surfactants were used during preparation, storage or experiments with the polymerases. Fluorescently labeled and

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unlabeled DNA oligodeoxyribonucleotides were purchased from Integrated DNA Technologies Inc.

### Fluorescence anisotropy assay

Equilibrium DNA binding experiments were performed with the following primer-template set: 63/70mer, 5'-TACGCA-GCGTACATGCTCGTGACTGGGATAACCGTGCCGTTT-GCCGACTTTCGAGCCGTTCCA-3' and 3'-ATGCGTCGC-ATGTACGAGCACTGACCCTATTGGCACGGCAAACG-GCTGAAAGCGTCGGCAGGTTCCCAAA-5'.

For fluorescence anisotropy assays, the primer was labeled at the 5' end with Rhodamine-X (ROX). This DNA has been used previously for salt dependence studies of *Taq* DNA binding (12), and contains the same DNA sequence used previously for kinetic studies of DNA binding of Klenow polymerase by Benkovic and associates (18) at its single-strand double-strand junction. The extended length of this DNA allows it to remain stable to higher temperatures. Titrations were performed in 10 mM Tris, 75 mM KCl, 5 mM MgCl<sub>2</sub>, pH 7.9 buffer at the indicated temperatures. The pH values of the buffers were adjusted at each experimental temperature.

The pH was adjusted by mixing Tris base and Tris-HCl. ROX-labeled DNA was titrated with increasing concentrations of protein and binding was monitored using the anisotropy signal change as the protein-DNA complex is formed (12). Fluorescence anisotropy measurements were performed using a FluoroMax-2 fluorometer equipped with an automated polarizer and regulated at the indicated temperatures. The excitation and emission wavelengths were 583 and 605 nm, respectively, with 8 nm band-pass and an integration time of 10 s. For all the equilibrium titrations, the DNA concentration used was 1 nM. In all the experiments the protein was titrated into fluorescently labeled DNA, with the total [DNA] <  $K_d$ . After each addition, the sample was equilibrated at the required temperature for 8 min and anisotropy was measured. The temperature was varied across the widest possible range for each polymerase. Low temperature titrations were performed down to 5°C. Limiting values at high temperatures were the point where well behaved, reproducible isotherms could no longer be obtained. Titrations at high temperatures were performed with a screw cap cuvette to minimize evaporation during the experiments, and at low temperatures nitrogen was flowed through the sample chamber to prevent condensation on the outside of the cuvette.

### Isothermal titration calorimetry

Titrations were performed as a function of temperature on a MicroCal VP-ITC for the binding of *Taq* and KlenTaq to the same 63/70mer DNA described above, without the fluorescent label. Titrations were performed in the same buffers as the fluorescence titrations. *Taq* or KlenTaq were titrated into 1 μM DNA. Each titration consisted of an initial 2 μl injection (not used for data analysis) followed by 31–36 subsequent 7 μl injections, except for the 50 and 60°C titrations for full-length *Taq*, which consisted of 24 subsequent 10 μl injections. The heat of dilution of the protein was obtained by titrating protein into the buffer. The actual heat of the reaction was determined after subtracting the heat of dilution of the protein. ITC

binding curves were analyzed using the single-site binding equation in the Microcal Origin software package.

### Data analysis

Equilibrium binding curves obtained using fluorescence anisotropy were fit to a standard single-site isotherm usable when the [DNA] <<  $K_d$ :

$$\Delta A = [\Delta A_T(E_T / K_d) / (1 + E_T / K_d)] \quad 1$$

where  $\Delta A$  is the change in fluorescence anisotropy,  $\Delta A_T$  is the total change in anisotropy,  $E_T$  is the total polymerase concentration at each point in the titration, and  $K_d$  is the dissociation constant for polymerase-DNA binding. It was shown previously that the polymerases bind the DNA with 1:1 stoichiometry (12). Titrations with low  $K_d$  values were also checked with a more general equation which does not assume equality between the total and free enzyme concentrations, as described previously (12). Fits to the titrations with the very tightest (lowest)  $K_d$  values give  $\leq 10\%$  variation in the fitted  $K_d$  values using the two different equations. All other titrations give, within error, identical fitted  $K_d$  values with equation 1 and the more general equation (12). All non-linear fitting was performed using the program KaleidaGraph (Synergy Software). Temperature dependencies of equilibrium binding were analyzed using an integrated form of the Gibbs-Helmholtz equation:

$$\Delta G_{(T)} = \Delta H_{\text{refT}} - T\Delta S_{\text{refT}} + \Delta C_p[T - T_{\text{refT}} - T \ln(T / T_{\text{refT}})] \quad 2$$

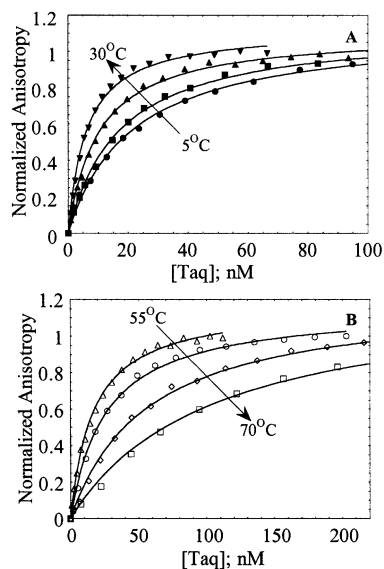
where  $\Delta G_{(T)}$  is the free energy at each temperature (dependent variable),  $T$  is the temperature in Kelvin (independent variable),  $\Delta C_p$  is the heat capacity, and  $\Delta H_{\text{refT}}$  and  $\Delta S_{\text{refT}}$  are the fitted van't Hoff enthalpy and entropy values at any chosen 'reference temperature'  $T_{\text{refT}}$ .

### Circular dichroism (CD) measurements

CD spectra were measured at room temperature (22°C) in an AVIV Model 202 CD spectrophotometer. A dual compartment mixing cuvette (Starna Cells) was used to record the spectra of protein + DNA before and after mixing. One compartment was filled with 3 μM DNA, the other with 3 μM KlenTaq.

## RESULTS

Direct equilibrium binding of *Taq* and KlenTaq polymerases to 63/70mer DNA was measured using a fluorescence anisotropy assay over a temperature range of 5–70°C. Selected titration curves are shown in Figure 1. Each titration curve fits well to a single-site binding isotherm, and it can be seen from the precision of the data that even modest shifts in  $K_d$  can be readily quantitated. The binding affinity increases with temperature until ~40–50°C, after which binding affinity decreases with increasing temperature. The titrations shown in Figure 1 illustrate the behavior of the titration curves in these increasing and decreasing portions of the temperature space. In Figure 2, the temperature dependence of the binding equilibrium is shown as a Gibbs-Helmholtz plot ( $\Delta G$  versus  $T$ ) for binding of *Taq* and KlenTaq polymerases to DNA. The observed non-linearity of  $\Delta G$  versus  $T$  results from a negative

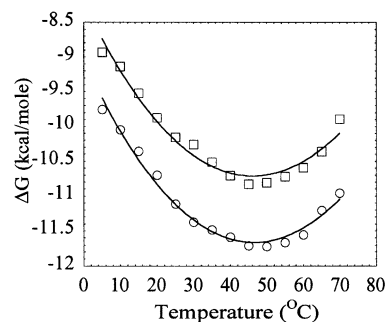


**Figure 1.** Equilibrium titrations of the binding of *Taq* polymerase to DNA monitored using fluorescence anisotropy. (A) Representative titrations of *Taq*–DNA binding at lower temperatures: 5 (circles), 10 (boxes), 20 (upright triangles) and 30°C (inverted triangles). (B) Representative titrations of *Taq*–DNA binding at higher temperatures: 55 (triangles), 60 (circles), 65 (diamonds) and 70°C (boxes).

heat capacity change ( $\Delta C_p$ ) associated with the binding process, and  $\Delta C_p$  can be calculated using the Gibbs–Helmholtz equation, which allows direct calculation of the van’t Hoff enthalpy ( $\Delta H_{vH}$ ) and entropy ( $\Delta S_{vH}$ ) as a function of temperature. The binding parameters at each temperature are listed in Table 1.

Figure 3 shows thermodynamic profiles for DNA binding of *Taq* and *Klentaq* polymerases, including the values of  $\Delta G$ ,  $\Delta H_{vH}$  and  $T\Delta S_{vH}$ , as a function of temperature. Plotted on this scale ( $\sim 20\times$  the scale in Fig. 2) it can be seen that the magnitudes of the temperature-dependent enthalpy and entropy changes are far larger than the temperature deviation of  $\Delta G$ . In other words, the  $\Delta H_{vH}$  and  $T\Delta S_{vH}$  are strongly temperature dependent. The binding of the polymerases exhibits enthalpy–entropy compensation: where the  $\Delta H$  and  $T\Delta S$  change in parallel with temperature (19,20). This thermodynamic profile is generally characteristic of sequence-specific DNA binding proteins (17,19,20).

The enthalpy of binding for *Taq* and *Klentaq* to the 63/70mer DNA was also measured calorimetrically. Titrations were performed under stoichiometric conditions to measure the heat of the reaction at several different temperatures, and representative titrations at high and low temperatures are shown in Figure 4. The buffer conditions were identical to those used to determine the temperature dependence of equilibrium binding. There are limitations in performing calorimetric titrations that are not a problem with the anisotropy measurements. For example, calorimetric titrations require 50–100-fold more protein and DNA per titration than do the fluorescence titrations. In addition, the binding enthalpy for *Taq*/*Klentaq* changes from positive to negative and thus passes through zero, so there is a temperature span near the middle of the binding range where the enthalpy change is not measurable calorimetrically using any reasonable quantities of



**Figure 2.** Gibbs–Helmholtz plot showing the temperature dependence of the free energy ( $\Delta G$ ) of DNA binding for *Taq* (circles) and *Klentaq* (boxes). Lines are the fits to the Gibbs–Helmholtz equation (equation 2) as described in Materials and Methods.

protein and DNA. Notwithstanding this, we calorimetrically determined the DNA binding enthalpies for *Taq* and *Klentaq* at several temperatures between 10 and 60°C. Data are reported in Table 2, and are shown in Figure 5 along with the  $\Delta H_{vH}$  dependence from Gibbs–Helmholtz analysis (from Fig. 2). While there is some variability in the absolute  $\Delta H$ s returned by van’t Hoff versus calorimetric analysis at any one temperature, the temperature dependencies of the calorimetric and van’t Hoff binding enthalpies (and the  $\Delta C_p$  values calculated from them) agree quite well. This agreement is an important confirmation of the thermodynamics, as differences between calorimetric and van’t Hoff enthalpies and heat capacities are observed in a large fraction of the systems that have been examined with both approaches and the origins of these common discrepancies has been the subject of ongoing debate for nearly a decade [see Liu and Sturtevant (21) and Horn *et al.* (22) for two of the most recent studies]. The temperatures  $T_H$  (where  $\Delta H$  is 0) and  $T_S$  (where  $\Delta S$  is 0) are  $\sim 32$  and  $\sim 45^\circ\text{C}$ , respectively, for *Taq* and *Klentaq*.  $T_H$  represents the temperatures at which  $K_d$  is minimum and  $T_S$  represents the temperature at which  $\Delta G$  is minimum (23).

To examine if DNA binding of the polymerase is associated with changes in protein or DNA structure in solution, we examined the CD spectra of the *Klentaq*–DNA complex relative to the isolated protein and DNA. Experiments were carried out in a dual compartment mixing cuvette, as described in Materials and Methods, to ensure that small spectral changes are exclusively due to complex formation. Data are shown in Figure 6 for the combined DNA + protein spectra before and after formation of the complex. Spectral signals above 240 nm in CD spectroscopy will be almost exclusively due to the DNA, while those below 240 nm are largely due to the protein. Figure 6 shows that there are small but easily observed conformational changes in both the DNA and the protein upon formation of the complex.

## DISCUSSION

We have examined the temperature dependence of the thermodynamics of binding of *Taq* and *Klentaq* polymerases to DNA. Equilibrium binding occurs with dissociation constants ( $K_d$ ) in the sub-micromolar range across a wide temperature span. The temperature dependence of the binding free energy (Gibbs–Helmholtz plot) is strongly non-linear,

**Table 1.** Thermodynamic parameters of DNA binding by *Taq* and KlenTaq DNA polymerases<sup>a</sup>

Temperature (°C)	$K_d$ (nM)	$\Delta G$ (kcal/mol)	$\Delta H$ (kcal/mol)	$\Delta S$ (kcal/mol K)	$T\Delta S$ (kcal/mol)	$\Delta C_p$ (kcal/mol K)
<i>Taq</i>						
5	21.3 ± 1.0	-9.76	+18.81	+0.102	+28.38	
10	17.3 ± 0.6	-10.05	+15.14	+0.089	+25.22	
15	13.8 ± 0.5	-10.36	+11.47	+0.076	+21.94	-0.73 ± 0.04
20	10.3 ± 0.4	-10.71	+7.81	+0.064	+18.63	
25	7.1 ± 0.2	-11.11	+4.14	+0.051	+15.26	
30	6.2 ± 0.3	-11.38	+0.47	+0.039	+11.81	
35	7.1 ± 0.2	-11.49	-3.2	+0.027	+8.31	
40	8.1 ± 0.3	-11.59	-6.86	+0.015	+4.75	
45	8.9 ± 0.4	-11.71	-10.53	+0.004	+1.11	
50	11.7 ± 0.6	-11.72	-14.2	-0.008	-2.54	
55	16.8 ± 0.6	-11.67	-17.86	-0.019	-6.26	
60	26.0 ± 1.4	-11.56	-21.53	-0.030	-10.07	
65	56.7 ± 2.4	-11.21	-25.2	-0.041	-13.92	
70	103.7 ± 6.5	-10.96	-28.87	-0.052	-17.82	
<i>KlenTaq</i>						
5	95.0 ± 1.7	-8.93	+18.62	+0.098	+27.36	
10	87.7 ± 1.8	-9.14	+15.06	+0.086	+24.25	
15	59.0 ± 0.8	-9.53	+11.5	+0.073	+21.08	-0.71 ± 0.06
20	43.3 ± 1.1	-9.87	+7.93	+0.061	+17.86	
25	35.6 ± 1.1	-10.16	+4.37	+0.049	+14.57	
30	39.8 ± 1.4	-10.26	+0.81	+0.037	+11.23	
35	34.6 ± 1.1	-10.51	-2.75	+0.025	+7.82	
40	33.3 ± 1.3	-10.71	-6.32	+0.014	+4.36	
45	35.8 ± 1.5	-10.83	-9.88	+0.003	+0.83	
50	48.3 ± 1.5	-10.81	-13.44	-0.008	-2.74	
55	71.4 ± 3.0	-10.72	-17.0	-0.019	-6.36	
60	111.4 ± 5.7	-10.59	-20.57	-0.030	-10.06	
65	199.7 ± 9.1	-10.36	-24.13	-0.041	-13.8	
70	497.5 ± 23.3	-9.89	-27.7	-0.051	-17.59	

<sup>a</sup> $K_d$  and  $\Delta G$  values are the experimental values determined from titrations at each temperature. The other thermodynamic parameters are calculated from the fit to the Gibbs–Helmholtz equation. Errors are the parameter value errors returned from the fits to equation 1 ( $K_d$  values) or equation 2 ( $\Delta C_p$  values).

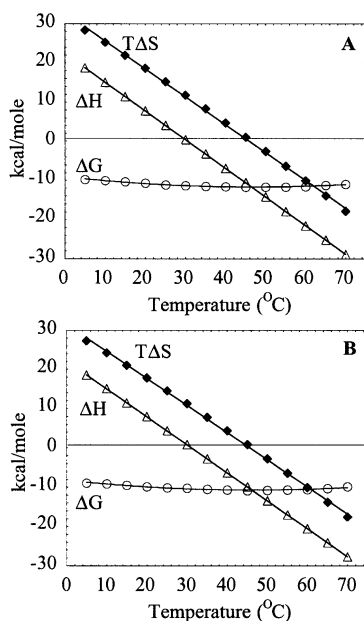
indicating a temperature-dependent binding enthalpy ( $\Delta H_{vH}$ ) and thus an associated  $\Delta C_p$  of binding. For comparison, absence of a  $\Delta C_p$  of binding would yield a linear dependence of  $\Delta G$  on temperature. Parallel determination of the enthalpy of binding versus temperature by isothermal titration calorimetry confirms that the binding of *Taq* and KlenTaq to DNA are associated with  $\Delta C_p$  values of  $-0.7$  to  $-0.8$  kcal/mol. These are substantial  $\Delta C_p$  values, and are in contrast to the absence of  $\Delta C_p$  generally associated with non-specific DNA–protein interactions (13–17).

Very few other thermophilic DNA binding proteins have had their DNA binding activity thermodynamically characterized. These include the non-specific binding of Sac7d from *Sulfolobus acidocaldarius* (24) and Sso7d from *Sulfolobus solfataricus* (16), and the site-specific binding of ORF56 protein from *Sulfolobus islandicus* (25). The site-specific binding of ORF56 has a  $\Delta C_p$  of  $-1.5$  kcal/mol (25). The non-specific binding of Sso7d and Sac7d have  $\Delta C_p$  values of  $-0.26$  kcal/mol and zero, respectively (16,24). The finding that the optimal DNA binding temperature is significantly below the optimal growth temperature for the thermophilic bacterium was also found for the DNA binding of ORF56 (25). An optimal binding temperature could not be determined for Sso7d, and Sac7d has no  $\Delta C_p$  of binding and hence has no minimum in its Gibbs–Helmholtz distribution (16,24).

*Taq* polymerase replicates DNA essentially non-sequence specifically, beginning at any single-stranded/double-stranded junction, and this non-specificity is a basis for *Taq*'s utility in

the PCR. While DNA structural elements such as mismatched base pairs, overhanging primers, gaps and nicks, etc., are known to have a significant influence over DNA polymerase binding and function, the DNA sequence dependence of the binding of prokaryotic DNA polymerases to matched primed DNA has not been studied in any detail. They are certainly not sequence specific in the same way that transcriptional regulators or restriction endonucleases are specific. Preferences for different sequences have been found for several non-specific DNA binding proteins (15,16), including Klenow polymerase (18), but the difference between the tightest and weakest binding sequences is up to about an order of magnitude, in contrast to the three to seven orders of magnitude differences between the specific and non-specific binding modes of site-specific DNA binding proteins (17). If any binding sequence specificity does exist for DNA polymerases, it is possible that the thermodynamic profile for DNA binding of a polymerase could be different for alternate DNA sequences.

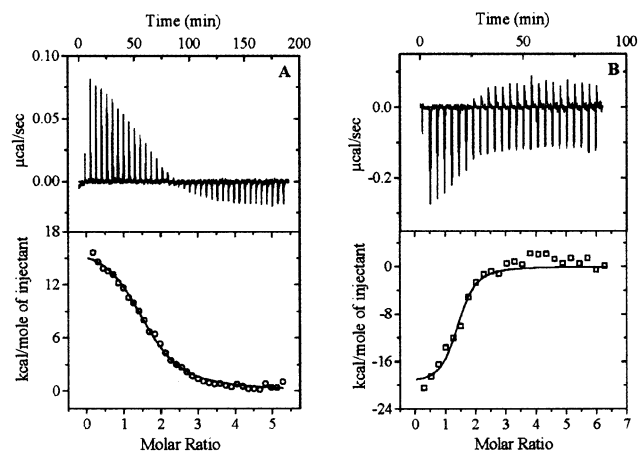
Is a measurable  $\Delta C_p$  of binding a common characteristic of proteins that bind DNA primarily non-specifically or that bind to specific DNA structures? Non-specific binding of DNA is generally correlated with a near zero  $\Delta C_p$  (reviewed in 17). Correspondingly, a negative  $\Delta C_p$  has been called a 'signature' of site-specific DNA binding (17). However, a few exceptions to this correlation have been reported, namely the  $\Delta C_p$  values reported for non-specific DNA binding of *E.coli* SSB, the Sso7d protein of *S.solfataricus*, and *E.coli* IHF (15,16,26). Our



**Figure 3.** Temperature dependencies of the thermodynamic parameters  $\Delta H$  (triangles),  $T\Delta S$  (diamonds) and  $\Delta G$  (circles) of DNA binding by (A) *Taq* and (B) *KlenTaq*.  $\Delta G$  values are the same data as in Figure 2, plotted here on a different scale to show its relationship to the  $\Delta H$  and  $T\Delta S$  parameters.  $\Delta H$  and  $\Delta S$  values at each temperature are derived from the non-linear analysis of the Gibbs–Helmholtz plot.

study of the binding of *Taq*/*KlenTaq* to DNA is the first characterization of  $\Delta C_p$  (along with  $\Delta H$  and  $\Delta S$  and their temperature dependencies) for the DNA binding of a DNA polymerase.

The non-specific protein–DNA interactions thus far found to have near zero  $\Delta C_p$  values are mostly sequence-specific binding proteins binding in their non-specific mode (*Sac7d* from *S.solfataricus* is an exception) (17,24). Three of the four proteins that have been found to bind DNA non-specifically with significant  $\Delta C_p$  values (*Taq*, *SSB* and *Sso7d*) differ from other non-specific binding proteins in two fundamental ways. *Taq*, *SSB* and *Sso7d* are proteins that primarily bind non-sequence specifically to DNA (although with some sequence preferences), and are proteins that show DNA structure specificity or preferences. These distinctions may be indicative of different subsets of non-specific DNA binding with different thermodynamic profiles. It may be that structure-specific DNA binding, like sequence-specific binding, will be found to be generally correlated with a larger  $\Delta C_p$ . As with sequence-specific binding, the formation of a tight complementary interface in a structure-specific DNA complex might be expected. On the other hand, as suggested previously in the case of *Sso7d*, a measurable  $\Delta C_p$  may be a general



**Figure 4.** Calorimetric titrations of *Taq* into DNA at (A) 10 and (B) 60°C. Shown here are the raw calorimetric data (top) and the fits to the integrated heats of the reaction (bottom).

characteristic of proteins that primarily bind non-specifically to DNA (16). However, until more primarily non-sequence-dependent binding proteins are examined, it is unclear how many thermodynamic/functional subclasses of such proteins might exist.

#### Conformational changes associated with complex formation

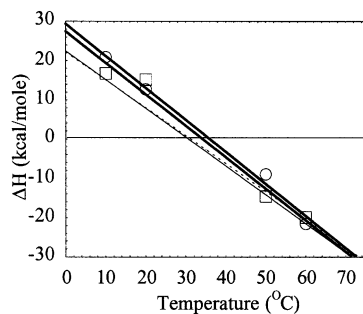
The CD spectrum of the *KlenTaq*–DNA complex when compared with the spectrum of the combined (but physically separated) DNA + polymerase clearly shows that the conformations of both the DNA and the protein change slightly upon complex formation. The CD spectrum at wavelengths above ~240 nm is due primarily to the DNA, and the 265–290nm spectrum in Figure 6 is characteristic of B-form DNA. CD spectral changes have been used to detect protein and DNA conformational changes upon binding (24,27), and a lack of spectral changes in this wavelength range has been used to demonstrate the absence of DNA conformational changes upon binding (28). The CD spectral changes shown in Figure 6, while small, demonstrate that the association of *KlenTaq* with DNA is not a simple rigid body association. Conformational changes of the protein upon DNA binding are also observed by crystallography and are discussed further below.

#### Possible molecular basis for the negative $\Delta C_p$ of DNA binding of *Taq*

The data in this study do not allow us to pinpoint the molecular contributions to the  $\Delta C_p$  of binding of *Taq* to DNA, but we can discuss some aspects of the possible correlations with the

**Table 2.** Calorimetric  $\Delta H$  and  $\Delta C_p$  values for *Taq* and *KlenTaq*

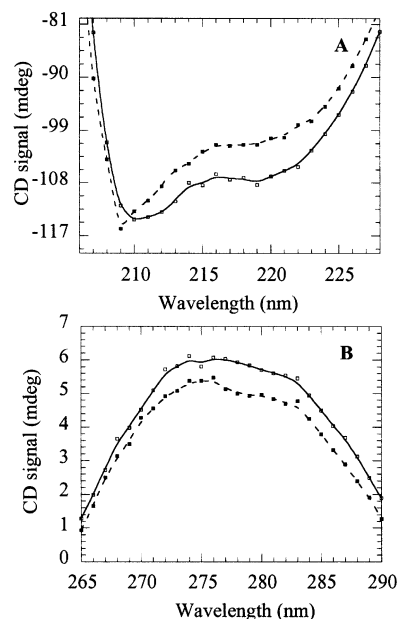
Temperature (°C)	<i>Taq</i> $\Delta H_{cal}$ (kcal/mol)	$\Delta C_{p,cal}$ (kcal/mol K)	<i>KlenTaq</i> $\Delta H_{cal}$ (kcal/mol)	$\Delta C_{p,cal}$ (kcal/mol K)
10	+16.73		+20.93	
20	+15.21	$-0.80 \pm 0.09$	+12.75	$-0.82 \pm 0.04$
50	-14.64		-8.93	
60	-19.95		-21.55	



**Figure 5.** Temperature dependence of the enthalpy change ( $\Delta H$ ) upon binding of *Taq* and KlenTaq to DNA. Calorimetric enthalpy ( $\Delta H_{\text{cal}}$ ) values obtained from the calorimetric titrations for *Taq* (boxes) and KlenTaq (circles) are shown along with linear fits to the calorimetric data used to obtain the calorimetric  $\Delta C_p$  (bold lines). For comparison, the van't Hoff enthalpy temperature dependencies derived from the Gibbs–Helmholtz analysis are also shown here as a thin solid line for *Taq* and a thin dashed line for KlenTaq (these two lines mostly overlap).

major proposed molecular models for  $\Delta C_p$  effects. For many protein–DNA interactions and protein folding reactions there is a correlation between  $\Delta C_p$  and surface area changes ( $\Delta \text{ASA}$ ) (29–34). This has proven a powerful connection between thermodynamic and structural information. However, there have also been a number of exceptions to this correlation (17,26,35–40) suggesting that such correlations may represent a specific subset of all reactions with a  $\Delta C_p$ . The exceptions often have the problem that the  $\Delta C_p$  for the process of interest is much larger than can be accounted for by burial of surface area. This is not the case for *Taq*/KlenTaq. A  $\Delta C_p$  of  $-0.765$  (mean of the Gibbs–Helmholtz and calorimetric  $\Delta C_p$ s), while unusually high for a non-sequence-specific protein–DNA interaction, is low compared with many sequence-specific binding proteins. Structure-based calculations of the surface area buried in the binding interface between *Taq* and DNA yield a value of  $2530 \text{ \AA}^2$  (41). If the entire change in surface area for the protein and DNA are included (i.e. not just the interface region), this total increases to  $3126 \text{ \AA}^2$  (V.J. LiCata, unpublished results). At least four different quantitative relationships between  $\Delta C_p$  and the sum of buried non-polar + polar surface area have been proposed (30–33). A correlation between  $\Delta C_p$  and buried surface area for *Taq*/KlenTaq can easily be achieved using any one of these relationships. However, all of these equations predict that a relatively high fraction of the buried surface area must be non-polar: the mean of all four relationships predicts that 82% of the buried surface area must be non-polar. Since protein–DNA interfaces are generally much more polar than interiors of proteins, it would seem that other linked processes may also be involved in the generation of the  $\Delta C_p$  of binding of *Taq* to DNA.

Another major proposed origin of negative  $\Delta C_p$  values for protein–DNA interactions are conformational changes of the DNA upon binding (38,42). Binding of TBP to the E4 promoter has been shown to be associated with large negative  $\Delta C_p$  that could not be explained by any significant burial of non-polar surfaces or conformational changes of the protein (38). It was proposed that the negative  $\Delta C_p$  might originate from the unwinding of the B-DNA helix, base unstacking and intercalation of phenylalanine side chains in the DNA kinks (38). A large negative  $\Delta C_p$  has also been reported for the



**Figure 6.** CD spectra for the KlenTaq–DNA complex (closed boxes) versus the free DNA + free protein (open boxes) at 22°C. Spectra were obtained using a dual compartment mixing cuvette and recorded before and after mixing. Shown here are the regions of the spectra showing differences. (A) The spectral region from 206 to 228 nm corresponding to signal primarily from the protein. (B) The spectral region from 265 to 290 nm corresponding to signal from the DNA. Spectra were collected in 1 nm steps and the lines are simply a smoothed interpolation of the stepwise spectral data.

interaction of *E.coli* SSB with single-stranded DNA (15). Subsequent studies showed that this  $\Delta C_p$  is partly due to adenine base unstacking and partly due to a linked protonation equilibrium (42,43). The CD spectra for KlenTaq binding to DNA in Figure 6 clearly show that there is some distortion of the DNA upon binding in the KlenTaq–DNA complex. The crystal structures of DNA bound to Klenow and KlenTaq (8,44,45) also show distortion of the DNA upon binding. Thus, structural changes in the DNA are also a likely contributor to the measured  $\Delta C_p$  of binding of *Taq* to DNA.

#### Low temperature DNA binding by *Taq* polymerase

Surprisingly, *Taq*/KlenTaq binds DNA with high affinity at temperatures as low as 5°C. This was unexpected since it has been shown that *Taq* is essentially catalytically inactive at room temperature (4,6). The enzyme is optimally catalytically active at 70–75°C (4,6). This indicates that catalysis but not binding involves a molecular process that can only occur at higher temperatures. The combination of high temperature stability with low temperature activity is rare in natural thermophilic proteins, and all but a very few thermophilic proteins are inactive or almost inactive at room temperature (reviewed in 46). This combination of properties can, however, be introduced into proteins by engineering or directed evolution (46).

In several studies, thermophilic proteins have been found to exhibit an increased molecular rigidity at room temperature, and this rigidity has been correlated with a decreased ability to undergo the conformational fluctuations required for catalytic

activity (47,48). Exceptions to this general correlation have also been observed (49). Although no data yet exist regarding the molecular rigidity of *Taq*, crystal structures have recently been solved for the binary complex of KlenTaq polymerase with DNA, and the ternary complex of KlenTaq with DNA and an incoming nucleotide (45). The binary complex structure corresponds to the interaction formed in our binding studies, the ternary complex reflects the enzyme performing catalytic polymerization. What is notable is that the structures of both complexes show significant conformational changes relative to the unbound polymerase (45). When KlenTaq binds DNA, the 'thumb' region rotates as a whole and moves in closer to the DNA (45). When the incoming nucleotide is added, the 'fingers' region of the polymerase on the opposite side of the DNA binding cleft performs a similar closure motion (45). The change associated with catalysis is somewhat larger in magnitude, based on the number of atoms involved and the distances they move, but the structural change associated solely with DNA binding is itself a substantial one. The CD spectral changes upon DNA binding (Fig. 6) confirm that in solution conformational changes occur in both the protein and the DNA. So, even though conformational flexibility is involved in the DNA binding process for *Taq*, it proceeds without a problem at low temperature.

The fact that *Taq* undergoes a conformational change upon DNA binding does not mean that it is not more rigid than mesophilic polymerases in general. In fact, the overall binding affinity of *Taq* for DNA was recently shown to be ~150× weaker than the affinity of Klenow polymerase for the same DNA under comparable solution conditions (12). This may be indicative of an overall higher rigidity of *Taq* polymerase relative to Klenow. Unlike the situation with the catalytic activity of many other thermophilic–mesophilic protein pairs, however, there is no 'corresponding states' temperature where the DNA affinity of *Taq* polymerase approximates that of Klenow polymerase.

While there have been numerous studies of the temperature dependencies of the enzymatic activity of thermophilic proteins, there have been very few direct substrate binding or substrate analog binding studies. It is interesting to note that in addition to *Taq*, the other thermophilic DNA binding proteins that have been characterized as a function of temperature, Sac7d, Sso7d and ORF56 from the *Sulfolobus* genus, which were discussed above, also bind DNA at temperatures below room temperature—although the unusual nature of this apparently common characteristic has not been noted previously. The DNA binding of Sac7d, Sso7d and ORF56 were studied from 10 to 40, 15 to 45 and 17 to 57°C, respectively (16,24,25). Thermophilic archaeobacterial flap endonucleases have also previously been shown to bind DNA at low temperature (50). Thus, it would seem based on the examples available thus far, that unlike the usual loss of catalytic activity, the DNA binding activity of thermophilic proteins is maintained at lower temperatures.

### Concluding summary

The temperature dependence of DNA binding by *Taq* polymerase provides the first such characterization of DNA binding by a thermophilic protein that is not from a *Sulfolobus* bacterium. The two most unusual features of the thermodynamics of *Taq*–DNA interactions are the low temperature

DNA binding of *Taq*, and the fact that its  $\Delta C_p$  and thermodynamic temperature profile are characteristic of sequence-specific DNA binding proteins. We have suggested that it is not *Taq* itself which is an exception to current empirical correlations, but that these correlations may need further refinement as our knowledge base on thermophilic and DNA binding proteins continues to expand.

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