Comparison of TATA-binding Protein Recognition of a Variant and Consensus DNA Promoters*

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Assembly of transcription pre-initiation complexes proceeds from the initial complex formed between “TATA” bearing promoter DNA and the TATA-binding protein (TBP). Our laboratory has been investigating the relationships among TATA sequence, TBP-TATA solution structure, recognition mechanisms, and transcription efficiency. TBP-TATA interactions have been modeled by global analysis of detailed kinetic and thermodynamic data obtained using fluorimetric and fluorometric techniques in conjunction with fluorescence resonance energy transfer. We have reported recently that TBP recognition of two consensus promoters, adenovirus major late (AdMLP; TATAAAG) and E4 (TATATAA), is well described by a linear two-intermediate mechanism with simultaneous DNA binding and bending. Similar DNA geometries and high transcription efficiencies characterize these TBP-TATA complexes. Here we show that, in contrast to the consensus sequences, TBP recognition of a variant sequence (C7: TATAAAGC) is described by a three-step model with two branching pathways. One pathway proceeds through an intermediate having severely bent DNA, reminiscent of the consensus interactions, with the other branch yielding a unique conformer with shallowly bent DNA. The resulting TBP-C7 complex has a dramatically different solution conformation than for TBP-DNACONS, and is correlated with diminished relative transcription activity. The temperature dependence of the TBP-C7 helical bend is postulated to derive from population shifts between the conformers with slightly and severely bent DNA.

For eukaryotic class II genes, the binary complex formed between the TATA-binding protein and a conserved promoter site, the TATA box, provides the foundation upon which the transcription pre-initiation complex is assembled (1–7). TBP binds productively to consensus (TATA(a/t)A(a/t)N) and diverse variant TATA sequences (8, 9), yielding relative transcription efficiencies ranging from <1 to 172 (9, 10).

The interactions of Saccharomyces cerevisiae TBP with promoters bearing two consensus sequences, adenovirus major late (TATATAAAG, AdMLP) and E4 (TATATAA), have been well characterized via extensive biochemical (11–22), crystallographic (23–28), and molecular dynamics (29–33) studies. Both promoters bind to TBP at rates significantly slower than diffusion limited (12, 13, 15–20), forming tightly bound complexes with similar co-crystal structures (23–27) and comparable DNA geometries in solution (34). Additionally, these two sequences yield high relative transcription activities (9, 10).

Our laboratory has been studying the detailed recognition mechanism of DNA promoters by S. cerevisiae TBP (15, 16, 20) and the solution geometries of the resulting TBP-TATA complexes (34, 35). Both lines of investigation utilize dye-labeled TATA-bearing oligomers and steady-state, stopped-flow, and time-resolved fluorescence techniques in conjunction with fluorescence resonance energy transfer (FRET). Global analysis of extensive real-time kinetic and thermodynamic data sets first revealed a linear three-step mechanism for the TBP-AdMLP reaction (16), with intermediate conformers having DNA bent to the same extent as in the final complex. These conformers are present at high mole fraction throughout the reaction and persist at equilibrium. The parallel investigation of TBP-DNA solution structures concurrently revealed a strong sequence dependence of the DNA helical bend in such complexes (34). Because the latter suggested TATA sequence-dependent recognition interactions, the mechanistic study was extended beyond AdMLP to include other sequences.

We first asked the question, “How do the detailed recognition processes of different consensus sequences by TBP compare?” The E4 sequence was chosen as the alternate consensus sequence, because functional differences between the TBP-AdMLP and TBP-E4 interactions had been identified previously using DNase I footprinting (18, 19). Extensive data collection and model fitting using global analysis showed the linear two-intermediate model to be common to the reaction of TBP with both strong promoters (20). The energetics of the partial reactions for the two promoters was very similar for the initial step, formation of the first intermediate conformer. Beyond that initial binding and bending event, however, the reaction progression differed substantially, with the TBP-E4 interaction nearly complete after the second step but with the TBP-AdMLP reaction continuing to undergo large energetic changes in the final step.

Having thus established a detailed comparison between the

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† The abbreviations used are: TBP, TATA-binding protein; FRET, fluorescence resonance energy transfer; TAMRA or T, carboxytetraethylrhodamine; F, fluorescein; T*C7dpx*F, double-labeled duplex containing the C7 sequence (TAMRA-5′-GGGCTATAAACCCAGG-3′-fluorescein); C7dpx*F, single-labeled duplex containing the C7 sequence; C7dpx, unlabeled duplex; E4 promoter sequence, GGGCTATATAAGG; ade-

novirus major late promoter sequence, GGGCTATAAAAAGG; TBP-DNA, binary complex formed between TBP and DNA.

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interactions of TBP with two consensus sequences, we have now examined TBP recognition of a variant sequence. The C7 sequence (TATAAACG) is a naturally occurring single base variant of the AdMLP sequence. TBP-bound C7 has a helical bend dramatically different from that in bound AdMLP that correlates with significantly reduced relative transcription efficiency (34). This solution geometry is highly sensitive to the presence and concentration of osmolytes (35), in contrast to that for the complexes bearing consensus sequences.

Detailed kinetic and energetic profiles for the TBP-C7 interaction have been determined using FRET-based measurements. A comprehensive comparison with the TBP-AdMLP and TBP-E4 profiles clearly distinguishes the recognition process by TBP of the variant sequence from those of the consensus sequences. The ensemble of TBP-C7 data is well described by a three-step model with two branching pathways. One of these pathways is unique to the variant sequence mechanism and yields a bound species with DNA bent only slightly. Along the other pathway, the variant reaction proceeds through an intermediate conformer bearing strongly bent DNA in a process remarkably reminiscent of that for the complexes bearing consensus sequences.

**EXPERIMENTAL PROCEDURES**

**DNA, Protein, and Solution Conditions—** Fourteen base fluorescein-labeled DNA oligonucleotide probes and the unlabeled complementary strands were synthesized and purified by Sigma-Genosys (The Woodlands, TX) as described previously (16, 20, 35). TAMRA and fluorescein were linked covalently to the 5′-end dye distance (15, 16, 34). The latter were scaled from 0 to 0.127 (15 °C), 0.216 (20 °C), 0.301 (25 °C), or 0.355 (30 °C), the temperature-dependent amplitude changes observed upon TBP-TC7dpx*F binding in steady-state FRET measurements. The theoretical response functions were determined as described (16, 20). The overall average squared residual, \( \sigma_{\text{global}}^2 \), derived from the weighted variance between the observed and theoretical points describing the shapes of the association (\( \sigma_{\text{on}}^2 \)), replacement (\( \sigma_{\text{off}}^2 \)), and equilibrium binding (\( \sigma_{\text{eq}}^2 \)) curves,

\[
\sigma_{\text{global}}^2 = \frac{1}{13} 2 \sigma_{\text{on}}^2 + 2 \sigma_{\text{off}}^2 + 3 \sigma_{\text{eq}}^2 \tag{1}
\]

with the coefficients reflecting the relative information content of each term. A fit with each term within its experimental error thus yields a value for \( \sigma_{\text{global}}^2 \leq 1 \). Error estimates for the optimal parameter values were obtained exactly as described (16).

The correspondence of the ensemble of TBP-C7 data was first tested against linear (Equation 2) and branching (Equation 3) two-step models,

\[
\begin{align*}
&\text{TBPA + DNA} \xrightarrow{k_1} \text{TBPA-DNA}_{\text{int}} \xrightarrow{k_2} \text{TBPA-DNA}_{\text{f}} \quad \text{Equation 2 (Branching)} \\
&\text{TBPA-DNA}_{\text{f}} \xrightarrow{k_3} \text{TBPA-DNA}_{\text{A}} \quad \text{Equation 3 (Linear)}
\end{align*}
\]

where the subscript "INT" denotes an intermediate conformer. Global analysis for either model yielded values for the four rate constants at 30 °C as well as the corresponding activation enthalpies. The quantum yields of the donor fluorescein in the two TBP-bound duplexes, relative to that of free \( ^{3}T^{\circledast}C_{\text{dpx}}*F \), were also determined. These quantum yield values indicate the relative extent of DNA bending in each binary complex, as they reflect the 5′-dye-3′-dye distance (15, 16, 34).

The data were further tested against various three-step mechanisms. First explored was a linear two-intermediate kinetic model,
shown previously to describe both the TBP-AdMLP and TBP-E4 reactions (16, 20). Determined in the analysis were values for the six rate constants at 30 °C, the corresponding activation energies, and the quantum yield for each TBP-bound conformer reflecting a temperature-dependent DNA conformational change. The parameters describing the probability distribution of the 5′ dye-3′ dye distance for free T°C7dpx*F were $R_{\text{free}} = 54.7 ± 0.1$ Å and $\sigma_{\text{free}} = 6.8 ± 0.2$ Å. These values were invariant over the experimental temperature range, consistent with the observed temperature independence of the T°C7dpx*F steady-state spectrum. In contrast, the values of $R_{\text{bound}}$ and $\sigma_{\text{bound}}$ were temperature-sensitive. These values are shown in Table I together with the corresponding bend angles. Successive decreases in $R_{\text{bound}}$ with increasing temperature indicate a temperature-dependent TBP-C7 conformational change. The change in the inter-dye distance from 53.1 Å at 15 °C to 50.4 Å at 30 °C corresponds to an increase of $\sim$1.7-fold in the DNA bend angle. As the bend angle for TBP-C7 increases with temperature, a corresponding decrease in $\sigma_{\text{bound}}$ is observed, consistent with similar results from sequence- and osmolyte concentration-dependent studies. This inverse correlation is attributed to increasing compatibility of duplex and protein structures at the interface, restricting the DNA helical mobility (34, 35).

**TABLE I**

| Temperature | $R_{\text{bound}}$ Å | $\sigma_{\text{bound}}$ | Two-kink bend$^a$ |
|-------------|-----------------------|------------------------|-------------------|
| 15          | 53.1 ± 0.1             | 11.4 ± 0.2             | 33.6 ± 0.8        |
| 20          | 51.6 ± 0.1             | 11.0 ± 0.2             | 47.4 ± 0.6        |
| 25          | 50.9 ± 0.1             | 10.2 ± 0.2             | 53.0 ± 0.6        |
| 30          | 50.4 ± 0.1             | 8.9 ± 0.2              | 58.3 ± 0.5        |

$^a$ A total of 48 decay curves, each on an average of three curves, were collected and analyzed. All curves were well described by biexponential decay, with a mean value for $\chi^2$ of 1.00 ± 0.07. Subsequent analysis was performed to determine $P(R)$ modeled as a shifted Gaussian (40). $P(R)$ values were refit to $P(R)_{\text{bound}} + P(R)_{\text{free}}$, weighed using $K_{\text{c}}$ to correct for the small amount (<5%) of free duplex.

The bend angle, $\alpha$, was determined using a two-kink bend model as described previously (34, 35), $\alpha = 2\cos^{-1}[L_{\text{free}}/(R_{\text{free}} - L_{\text{free}})]$, with $L_{\text{free}} = 20.4$ Å. The $R_{\text{free}}$ value of 54.7 ± 0.1 Å is invariant with temperature through this range.

These data were well fit by linear regression using the van’t Hoff equation, yielding $K_s$ values, in μM$^{-1}$, of 10.3 ± 5.0 at 15 °C, 17.2 ± 5.3 at 20 °C, 28.2 ± 8.7 at 25 °C, and 45.4 ± 20.3 °C at 30 °C. The affinity of TBP for C7 is $\sim$3X of and $\sim$9–18X lower than for AdMLP and E4, respectively (Fig. 1, inset (16, 20)). In contrast with TBP-AdMLP and TBP-E4, a temperature-dependent decrease was observed in the fluorescein/TAMRA steady-state emission peak ratio upon saturation of T°C7dpx*F with TBP, with values of 12.7 ± 0.6% at 15 °C, 21.6 ± 1.2% at 20 °C, 30.1 ± 1.4% at 25 °C, and 33.5 ± 1.9% at 30 °C.

**End-to-End Distance Distributions, P(R), and TBP-bound C7 Solution Bend Angles**—The parameters describing the probability distribution of the 5′ dye-3′ dye distance for free T°C7dpx*F were $R_{\text{free}} = 54.7 ± 0.1$ Å and $\sigma_{\text{free}} = 6.8 ± 0.2$ Å. These values were invariant over the experimental temperature range, consistent with the observed temperature independence of the T°C7dpx*F steady-state spectrum. In contrast, the values of $R_{\text{bound}}$ and $\sigma_{\text{bound}}$ were temperature-sensitive. These values are shown in Table I together with the corresponding bend angles. Successive decreases in $R_{\text{bound}}$ with increasing temperature indicate a temperature-dependent TBP-C7 conformational change. The change in the inter-dye distance from 53.1 Å at 15 °C to 50.4 Å at 30 °C corresponds to an increase of $\sim$1.7-fold in the DNA bend angle. As the bend angle for TBP-C7 increases with temperature, a corresponding decrease in $\sigma_{\text{bound}}$ is observed, consistent with similar results from sequence- and osmolyte concentration-dependent studies. This inverse correlation is attributed to increasing compatibility of duplex and protein structures at the interface, restricting the DNA helical mobility (34, 35).

**TBP-C7 Association Kinetics**—The binding and bending of T°C7dpx*F by TBP was monitored in real-time as a function of temperature and protein concentration using fluorescence stopped-flow and FRET. A total of 40 curves were collected, yielding a set of eight averaged curves, each a unique combination of temperature (15–30 °C) and TBP concentration (200–800 nM). All curves were very well represented by biexponential decay (15, 16). The overall change in amplitude at each temperature was consistent with the corresponding steady-state change obtained for the binding isotherms. The association of TBP with C7 is notably slower and more biphatic than with either consensus promoter (Fig. 2 (16, 20)).

**TBP-C7 Dissociation Kinetics**—A solution of TBP-T°C7dpx*F complex was challenged with high concentrations of unlabeled C7 duplex and dissociation from all bound species monitored via changes in steady-state emission. The dependence of the TBP-C7 dissociation kinetics on the unlabeled DNA concentra-
tion revealed both passive replacement and facilitated displacement processes, as previously observed for TBP-E4 but not TBP-AdMLP (20). The pure replacement curve was extracted from the set of decays collected at each temperature using two procedures. A Taylor series expansion about an intermediate from the set of decays collected at each temperature using two TBP, TBP-C7 stopped-flow association kinetic curves (inset) for 20 nM TpC7 dpx + F reacting with 400 nM TBP at 15 °C, 20 °C, 25 °C, and 30 °C. Shown are the normalized biexponential decays corresponding to the observed progress curves.

The nature of the biphasicity characterizing the kinetics of C7 replacement from the TBP-bound complex differs markedly from that of the consensus sequences (Fig. 3). The slow phase eigenvalue, 0.0010 ± 0.0002 s−1, and its essential invariance with temperature are nearly identical with λslow for AdMLP and E4 replacement. However, this phase ranges in amplitude from only 11 to 17%, in sharp contrast to the dominance of the slower binding and in-...
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**Thermodynamic Pathway for the TBP-C7 Reaction Pathway**—The thermodynamic profile for TBP-C7 association is shown in Fig. 6 for the three-step branching model (Equation 7). Free energy changes relative to (TBP + DNA) for TBP-DNA\textsubscript{A, SHALLOW}, TBP-DNA\textsubscript{B, SEVERE}, and TBP-DNA\textsubscript{C, SEVERE} are -9.25 [-9.38, -9.15], -9.85 [-9.88, -9.75], and -8.95 [-8.96, -8.86] kcal mol\textsuperscript{-1}, respectively. Formation of TBP-DNA\textsubscript{A, SEVERE} is entropy-driven and endothermic under the experimental conditions with $\Delta S^0 = 150.4$ [145.6, 156.1] cal K\textsuperscript{-1} mol\textsuperscript{-1} and $\Delta H^0 = 35.9$ [34.9, 36.7] kcal mol\textsuperscript{-1}. This branch of the reaction pathway proceeds through two large energetic barriers. In contrast, formation of TBP-DNA\textsubscript{A, SHALLOW} is slightly exothermic with $\Delta S^0 = 27.0$ [16.0, 38.5] cal K\textsuperscript{-1} mol\textsuperscript{-1} and $\Delta H^0 = -1.2$ [-2.6, -0.7] kcal mol\textsuperscript{-1}.

**DISCUSSION**

Extensive kinetic and thermodynamic data sets describing the interaction of TBP and C7 have been shown to correspond within experimental error to a three-step branching model. The consequent energetic and kinetic profiles reveal the partial reactions that constitute the recognition mechanism of TBP for this variant promoter sequence. This work makes possible a detailed comparison of TBP interactions with the variant C7 sequence and two strong consensus promoter sequences, AdMLP and E4, characterized previously.

The interactions of *S. cerevisiae* TBP with the consensus AdMLP and E4 promoters have been shown previously to be accommodated by a three-step linear model (Equation 4) (16, 20). This model cannot accommodate the TBP-C7 interaction. Features common to both consensus reactions include a linear progression through two intermediates having fully bent DNA, the first intermediate species present at high mole fraction throughout the reaction and at equilibrium, and the second intermediate present at low mole fraction. The reactions proceed through very similar entropic and enthalpic changes during the first step but differ significantly for the second and third steps. These changes lead ultimately to very similar TBP-DNA structures in solution and co-crystals, both with the DNA helix severely bent (23–28, 34, 35).

How do the partial recognition reactions compare for the variant and consensus sequences? Two striking differences present themselves. Most importantly, TBP binding with the C7 variant yields complexes having DNA with different bends, one with DNA bent to only approximately half the extent of that in the other complexes. This result contrasts with the consensus reactions in which TBP induces exclusively the final, severe DNA bend in all bound species. In addition, the variant interaction proceeds via two branching pathways, one in which TBP-DNA\textsubscript{A, SEVERE} is formed directly rather than through an intermediate species.

On the other hand, the interaction with the variant sequence along the other branch is remarkably reminiscent overall of the reaction with the consensus sequences. Binding and DNA bending are simultaneous, proceed through an intermediate DNA species, and yield conformers with strongly bent DNA. The initial binding/bending event for both variant and consensus promoters (TBP + C7 → TBP-C\textsubscript{7, SEVERE} and TBP + DNA → I\textsubscript{1}, respectively) occurs via a large energetic barrier together with the largest stepwise increase in entropy. Likewise, the thermodynamic changes characterizing the second step of this pathway for C7 (TBP-C\textsubscript{7, SEVERE} → TBP-C\textsubscript{7, C, SEVERE}) follow a pattern very similar to those for the combined second and third steps of the TBP-AdMLP and TBP-E4 interactions (I\textsubscript{1} → I\textsubscript{2} → I\textsubscript{3}).
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Table II
Globally derived TBP-C7 kinetic and thermodynamic parameter values corresponding to the three-step model with two branching pathways (Equation 7). Values shown are for 25 °C and 1 M standard state for each “step” i, along the reaction pathway (Equation 7).

| i | $k_i$ | $\Delta H_i^{\circ}$ | $\Delta S_i^{\circ}$ | $\Delta G_i^{\circ}$ |
|---|---|---|---|---|
| 1 | 0.169 [0.151, 0.207]$^a$ | 23.9 [22.4, 25.6] | 18.1 [13.1, 24.1] | 18.5 [18.4, 18.6] |
| 2 | 1.02 [0.961, 1.30] μM$^{-1}$ s$^{-1}$ | 22.7 [19.8, 24.9] | 45.1 [35.6, 52.6] | 9.3 [9.1, 9.3] |
| 3 | 1.23 [1.13, 1.29] μM$^{-1}$ | 40.8 [40.2, 43.0] | 106.2 [104.1, 113.4] | 9.1 [9.1, 9.2] |
| 4 | 0.0736 [0.0716, 0.0854] | 10.1 [9.0, 11.5] | −29.8 [−33.3, −24.9] | 19.0 [18.9, 19.0] |
| 5 | 0.00685 [0.0058, 0.00115] | 34.5 [28.5, 37.0] | 42.6 [32.3, 51.2] | 21.8 [21.5, 21.9] |
| 6 | 0.00299 [0.00277, 0.00503] | 29.3 [24.7, 31.7] | 28.2 [13.5, 36.7] | 20.9 [20.6, 20.9] |

$^a$ Numbers in brackets represent the upper and lower error bounds corresponding to the 68% confidence region, determined as described previously (16). $k_2$ and $k_3$ are second order rate constants with units of μM$^{-1}$ s$^{-1}$; $k_1$ and $k_4$ through $k_6$ are first order rate constants. A quantum yield value of 0.985 [0.970, 0.987] is obtained for TBP-DNA,SHALLOW and 0.626 [0.599, 0.630] for TBP-DNA,SEVERE and TBP-DNA,SEVERE. These relative quantum yield values reflect the extent of DNA bending (16).

Figure 5. The mole fraction of each species along the TBP-C7 reaction pathway (Equation 7) at 15 °C (A), 20 °C (B), 25 °C (C), and 30 °C (D), generated from the globally derived parameters using saturating TBP. The time-dependent mole fraction of each species varies as a function of temperature, as shown here, and as a function of [TBP].

TBP-DNA,CONSENSUS (Fig. 6). The TBP-C7 reaction has only one intermediate along the path with a severe bend, unlike that for AdMLP and E4. This result may not reflect a real difference between the recognition processes for the two types of promoters but, rather, that meaningful determination of two additional rate constants and one additional quantum yield is not possible from the data. The dominance of TBP-DNA,SHALLOW over the course of the reaction and its persistence at equilibrium, much like I₁ for the strong promoters (16, 20), reflects kinetic similarities between the variant and consensus partial reactions. A prevalent TBP intermediate, corresponding closely both structurally and kinetically to I₁, further implicates this conformer as a biologically relevant species to which transcription factors may bind in subsequent steps of pre-initiation complex assembly (16, 20).

The atomic resolution TBP-C7 co-crystal structure revealed a C:G Hoogsteen base pair at position 7 (28). This adaptation was unique among twelve such sequences investigated and apparently allowed the C7 helix to conform to the binding site to yield a complex with strongly bent DNA. The branching pathways of the TBP-C7 interaction may correlate with this potential to form an alternate base pairing. In this view, a steric incompatibility between the exocyclic NH₂ of guanine and Leu-72 of TBP associated with Watson-Crick base pairing at position 7 interferes with successful intercalation of the 3'-phenylalanine pair, yielding a bound conformer with only partially bent DNA. This scenario is consistent with the negative enthalpic change and very slight entropic increase leading to formation of TBP-DNA,SHALLOW. On the other hand, the “right” branch may reflect formation of TBP-C7,SEVERE via the Hoogsteen base
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FIG. 6. A graphical representation of the thermodynamic changes associated with each step along the TBP-C7 (solid line) reaction pathway (Equation 7) at 25 °C and 1 M standard state for the enthalpy of activation, entropy of activation, and free energy of activation. TS denotes the transition state species. Also shown are the thermodynamic changes for TBP-AdMLP (dotted line) and TBP-E4 (dashed line) along the linear two-intermediate pathway (Equation 4). The I1 conformer in Equation 4 corresponds to TBP-DNA_{shallow} in the figure. The final two steps characterizing the consensus promoter pathways (I1 → I2 → TBP-DNA_{consensus}) have been combined and are represented by TBP-DNA_{shallow} → TBP-DNA_{severe}, and all associated transition state species have been deleted so that only corresponding species are compared.

FIG. 7. TBP-T*C7_dpx*F dissociation kinetic data obtained at 15 °C using 35.5 μg unlabeled DNA (○) and the corresponding pure replacement curve (×). The fast phase of the replacement process for bound C7 occurs from two species, TBP-C7_{A, shallow} and TBP-C7_{B, severe}. The fast phase replacement rate is approximately a mole fraction-weighted average of k_s and k_p with an amplitude reflecting the sum of the fractional populations of the two species at equilibrium. The slow replacement rate derives from TBP-C7_{B, severe → TBP + DNA} with a rate constant k_p. To determine the predominant species from which dissociation occurs, theoretical dissociation curves were generated as described (20), including displacement from either TBP-C7_{A, shallow} (dashed line) or TBP-DNA_{B, severe} (solid line). Due both to the very low equilibrium population of TBP-C7_{B, severe} and a slow rate constant of formation (k_f), this conformer is not a significant participant in the displacement process. Accommodation of the dissociation data to within error at all temperatures can result from displacement only from TBP-DNA_{B, severe} (solid line). In contrast, displacement only from TBP-C7_{A, shallow} yields large errors (residuals 1.5–4× error) and systematic deviation of the calculated from the observed curves. A rapid rate of displacement from TBP-C7_{A, shallow} alone is required to be added to the pure replacement curve for the sum to mimic the fast phase of the observed dissociation. However, rapid removal of this predominant reaction species at 15 °C precludes significant contributions of TBP-C7_{B, severe} to overall dissociation, thus eliminating the slow replacement phase and producing a severe lack of fit (dashed line). The first 150 s of the TBP-C7 dissociation reaction at 15 °C and the simulated results are shown in the inset.

The intermediate conformers in the displacement reaction and further support the proposal that TBP displacement by regulatory proteins may occur from these species (20).

TBP binding to C7 in solution induces a bend in the DNA helical axis with an average apparent angle significantly less than that of bound AdMLP and E4 (34). The results of model fitting reported herein show that the C7 bend derives from a weighted average of the bends in three TBP-bound species present at equilibrium: one conformer with DNA bent only slightly, TBP-C7_{A, shallow}, and two conformers with DNA bent severely, TBP-C7_{B, severe} and TBP-C7_{C, severe}. The enthalpy change corresponding to the transition from a shallow bend to a severe DNA bend in the complex is 37.1 kcal mol$^{-1}$, with ΔS° = 123.4 cal K$^{-1}$ mol$^{-1}$. TBP-C7_{A, shallow} and TBP-C7_{B, severe} dominate the reaction even at equilibrium, with the mole fraction ratios of the three species dependent upon both temperature and TBP concentration.

The solution bend angle for TBP-bound C7 is temperature-dependent (Table I), in contrast to those of bound AdMLP and E4 (20, 34). This variability was attributed to temperature-dependent population shifts between complexes with slightly and strongly bent DNA. The corresponding probability distributions were analyzed using a two-state model with the value of R_{shallow} fixed at 53.3 Å as described (34, 35). The data were well accommodated and yielded a value for R_{severe} of 50.1 Å. These mean distances correspond to DNA bend angles for the two bound conformers of −30° and −60°. This two-state model has been shown previously to accommodate the sequence and
osmolyte concentration dependence of TBP-bound DNA bend angles. The model is now extended to include the temperature dependence of the bound C7 bend, with the bend angle for the severely bent DNA somewhat smaller than that obtained previously.

Global analysis of the collective TBP-C7 kinetic and thermodynamic data was conducted with the assumption that the microscopic $\Delta C_P^\text{rxn}$ term associated with each intrinsic $\Delta H^\circ$ is negligible over the temperature range of the FRET measurements. The finding of multiple TBP-C7 species with different structural and energetic properties led us to investigate the contributions of temperature-dependent shifts in the equilibrium populations of these conformers to an overall heat capacity change for the binding reaction (46). The experimental equilibrium constants determined directly using steady-state FRET are shown in Fig. 8 together with the best least-squares fit to a straight line. The theoretical equilibrium constants corresponding to Equation 7, $K_{eq}^\text{apparent}$, were generated from the values of the rate constants and activation energies (solid line in Fig. 8). Both lines accommodate the data over the experimental temperature range, although the kinetic model assumed neither heat capacity changes for the individual reaction steps nor a van't Hoff correspondence. $\Delta C_P^\text{rxn}$, the heat capacity change for the reaction corresponding to $K_{eq}^\text{apparent}$, is not constant with temperature from 0 to 40 °C (Fig. 9), with a maximum value of 1.58 kcal K$^{-1}$ mol$^{-1}$ at 18 °C. This $\Delta C_P^\text{rxn}$ derives solely from shifts in the populations of conformers with shallowly and severely bent DNA that have differing values of $\Delta H^\text{rxn}$, referenced to free TBP + DNA.

Conclusions—This work further defines the relationships among TATA box sequence, TBP-TATA solution structures, and TBP-TATA recognition mechanisms. A single base pair substitution in the consensus AdMLP TATA sequence has dramatic functional consequences, yielding kinetics for association and dissociation that differ significantly from those of the parent promoter and another strong promoter, E4. Global analysis reveals a branching mechanism for the TBP-C7 interaction having a partial reaction that is absent from the consensus interactions. The solution structure of the TBP-C7 complex likewise differs from those of the consensus sequences and is temperature-dependent. A two-state model has now been shown to accommodate the dependence of TBP-TATA bend angles on sequence, osmolyte concentration, and temperature.

FIG. 8. Analysis of heat capacity changes deriving from temperature-dependent shifts in TBP-DNA

FIG. 9. The predicted heat capacity change for the reaction modeled as in Equation 7 with $\Delta C_P^\text{rxn} = 0$ for each step in the mechanism. The variations in the value of $\Delta H^\text{rxn}$ among the TBP-C7 conformers with differently bent DNA give rise to values of $\Delta H^\text{rxn}$ that are strongly temperature dependent, in contrast to the usual calorimetric assumption.

These functional and structural differences in the TBP-C7 variant complex correspond to diminished transcription efficiency relative to consensus binary complexes. Studies are currently underway to determine whether TBP recognizes variant TATA sequences similarly or uniquely.

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