Native Human TATA-binding Protein Simultaneously Binds and Bends Promoter DNA without a Slow Isomerization Step or TFIIB Requirement*

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The association of TATA-binding protein (TBP) with promoter DNA is central to the initiation and regulation of eukaryotic protein synthesis. Our laboratory has previously conducted detailed investigations of this interaction using yeast TBP and seven consensus and variant TATA sequences. We have now investigated this key interaction using human TBP and the TATA sequence from the adenovirus major late promoter (AdMLP). Recombinant native human protein was used together with fluorescently labeled DNA, allowing real time data acquisition in solution. We find that the wild-type hTBP-DNA reaction is characterized by high affinity (\(K_d = 5 \text{ nM}\)), simultaneous binding and DNA bending, and rapid formation of a stable human TBP-DNA complex having DNA bent \(-100^\circ\). These data allow, for the first time, a direct comparison of the reactions of the full-length, native human and yeast TBPs with a consensus promoter, studied under identical conditions. The general reaction characteristics are similar for the human and yeast proteins, although the details differ and the hTBP*-induced bend is more severe. This directly measured hTBP*-DNA interaction differs fundamentally from a recently published hTBP*-DNA model characterized by low affinity (\(\mu\text{M}\)) binding and an unstable complex requiring either a 30-min isomerization or TFIIB to achieve DNA bending. Possible sources of these significant differences are discussed.

The recognition and binding of promoter TATA sequences (DNA\(_{TATA}\)) by the TATA-binding protein (TBP) is central to the initiation and regulation of gene transcription in eukaryotes. The importance of the TBP-DNA\(_{TATA}\) interaction has prompted extensive biochemical and biophysical investigations, which have revealed complex binding mechanisms and dramatic TBP-induced helical distortion, including DNA\(_{TATA}\) bending and unwinding (see Ref. 1 and references therein and Refs. 2–11). The extent of the DNA\(_{TATA}\) bending is TATA sequence-dependent in solution (8, 9, 12, 13). Because these collective results have been obtained using different TBPs, full-length and truncated proteins, and diverse experimental conditions, key issues relating to the behavior and structure of TBP-promoter complexes remain unresolved.

The 180-residue C-terminal DNA binding domains (CTD) of the TBPs from yeast (\(y\)TBP), human (hTBP), and Arabidopsis thaliana (\(a\)TBP) are highly conserved, with greater than 80% homology and with generally conservative substitutions in the remainder. The pseudo-symmetric TBCD contacts the duplex along the distorted minor groove via interactions that are largely non-polar and hydrophobic. The amino acid residues involved in these DNA contacts are nearly 100% conserved, with only a single variation in which Arg-204 in hTBP complexes to Lys-110 in \(y\)TBP and Lys-68 in \(a\)TBP (2). The N-terminal domain is, in contrast, heterogeneous between the yeast (60 amino acids (14)) and human (159 amino acids (15)) proteins and severely truncated in the \(A.\) thaliana protein (18 amino acids (16)).

How these similarities and differences among TBP molecules from different species affect the TBP-DNA\(_{TATA}\) interaction and structure remains an open question. A limited answer has emerged from crystallographic studies on the C-terminal domains of \(y\)TBP and hTBP and the (analogous) full-length aTBP, bound to DNA\(_{TATA}\). The structures of these three TBP-DNA complexes are essentially identical in the crystalline form (2, 17, 18) and are TATA sequence-independent at least for \(y\)TBP (6). Beyond these results, however, comparisons of the behavior of \(y\)TBP, hTBP, and aTBP with DNA\(_{TATA}\) and the structures of the resulting binary complexes must be inferred from unrelated experiments conducted in different laboratories using any one of these three proteins, either as the full-length or truncated C-terminal form. In addition, although a clear understanding of the behavior of the human protein is of obvious interest, most of the work done to date has utilized Saccharomyces cerevisiae, or yeast, TBP. The extent to which these latter results also hold true for hTBP is currently largely a matter of speculation.

The recognition mechanisms of TBP for promoter DNA and the solution structures of TBP-DNA complexes have been primary research interests in our laboratory for several years (4, 7–11). Our focus has been 2-fold: 1) identifying and comparing detailed kinetic models for TBP binding to consensus (TATA\(_a/\tilde{a}/N\)) and variant promoter TATA sequences, and 2) evaluating the solution structure of the TBP-DNA\(_{TATA}\) complex.

This paper is available on line at http://www.jbc.org

Received for publication, May 17, 2003
Published, JBC Papers in Press, June 5, 2003, DOI 10.1074/jbc.M305201200
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These studies utilize DNA duplexes that are labeled with fluorescent dyes that serve as a donor-acceptor pair for Förster resonance energy transfer (FRET). The methodologies employed in these biophysical studies allow the collection of kinetic data in real time as well as precise measurements of inter-dye distances that correspond to the extent of helical bending in the TBP-DNA complexes.

Our prior work has been conducted using full-length S. cerevisiae TBP. Function studies conducted using three TATA sequences have shown multistep interaction mechanisms overall with concurrent binding and DNA bending (7, 10, 11). The associated structure studies have revealed TATA sequence-dependent DNA bend angles for yTBP-DNA complexes in solution, with bends varying from ~30 to ~80° for the seven sequences examined. These bend angles are strongly correlated with relative transcription activity (8). The sequence dependence of the yTBP-induced DNATA bend angles in solution complexes is in direct contrast to the invariance of 11 crystal-line yTBPCTD-DNATA complexes: all of these complexes, in which the TATA sequence substitutions were identical or similar to those in the solution studies, had essentially identical crystal structures with ~80° DNA bend angles (6). In solution, only consensus TATA sequences are bent by yTBP as severely as in the crystal structures in the absence of high osmolyte concentrations (9). These functional and structural differences highlight the fact that generalizations cannot safely be made about the behavior and architecture of TBP-promoter complexes between full-length and truncated proteins, between complexes in solution and in crystals, or among TBPs from diverse species and emphasize the need for clear comparative studies.

To directly address this issue, we have expanded our biophysical studies to include the interaction of full-length wild-type human TBP with promoter DNA. The work reported herein includes the binding affinity and kinetics of hTBP with DNA bearing the adenovirus major late promoter (AdMLP) and is, to our knowledge, the first published kinetic data in real time for this interaction. In addition, we estimate the bend angle induced in this consensus sequence by hTBP in solution. Our results show that hTBP rapidly and simultaneously bends and binds promoter DNA and induces a bend angle even larger than that induced by yTBP. This reaction is broadly comparable with those with the yeast protein, although the mechanistic details clearly differ, and the distortion in the helical trajectory appears to be significantly larger in the human protein-DNA complex.

Our results differ sharply with the inferences drawn from the electrophoretic mobility shift assay studies of Zhao and Herr (19). They recently proposed a model in which full-length wild-type hTBP binds initially to consensus DNAAML to yield an unstable complex having unbent DNA, with introduction of the helical bend occurring only after ~30 min. The significant differences between their results and ours are discussed.

EXPERIMENTAL PROCEDURES

Materials—The 14-nucleotide DNA oligomer bearing the AdMLP TATA box sequence was labeled on the coding strand at the 3′ and 5′ ends with TAMRA and fluorescein, respectively (TAMRA-CGCTTATAAAAGGCG-fluorescein, T-ML-F) attached via 6-carbon linkers. This probe and the corresponding probes having only the fluorescein label were synthesized and highly purified by TriLink Biotechnologies, Inc. (San Diego, CA). The unlabeled complementary strand was also obtained from TriLink. These same probes have been fully characterized and used extensively in previous studies of yeast TBP-DNA binding (4, 7–11).

Recombinant human TBP, containing only the native amino acid sequence, was expressed in the Escherichia coli strain BL21(DE3) and purified, with slight modifications, as described (20). In the purification, non-ionic detergents were omitted, thus avoiding any potential complications from abnormal association/aggregation, such as those seen previously with yeast TBP (21). Protein purity was analyzed using SDS-PAGE with Coomassie stain, and the mass of the protein was determined using matrix-assisted laser desorption ionization/time of flight mass spectrometry. The protein activity was determined as described (8) using a fluorescence assay with stoichiometric concentrations of protein and DNA. Briefly, equimolar concentrations of duplex DNA and protein were mixed at concentrations ~Kd and the fractional saturation determined from the steady-state spectra. The ratio of the observed to predicted binding gave the fraction of active protein. The concentrations reported are for active protein. All experiments were conducted using ~800 nM protein, concentrations at which protein dimerization is negligible (2). All reactions were conducted at 20 ± 0.05 °C in 7.4, 100 mM Tris, pH 7.4, 100 mM KCl, 1 mM CaCl2, 2.5 mM MgCl2, 5 mM dithiothreitol, 5 μM leupeptin, and 10% glycerol.

Theory of FRET—Förster developed the basic theory for the phenomenon of the resonant transfer of energy between a suitable donor fluorophore and acceptor chromophore (22–24). Stryer and Haugland (25) elegantly confirmed these principles experimentally and Webber advanced the ideas, techniques, and instrumentation for applications to biomolecular systems (reviewed in Ref. 26). An extensive body of literature established since that time has further addressed FRET theory and its application in myriad biomolecular systems. Very briefly, quantum mechanical coupling of the electronic transitions in two fluorophores (reflected in the overlap of the donor emission and acceptor absorption spectra) permits excited state energy in the donor molecule to be transferred directly to the excited state of the acceptor molecule without emission and re-absorption of a photon. The probability of such transfer depends on the extent to which the transition moments of these two molecules assume the appropriate relative orientations. The rate of the energy transfer process depends on the inverse sixth power of the distance separating the two molecules, conferring an exquisite dependence of the transfer rate on the inter-dye distance.

The extent to which energy is transferred is readily observable in the fluorescence emission spectra of donor and acceptor dyes attached to biomolecules; efficient energy transfer decreases the emission intensity of the donor and increases that of the acceptor. We have discussed in detail the basics of FRET, as it is used in the present work, utilizing DNA labeled with 3′-fluorescein (donor) and 5′-TAMRA (acceptor), in a number of related studies (4, 7–11, 27–29).

Instrumentation, Data Acquisition, and Analysis—Steady-state spectra were obtained using aPhoton Technologies, Inc. (Lawrenceville, NJ) model A-1010 steady-state fluorimeter together with a Coherent (Santa Clara, CA) model INN-DA-100 argon laser ion tuned to 488 nm with the power set at 15–20 milliwatts. DNA duplex was formed using 8 nM T-ML-F with 5× complement, shown in separate experiments to yield full hybridization of the labeled strand. The titration was conducted using seven concentrations of hTBP ranging from 0 to 175 nM. Each successive titration point was obtained using a new duplex preparation in order to minimize any effect of protein present. Spectra were collected from 500 to 600 nm and base line corrected at each hTBP concentration. Determinations of the corresponding fractional DNA saturation, the equilibrium association constant (Kd), and the titration end point have been described (7).

Fluorescence stopped-flow data were collected and analyzed exactly as described (7, 10, 11) using 20 nM T-ML-F* and 140 nM hTBP (concentrations after mixing). Briefly, a customized stopped-flow device having a 1.8-missed dead time was used to collect a total of 2000 points/curve over ≤40 s with the time constant = 12.24 ms and the fluorescein emission isolated using a 520-nm interference filter. Replicate curves were corrected for background and very well fit to a tri-exponential decay model. The slowest phase, which has been shown to correspond to fluorescein photobleaching (4, 7), was extracted, and the resulting decay parameters were averaged.

Previous mechanistic studies using yTBP were conducted in buffer identical to that in the present study but without 10% glycerol. To compare unambiguously, the comparison between these and those obtained previously using yTBP, the binding isotherm and stopped-flow kinetics were re-collected for the yTBP-T-ML-F interaction in the presence and absence of 10% glycerol using 200 nM yTBP.

RESULTS

The functional and structural characteristics of the interaction of hTBP with the AdMLP consensus TATA sequence (TATAAAG) have been investigated in solution using full-
length, wild-type protein and fluorescently labeled DNA. The protein was expressed and purified without tagging or exposure to non-ionic detergents. SDS-gel electrophoresis showed purity >99% and an estimated mass from the relative mobility of ~38 kDa (Fig. 1), consistent with that of the native protein. The mass obtained from matrix-assisted laser desorption ionization/time of flight spectral analysis confirmed that the purified protein was full length. The fluorescence assay showed the DNA binding activity to be ≥70%, slightly lower than the activity of the yTBP determined previously using the same assay and independently confirmed by DNase footprinting. A full characterization of the protein will be reported elsewhere.

The 14-bp duplex DNA (5'-MLdpx*F, top strand TAMRA-5'-CGCTATAAAAGGC-3'-fluorescein) and similar probes have been used extensively in our studies with yTBP (4, 7–11, 27, 29). The fluorescence emission from the two dyes directly signals changes in the distance separating the TAMRA and fluorescein dyes, due to the corresponding changes in FRET (Fig. 2), and has been shown in our previously published studies to be highly sensitive to changes in the 5'-3' distance as the duplex is bound and bent by TBP (details in the legend for Fig. 2).

Steady-state spectra are shown in Fig. 2 for 5'-MLdpx*F alone in solution (A) and hTBP-bound (B). The latter was obtained within 1 min following addition of the hTBP to the DNA. The dramatic difference in the signal between the unbound and bound DNA derives from a large decrease in the distance between the 5'-TAMRA and the 3'-fluorescein and has been shown to directly reflect the extent of helical bending induced by the protein (8, 9). Both spectra were monitored for an additional 60 min and yielded superimposable spectra over this period (Fig. 2, A and B). This observation is particularly important for the TBP-DNA complex (Fig. 2B) because it demonstrates the invariance of the conformation of the hTBP-bound duplex throughout a 1-h period. These spectra are sufficiently sensitive to changes in the DNA structure that changes of even a few degrees in the helical bend of the protein-bound DNA can be precisely distinguished (28, 29). In addition, such superimposable spectra reflect the stability of the DNA and protein preparations.

Titrations of 5'-MLdpx*F with hTBP were conducted using steady-state emission spectra. The fractional DNA saturation determined from the spectral changes observed at each point was used to construct the equilibrium binding isotherm (Fig. 3). These data reveal the high affinity of the human protein for this duplex, with a dissociation equilibrium constant of 5 nM at 20 °C. By comparison, yeast TBP binds the AdMLP sequence with $K_d = 21$ nM (10), a value confirmed in the present study to be independent of the presence or absence of 10% glycerol. The percent decrease in the ratio of the peak emission intensities at full DNA saturation was calculated to be ~60%. This spectral change upon hTBP binding is significantly larger than the 44 ± 1.5% decrease obtained with yTBP binding to the same labeled DNA duplex. The corresponding control experiments show that the emission of the donor-only labeled duplex, MLdpx*F, is invariant for the unbound and TBP-bound duplex for both the human and yeast proteins. The implications of these observations for helical bending are discussed below.

The kinetic trace of hTBP binding to the AdMLP duplex was monitored using fluorescence stopped-flow (Fig. 4, closed cir-
ingly, the overall pattern that is emerging is one of unique recognition pathways. The three yTBP-DNATATA interactions have been shown for the yTBP-DNATATA interactions to arise from the presence of at least two intermediate species, both of which have fully bent DNA. Notably, the two phases observed during the course of TBP-DNA association do not reflect partial bending of the DNA followed by completion of bending; rather, the ordinate in Fig. 4 reflects a progressive increase in the time regime (8, 10). Time-resolved decays for the fluorescein emission due to the decreased inter-dye distance and the corresponding increase in the rate of energy transferred away from the FRET donor. The equilibrium binding constant obtained from the data in Fig. 3 predicts ~96% saturation of the DNA for these concentrations, in excellent agreement with the observed amplitude decrease of 53%. The theoretical curve (solid line) was generated from the optimal parameter values describing the two phases that reflect the association reaction and excludes the trivial photobleaching of fluorescein. The poor fit of these hTBP-DNA reaction kinetics to a mono-exponential decay model (dotted line) emphasizes the multiphasic nature of this process. The kinetic curves for T*MLdpx*F reacting with hTBP (solid line) and yTBP (dashed line) are compared in the inset. The latter curve was generated from the previously determined rate constants (7) for 140 nm protein at 20 °C. Control experiments in the present study showed no difference within error for the yTBP-DNA kinetic traces in the presence or absence of 10% glycerol.

The central finding of this work is that full-length wild-type human TBP rapidly binds to and concurrently bends the AdMLP consensus promoter, forming a stable hTBP-DNA complex. This result is consistent with the results that we have obtained for native yeast TBP binding consensus and variant TATA sequences, which show multiphasic association occurring with overall rates generally comparable with those obtained herein and which show binding and bending to be in separable processes (4, 7, 10, 11). The multiphasic kinetics have been shown for the yTBP-DNA_hTBP interactions to arise from the presence of at least two intermediate species, both of which have fully bent DNA_hTBP. Notably, the two phases observed during the course of TBP-DNA association do not reflect partial bending of the DNA followed by completion of bending; rather, the ordinate in Fig. 4 reflects a progressive increase in the “average” bend angle for the entire DNA population as unbound duplex binds to and is bent by the TBP.

With yTBP, even single base pair substitutions in the TATA sequence result in significant differences in the binding isotherms, kinetics (7, 10, 11), and the degree of the TBP-induced DNA bend angle in solution (9, 11). These differences reflect unique recognition pathways. The three yTBP-DNA_TATA interactions examined in most detail differ in the kinetics and thermodynamics of the partial reaction steps and in the concomitant uptake or release of salt and water. We have proposed that these variations may derive in part from differences in the inherent flexibility of the sequences and the corresponding compatibility along the protein-DNA interface. However, the common theme remains that yTBP-promoter recognition proceeds with simultaneous TBP-DNA binding and helical bending to rapidly form very stable complexes.

DISCUSSION

Our laboratory has conducted solution studies of TBP-DNATATA function and structure for >10 different TBP-DNA combinations. Previous studies were conducted using consensus and variant TATA sequences, all with the same preparation of S. cerevisiae TBP. In the present study we have done just the opposite; we have used human rather than yeast TBP, together with one of the promoter-bearing DNA duplexes from the previous studies, T*MLdpx*F. These combinations afford very clear comparisons of the bending interactions and solution structures of TBP-DNA_TATA complexes. Perhaps not surprisingly, the overall pattern that is emerging is one of “variations on a theme.”

With yTBP, even single base pair substitutions in the TATA sequence result in significant differences in the binding isotherms, kinetics (7, 10, 11), and the degree of the TBP-induced DNA bend angle in solution (9, 11). These differences reflect unique recognition pathways. The three yTBP-DNA_TATA interactions examined in most detail differ in the kinetics and thermodynamics of the partial reaction steps and in the concomitant uptake or release of salt and water. We have proposed that these variations may derive in part from differences in the inherent flexibility of the sequences and the corresponding compatibility along the protein-DNA interface. However, the common theme remains that yTBP-promoter recognition proceeds with simultaneous TBP-DNA binding and helical bending to rapidly form very stable complexes.
donor in the absence and presence of TAMRA acceptor are used to obtain the mean 5‘–3‘ distance, for both the unbound and TBP-bound duplex, from which model-dependent bend angles may then be calculated. The present study employs one of these same probes, and it is possible to combine the time-resolved yTBP-AdMLP data with the steady-state hTBP-AdMLP data to estimate a bend angle for the latter; we know the mean end-to-end distance of unbound T′-MLdup,F and also that the width of this inter-dye distance distribution, σ, is nearly invariant for unbound and TBP-bound duplex (8). Furthermore, both the time-resolved and steady-state emission of the duplex labeled only with the donor fluorescein, MLdup,F, remain unchanged upon addition of saturating amounts of hTBP. Consequently, the difference in the steady-state emission of T′-MLdup,F unbound and hTBP-bound may be attributed to a change in the inter-dye distance due to increased FRET with bending. Finally, we know the steady-state intensity changes for this reaction then proceeds along one of two pathways: either this hTBP-DNAunbent complex is, along one reaction pathway, followed by the very slow introduction of a helical bend. Because the latter is a first-order process, the rate of this bending would be independent of the relatively high reactant concentrations in the cell and would presumably proceed at the rate reported by Zhao and Herr (19), over the course of ∼30 min. The TBP-DNA complex having bent DNA is almost certainly the transcriptionally active form, yielding the architectural scaffolding necessary for the assembly of subsequent transcription factors. The proposed isomerization process would therefore be, overwhelmingly, the rate-limiting step in formation of hTBP-DNAbent along this pathway. Realistically, any hTBP thus bound would be effectively trapped and unavailable for nucleating assembly of the transcription machinery. The pathway in which TFIIb binds to hTBP-DNAbent can likewise reasonably be neglected since this complex would be available for assembly of the pre-initiation complex only after ∼30 min following the initial binding of hTBP. This kinetic trapping would eliminate such complexes from participating in the cellular response to the need for synthesizing protein.

According to the alternative reaction pathway, TFIIb would bind to hTBP-DNAunbent and induce a helical bend. The hTBP-DNAunbent complex reported by Zhao and Herr (19) is 31689

The implications of such a mechanism for cellular functioning in humans are significant. In this model, formation of the hTBP-DNAunbent complex is, along one reaction pathway, followed by the very slow introduction of a helical bend. Because the latter is a first-order process, the rate of this bending would be independent of the relatively high reactant concentrations in the cell and would presumably proceed at the rate reported by Zhao and Herr (19), over the course of ∼30 min. The TBP-DNA complex having bent DNA is almost certainly the transcriptionally active form, yielding the architectural scaffolding necessary for the assembly of subsequent transcription factors. The proposed isomerization process would therefore be, overwhelmingly, the rate-limiting step in formation of hTBP-DNAbent along this pathway. Realistically, any hTBP thus bound would be effectively trapped and unavailable for nucleating assembly of the transcription machinery. The pathway in which TFIIb binds to hTBP-DNAbent can likewise reasonably be neglected since this complex would be available for assembly of the pre-initiation complex only after ∼30 min following the initial binding of hTBP. This kinetic trapping would eliminate such complexes from participating in the cellular response to the need for synthesizing protein.

Along the other proposed reaction pathway, TFIIb would bind to hTBP-DNAunbent and induce a helical bend. The hTBP-DNAunbent co-crystal structure shows that the bend in the TATA sequence results from the partial intercalation of pairs of phenylalanine residues of the hTBP into the DNA helix, with the minor groove of the DNA fully interfaced with the protein-binding site. In addition, we know from crystallography that the conformation of the TBP-TATA is very similar before and after DNA binding. Furthermore, the TFIIb contacts the DNA helix both upstream and downstream of TBP, contacts facilitated by the TBP-induced bending of DNA. These facts taken together make it difficult to envision a mechanism whereby TFIIb could induce the DNA bend in a pre-formed TBP-DNAunbent complex. In addition, we observe stable complexes with fully bent DNA-TATA within 150 s of the addition of relatively low concentrations of hTBP in the absence of TFIIb. A model in which hTBP-DNAunbent complexes are kinetically trapped in a transcriptionally inactive form unless and until TFIIb binds and induces a bend in the DNA is thus called into question.

Zhao and Herr (19) cite precedent for this slow-bending model with a previously proposed two-step mechanism for S. cerevisiae TBP-DNA binding (30). This comparison is inaccurate. First, Hoopes et al. (30) reported formation of a stable yTBP-DNA_TATA complex with an association half-time of ∼1 min. This result is in good agreement with our data but inconsistent with that reported by Zhao and Herr (19) for hTBP. Second, Hoopes et al. (30) stated specifically that their data were not consistent with a process that included a rate-limiting isomerization step. Finally, the data that supported this yTBP model have been shown to be more appropriately interpreted as simply formation of a transient encounter complex leading to successful binding rather than as a true two-step process (7). In fact, the hTBP-DNA data reported by Zhao and Herr (19) differ strikingly from the data obtained for yTBP-DNA by both Hoopes et al. (30) and our laboratory, and their proposed model differs fundamentally from the models for yTBP recognition of three TATA sequences proposed previously by our laboratory (4, 7, 10, 11).

Several observations raise concerns about the results upon which the Zhao and Herr model is predicated. An inspection of their published gel results shows that only a very small fraction (∼1–5%) of the native hTBP used in these experiments was active in DNA binding (see Figs. 1–7 in Ref. 19). A dissociation equilibrium binding constant \( K_d \) can be calculated from this
fraction of binding and the reported concentrations, yielding a $K_d$ value between 0.5 and 2.0 $\mu$M. This value reflects binding affinity that is about 3 orders of magnitude lower than ours and is in the range usually associated with nonspecific binding. Such low affinity raises the possibility that the DNA-binding properties of the small fraction of active hTBP present in those experiments may have been compromised. Such altered functionality might also be expected to alter the ability of the protein to induce helical bending, since we know of no example of such low affinity binding that results in bent DNA.

One potential area of agreement between our results and those of Zhao and Herr (19) involves their mutant hTBPs. They identified a group of hTBP molecules having single residue substitutions that rapidly bound and bent promoter DNA, behavior that is in good agreement with our data for wild-type hTBP. One possible explanation for this result is that the non-native residues remaining at the thrombin cleavage site of their GST-fusion protein interacted adversely with the wild-type but not mutant TBP. If their mutations were in fact functionally innocuous, these modified proteins would then display normal DNA binding/bending activity. Whatever the explanation, it is clear from their results that their mutant hTBPs show much higher DNA binding activity and form far more stable complexes with DNA than does their native hTBP.

The data presented herein show unambiguously that native hTBP binds to and concurrently introduces a severe bend in $T^*ML_{dpse}^*F$ in a reaction that is in general accord with that of yTBP. The agreement between the stopped-flow and steady-state amplitudes shows that the processes of binding and bending are coincident, a particularly clear result since ML_{dpse}^*F shows no change. Thus, the concomitant binding and bending behavior that is in good agreement with our data for wild-type hTBP.

However, the differences in affinity and in the stopped-flow curves for hTBP and yTBP binding to AdMLP, together with the increased severity of the hTBP-induced bend, suggest that the recognition mechanism of these two proteins differ. Because the multiphasic association kinetics for the hTBP-AdMLP reaction are reminiscent of those for the yeast protein, it seems reasonable to hypothesize that the hTBP-DNA recognition pathway will include formation of at least one intermediate species. A detailed description of each partial reaction along this pathway requires collection and analysis of extensive thermodynamic and kinetic data sets, work that is now beginning. Because our investigations with human and yeast TBPs use the identical labeled promoter DNA and the wild-type, full-length proteins, these solution studies yield a detailed and dependable comparison of the way in which these two proteins recognize a consensus TATA sequence.

Acknowledgments—We thank Professors Mark Grier and Michael Brenowitz for reading and commenting on the manuscript.

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J. Biol. Chem. 2003, 278:31685-31690.
doi: 10.1074/jbc.M305201200 originally published online June 5, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M305201200

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