DNA Sequence-dependent Differences in TATA-binding Protein-induced DNA Bending in Solution Are Highly Sensitive to Osmolytes*

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The complex formed between the TATA-binding protein (TBP) and the “TATA box” of eukaryotic class II promoters is the foundation for assembly of the complex to which RNA polymerase II is ultimately recruited. TBP binds productively to canonical and diverse variant TATA sequences with >100-fold differences in transcription efficiency. Co-crystals of canonical sequences and >11 variant sequences bound to various TBP molecules all have ≈80° bends. In contrast, the bend angles for TBP-TATA complexes in solution, derived from distance distributions, are ≈80° for a canonical sequence but range from 30° to 62° for five variant sequences (1). We show in this study that the osmolytes used to crystallize TBP-TATA complexes induce profound increases in the DNA bends of two transcriptionally active TBP-bound variant sequences to a common angle of ≈80° but have little effect on a transcriptionally inactive variant. The effect of osmolyte on the TBP-induced DNA bend of a variant TATA box sequence is also manifest in the kinetics of association, demonstrating a functional consequence of an osmolyte-induced structural change.

The complex formed between the TATA-binding protein (TBP) and eukaryotic class II promoters of consensus sequence TATA(a/t)A(a/t)N is the foundation for assembly of the preinitiation complex to which RNA polymerase II is ultimately recruited. TBP binds productively to canonical and diverse variant TATA sequences with >100-fold differences in transcription efficiency (2). A key feature of the solution geometry of the adenovirus major late promoter (AdMLP) and five variant sequences bound to Saccharomyces cerevisiae TBP has been determined using time-resolved fluorometry coupled with Förster resonance energy transfer (FRET) (1). The mean end-to-end distances in these six TBP-bound sequences vary widely and correspond to TBP-induced bends of 76° for the consensus AdMLP sequence and from 30° to 62° for the variant sequences. High resolution co-crystal structures of canonical TATA sequences bound to S. cerevisiae (3), Arabidopsis thaliana (4), and human (5, 6) TBPs are extremely similar. These binary structures are characterized by ≈80°-induced bends in the DNA helical axes, in excellent accord with the solution bend angle obtained subsequently for AdMLP using FRET. However, in contrast to the bends determined using FRET, the co-crystal structures of 11 TATA sequence variants (including those examined by FRET) bound to A. thaliana TBP are all very similar, with the DNA bent ≈80° as in the strong promoters (6, 7).

Because assembly of the transcription complex proceeds by sequential structural alterations, a clear picture of the relevant discrete structures is fundamental to an accurate understanding of these processes. The present study was therefore undertaken to further investigate the relationship between the solution and co-crystal structures of TBP-promoter complexes.

Osmolytes used as crystallizing agents for TBP-TATA complexes were postulated to induce in the bound variant sequences severe bends approaching those observed in the strong promoters. End-to-end distance distributions were therefore obtained using FRET fluorometry for the reference AdMLP and three variant sequences, free and bound to S. cerevisiae TBP, in solutions containing 0–3 M ethylene glycol or 3.5 M glycerol. Determination of the corresponding solution bend angles revealed little change for TBP-bound AdMLP upon addition of osmolyte. In contrast, osmolytes induced profound increases in the bend angles of the transcriptionally active variant sequences, to a common angle of ≈80° but little change in the solution geometry of the transcriptionally inactive variant (T → A substitution at position 3). The conformation of the DNA within TBP-TATA complexes appears to be conserved only in the presence of osmolytes.

EXPERIMENTAL PROCEDURES

The top strands of each DNA duplex (denoted T14-mera*F) are labeled via 6-carbon linkers with 3'-fluorescein and 5'-TAMRA, constituting a FRET donor-acceptor pair. The labeled and unlabeled DNA as well as the full-length S. cerevisiae TBP were as described previously (1, 8–11), with the specific DNA sequences shown below in Table I. Studies were conducted in 10 mM Tris-Cl (pH 7.4), 100 mM KCl, 2.5 mM MgCl2, 1 mM CaCl2, and 1 mM dithiothreitol. Ethylene glycol was added to 0.5–3 M (0.5–3.8 osmolal) and glycerol to 3.5 M (5.3 osmolal) as noted. All measurements were made at 30 °C.

Detailed theoretical discussions of Förster resonance energy transfer relevant to this study have been published previously (1, 9, 11, 12 and references therein). All instrumentation, data acquisition, and analyses and error estimates were exactly as described (1). R and σ for AdMLP and C7 in 3 M ethylene glycol derived from four composite curves (each
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The mean 5'-dye-3' dye distance, $\bar{R}$, and corresponding $\sigma$ for the probability distribution characterizing the AdMLP, C7, and T6 duplexes free and TBP-bound at 30 °C, in the absence and presence of osmolyte

| Sequence     | Buffer* | 3 M Ethylene glycol | 3.5 M Glycerol |
|--------------|---------|---------------------|---------------|
|              | AdMLP   | C7                  | T6            |
|              | TATAAAG | TATAAAAG            | TATAAAG       |
| $R_{\text{free}}$ (Å) | 54.4 ± 0.1 | 54.7 ± 0.1           | 54.3 ± 0.1    |
| $\sigma_{\text{free}}$ (Å) | 8.1 ± 0.1  | 6.8 ± 0.2            | 4.3 ± 0.2     |
| $R_{\text{bound}}$ (Å) | 47.1 ± 0.1 | 51.2 ± 0.1           | 52.5 ± 0.3    |
| $\sigma_{\text{bound}}$ (Å) | 8.5 ± 0.1  | 10.4 ± 0.1           | 10.5 ± 0.3    |
| $K_{b}$ (μM$^{-1}$) | 168 (147, 191) | 60 (51, 70)         | 29 (25, 33)   |

$\dagger$ Not determined.

Errors reported are the standard errors of the mean for a parameter corresponding to a specific model (Fig. 1) and do not imply that changes in trajectory of the DNA helical axes are being measured in solution to accuracy < 1°.

The solution bend angles corresponding to the model depicted in Fig. 1 for TBP-bound AdMLP, C7, and T6 in the absence and presence of osmolyte

|          | AdMLP | C7    | T6    |
|----------|-------|-------|-------|
| Buffer   | 76.2° ± 0.2° | 52.3° ± 0.3° | 36.9° ± 0.2° |
| 3 MEG    | 83.5° ± 0.1° | 77.6° ± 0.1° | 78.2° ± 0.3° |
| 3.5 M Glycerol | †     | †     | †     |

$\dagger$ Not determined.

RESULTS AND DISCUSSION

End-to-End Distance Distributions for Free and TBP-bound AdMLP and Two Variant Sequences—The TBP-induced bend in DNA bearing the AdMLP promoter sequence and DNA bearing an A → C substitution at position 7 (C7) and an A → T substitution at position 6 (T6) was probed using FRET fluorometry. The mean end-to-end distance for each sequence free ($R_{\text{free}}$) and TBP-bound ($R_{\text{bound}}$) and the corresponding $\sigma$ for the distance distribution in buffer, buffer plus 3 M ethylene glycol, and buffer plus 3.5 M glycerol are listed in Table I.

The effect of osmolytes on the unbonded duplexes was similar. The values of $R_{\text{free}}$ for those duplexes were within 2% for each solution condition and increased by only ~0.8 Å in ≥3 M osmolyte. The slight increases observed in $R_{\text{free}}$ may be attributable to the increase in charge-charge repulsion along the DNA backbone resulting from the decrease in the dielectric constant of the solution due to osmolyte (13). The values of $\sigma_{\text{free}}$ for the unbonded duplexes also increased in the presence of osmolyte, from 7% to 42%, the latter for the T6 variant. The breadth of the distribution derives both from the motion of the dye linkers and from relatively slow fluctuations in the duplex. Because the linkers are invariant among these oligomers, the observed increases in $\sigma_{\text{free}}$ are believed to reflect osmolyte-dependent increases in the deformability of the free duplexes. The determination of the relative contributions of the linker arms and the duplex DNA to $\sigma_{\text{free}}$ is the subject of active investigation.

Remarkably, osmolytes affect complexes of TBP with the three DNA sequences very differently. In the absence of osmolyte, $R_{\text{bound}}$ is significantly shorter for bound AdMLP than for either variant (Table I). In contrast, in the presence of ≥3 M osmolyte, the values of $R_{\text{bound}}$ determined for the C7 and T6 variants approach that of the strong parent promoter, due to the significant decreases in the end-to-end distance for both variants.
TABLE III

| [EG] (M) | 0 | 0.5 | 1 | 2 | 3 |
|---------|---|----|---|---|---|
| \(R_{\text{free}}\) (Å) | 54.3 ± 0.1 | 54.8 ± 0.1 | 55.1 ± 0.2 | 55.6 ± 0.1 | 55.5 ± 0.2 |
| \(\sigma_{\text{free}}\) (Å) | 4.3 ± 0.2 | 5.0 ± 0.3 | 5.4 ± 0.1 | 6.3 ± 0.3 | 6.1 ± 0.1 |
| \(R_{\text{bound}}\) (Å) | 52.5 ± 0.1 | 50.8 ± 0.1 | 49.8 ± 0.1 | 48.3 ± 0.1 | 47.6 ± 0.1 |
| \(\sigma_{\text{bound}}\) (Å) | 10.5 ± 0.3 | 10.1 ± 0.1 | 9.3 ± 0.1 | 8.8 ± 0.1 | 8.7 ± 0.1 |
| Bend angle | 36.9° ± 0.2° | 55.7° ± 0.3° | 63.9° ± 0.4° | 74.9° ± 0.2° | 78.2° ± 0.3° |

Similarly, the value of \(\sigma_{\text{bound}}\) for TBP-bound AdMLP is unchanged by addition of osmolyte, whereas \(\sigma_{\text{bound}}\) for both variant sequences decreases significantly. Identical results were obtained for C7 in ethylene glycol and in glycerol. The implications of these changes are discussed below.

Control experiments were conducted to ensure that the changes in the value of \(\bar{R}\) did not derive from osmolyte-dependent changes in \(R_0\). The semi cone angles, which are a measure of the rotational wobble of the fluorophores, determined in 3 M ethylene glycol for free and TBP-bound M\(_{\text{AdMLP}}^*\) F and T\(_{\text{AdMLP}}^*\) F did not differ significantly from those determined in the absence of osmolyte (1), ranging from 56° to 68° with a mean value of 64° and an average error of ±7°. The fast and slow rotational correlation times for these four cases were as follows: \(\tau_{\text{fast}} = 0.15 ± 0.03\) ns, \(\tau_{\text{slow,free}} = 5 ± 2\) ns, and \(\tau_{\text{slow,bound}} = 23 ± 2\) ns, indistinguishable from those determined in the absence of ethylene glycol (1). These values taken together reflect a high degree of rotational freedom in 3 M ethylene glycol for both dyes for the free and TBP-bound duplexes. Because the conformations of the bound AdMLP, T6, and C7 duplexes in osmolyte-containing solutions are very similar to each other as well as to those in the corresponding co-crystals, these controls were deemed sufficient to justify using \(\alpha^2 = 2/3\) in all calculations of \(R_0\) for the duplexes in osmolyte.

The overlap integrals determined for free AdMLP and T6 in 3 M ethylene glycol were identical, with \(R_0 = 60.9\) Å. The 3′-fluorescein emission and 5′-TAMRA absorption spectra were likewise invariant for TBP-bound AdMLP and T6, with \(R_0 = 61.1\) Å. For free T6 in 0.5, 1, and 2 M ethylene glycol, \(R_0 = 61.1\), 61.0, and 61.0 Å, respectively. The corresponding values for TBP-bound T6 were 61.2, 61.2, and 61.1 Å. Because the overlap integrals were nearly sequence- and osmolyte-independent, the integral determined for free and bound T6 in 3 M ethylene glycol was used to calculate \(R_0\) values of 60.3 and 60.5 Å for free and bound C7 in 3.5 M glycerol (\(\eta = 1.3704\)). These results confirm that the observed changes in the values of \(\bar{R}\) do not derive from osmolyte-dependent changes in dye mobility or \(R_0\).

In addition, the osmolyte independence of the slow phase of the anisotropy decays, which correspond to the rotational correlation times for the TBP-DNA complex, suggests no osmolyte-induced aggregation of the protein. This conclusion is further supported by ultracentrifugation studies using 5% glycerol (14) that show no effect of the osmolyte on TBP aggregation and in 10% glycerol\(^2\) that show a limited decrease in TBP oligomers. Finally, the reference AdMLP sequence serves as an effective internal control. The similarity of \(P(R)\) for the AdMLP-TBP complex with and without osmolyte suggests that the large changes in \(P(R)\) observed for bound T6 and C7 do not derive from osmolyte-induced TBP aggregation.

Solution Bend Angles for TBP-bound TATA Duplexes in the Absence and Presence of Osmolytes—The bend angle for TBP-bound T\(_{\text{AdMLP}}^*\) F and C\(_{\text{AdMLP}}^*\) F obtained from the ratio of \(R_{\text{bound}}/R_{\text{free}}\) using the bend model shown in Fig. 1 is in excellent accord with the co-crystal structure (1). This model has two kinks symmetrically positioned to coincide with the phenylalanine intercalation sites observed in the co-crystal structure with the angle, \(\alpha\), determined according to the relationship

\[
\alpha = 2 \cos^{-1} \left( \frac{R_{\text{bound}} - L_2}{R_{\text{free}} - L_2} \right) \quad (\text{Eq. 1})
\]

Fig. 2. The inverse relationship between the breadths of the distributions and the osmolyte-dependent bend angles for TBP-bound T6. [EG] is the concentration of ethylene glycol from 0 to 3 M (0–3.8 osmolar). To minimize the contribution from the tethers, \(\sigma_{\text{diff}}\) is defined as \(\sigma_{\text{bound}}^2 - \sigma_{\text{free}}^2\). A very similar correspondence is shown in Fig. 3 of the accompanying paper for \(\sigma_{\text{diff}}\) versus the sequence-dependent bend angles.

Solution Bend Angles for TBP-bound TATA Duplexes in the Absence and Presence of Osmolytes—The bend angle for TBP-bound T\(_{\text{AdMLP}}^*\) F obtained from the ratio of \(R_{\text{bound}}/R_{\text{free}}\) using the bend model shown in Fig. 1 is in excellent accord with the co-crystal structure (1). This model has two kinks symmetrically positioned to coincide with the phenylalanine intercalation sites observed in the co-crystal structure with the angle, \(\alpha\), determined according to the relationship

\[
\alpha = 2 \cos^{-1} \left( \frac{R_{\text{bound}} - L_2}{R_{\text{free}} - L_2} \right) \quad (\text{Eq. 1})
\]

with \(L_2\) defined as in Fig. 1.

The solution bend angles corresponding to this model for the three TBP-bound duplexes in the absence and presence of osmolyte are shown in Table II. The bend for the bound AdMLP duplex is similar, −80°, in buffer and in osmolyte. The solution bend angles for the bound C7 and T6 variants in buffer, 52.3° and 36.9°, respectively, are much smaller than for the strong promotor sequence, in distinct contrast to the −80° bends observed for both variants in their co-crystals (7). However, upon addition of osmolyte, the solution bends for both variants increase dramatically (48 and 112%, respectively), to closely resemble the −80° bend of the consensus sequence. For the T6/TBP and C7/TBP complexes, the ethylene glycol-induced change is sequence-independent and, for the C7/TBP complex, the osmolyte-induced change is osmolyte species-independent.

To induce crystallization, co-crystals are grown in solutions of osmolytes such as ethylene glycol + glyceraldehyde or polyethylene glycol (3) or glyceraldehyde (4, 7) at osmolalities commensurate with those used herein. The differences in geometry observed for the TBP-bound variant TATA sequences in buffered solutions and in co-crystals thus appear to be attributable to the presence of the osmolytes used in crystallization.

As the bend angles for the bound variants increase in the presence of osmolyte to approach that of the consensus sequence, \(\sigma_{\text{bound}}\) decreases correspondingly. These observations are consistent with our hypothesis that the observed inverse correlation between \(\sigma_{\text{bound}}\) and the solution bend angle for TBP-bound sequences is attributable to increased restriction of

\(^2\) S. Morris and M. Brenowitz, personal communication.
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Fig. 3. $\Delta G^\circ$ for the conversion of conformer$_{\text{TL}}$ to conformer$_{\text{ML}}$ for TBP-T6$_{\text{dpx*F}}$. The TBP-bound T6 variant in the absence of osmolyte has a bend angle approximately half that of AdMLP. The successive increases in the observed bend (Table III) from 0 to 3 M ethylene glycol (EG) are attributable, within the context of the two-state model, to small energetic changes that alter the population distribution to favor conformer$_{\text{ML}}$.

Conformer$_{\text{ML}}$ (reference)

Conformer$_{\text{TL}}$

Normalized Fluorescence

Time (sec)

0 10 20 30

0 M 0.5 M 1 M 2 M 3 M

[EG]:

-0.70 kcal/mol Conformer$_{\text{TL}}$

-0.06 kcal/mol Conformer$_{\text{TL}}$

1.32 kcal/mol Conformer$_{\text{TL}}$

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DNA helical motion with increased complementarity of the protein-DNA interface (1).

Minimal Effect of Osmolytes on the TBP-bound A3 Variant—Patigkoglou et al. (7) reported that all attempts to crystallize TBP bound to the A3 variant were entirely unsuccessful. Because only complexes with ~80° bends have been crystallized, we hypothesized that osmolytes would not induce the AdMLP-like structure in TBP-bound A3, the only sequence not known to occur naturally of those investigated in this and the accompanying studies. The effect of osmolytes on $R$ for A3 was therefore estimated from a mixture containing approximately equal amounts of free and TBP-bound A3 duplex in 3 M ethylene glycol, because the affinity of TBP for A3 is too low to achieve near-saturation of the DNA. Assuming the same $K_a$ as for buffer alone, the value of $R_{\text{bound}}$ calculated for TBP-A3 in osmolyte was $53.1 \pm 0.2$ Å, consistent with a bend angle of $43 \pm 7^\circ$,4 in keeping with our hypothesis. This estimate is an upper limit on the A3 bend angle, because osmolytes generally increase $K_a$. For instance, a 3-fold increase in $K_a$ would give a bend angle of $36 \pm 8^\circ$.

A Two-state Model Relating the T6 Bend Angle and Osmolyte Concentration—The ethylene glycol concentration dependence of $R$ and $\sigma$ for free and TBP-bound T*T6$_{\text{dpx*F}}$ is shown in Table III together with the corresponding bend angles. The successive decreases in the end-to-end distance of bound T6 as osmolyte concentration increases from 0 to 3 M correspond to an overall 2-fold increase in the bend angle for bound T6, with 86% of the total change occurring by 2 M. The linear decrease in the breadth of the distribution as $\alpha$ increases is 94% complete by 2 M (Fig. 2) and further confirms the thesis that the duplex is constrained as it conforms to the protein binding site.

In the accompanying paper (1), a two-state model provided insight into the close relationships among TATA sequence, solution bend angle, and transcription activity. The relationship between the T6 bend angle and osmolyte concentration has been considered in the same manner. In this model, the TBP-T6 complex exists in two forms, conformer$_{\text{ML}}$ and conformer$_{\text{TI}}$ (Fig. 4A in Ref. 1). The DNA in conformer$_{\text{TI}}$ is bent ~80° as for the consensus sequence, with the DNA in the transcriptionally inactive conformer$_{\text{TI}}$ much less bent. The population distributions of conformer$_{\text{ML}}$ and conformer$_{\text{TI}}$ are osmolyte concentration-dependent, with the equilibrium shifting toward conformer$_{\text{ML}}$ as osmolyte concentration increases.

The probability distributions, determined for bound T6 at all five osmolyte concentrations, were analyzed globally to obtain $P(R)$ for conformer$_{\text{TI}}$, where $i$ specifies osmolyte concentration and all else was as described (1). The set of all five $P(R)$ values was very well described by the two-state model, with a correlation coefficient of 0.999 for the analysis. The values obtained for the two fitted parameters were $R_{\text{TI, bound}} = 53.1 \pm 0.1$ Å and $\sigma_{\text{TI, bound}} = 9.9 \pm 0.1$ Å. Bend angles corresponding to the two-state model were then calculated as described (1) at each osmolyte concentration; these angles differ from those in Table III by only $1.6 \pm 0.8^\circ$.

The FRET measurements yield $P(R)$ values for these complexes that vary both with sequence (1) and with osmolyte concentration. Remarkably, this two-dimensional data set is well fit in its entirety by only two parameters corresponding to the two-state model, $R_{\text{bound}}$ and $\sigma_{\text{bound}}$ for conformer$_{\text{TI}}$. The osmolyte concentration-dependent analysis alone yields $R_{\text{TI, bound}} = 53.1$ Å and $\sigma_{\text{TI, bound}} = 9.9$ Å, and the sequence-de-
dependent analysis alone yields $R_{\text{TL, bound}} = 53.3$ Å and $\sigma_{\text{TL, bound}} = 9.9$ Å. Thus, only two conformations of the TBP-DNA binary complex are sufficient to account for both the osmolyte concentration- and sequence-dependent solution conformations of these TBP-bound TATA sequences, as well as the sequence dependence of transcription efficiency.

The free energy required to convert conformer $\text{TI}$ to conformer $\text{ML}$ is sequence-dependent due to variations in contacts, interactions, and solvation along the minor groove/protein interfaces, and inherent ease of deformability. The osmolyte concentration dependence of the free energy required to convert conformer $\text{TI} \rightarrow$ conformer $\text{ML}$ for the TBP-T6 complex is shown in Fig. 3. For this sequence variant, conformer $\text{TI}$ is energetically favored in the absence of osmolyte, whereas conformer $\text{ML}$ is more stable in 3 M ethylene glycol. The largest energy difference between conformer $\text{ML}$ and conformer $\text{TI}$, 1.32 kcal/mol, is only 0.17 kcal/mol per base pair. The differences in the solution and co-crystal structures thus result from very small differences in $\Delta G$.

The A3 results may also be considered within the context of the two-state model. The mole fraction of conformer $\text{ML}$ predicted to be present in a solution of TBP and A3 approaches zero at all TBP concentrations explored, assuming the $K_a$ measured in buffer. Co-crystals would be unlikely from such a solution, because crystallization has been reported only for a conformer $\text{ML}$-like structure. The solution and crystallographic results thus present a coherent perspective considered either directly or within the context of the two-state model.

**Osmolyte Concentration-dependent Changes in TBP + C7 Stopped-flow Association Kinetic Curves**—The association binding kinetics of AdMLP-TBP have been previously investigated in our laboratory using FRET stopped-flow (10, 11). Analogous initial association curves have been obtained for TBP binding to the C7 variant in 0–3 M ethylene glycol (Fig. 4). In the absence of osmolyte, the TBP association kinetics are remarkably dependent on the C7 substitution in the canonical sequence (compare the uppermost, C7, and lowest, AdMLP, curves in Fig. 4). In the presence of osmolyte, however, the kinetic trace for TBP binding to C7 changes dramatically to approach that of AdMLP. The trace for the reference AdMLP sequence is nearly unchanged in the presence and absence of osmolyte.4

The sequential changes in binding as osmolyte concentration is increased appear to correspond to the osmolyte-induced changes in the solution conformation of the TBP-C7 complexes. A detailed analysis of the kinetics of TBP binding the C7 variant is in progress and will be published elsewhere. Such strong coupling between structure and function for the TBP-TATA complex further validates the central role of the conformation of this binary complex in the process of transcription.

**Conclusions**—This work establishes the osmolyte dependence of the DNA bend angles in transcriptionally active variant DNA-TBP complexes. The large body of work to date investigating the TBP-DNA binary complex, multiprotein transcription complexes, and transcription efficiency has been done using a wide range of osmolyte species and concentrations. The interpretation of these collective results is complicated by recognition of the effects of osmolytes on the TBP-DNA conformation. This complexity is particularly apparent in light of the very small energetic differences we have found between the two putative conformers for a given sequence, because the effects of binding of other proteins on the TBP-DNA conformation in osmolyte is not known. The sequence-dependent differences in association binding suggest that TBP-DNA kinetics may also play a significant role in ultimately determining transcription activity. We have suggested that a stable intermediate conformer in the TBP-DNA binding pathway may be the binary complex to which subsequent proteins bind (11). Transcription efficiency would then depend on both the solution conformation and the concentration time profile of the intermediate species.

Recent reviews have summarized the role of osmolytes in preferential solute-solvent interactions with macromolecules and the associated changes in ligand equilibria and conformational changes (15–17). We have begun a detailed study of osmolyte effects on the structures and populations of the intermediate species detected along the TBP-DNA binding pathway.

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