Regulation of activity of the yeast TATA-binding protein through intra-molecular interactions

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Dimerization is proposed to be a regulatory mechanism for TATA-binding protein (TBP) activity both *in vitro* and *in vivo*. The reversible dimer-monomer transition of TBP is influenced by the buffer conditions *in vitro*. Using *in vitro* chemical cross-linking, we found yeast TBP (yTBP) to be largely monomeric in the presence of the divalent cation Mg^{2+}, even at high salt concentrations. Apparent molecular mass of yTBP at high salt with Mg^{2+}, run through a gel filtration column, was close to that of monomeric yTBP. Lowering the monovalent ionic concentration in the absence of Mg^{2+}, resulted in dimerization of TBP. Effect of Mg^{2+} was seen at two different levels: at higher TBP concentrations, it suppressed the TBP dimerization and at lower TBP levels, it helped keep TBP monomers in active conformation (competent for binding TATA box), resulting in enhanced TBP-TATA complex formation in the presence of increasing Mg^{2+}. At both the levels, activity of the full-length TBP in the presence of Mg^{2+} was like that reported for the truncated C-terminal domain of TBP from which the N-terminus is removed. Therefore for full-length TBP, intra-molecular interactions can regulate its activity via a similar mechanism.

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1. Introduction

TATA-binding protein (TBP) is known to be the central component of the transcription complex for all the three classes of eukaryotic genes (Rigby 1993; Burley and Roeder 1996). TBP gene is essential and, barring a few examples, almost all genes need TBP for transcription and nucleation of pre-initiation complex (Hernandez 1993; Bell and Tora 1999). The accessibility and binding of TBP to the promoter can be regulated by a number of protein factors. However, the demonstration of TBP existing as dimer (Icard-Liepkalns 1993) and DNA-binding capability of the multimeric form of the protein (Jupp *et al* 1993) has given a new dimension to the known, existing mechanisms of promoter binding and transcription regulation involving TBP. It was suggested that TBP dimerizes to protect itself from inactivation (Jackson-Fisher *et al* 1999a), and also to avoid unregulated gene expression in turn (Jackson-Fisher *et al* 1999b). It was further demonstrated that the conversion of dimer to monomer is regulated by TFIIA (Coleman *et al* 1999), a factor known to stabilize the TBP-DNA complex, which facilitates the rapid loading of the protein onto the promoter by causing the dimers to dissociate. Studies on the biochemistry of the dimerization process have gained importance due to the possibility of involvement of TBP dimers in gene regulation.

Two TBP molecules (lacking the N-terminal tail) come together and interact through their concave DNA binding surfaces, in a unit cell of a TBP crystal (Nikolov *et al* 1992; Chasman *et al* 1993). Largely hydrophobic nature (Nikolov *et al* 1996) of the DNA binding surface of this saddle-shaped molecule suggests the holding together of two molecules through it will be resistant to change in

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Abbreviations used: BMH, Bismaleimidohexane; TBP, TATA-binding protein; yTBP, yeast TBP.

ionic concentrations of the medium. The observed existence of dimers even at high salt condition (Coleman *et al* 1995), confirms the interaction to be largely hydrophobic. The binding of TBP to TATA DNA is also salt sensitive, and the optimal concentration differs for the variants of the TATA box (Petri *et al* 1995; Wong and Bateman 1994). DNA-protein interactions generally show sensitivity to salt, and a divalent cation like Mg^{2+} is generally included in binding buffers together with monovalent cation. Combination of these two ions (monovalent and divalent) finally determines the ionic condition for complex formation. Mg^{2+} itself can bind DNA and its effect on binding of lac repressor to the operator has been previously interpreted as a competition for the repressor (Record *et al* 1977). Stabilizing effects of transcription factors, TFIIA and TFIIB, on TBP-DNA complex vary as reported previously, depending on the presence of suboptimal (0·5 mM) or optimal (7 mM) $MgCl_2$ in the binding buffer (Imbalzano *et al* 1994). We have studied the effect of Mg^{2+} on yeast (yTBP) dimerization under different ionic conditions and found that although TBP dimers/multimers were seen at all salt concentrations, tested *in vitro*, monomers predominated in the presence of Mg^{2+}, suggesting Mg^{2+} can suppress the dimerization significantly. Several-fold enhancement of TBP-TATA interaction by Mg^{2+} is seen because it helps to keep TBP molecules active.

2. Materials and methods

2.1 *TBP preparation*

Overexpression clones having either full length untagged or 6 × His tagged at C-terminus (also having a kinase tag at its N-terminus) yTBP were gifts from G Kassavetis, USA. The protein was purified according to Buratowski *et al* (1988) with the change that heparin-Sepharose column was preceded by DEAE-Sephacel column. The pure (single band in SDS-PAGE on silver staining) yTBP was stored in buffer T [30 mM Tris-Cl (pH 8·0); 5% glycerol; 1 mM EDTA; 1 mM PMSF; 1 mM DTT] + 0·4 M KCl. Full-length yTBP with 6 × His tag was partially purified over heparin-Sepharose before binding to and eluting from Ni^{2+}-NTA agarose (Qiagen) affinity resin. TBP used in this study was a full length protein.

2.2 *Gel mobility shift assay*

TBP-TATA complex was formed in 20 µl reaction volume having 30 mM HEPES-NaOH (pH 7·5), 7% glycerol, 2 mM DTT, 100 µg/ml BSA, 100 ng poly[dG-dC], 0·1 M NaCl, 3·6 pmol of TBP and 100 fmol of a 75 bp radiolabelled and gel purified DNA fragment having a sequence TATAAAAA at its centre. This DNA having the Adenovirus major late promoter was PCR amplified from the plasmid pMLΔ50 (a gift from K P Kumar, New Jersey, USA). Binding was allowed in the presence of indicated amounts of $MgCl_2$, for 1 h at 25°C and loaded under running condition on a 6% polyacrylamide gel (containing 7% glycerol) to resolve the complexes. The gel was cast in TGM [25 mM Tris-Cl (pH 8·0), 190 mM glycine and 5 mM $MgCl_2$] buffer having 7% glycerol and pre-run in TGM buffer at 30 mA for 1 h before loading the samples. Radioactivity in free and complexed DNA was measured in dried gel using a Fuji PhosphorImager.

2.3 Crosslinking reactions

Purified C-terminal 6 × His tagged TBP bearing an N-terminal kinase tag was labelled using the *g*-[P^{32}]-ATP and catalytic subunit of protein kinase A (PKA) from bovine heart (Boehringer Mannheim) according to supplier's protocol. The unused label was removed through a Sephadex gel filtration column equilibrated in 30 mM HEPES-NaOH (pH 7·5), 5% glycerol, 1 mM DTT and 0·1 M NaCl.

TBP was crosslinked in a 20 µl volume using a cysteine-specific cross-linker Bismaleimidohexane (BMH, from Pierce), for 30 min at 25°C. Reaction mixture had 30 mM HEPES-NaOH (pH 7·5), 0·5 mM DTT, 7% glycerol, 0·1 M NaCl and 1 mM BMH. BMH was quenched by the addition of protein sample loading buffer, having 200 mM *b*-mercaptoethanol and products were resolved on 10% SDS-PAGE.

2.4 *Gel filtration*

Superdex-75 HR 10/30 (Pharmacia) FPLC column (fractionation range of 3–70 kDa for proteins) was used for checking the size of TBP (27·5 kDa) and its oligomers. The column was first calibrated with different commercially available molecular weight markers. Two-hundred µl of 2·28 µM freshly prepared TBP stock was injected into the column pre-equilibrated with H buffer (25 mM HEPES-KOH, pH 7·5) having different ion concentrations for different run conditions.

2.5 In vivo *crosslinking*

Yeast cells (strain BJ926 of *Saccharomyces cerevisiae*) were grown to 0·6 $OD_{600\,nm}$ in 500 ml enriched YEPD medium and divided into two equal parts. Cells were spheroplasted and suspended in 1 M sorbitol having YEPD and 2 mM BMH was added to one of them while the other was mock treated with DMSO. After incubation at 30°C for 1 h, spheroplasts were washed with 1 M sor-

bitol having *b*-mercaptoethanol and further processed according to Taggart and Pugh (1996). Lysates were precleared through protein A-Sepharose-linked non-specific serum. The flowthrough was incubated overnight with polyclonal anti-TBP antibody purified over TBP-affinity column. TBP-affinity column was made by covalently attaching pure TBP to CNBr-activated Sepharose CL4B (Sigma) according to Supplier's protocol. Western blot of the immunoprecipitate was probed with the same affinity purified but radiolabelled anti-TBP antibody.

3. Results

3.1 *Positive effects of Mg^{2+} on TBP activity*

In a gel-shift assay for TBP-TATA complex formation, increasing amounts of Mg^{2+} resulted in more complex formation (figure 1A). Quantitation of complexes and free DNA showed that 80% of DNA remained free in the absence of Mg^{2+} as compared to ~ 64% at 5–7·5 mM or 48% at 10 mM $MgCl_2$. Therefore, it is possible that on increasing the Mg^{2+} from zero to 20 mM (when only 45% DNA remained free) the additional 35% complex was formed due to availability of greater number of active TBP molecules. This can be achieved either through activation of the existing monomers or through conversion of more dimers into active monomers by the increasing Mg^{2+} ions.

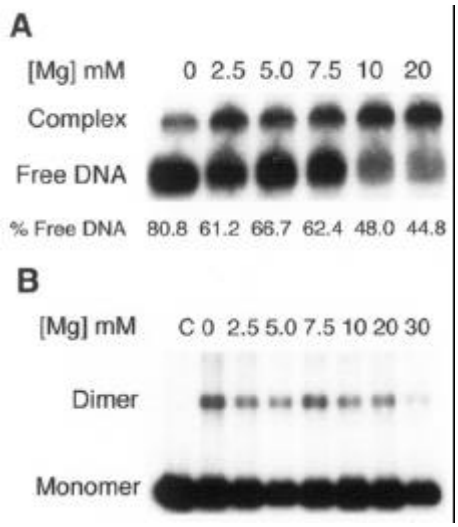

Figure 1. Effect of Mg^{2+} on TBP activity. **(A)** Gel shift assay for TBP-DNA complex formation. DNA and TBP at 1 : 8 molar ratio were allowed to form a complex in the presence of 0·0, 2·5, 5·0, 7·5, 10·0 or 20·0 mM $MgCl_2$ (lanes 1–6 respectively) and resolved by native PAGE as given under § 2. Percent free DNA in each lane is given at the bottom of the gel. **(B)** TBP dimerization at low TBP concentration. Radiolabelled TBP (150 nM TBP) was cross-linked at 0·1 M KCl (lanes 2–8) in the presence of 0–30 mM $MgCl_2$ as marked and products were resolved by SDS-PAGE as given under § 2. Lane 1 is uncross-linked TBP control 'c'. All the bands above dimer are marked as multimers.

Dimer dissociation is shown to govern the kinetics of DNA binding (Coleman and Pugh 1997) and yTBP binds DNA as monomer (Horikoshi *et al* 1990). We used a chemical cross-linking method to find if the increased complex formation was due to the effect of Mg^{2+} directly on TBP dimerization. Using analytical sedimentation equilibrium at 120 mM KCl with or without 4 mM $MgCl_2$, dimeric population was not detected previously, even at higher TBP concentrations (Daugherty *et al* 1999, 2000). To increase the sensitivity of dimer detection at low TBP amounts used under conditions of the gel-shift assay, we used TBP radiolabelled at the N-terminus. Similar to unlabelled TBP, dimerization of this TBP also showed total protein concentration dependence (not shown). Cross-linking was complete within 1 min and further incubation with cross-linker did not affect the amount of cross-linked dimers. Under the conditions of figure 1B, where bulk of TBP molecules were monomeric, increasing Mg^{2+} levels showed an apparent decrease in dimer level. However, quantitation of TBP in each lane (to take care of the loading differences) showed that increasing Mg^{2+} did not change the dimer population significantly (figure 2A). Chemical cross-linking under these conditions also detected a minor fraction of oligomers higher than the dimer, which did not show detectable change in response to change in Mg^{2+} concentration upon quantitation, as seen in figure 2A. Thus, the increase in TBP-TATA interaction under the conditions of gel-shift assay was not accompanied by a significant change in dimer level.

Effect of Mg^{2+} shown in figure 1 was followed at 0·1 M KCl. However, the TBP-DNA binding is affected by monovalent ion concentration (Wong and Bateman 1994; Petri *et al* 1995), and dimerization depends on KCl concentration (Coleman *et al* 1995). Therefore, we also checked the Mg^{2+} effect on dimerization at different salt concentrations. BMH crosslinking of TBP showed that at every salt concentration tested, 3 mM $MgCl_2$ resulted in a lower dimeric population (figure 2B), the effect being least pronounced at 0·3 M KCl and maximum at 0·5 M KCl when dimer seems to be negligible in the presence of Mg^{2+}. In the absence of Mg^{2+}, the dimer level showed a biphasic pattern: a depression to lowest at 0·3 M KCl and then an increase; increase with high salt signifying predominance of hydrophobic interactions. A biphasic pattern of yTBP self-assembly dependence on ionic strength with a transition point at 0·3 M KCl was reported previously (Daugherty *et al* 1999, 2000). At lower TBP concentration (150 nM in figure 2A), due to lower dimer propensity, effect of 2·5 mM $MgCl_2$ on dimer population

at 0·1 M KCl was not visible. However, at higher dimer levels (figure 2B, ~ 22 times more TBP) at 0·1 M KCl, dimer population was reduced 2·8 times in the presence of 3 mM $MgCl_2$, suggesting that even low level of $MgCl_2$ (3 mM) lowers the TBP dimer/monomer ratio. Increasing level of Mg^{2+} did not show significantly different amounts of dimer in figure 2A. The chemical reactivity of BMH does not get affected by the presence of Mg^{2+} because even in its absence the same level of cross-linking could be observed (figure 2A). Cross-linking can be carried out even in the presence of 5–10 mM EDTA. Therefore, the observed effects of Mg^{2+} are directly on dimer levels.

The suppressive effect of Mg^{2+} on dimerization was more pronounced at higher TBP concentrations (higher dimer levels) for every salt concentration (figure 2B). The dimer population detected by BMH cross-linking at 0·1 M KCl was only ~ 11% for 3·42 µm (figure 2B at 3 mM $MgCl_2$) and only 5–7% for 150 nM TBP (figure 2A, at 5 mM $MgCl_2$), but a trend to dimerize in the absence of Mg^{2+} was evident.

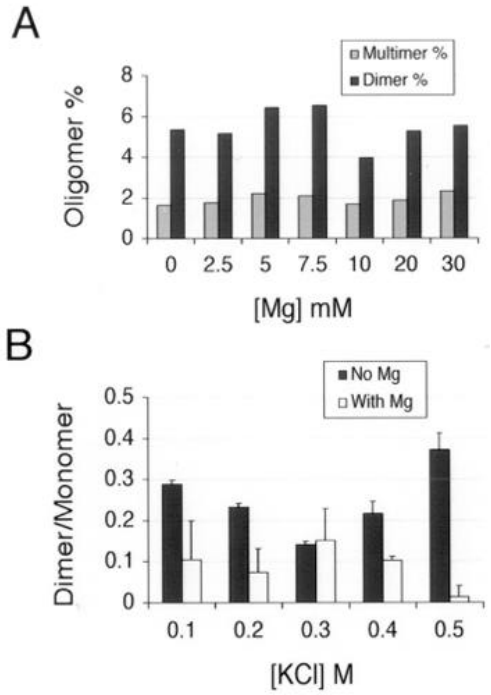

Figure 2. Effect of Ionic strength on TBP dimerization. (**A**) Effect of Mg^{2+} on low dimer levels. Multimer represents sum of all the oligomeric sizes above dimers. Data was obtained by PhosphorImager quantitation of the gel in figure 1B. Values represent the percent of the total TBP amount in each lane and average of two independent estimations. (**B**) Effect of salt on TBP dimerization at higher TBP concentrations. Freshly prepared TBP stock (at 0·4 M KCl) was successively dialysed against 0·3, 0·2 and 0·1 M salt containing buffers; samples at each of the dialysis steps were removed and stored. Completion of dialysis to each salt was confirmed by conductivity measurements and TBP amount was estimated in each. The 0·5 M KCl TBP was obtained by adding the salt externally to the stock. Equal amounts of TBP (2·6 µM) having different salt concentrations were used for BMH cross-linking, with and without 3 mM $MgCl_2$, in a total reaction volume of 20 µl, taking care to keep the final salt concentration same as that of each TBP stock. The protein bands were silver stainined, scanned and quantitated using the Gene Tools for Syngene programme [Syngene (A division of Synoptics Ltd., Cambridge, UK)] to get the amounts of monomeric and dimeric species in each lane. Data given was obtained from more than three independent estimations in each case.

3.2 *Effect of Mg^{2+} on TBP conformation*

BMH cross-linking is more useful for getting the relative levels of dimers/monomers. Therefore in order to directly visualize dimerization, TBP was run through a gel filtration column (figure 3). TBP was either exposed or not exposed to 5–10 mM $MgCl_2$, before loading on a column equilibrated in the same salt containing buffer with or without Mg^{2+}. Depending on the column equilibration buffer, TBP eluted at slightly different positions (table 1). When Mg^{2+} was included in column buffer, its effect was seen within 20–25 min, time in which TBP comes out of Superdex-75 column and a different TBP population was seen (table 1). TBP in 0·4 (or 0·8) M KCl injected to column in the same salt buffer showed a molecular mass of 30·19 kDa (rows 1 or 2, table 1), as opposed to the same coming out as 52·78 kDa protein from a column equilibrated with 0·1 M KCl (row 6, table 1, figure 3B). Thus, in the absence of Mg^{2+}, reducing the salt during the column run favoured TBP dimerization. In contrast, TBP pre-exposed to 5 mM $MgCl_2$ showed a monomeric mass of 23·71 kDa (figure 3C), when injected to column run in 0·1 M KCl (cf. rows 7, 8, 9 table 1). In the presence of Mg^{2+}, lowering of salt did not result in dimerization. Thus, it appears that yTBP is largely monomeric, and in low ionic environment it may dimerize if not protected by Mg^{2+}. If diluted to lower than 0·1 M KCl in the absence of Mg^{2+} or DNA, the dimerization becomes irreversible, resulting in inactivation of the protein (not shown).

Dimer interface is dominated by hydrophobic interactions therefore we increased the salt concentration further to 1 M KCl. When TBP with or without pre-exposure to 5 mM $MgCl_2$ was injected into a column equilibrated with 1 M KCl and 5 mM $MgCl_2$, a proper dimer was still not seen. TBP pre-incubated with Mg^{2+} was largely a 30·19 kDa species, with a minor fraction at 45·08 kDa. The mixture of three species seen without prior exposure to $MgCl_2$ (figure 3C) could be related to the 30·19 kDa species predominant at 0·4 M KCl and all salt concentrations above it (table 1). An average size of the three species, 31·57 kDa is also very close to 31·6 kDa reported by

Campbell *et al* (2000) for yTBP eluting from a gel filtration column in high salt and 5 mM $MgCl_2$. All the peaks collected from each column were found to be of the same size on gel analysis (SDS-PAGE), and as compared to TBP monomer (mol. wt. 27·5 kDa), they also have a mass less than the expected dimeric size (~ 54–55 kDa). One pure protein like TBP can not have different sizes as suggested by the different apparent molecular mass sizes in table 1. Therefore, all these species of closely related molecular masses could be various conformers of TBP, differing in their bulk (compactness) arising out of the same stock, which is 99·9% pure (single band on SDS-PAGE and silver staining).

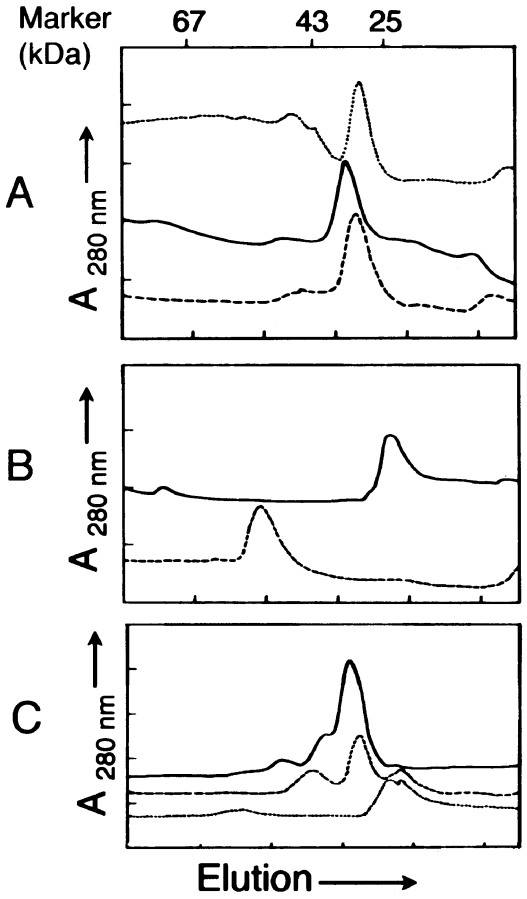

Figure 3. The FPLC gel filtration profiles of TBP under different ionic conditions. **(A)** TBP at high salt, also run in high salt. TBP in T + 0·4 M KCl buffer was incubated with 10 mM $MgCl_2$ for 75–120 min at 25°C and injected into column run in (—), T + 0·4 M KCl or (- - -), T + 0·4 M KCl + 10 mM $MgCl_2$ or (. . . . .), T + 0·8 M KCl. **(B)** TBP stored at high salt and run into lower salt. (- - -), TBP in T + 0·4 M KCl buffer injected into column run at T + 0·1 M KCl buffer; (—), TBP in H + 0·3 M KCl + 5 mM $MgCl_2$ buffer injected into column run at H + 0·1 M KCl buffer. **(C)** 5 mM Mg^{2+} affects TBP conformation. (. . . . .), TBP in H + 0·3 M KCl + 5 mM $MgCl_2$ buffer injected into column run at H + 0·1 M KCl + 5 mM $MgCl_2$ buffer; (- - -), TBP in H + 1 M KCl + 5 mM $MgCl_2$, run at 1 M KCl and 5 mM $MgCl_2$; (—), TBP in H + 1 M KCl buffer run at 1 M KCl and 5 mM $MgCl_2$.

3.3 *yTBP dimers* in vivo

At high protein concentrations (μM range) and lower salt amounts, TBP tends to dimerize and Mg^{2+} suppresses this dimerization. At lower yTBP concentrations *in vitro*, the dimer level is low making it difficulty to see. However, if TBP concentration in yeast cells is ~ 6·3 μM (Daugherty *et al* 1999), there could be a strong possibility of its dimerization *in vivo*. We looked at the dimer levels in normal yeast cells directly by using a radioactive detection method and found that a fraction of normal TBP in yeast can also be dimeric. As shown in figure 4, we estimated through an *in vivo* immunodetection experiment that out of total TBP population in a log phase yeast cell, up to 20% population could dimerize reproducibly. The antibody heavy chain (used for immunoprecipitation), which co-migrates with the TBP dimer species (both ~ 50 kDa) on an SDS-PAGE, appears on probing the Western blot with the radiolabelled antibody (lanes 2 and 3, figure 4). This is a common problem in immunoprecipitation experiments and was reported by several others also before (Lescure *et al* 1994). However, compared to lane 2, a visibly lower signal at the monomer position in lane 3 can not be ignored. But the band at dimer position in lane 3 was not reduced simultaneously. On the contrary, it showed an increase in intensity. Thus, cross-linking clearly resulted in loss of monomer and increase in signal at antibody heavy chain position could be only because of contribution from the dimer to the intensity. The antibody band served as internal control for sample recovery. Assuming same signal contribution from the antibody in both the lanes 2 and 3, we quantified the blot by PhosphorImaging. However small, the increase in the intensity of the band at dimer position from lane 2 to 3, turned out to be reproducibly the same as the decrease in that for monomer position, indicating that the observed increase was due to the authentic dimer which appeared only after chemical cross-linking.

The results in this study have shown that the dimer level *in vitro* is sensitive to TBP and ionic concentration of the solution, and the divalent ion Mg^{2+} plays an important role in TBP activity. Unlike human TBP, yeast TBP is largely monomeric *in vitro* as well as *in vivo*.

4. Discussion

4.1 *Mg^{2+} and TBP activity*

Large protein-DNA complexes can be resolved better (Zerby and Lieberman 1997) in gels containing Mg^{2+}. It

Table 1. Various conformers of TBP under different column conditions.

Sl. No.	Inject buffer	Column buffer	Molecular mass (kDa)	Where
1.	0·4 M KCl	0·4 M KCl	30·19	Not shown
2.	0·8 M KCl	0·8 M KCl	30·19	Not shown
3.	0·4 M KCl + 10 mM $MgCl_2$	0·4 M KCl	30·19	Figures 3A, 5A
4.	0·4 M KCl + 10 mM $MgCl_2$	0·8 M KCl	27·54	Figure 3A
5.	0·4 M KCl + 10 mM $MgCl_2$	0·4 M KCl +10 mM $MgCl_2$	28·84	Figures 3A, 5B
6.	0·4 M KCl	0·1 M KCl	52·78	Figure 3B
7.	0·4 M KCl	0·1 M KCl + 5 mM $MgCl_2$	45·18 + 35·48	Not shown
8.	0·3 M KCl + 5 mM $MgCl_2$	0·1 M KCl	23·71	Figures 3B, 5C
9.	0·3 M KCl + 5 mM $MgCl_2$	0·1 M KCl + 5 mM $MgCl_2$	23·45	Figure 3C
10.	1·0 M KCl	1·0 M KCl + 5 mM $MgCl_2$	39·81 + 29·51 + 25·40	Figure 3C
11.	1·0 M KCl + 5 mM $MgCl_2$	1·0 M KCl + 5 mM $MgCl_2$	30·19 + 45·08	Figure 3C

Results of gel filtration column experiments are summarized. TBP in rows 3–5, 8, 9 and 11 was incubated with given Mg^{2+} for 75–120 min at 25°C before injecting into the column pre-equilibrated with the given buffer.

Figure 4. Dimers of yeast TBP *in vivo*. Western blot of yeast TBP immunoprecipitated from spheroplasts after BMH (lane 3) or mock (lane 2) cross-linking as described under § 2. Lane 1 is control showing cross-linking with recombinant yTBP *in vitro*. Dimer population was estimated by phosphorImager quantitation of the blot.

is also known that Mg^{2+} is required in the gel of the gel-shift assay to see a TBP-TATA complex better. Reasons for these positive effects of Mg^{2+} are not known. Our results have shown a direct effect of 5–10 mM $MgCl_2$ on TBP dimerization and TBP-TATA complex formation. However, at low TBP amounts used for gel shift assay, dimeric TBP fraction was found to be very low, suggesting that the enhancing effect of Mg^{2+} on TBP-TATA complex formation is also due to its effect on some additional parameters. Gel filtration data (figure 3, table 1) revealed that this is due to Mg^{2+} related conformational changes of TBP as discussed below. Direct response of both yTBP dimer population, and molecular masses of its conformers to change in Mg^{2+} levels shows a correlation between the two. Together, they contribute to the enhancement of TBP-TATA complex formation by Mg^{2+}, observed in gel-shift assay. Results also point out that while dimerization of yTBP increases with increasing salt concentration, indicative of hydrophobic interaction; absence of a dimeric peak in gel filtration column could mean that yTBP at high salt has intrinsically low dimer level which is further suppressed in the presence of Mg^{2+}. Single tryptophan of yTBP is additional cause of the failure to detect the dimer if present in low amounts. Suppression of the dimerization and a TBP monomer of probably an altered conformation in the presence of Mg^{2+}, as discussed below, may also be the reason why in some of earlier reports, yTBP dimer was not seen (Daugherty *et al* 1999, 2000; Campbell *et al* 2000).

4.2 *N-terminus and TBP conformers*

As given under the model given in figure 5, we suggest these conformational isomers arise probably as a result of mild opening up of the TBP structure whereby the unstructured N-terminus moves away from highly structured C-terminus, rather than a destructive unfolding of the compact and organized bulk of the molecule. Slight unfolding of the protein through N-terminus was documented before (Perez-Howard *et al* 1995) using fluorescence spectroscopy. Either higher temperature or 1 M guanidinium hydrochloride – both of which can cause denaturation and complete unfolding of a protein – resulted in a large red-shift of fluorescence of single tryptophan present in N-terminus of yTBP (Perez-Howard *et al* 1995). A similar red-shift was observed upon DNA binding, indicating that TBP in active state undergoes a conformational change in which N-terminus is more exposed to solvent. The presence of N-terminus enhances the multimerization of the mouse TBP (Kato *et al* 1994; in the absence of $MgCl_2$) but inhibits dimerization of yTBP (Campbell *et al* 2000; in the presence of $MgCl_2$). Thus, it is possible that in the presence of Mg^{2+}, N-terminus of yTBP is disposed such that it makes the overall molecular structure less compact and prevents two TBP mole-

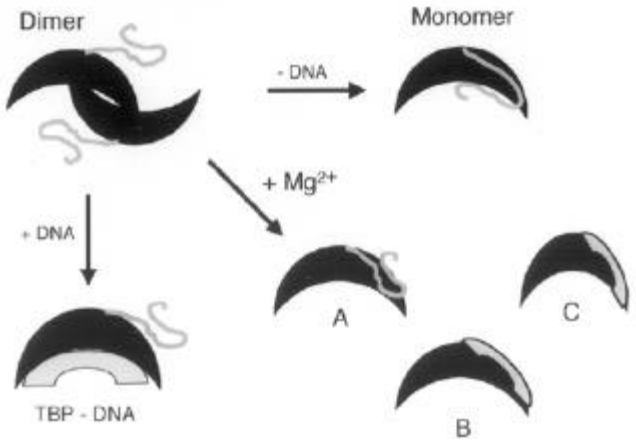

Figure 5. Model showing various conformational states of TBP. C-terminal domain of TBP is shown as black moon, and DNA as broad and filled arc. Since its position has not been defined yet by the crystal structure, the grey coloured N-terminus is shown completely disordered and freely hanging in 'TBP-DNA' complex as well as the 'dimer'. In the absence of DNA, the N-terminus in 'monomer' can block the DNA-binding as well as dimerization surfaces. However, in the presence of Mg^{2+}, depending on the monovalent salt concentration, N-terminus can get variously positioned with respect to the C-terminal domain giving the molecules A or B or C, as given in the table 1 and explained in the text. In all three of them, N-terminus does not block the DNA binding surface but interacts with lateral surface of the molecule, blocking the dimerization. In doing so, it may become more organized or compact; shown as grey arcs in (B) and (C).

cules to self associate, resulting in less dimer formation and more DNA binding. Crystal structures of TBP have not defined the position of N-terminus, although there had been suggestions that it folds back to block DNA binding surface (monomer in figure 5), since its deletion makes TBP-DNA complex more stable (Lieberman *et al* 1991). Conformational changes involving N-terminus of yTBP are also suggested in the formation of a stable TBP-TATA complex and N-terminus is proposed to change both the shape and size of TBP-DNA complex (Kuddus and Schmitz 1993). However, as depicted in figure 5, smaller N-terminus of yTBP may not reach the concave under-surface and may be interacting with a lateral surface of TBP C-terminal saddle, proposed as dimerization interface by crystallization studies and thus interfere with dimerization process (molecules A–C). Response of yTBP dimerization to Mg^{2+} suggests that Mg^{2+} promotes this interference by N-terminus in the absence of DNA and shield the DNA-binding surface of the protein at the same time.

On the basis of our results and reported facts, we think in the presence of high salt and Mg^{2+}, TBP monomers have their N-termini variously disposed; resulting in a different shape and volume (bulk size) of the molecule in each case. yTBP was reported to have a conformational flexibility (Chasman *et al* 1993) and large domain movements in the TBP monomer were detected by molecular simulations (Miaskeiwicz and Ornstein 1996). Mg^{2+} is also known to affect macromolecular conformation (Madore *et al* 1999). Thus, it is not surprising that we observed several monomeric conformers of yTBP. The observed response of yTBP oligomerization to ionic strength has also suggested an involvement of the N-terminus in modulating the tetramer and octamer formation (Daugherty *et al* 2000). Our results suggest that high ionic strength promotes dimerization due to hydrophobic interactions of the interface contributed by the DNA binding surface. But Mg^{2+} probably acts upon other region of the dimerization interface where it helps place N-terminus stably; keeping it away from DNA binding surface of yTBP and thus preventing another TBP molecule to come close enough for giving a stable dimer. By analogy, this TBP surface may also be the same as the inhibitory DNA binding region on the solvent-exposed surface of human TBP (Zhao and Herr 2002). TFIIA promotes dissociation of yTBP dimers (Coleman *et al* 1999) and a possible interaction of the N-terminus with this hTBP region, which overlaps TFIIA interaction surface was also reported (Zhao and Herr 2002).

4.3 *TBP activity* in vivo

Physiological relevance and involvement, if any, of TBP dimer-monomer transition in its activity are not demonstrated conclusively yet. Others have reported the presence of yTBP dimers *in vivo* after overexpression and other indirect methods because of difficulty in identifying and measuring the cross-linked *in vivo* dimers at normal TBP levels in yeast cells (Jackson-Fisher *et al* 1999; Campbell *et al* 2000). Our results using direct methods also suggest only a low possibility of TBP dimerization at its normal physiological level. However, demonstration that a small fraction of yTBP can dimerize *in vivo* also, suggests a two-way mechanism for TBP action *in vivo*. Physiological concentration is usually taken as 10–80 mM for Mg^{2+} and ~ 0·2 M for monovalent cations. We could see ~ 23% TBP dimer in the absence of, and 6–7% dimers in the presence of, 3 mM Mg^{2+} and 0·2 M KCl *in vitro*. However, at 5–6 μM *in vivo* concentration of TBP, we found only ~ 20% or less TBP to dimerize – a level similar to that for 2·6 μM TBP at 0·2 M KCl *in vitro*. Thus, there could be a TBP-binding effector *in vivo*, probably working like Mg^{2+} *in vitro*, suppressing dimer formation from the larger TBP pool. However, if out of 30–50,000 TBP molecules per cell (Lee and Young 1998), ~ 20% are capable of dimerizing, then this 20% may represent a fraction in which the DNA-binding sur-

face is not blocked by any other mechanism. This 20% fraction amounting to 6–10,000 molecules should be suffficient to bind all the 6000 promoters of yeast cells (Goffeau *et al* 1996), should the need arise. Thus the dimeric fraction may represent a TBP pool which is kept in a binding competent form for a quick action (for example in case of genes which are rapidly inducible). This pool fraction could be subject to regulatory interactions of factors like TFIIA (Coleman *et al* 1999) or even transcriptional activators, which may act like Mg^{2+} *in vitro*, as seen here.

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