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 Mg²⁺, even at high salt concentrations. Apparent molecular mass of yTBP at high salt with Mg²⁺, run through a gel filtration column, was close to that of monomeric yTBP. Lowering the monovalent ionic concentration in the absence of Mg²⁺, resulted in dimerization of TBP. Effect of Mg²⁺ 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 Mg²⁺. At both the levels, activity of the full-length TBP in the presence of Mg²⁺ 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.

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 TFIIIB, 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 (\(\gamma\)TBP) 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) \(\gamma\)TBP 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-Sephalac column. The pure (single band) in SDS-PAGE on silver staining \(\gamma\)TBP 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 \(\gamma\)TBP 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 pMLA50 (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 γ[-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 β-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\(_{600}\) 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-