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Improved method for soluble expression and rapid purification of yeast TFIIA

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ABSTRACT

In vitro transcription systems have been utilized to elucidate detailed mechanisms of transcription. Purified RNA polymerase II (pol II) and general transcription factors (GTFs) are required for the *in vitro* reconstitution of eukaryotic transcription systems. Among GTFs, TFIID and TFIIA play critical roles in the early stage of transcription initiation; TFIID first binds to the DNA in transcription initiation and TFIIA regulates TFIID's DNA binding activity. Despite the important roles of TFIIA, the time-consuming steps required to purify it, such as denaturing and refolding, have hampered the preparation of *in vitro* transcription systems. Here, we report an improved method for soluble expression and rapid purification of yeast TFIIA. The subunits of TFIIA, TOA1 and TOA2, were bacterially expressed as fusion proteins in soluble form, then processed by the PreScission protease and co-purified. TFIIA's heterodimer formation was confirmed by size exclusion chromatography–multiangle light scattering (SEC–MALS). The hydrodynamic radius (R_h) and radius of gyration (R_g) were measured by dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS), respectively. The R_g/R_h value implied that the intrinsically disordered region of TOA1 might not have an extended structure in solution. Our improved method provides highly purified TFIIA of sufficient quality for biochemical, biophysical, and structural analyses of eukaryotic transcription systems.

1. Introduction

Transcription initiation from an accurate position by RNA polymerase II (pol II) requires multiple general transcription initiation factors (GTFs): TFIID, TFIIA, TFIIB, TFIIF, TFIIE, and TFIIH [1,2]. *In vitro* transcription systems have been utilized to elucidate the detailed mechanisms underlying the transcription reaction [3]. Among GTFs, TFIID and TFIIA have important roles in the early stage of transcription initiation [4]. TFIID first binds to an AT-rich sequence, known as the TATA box, located upstream of the transcription start site [5,6]. Since the recognition of the TATA box by TATA box-binding protein (TBP), a DNA-binding subunit of TFIID [7], is a critical step for transcription initiation, TBP's activity is negatively or positively regulated in several ways. For instance, TBP's DNA binding activity is negatively regulated by its dimerization [8] or its interaction with TBP-associated factor 1 (TAF1), another subunit of TFIID [9,10]. These negative regulations are released by TFIIA, which is a heterodimer of TOA1 and TOA2 in yeast. TFIIA binds directly to TBP and destabilizes the TBP-TBP dimer and the TBP-TAF1 complex, facilitating TBP-DNA interaction [11,12].

Recent functional studies indicated that TFIIA has additional roles in the transcription reaction. Pol II and GTFs are involved in the synthesis of not only messenger RNA [1,2] but also noncoding [13] and enhancer RNAs [14], implying that TFIIA may be involved in the synthesis of these RNAs. Since the transcriptional regulations of noncoding and enhancer RNAs are still vague, reconstitution of the *in vitro* transcription system is necessary for further study of the

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synthesis of noncoding and enhancer RNAs. However, *in vitro* transcription analyses have been hampered by the lack of a rapid purification method for TFIIA. Since rapid purification methods for pol II and other GTFs have been established, the purification of TFIIA is thought to be a bottleneck in the reconstitution of the *in vitro* transcription system. Bacterial expressions of yeast TOA1 and TOA2 result in an insoluble form [15], and purification of TFIIA requires the time-consuming steps of denaturing and refolding (left panel in Fig. 1A). An improved method of purifying TFIIA is therefore awaited.

Here, we report an improved method for soluble expression and rapid purification of yeast TFIIA. Bacterial expression is performed by a polyprotein strategy [16], in which the subunits of TFIIA are expressed as a fusion protein, which is then processed by a protease. Our improved method purified the TFIIA complex at ~90% purity. We confirmed a heterodimer formation of purified TOA1 (33.5 kDa) and TOA2 (14.0 kDa) by the equimolar ratio of two proteins in gel filtration chromatography and its molecular weight (45.4 kDa) *via* size exclusion chromatography–multiangle light scattering (SEC–MALS) analysis. Next, the purified sample showed monomodal size distribution and monodispersity in dynamic light scattering (DLS) analysis. Finally, the hydrodynamic radius (R_h) (38.7 Å) and the radius of gyration (R_g) (38.1 Å) were measured by DLS and small-angle X-ray scattering (SAXS), respectively. Compared to the theoretical value of R_g/R_h calculated by using the crystal structure of TFIIA (1.01), the measured value of R_g/R_h (0.984) implied that a long intrinsically disordered region of TOA1 might not adopt an extended structure in the solvent. Our improved method is valuable for biochemical, biophysical, and structural analyses of eukaryotic transcription systems.

2. Material and methods

2.1. Vector cloning and subcloning

Synthetic DNA encoding the full length of the TOA1-TOA2 fusion protein was purchased from Eurofin (Luxembourg). The synthesized TOA1-TOA2 gene, whose codons were optimized for expression in *Escherichia coli*, was subcloned into a pET47b vector by using SmaI and NotI sites. pET47b-TOA1-TOA2 plasmid was designed to express MAHHHHHHSAA - LEVLFQGP - TOA1 - GGSGGS - LEVLFQGP - GGSGGS - TOA2 proteins (the full sequence of the TOA1-TOA2 fusion protein is shown in Supplementary Fig. S1). The purified TOA1 and TOA2 contained linker sequences after PreScission cleavage: GP at the N-terminal end of TOA1, GGSGGSLEVLFQ at the C-terminal end of TOA1, and GPGGSGGS at the N-terminal end of TOA2.

2.2. Expression and purification of yeast TFIIA

The pET47b-TOA1-TOA2 plasmid was introduced into *E. coli* BL21(DE3) cells and grown in LB + 2% glucose medium with 30 μg/mL kanamycin. Protein expression was induced at culture $OD_{600} = 0.6–0.8$ with 0.4 mM isopropyl β-D-thiogalactoside (IPTG). After 3 h of induction, cells were harvested by centrifugation at 4000 rpm for 10 min at 4 °C. Collected cells were frozen in liquid nitrogen and stored at −30 °C. The following steps were performed at 4 °C. Cells were resuspended in 10 mL of suspension buffer (20 mM Tris-HCl (pH 7.5), 10% glycerol, 500 mM NaCl, 50 mM 2-mercaptoethanol) per liter of culture. Cells were sonicated for 60 s (repeated three times), and the lysate was clarified by centrifugation at 15,000 rpm for 30 min at 4 °C. A HisTrap HP column (GE Healthcare) was pre-equilibrated in suspension buffer, and the lysate was applied to the column. The column was sequentially washed with more than 10 column volumes of Buffer A (20 mM Tris-HCl (pH 7.5), 10% glycerol, 100 mM NaCl, 50 mM 2-mercaptoethanol) containing 20 mM and 50 mM imidazole. A linear gradient of imidazole was then applied from 50 mM to

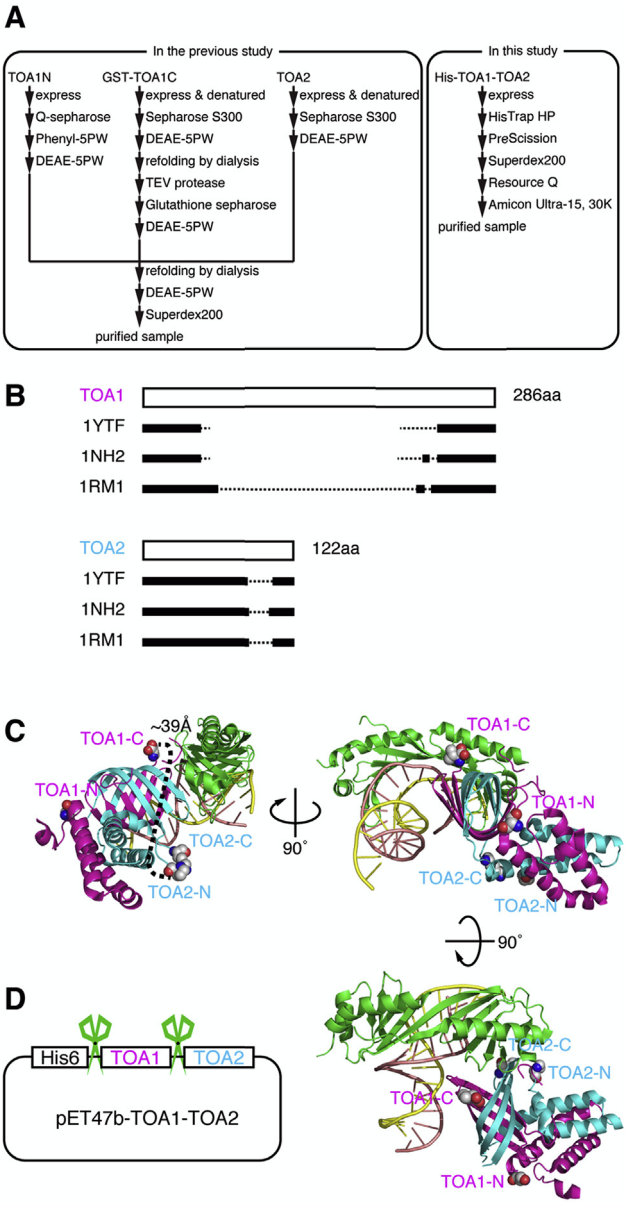

Fig. 1. Vector design for the expression of TOA1 and TOA2 as a fusion protein.
(A) Comparison of the purification scheme of TFIIA. The purification scheme in the previous study is shown at left [15]. That of the present study is shown at right.
(B) Schematic diagram of the TOA1 and TOA2 subunits of TFIIA. White boxes are full-length TOA1 and TOA2. In the crystal structures, the corresponding regions of the black bar are determined (PDB codes: 1YTF, 1NH2, and 1RM1). Dotted lines show the structurally missing region in the crystal structure. TOA1N, TOA1C, and TOA2 were used for the crystal structure determination in 1YTF and 1NH2. The full length of TOA1 and that of TOA2 were used for the crystal structure determination in 1RM1, but the experimental procedure has not been published yet.
(C) The crystal structure of yeast TBP-DNA-TFIIA complex (PDB code: 1RM1). TBP, TOA1, and TOA2 are shown in green, magenta, and cyan, respectively. The N- and C-terminal ends of TOA1 and TOA2 are shown in the sphere. The distance between the C-terminal of TOA1 and the N-terminal of TOA2 is ~39 Å.
(D) Schematic diagram of the key feature of the expression vector used to express yeast TOA1 and TOA2. Two proteins are fused and expressed as His6-tagged proteins. The His6-tag and each protein can be processed by PreScission protease. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)