A Novel Zinc Finger Structure in the Large Subunit of Human General Transcription Factor TFIIE

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Human TFIIE (hTFIIE) is a heterotrimer consisting of two α (hTFIIEα; 57 kDa, 439 aa) and two β (hTFIIEβ; 34 kDa, 291 amino acids) subunits (10–13). Both subunits possess several structural motifs and characteristic sequences. Although hTFIIE has been functionally characterized, little is known about its structure, especially on hTFIIEα (14–16). Main reason is its low solubility. Although high concentration of protein sample should be prepared for the structural studies, each hTFIIE subunit aggregates and precipitates at the concentration higher than 10 mg/ml. Thus we have been focusing on dissecting functional domains in each subunit. To identify core regions in hTFIIEα, limited proteolyses were performed and a highly conserved region (residues 113–174) containing a zinc finger motif, which is essential for transcription activity of TFIIE (14, 17) was isolated (Fig. 1). In this study, we have

The zig finger domain in the large subunit of TFIIE (TFIIEα) is phylogenetically conserved and is essential for transcription. We determined the solution structure of this domain by using NMR. It consisted of one α-helix and five β-strands, showing novel features distinct from previously determined zinc-binding structures. We created point mutants of TFIIEα in this domain and examined their binding abilities to other general transcription factors as well as their transcription activities. Four Zn2+-ligand mutants, in which each of cysteine residues at positions 129, 132, 154, and 157 was replaced by alanine, possessed no transcription activities on a linearized template, whereas, on a supercoiled template, interesting functional asymmetry was observed: although the C-terminal two mutants abolished transcription activity (<5%), the N-terminal two mutants retained about 20% activities. The N-terminal two mutants bound stronger to the small subunit of TFIIF than the wild type and the C-terminal two mutants were impaired in their binding abilities to the XPB subunits of TFIIF. These suggest that the structural integrity of the zinc finger domain is essential for the TFIIE function, particularly in the transition from transcription initiation to elongation and the conformational tuning of this domain for appropriate positioning of TFIIF, TFIIF, and polymerase II would be needed depending on the situation and timing.

In eukaryotes, transcription initiation of protein-coding genes requires RNA polymerase II (pol II) and its auxiliary five general transcription factors, TFIIB, TFIID, TFIIE, TFIIF, and TFIIF (1, 2). Pol II and general transcription factors assemble together on a promoter DNA to form a preinitiation complex (PIC). TATA box-binding protein, a DNA binding subunit of TFIID, binds first to the TATA box. TFIIB then binds and recruits pol II to the complex (3). Pol II comes with TFIIE, which is important for accurate location of pol II in the complex around the transcription initiation site (4). Finally, TFIIE and TFIIF are incorporated to complete PIC formation (5).

During PIC formation, TFIIE interacts with various general transcription factors, pol II, and promoter DNA, recruits TFIIE into the PIC, and regulates the enzymatic activities of TFIIE; serine kinase of the C-terminal domain of the largest subunit of pol II, DNA-dependent ATPase, and DNA helicase activities (5–7). At transcription initiation, TFIIE binds both to pol II near its active site and to promoter DNA (−10 bp upstream (−10) from the transcription initiation site (+1), where the promoter melting starts (8). There TFIIE conducts pol II to sit at the initiation site, makes pol II processive upon C-terminal domain phosphorylation by stimulating C-terminal domain kinase activity of TFIIE, and simultaneously assists a helicase subunit XPB of TFIIE to start melting at the −10 position. At the transition from initiation to elongation, TFIIE also plays a direct role in promoter clearance by regulating kinase and DNA helicase activities of TFIIF (6, 9).
determined the solution structure of this core domain (hTFIIExc) of hTFIIEx by NMR and provided insight into its role for the TFIIEx function.

**EXPERIMENTAL PROCEDURES**

**Construction of hTFIIEx Expression Plasmid—**For subcloning the nucleotide sequence of the hTFIIEx corresponding to the amino acid residues 113–174 into a bacterial expression vector, the oligonucleotide ES17 (5'-GTTGATCAAGATGGATGACGCGATGAGAGAG-3') was designed to create an Ndel site (underlined) and to add the methionine codon before the RI13 residue and the nucleotide ES18 (5'-GAAAGCACGATCAGCATTTTTGTGACCATTGC-3') was designed to create a BamHII site (underlined) right after the stop codon (bold letters). This nucleotide fragment was amplified by PCR using two oligonucleotides (ES17/ES18) and digested with NdeI and BamHII, and subcloned into the NdeI and BamHII restriction sites of the pBluescript SK(-) digested with NdeI and BamHII, and subcloned into the pBluescript SK(-).

**Expression of hTFIIEx—**The hTFIIEx plasmid was transformed into Escherichia coli BL21(DE3)pLysS (Novagen). The cells were grown at 37 °C in LB or in M9 minimal media containing [15N]ammonium chloride. The medium was changed to 50 mM Tris-HCl (pH 8.0), 30 mM ZnCl2, 5 mM 1,4-dithiothreitol containing 50 mM NaCl. The sample was digested with thrombin for 16 h at 25 °C to remove the His6 tag, then, loaded onto the Ni-NTA agarose column equilibrated with buffer B (20 mM potassium phosphate (pH 7.0), 30 mM ZnCl2) containing 200 mM NaCl. hTFIIExc was passed over the column, concentrated using Centriprep (Amicon) and applied on Superdex30 (Amersham Biosciences) equilibrated with the buffer (20 mM potassium phosphate (pH 7.0), 30 mM ZnCl2, 5 mM 1,4-dithiothreitol) containing 50 mM NaCl. The final fractions were used for analyses.

**Statistics for structure calculations**

**Root mean square deviations from experimental restraints**

| Distance (Å)         | 0.0091 ± 0.0005 |
|----------------------|-----------------|
| δH bond              | 0.030 ± 0.038   |
| Angles (°)           | 0.0117 ± 0.0000 |
| Improper (°)         | 0.39 ± 0.00     |

**Final energies (kcal mol⁻¹)**

| E total              | 102.8 ± 1.3     |
| E bond               | 2.9 ± 0.1       |
| E angle              | 80.0 ± 0.8      |
| E van der Waals      | 3.9 ± 0.4       |
| E NOE                | 0.0 ± 0.0       |
| E dihedral           | 11.4 ± 0.2      |
| E improper           | 4.6 ± 0.4       |

**Coordinate precision (Å) (residues 126–164)**

- Backbone atoms: 0.23 ± 0.03 Å
- All atoms: 0.64 ± 0.04 Å

**Ramachandran plot statistics (%):**
- Residues in most favored regions: 74.0%
- Residues in additionally allowed regions: 23.2%
- Residues in generously allowed regions: 2.8%
- Residues in disallowed regions: 0.0%

**NMR Spectroscopy—**Protein concentration for NMR experiments is about 3–4 mM in the buffer (20 mM potassium phosphate (pH 6.0), 30 mM ZnCl2, 5 mM 1,4-dithiothreitol, 50 mM NaCl) dissolved in water. All NMR experiments were carried out at 32 °C on either Bruker DRX-500, DRX-600, AVANCE-600, or AVANCE-600 spectrometer. Backbone and side-chain resonances were assigned using the following experiments: CBCA(CO)NH, CBCANH, HN(CA)CO, HNCO, DQF-COSY, TOCSY, BBHSY, BBH(CO), 15N-edited TOCSY, HSQC, HCH-COSY, HCH-TOCSY (18), (HB)CB(CGCD)HD, (HB)CB(CGCD)CE, (CB)C(CGCD)H, (CB)C(HD)CD, and (CGCD)CE(CD). Statistics for structure calculations

**TABLE I**

| Oligonucleotide sequences used for point mutations |
|--------------------------------------------------|
| 5'-GTGTACCATATGAGAATTGAGACCGATGAGAGAG-3' |
| 5'-GTGTACCATATGAGAATTGAGACCGATGAGAGAG-3' |
| 5'-GTGTACCATATGAGAATTGAGACCGATGAGAGAG-3' |
| 5'-GTGTACCATATGAGAATTGAGACCGATGAGAGAG-3' |
| 5'-GTGTACCATATGAGAATTGAGACCGATGAGAGAG-3' |
| 5'-GTGTACCATATGAGAATTGAGACCGATGAGAGAG-3' |
**Construction of hTFIIEα Point Mutants**—By using the site-directed mutagenesis kit Mutan-K (TaKaRa) with the wild type hTFIIEα DNA plasmid as a template, various oligonucleotide-mediated point mutants were created (31). A restriction enzyme site was placed in an oligonucleotide to select for properly mutated plasmids as described elsewhere, and the mutants were then checked by sequencing using an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The oligonucleotides used for mutation were listed in Table II. Created enzyme sites were underlined, and the enzyme names were written in parentheses. Mutated sequences were written in bold, and each mutated codon is boxed. For defining the transcription activity of 24 ng of wild type hTFIIEα as 100%.

**GST-Pull-down Assay**—GST fusion proteins were performed as previously described (31). The bound proteins were detected by Western blotting with anti-hTFIIEα mouse monoclonal antibody (1:5,000 dilution) (Medical Biological Laboratory). Signals were detected using the enhanced chemiluminescence (ECL) detection system (Amerham Biosciences) using RX-U film (Fuji Film).

**RESULTS**

**Identification of the Core Domain of hTFIIEα**—hTFIIEα is highly conserved among eukaryotes. In addition, TFE from archaea has recently been found to be a homolog of the N-terminal half of hTFIIEα (32–34). To identify the structural domain, the recombinant hTFIIEα was digested by limited proteolysis. Three structural domains consisting of the amino acid residues 113–174, 276–323, and 283–323 and two structural domains consisting of the amino acid residues 106–188 and 302–342 were identified by trypsin and chymotrypsin digestions, respectively, followed by mass spectrometries. Finally, a central core domain (hTFIIEα) was identified to consist of the amino acid residues 113–174 containing a putative zinc finger motif (Fig. 1). hTFIIEα was expressed in *E. coli* BL21(DE3)pLysS, purified, and subjected to conventional multidimensional NMR measurements.

**Overview of the Core Domain Structure**—Almost all signals for each amino acid of hTFIIEα could be assigned except for the β protons of the N-terminal Arg-113. The hTFIIEα has the compact structure consisting of one short α-helix and five β-strands (Fig. 2, A and B). S1 strand (residues 128–129), t1 turn (residues 130–133), S2 strand (residues 134–136), α-helix (residues 138–144), S3 strand (residues 145–146), loop (residues 147–150), S4 strand (residues 151–154), t2 turn (residues 155–158), and S5 strand (residues 159–164). The three β-strands (S1, S2, and C-terminal half of S5) and the other three β-strands (S3, S4, and N-terminal half of S5) form antiparallel β-sheets. Fig. 2C shows that two β-sheets separated by the α-helix are symmetrical, including the positions of cysteine residues in the topology diagram. Zn2+ coordinates to Cys-129 and Cys-132 in the t1 turn and Cys-154 and Cys-157 in the t2 turn. The H-D exchange rates of amide protons of Val-131,
FIG. 2. The hTFIIExo structure. A, the superposition of a family of the final 20 NMR structures. Structures are superposed by minimizing the r.m.s.d. of backbone atoms of residues 126–164. B, ribbon representation of the mean structure. Five β-strands, one α-helix, and two turns are indicated by S1–S5, H1, t1, and t2, respectively. Zn²⁺ are shown as pink spheres. C, topology diagram. First and second β-sheets are colored yellow and green, an α-helix is colored light blue. Four Cs on the turns indicate Zn²⁺-conjugating cysteines. D, zinc-binding site. A pink sphere indicates Zn²⁺. Residues involved in Zn²⁺ coordination (indicated by dotted red lines) and bifurcated hydrogen bonds (indicated by dotted blue lines) are shown. E, hydrophobic core. Residues involved in the hydrophobic core and the zinc-binding site are shown with the side chains. Zn²⁺-coordinating cysteines are shown in green. Five phenylalanines are shown in red. The others are shown in blue. F, electrostatic potential on the molecular surface. Positive potential is colored blue, and negative potential is colored red. Conserved negative potential cluster are indicated by arrows.
Cys-132, Phe-156, and Cys-157 were found to be relatively slow. Judging from the structure, hydrogen bonds could be formed in Cys-129: S/H9253/H11002 Val-131: HN, Cys-132: HN and Cys-154: S/H9253/H11002 Phe-156: HN, respectively (Fig. 2D). These bifurcating hydrogen bonds are consistent with the hydrogen bond pattern between S/H9253 of i residue and HN of i+2, i+3 residues observed in many zing finger domains (35–38). In the H-D exchange experiment almost all of amide protons were exchanged with deuterium within 40 min, so that little hydrogen bond information was obtained. We could directly observe more hydrogen bonds in the β-sheets by measuring trans-hydrogen bond 3hJNC couplings (39) (Supplemental Fig. S4).

The coordination of Zn2+ to four cysteine residues and the auxiliary two bifurcated hydrogen bonds fix the t1 and t2 turns rigidly, forming two anti-parallel β-sheets approach around one side of the α-helix. Fig. 2E shows that a hydrophobic core is formed by hydrophobic amino acids: Ala-141, Leu-144, and Val-161 as well as five phenylalanine residues that are piled up each other and arranged like a capital character, L. This aromatic-aromatic interaction network seems to contribute significantly to the stability of the structure. These residues are well conserved among species (Fig. 1). Electrostatic potential on the molecular surface shows that the hTFIIEα has a negative potential cluster, which is formed by conserved Asp-138, Glu-
160, Glu-162, Glu-163, Asp-164, and Glu-165, and is extensively located on a concave surface opposite to the zinc-binding site (Fig. 2F).

N-terminal and C-terminal regions were disordered due to poor distance restraints. Nevertheless only about 40 amino acids can architect the rigid structure. Actually, 15N-T1, T2, and 13N-{1H}heteronuclear NOE values revealed flexibility of backbone in the N-terminal 13 and C-terminal 11 residues (Fig. 3).

Novel Structural Features of the Zinc Finger Motif—Once hTFIIE\textsubscript{oc} was predicted to form a zinc-ribbon structure as observed in the C-terminal domain of transcription elongation factor TFIIS (TFIIS\textsubscript{c}) (35), the N-terminal domain of TFIIB (TFIIB\textsubscript{n}) (36), and the C-terminal domain of RPB9 subunit of pol II (RPB9\textsubscript{c}) (37). However, hTFIIE\textsubscript{oc} holds an entirely different structure from so-called zinc-ribbon structures (Fig. 4A) (35–38, 40–42). The topology of TFIIS\textsubscript{c}, TFIIB\textsubscript{n}, and RPB9\textsubscript{c} are \(\beta\beta\beta\), \(\beta\beta\beta\), and \(\beta\beta\beta\) \((\beta\beta\beta\text{-strand})\), respectively, which form anti-parallel \(\beta\)-sheets, whereas the topology of hTFIIE\textsubscript{oc} is more complicated as \(\beta\beta\alpha\beta\beta\beta\) \((\alpha\alpha\text{-helix})\), forming two anti-parallel \(\beta\)-sheets separated by one \(\alpha\)-helix. However, architectures of the zinc-binding sites of these proteins are very similar in each other (Fig. 4B). Comparing the backbone structures of two turns (CXXCX and CXXCXX, 12 amino acid residues), root mean square deviations of hTFIIE\textsubscript{oc} to TFIIS\textsubscript{c}, TFIIB\textsubscript{n}, and RPB9\textsubscript{c} are 0.78, 0.97, and 0.74 \(\AA\), respectively. Although we searched similar structures of hTFIIE\textsubscript{oc} by using the DALI server (43), nothing has been identified. So, we considered that hTFIIE\textsubscript{oc} holds an entirely novel type of zinc finger structures.

Functional Roles of hTFIIE\textsubscript{oc} in Transcription—For functional analyses of hTFIIE\textsubscript{oc}, we substituted each of the four Zn\(^{2+}\)-binding cysteine residues at 129, 132, 154, and 157 with alanine, C129A, C132A, C154A, and C157A, as well as two highly conserved acidic residues, Glu-140 and Asp-164 on the negatively charged surface, with alanine or lysine, E140A, E140K, E140T, E140D, D164A, and D164K. All these mutants of hTFIIE\textsubscript{oc} with His\(_{8}\) at the N terminus were expressed in \(E. \ coli\), purified through Ni-NTA-agarose column, and subjected to SDS-PAGE (Fig. 5A). The effects of these point mutants on basal transcription by using a supercoiled adenovirus major late pMLA-50(C\(_{2}\)AT) template were analyzed (Fig. 5, B and C). Although C154A and C157A abolished transcription activity (<5%), C129A and C132A retained 12 and 25% activities, respectively (Fig. 5B). On the other hand, the acidic residue mutants were active in transcription; E140A possessed almost the same activity as the wild type, E140K was also active but with the reduced activity of 50%, and D164A and D164K mutants possessed higher activities at -170 to -180% than the wild type (Fig. 5C).

To dissect the effects of these mutants on the transcription step more precisely and to see the transition activity from transcription initiation to elongation, a linearized template was used (44). Most of the transcription activities of the mutants on a linearized template were similar to the activities on a supercoiled template except that the activities of C129A and C132A were completely abolished (Fig. 5D), and the mutant D164A showed further augmented activity of 370% (Fig. 5E). These suggest that hTFIIE\textsubscript{oc} can be functionally dissected into two; the C-terminal half involves in transcription initiation alone, but the N-terminal half involves not only initiation but also the transition from initiation to elongation. Although Asp-164 is conserved in eukaryotes as well as even in archaea (Fig. 1), substitutions to alanine and lysine residues, D164A and D164K, rather stimulated transcription both on supercoiled and linear templates (Fig. 5, C and E).

Effects of Mutations on Binding to the General Transcription Factors—We next investigated the binding abilities of these mutants to the general transcription factors by GST-pull-down assays (Fig. 6, A and B). All mutants as well as the wild type gave the similar binding patterns except that C129A and C132A bound to TFIIF\(_{\beta}\) about 3-fold stronger than the wild type (Fig. 6A, lane 6) and that C154A completely lost and C157A reduced their binding abilities to the XPB subunit of TFIIF\(_{H}\) (Fig. 6B, lane 3). The bindings of the mutants to intact pol II were also tested but all, including the wild type failed to bind (data not shown). The stronger binding to TFIIF\(_{\beta}\) may contribute to the transcription initiation activities of C129A and C132A on a supercoiled template retained ~12–25%. TFIIF is important for transcription initiation and thus will be able to substitute for a part of TFIIE\textsubscript{oc} function. The reduced binding of C154A and C157A to XPB of TFIIF\(_{H}\) may cause abolished transcription on a supercoiled template; C154A...
and C157A could not recruit XPB at the region on the promoter where promoter melting starts, and thus these mutants are defective in transcription initiation, because XPB functions for promoter opening utilizing its DNA helicase activity.

The Asp-164 mutants of hTFIIE\(\alpha\)/H9251 c, D164A and D164K, did not show so much difference from the wild type in binding to general transcription factors except that both bound more strongly to the XPB and XPD subunits of TFIIH. In addition, D164A bound to the p44 and Cdk7 subunits of TFIIH (Fig. 6, A and B). It is intriguing to consider that three TFIIH subunits (XPD, p44, and Cdk7) have been reported to be involved in the transition stage from initiation to elongation. This fits to the present experiment that D164A was more active in transcription, especially, on a linearized template (3.7-fold of the wild type activity) (Fig. 5E) and indicates that TFIIH may bind more rigidly to D164A than the wild type hTFIIE\(\alpha\) and contributes to the transition stage to elongation.

**DISCUSSION**

To elucidate the functions of hTFIIE\(\alpha\), we investigated the effects of several point mutants on transcription and on the binding abilities to the general transcription factors (Figs. 5 and 6). For transcription activity assay we used the supercoiled and linearized templates of adenovirus major late promoter. Requirement of TFIIE and TFIIH for the promoter melting depends on promoter DNA (45–48). On the supercoiled template, TFIIE can melt the promoter independently of TFIIH. However, on the linearized template both TFIIE and TFIIH are necessary for the transition activity from the initiation to elongation. In mutants of Zn\(^{2+}\)/H11001 ligand cysteine residues at 129, 132, 154, and 157, the interesting finding was the functional asymmetry in hTFIIE\(\alpha\). The N-terminal two mutants C129A and C132A retained 12 and 25% activities in transcription with the supercoiled template, whereas the C-terminal two mutants C154A and C157A almost completely abolished the transcription activities (Fig. 5B). On the other hand, all of the cysteine
mutants showed no transcription activities with the linearized template (Fig. 5D). It is suggested that a defect in transcription initiation should cause no transcription activity with a supercoiled template, whereas a defect in the transition from initiation to elongation could cause some transcription activities with a supercoiled template, but not at all with a linearized template. So C154A and C157A would be deficient in transcription initiation, whereas C129A and C132A would be somewhat deficient in initiation but significantly deficient in the transition stage. The N-terminal zinc finger mutants C129A and C132A showed stronger binding to TFIIFβ and the C-terminal mutants C154A and C157A, on the other hand, showed diminished binding to XPB (Fig. 6A, lane 6, and Fig. 6B, lane 3). It is noteworthy that the TFIIE deletion mutants of the zinc finger domain still possessed the stimulation activity of TFIIE-mediated C-terminal domain phosphorylation of pol II in the presence of template DNA and other general transcription factors but not in the absence (14). Thus, the structural integrity of hTFIIEαc might be essential for the TFIIE function, the conformational tuning of hTFIIEαc for appropriate positioning of TFIIEβ, TFIIF, and pol II would be needed depending on the situation and timing.

The functional meaning of Asp-164 is still under consideration, because it is still difficult to explain the reasons why the D164A and D164K mutants showed stronger transcription than the wild type. Because Asp-164 is well conserved among species, this acidic character may be essential for association with some ubiquitous transcription factors, for example, TRAP/Mediator, cofactors, and transcription elongation factors (49). One candidate is p100, a coactivator interacting with ERBNA2 and STAT6, because this protein was reported to bind to both subunits of TFIIE (50, 51). p100 possesses the Tudor domain, which is supposed to bind to the methylated peptides (52). Recently, transcriptionally active pol II with Ser-5 phosphorylation of the heptapeptide repeat of the largest subunit was found to recruit histone H3 Lys-4-specific methyltransferase Set1 (reviewed in Ref. 53). Thus, it is intriguing to speculate that p100 and TFIIE work for the bridges to connect between methylated histones and pol II. Further studies are necessary to confirm those possibilities for the functional roles of hTFIIEαc.
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