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Conformational States of *Xenopus* Transcription Factor IIIA[†]

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ABSTRACT: The conformation of *Xenopus* transcription factor IIIA (TFIIIA) free in solution, bound to 5S RNA in the 7S particle, depleted of zinc, or bound to plasmid DNA was analyzed by (1) trypsin digestion and electrophoretic analysis of proteolytic fragments or (2) measurement of the fluorescence of TFIIIA mildly derivatized with *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (IAEDANS). TFIIIA free or complexed with 5S RNA has a similar conformation as judged (a) by trypsin-dependent generation of similar metastable 20-kDa domains (corresponding to the N-terminal half of the protein) or (b) by the negligible change in AEDANS–TFIIIA fluorescence when free or bound to 5S RNA. When TFIIIA binds plasmid DNA, its N-terminal half becomes hypersensitive to trypsin digestion, indicating a structural change in this region of the protein upon interaction with DNA. Quenching of AEDANS–TFIIIA fluorescence is observed upon interaction of the protein with plasmid DNA, a result also indicative of a conformational change upon protein–DNA interaction. Removal of zinc from TFIIIA by EDTA chelation results in (a) increased proteolysis of this 20-kDa domain, indicating a structural change in the N-terminal half of the protein upon zinc removal, and (b) large enhancement of AEDANS–TFIIIA fluorescence. EDTA chelation of TFIIIA bound to 5S RNA in the 7S particle, a procedure which does not deplete all zinc from the protein, neither increases the trypsin sensitivity of the 20-kDa domain nor alters appreciably the fluorescence of AEDANS–TFIIIA. These results indicate that zinc is involved in maintaining the native conformation of at least the N-terminal half of the protein.

Elucidating the structure and function of eukaryotic gene regulatory proteins is necessary for understanding gene expression and cell differentiation. *Xenopus* transcription factor IIIA (TFIIIA)[1] regulates 5S RNA synthesis during oogenesis in *Xenopus laevis* by binding to an intragenic control region (ICR) of the 5S RNA gene (Engelke et al., 1980; Sakonju et al., 1980; Bogenhagen et al., 1980). Specific DNA binding by TFIIIA is initiated by the interaction of the N-terminal portion of the protein with the 3′ region of the ICR (Miller et al., 1985; Smith et al., 1984; Fiser-Littell et al., 1988; Vrana

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[1] Abbreviations: TFIIIA, transcription factor IIIA from *Xenopus laevis* immature oocytes; ICR, internal control region of the *Xenopus laevis* oocyte 5S RNA gene; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IAEDANS, *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid.

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et al., 1988). In *Xenopus laevis* oocytes, TFIIIA is found complexed with 5S RNA in a stable 7S particle (Picard & Wignez, 1979; Pelham & Brown, 1980; Honda & Roeder, 1980). As isolated from immature oocytes, free TFIIIA and TFIIIA in the 7S particle contain zinc, and the protein requires the metal for specific binding to the ICR of the 5S RNA gene (Hanas et al., 1983a). Endogenous zinc in free TFIIIA is loosely bound and easily removed by brief exposure to EDTA; when TFIIIA is bound to 5S RNA in the 7S particle, two to three zinc atoms in the protein are resistant to EDTA chelation (Hanas et al., 1983a).

Zinc in the 7S particle is coordinated between two cysteines and two histidines (Diakun et al., 1986). Analysis of the amino acid sequence of TFIIIA (Ginsberg et al., 1984) revealed 9-11 Cys-Cys-His-His repeat units, each having the potential to coordinate zinc via two cysteines and two histidines (Miller et al., 1985; Brown et al., 1985). Potential metal binding sites have been found in the coding sequences of a variety of eukaryotic gene regulatory proteins (Berg, 1986), a number of which have been shown to require zinc for function (Johnston, 1987; Sabbah et al., 1987; Kadonaga et al., 1987). A key question concerning TFIIIA and related zinc binding proteins is what role(s) do(es) zinc play in protein structure and function. Results obtained from this study indicate that zinc is necessary for maintaining the proper conformation of the N-terminal half of TFIIIA required for specific DNA binding.

Experimental Procedures

Isolation of 7S Particles and TFIIIA from Xenopus laevis. Immature ovaries, isolated from 4–5-cm *Xenopus laevis* (Nasco, Fort Atkinson, WI), were homogenized in 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM dithiothreitol (DTT), and 0.2 mM phenylmethanesulfonyl fluoride. 7S particles, comprised of TFIIIA and oocyte 5S RNA, were isolated by glycerol gradient centrifugation of ovarian homogenates followed by DEAE ion-exchange chromatography of the 7S fractions (Hanas et al., 1983b). TFIIIA in the 7S particle was liberated from 5S RNA by digesting the particle [100 μg/mL in buffer A (20 mM Tris-HCl, pH 7.5, 70 mM NH_4Cl, 7 mM $MgCl_2$, 0.1% Nonidet 40, and 0.5 mM DTT) or buffer B (20 mM Tris-HCl, pH 7.5, 320 mM KCl, 1.5 mM MgCl2, and 0.5 mM DTT)] with RNase A (40 μg/mL, Calbiochem) for 30 min at 23 °C. Protein amounts were determined by the method of Bradford (1976) using bovine serum albumin as the standard.

EDTA Treatment of 7S Particles and TFIIIA. Brief exposure of TFIIIA to EDTA has been previously shown to remove all the endogenous zinc in the protein whereas exposure of TFIIIA in the 7S particle to EDTA leaves two to three zinc atoms bound to the protein (Hanas et al., 1983a). Five micrograms of 7S particles or TFIIIA freed from 5S RNA in the 7S particle by RNase digestion was suspended in 50 μL of buffer A. Na_2EDTA was then added to the TFIIIA samples at a final concentration of 3 mM. The samples were incubated at 23 °C for 10 min. The presence of zinc in free TFIIIA, TFIIIA bound to 5S RNA in the 7S particle, or TFIIIA in the 7S particle treated with EDTA was inferred from the ability of these proteins to bind specifically to the 5S RNA gene as assayed by DNase I protection (Galas & Schmitz, 1978). Conversely, the lack of zinc in free TFIIIA exposed to EDTA was inferred from the inability of the apoprotein to protect the ICR of the 5S RNA gene from DNase I digestion.

Trypsin Proteolysis of 7S Particles and TFIIIA: Denaturing Polyacrylamide Gel Electrophoresis. Under limited proteolysis conditions, trypsin preferentially degrades the C-terminal half of TFIIIA (Smith et al., 1984). 7S particles or TFIIIA was treated as described in the figure legends at a concentration of 100 μg/mL protein in buffer A. Trypsin (Sigma Chemical Co.), at final concentrations of 10 or 15 μg/mL, was added to the TFIIIA samples followed by incubation for 25 min at 23 °C. Proteolysis was quenched upon addition of an equal volume of electrophoresis buffer (100 mM Tris-HCl, pH 6.8, 100 mM DTT, 0.2% SDS, and 10% glycerol) and incubation for 10 min at 95 °C. Denatured samples were electrophoresed on 15% polyacrylamide gels as described previously (Hanas et al., 1983), stained with 0.2% Coomassie blue, destained with methanol/acetic acid (40%/7.5%), and photographed on a white light box with Polaroid 665 film. After SDS-PAGE, the fluorescence of intact AEDANS–TFIIIA (modification described below) or of AEDANS–TFIIIA subjected to trypsin proteolysis was photographed through an orange gelatin filter on a 360-nm UV light box with Polaroid 665 film.

Table I: Spectrophotometric Quantitation of AEDANS Bound to the 7S Particle[a]

compound	μmol/L	absorbance[b]	[AEDANS]/[7S particle]
AEDANS-2ME[c]	1.0	0.004	
AEDANS-2ME	2.0	0.008	
AEDANS-2ME	3.0	0.011	
2ME	2.0	0.000	
AEDANS-7S particle	1.0	0.004	1.0
AEDANS-7S particle	2.0	0.008	1.0
7S particle	2.0	0.000	

[a] Modification of TFIIIA in the 7S particle with IAEDANS and spectrophotometric quantitation of AEDANS adducts were described under Experimental Procedures. [b] Absorbance of AEDANS adducts in buffer B was determined at 340 nm through a 1-cm path length. [c] IAEDANS (10^{-4} M) was reacted with 2-mercaptoethanol (2ME, 2×10^{-4} M) for 1 h at 23 °C in buffer B (no DTT).

Modification of TFIIIA with IAEDANS and Fluorescence: Spectrophotometric Measurements. Labeling TFIIIA in the 7S particle with IAEDANS and removal of unreacted probe were performed as described previously for ribosome modification (Hanas & Simpson, 1985). 7S particles, at a concentration of 5×10^{-6} M in buffer B (0.1 mM DTT), were reacted with 5×10^{-4} M IAEDANS (Sigma Chemical Co.) for 15 min at 23 °C. Unreacted probe was removed by gel chromatography (Sephadex G-25 medium) in buffer B. Spectrophotometric quantitations of AEDANS bound to the 7S particle and of other compounds (see Table I) were carried out at 23 °C in a Gilford Model 240 spectrometer with a digital display. Fluorescence measurements were conducted in a Farrand Mark I spectrofluorometer (digital display) with a 200-μL quartz microcell at 23 °C. Static readings were recorded at 360-nm excitation and 480-nm emission (5-nm slits), and emission spectra (excitation at 360 nm) were plotted with a Kipp & Zonen X–Y recorder.

Results and Discussion

Trypsin Digestion of TFIIIA Free or Complexed with 5S RNA in the 7S Particle. The amino acid sequence of *Xenopus* TFIIIA contains a large number of lysine and arginine residues (43 lysines and 20 arginines, Ginsberg et al., 1984). Cleavage by trypsin at these many sites is likely to be dependent upon the three-dimensional structure of TFIIIA and, therefore, a possible way to detect conformational changes in this protein. The distribution of potential trypsin cleavage sites on a linear projection of the TFIIIA amino acid sequence is depicted in Figure 1. Lysyl–prolyl (located at residues 40, 41 and 326, 327) and arginyl–prolyl (residues 250, 251 and 289, 290) bonds are refractory to trypsin digestion (Hill, 1965) and are not

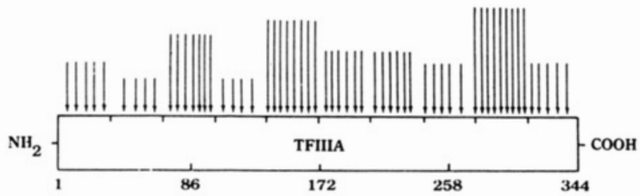

FIGURE 1: Distribution of potential trypsin-sensitive sites on TFIIIA. The 344 amino acids of TFIIIA (from the N to the C terminus) are subdivided every 86 amino acids (lower divisional markings and numbers) and also every 34 amino acids (upper divisional markings). Arrows clustered between the upper markings indicate the relative positions of arginine and lysine residues in that 34 amino acid segment in the primary structure of TFIIIA (Ginsberg et al., 1984). The length of the arrows is proportional to their number in the segments.

included in this distribution. In Figure 1, potential trypsin cleavage sites are distributed fairly evenly from the N to C termini with the N-terminal half of the protein (amino acids 1–172) containing 28 potential sites and the C-terminal half (173–344) containing 31 potential sites. *Xenopus* TFIIIA has a molecular mass of 40 kDa, and upon digestion of the protein in the 7S particle with trypsin, a 20-kDa fragment is generated (Smith et al., 1984). Lanes 2 and 3 in Figure 2A illustrate SDS–PAGE of trypsin-digested 7S particles; the 20-kDa fragment generated corresponds to the N-terminal half of TFIIIA (Miller et al., 1985); smaller fragments of TFIIIA generated by trypsin digestion (molecular masses of about 3 kDa) migrate at the ion front in this gel system. If the 5S RNA is removed from TFIIIA in the 7S particle by digestion with RNAse A (13-kDa band), trypsin proteolysis of the free protein results in similar generation of the 20-kDa domain (lanes 4 and 5, Figure 2A). We conclude that the conformation of the N-terminal half of TFIIIA, as assayed by trypsin proteolysis, does not change appreciably when free or bound to 5S RNA. The generation of a metastable 20-kDa domain by digestion of TFIIIA with trypsin also suggests that the N-terminal half of the protein has a more compact structure than the C-terminal half since potential sites for trypsin proteolysis are distributed between the two halves of the protein (Figure 1). Because trypsin extensively digests the C-terminal half of TFIIIA (3-kDa-size fragments or less), the assay is not useful for detecting structural changes in this region.

Trypsin Digestion of TFIIIA Bound to DNA. Upon TFIIIA binding to DNA, the N-terminal half of the protein becomes hypersensitive to trypsin proteolysis as evidenced by the loss of the 20-kDa fragment (lanes 4–7, Figure 2B). The proteolysis of the N-terminal half of TFIIIA upon DNA binding is dependent upon trypsin and is not the result of an autodigestion process in the presence of DNA alone (data not shown). The results in Figure 2B indicate a structural change in the N-terminal half of TFIIIA upon DNA binding. Although this structural change takes place upon TFIIIA binding to plasmid DNA containing a *Xenopus* 5S RNA gene (pXbs201), most of the protein under these conditions is probably bound to nonspecific DNA of this plasmid. The conformational change induced in TFIIIA by DNA binding may extend into the C-terminal portion of the protein and go undetected by this proteolysis assay. An opening and/or extension of the N-terminal region of the protein over the DNA template would be consistent with increasing the trypsin sensitivity by exposing more sites. Tandemly arranged DNA binding domains (fingers) may be especially suited for such dynamic changes in protein structure. This change in conformation may be necessary for interactions between the C-terminal region of TFIIIA and RNA polymerase III and/or other transcription factors (Vrana et al., 1988). Such an

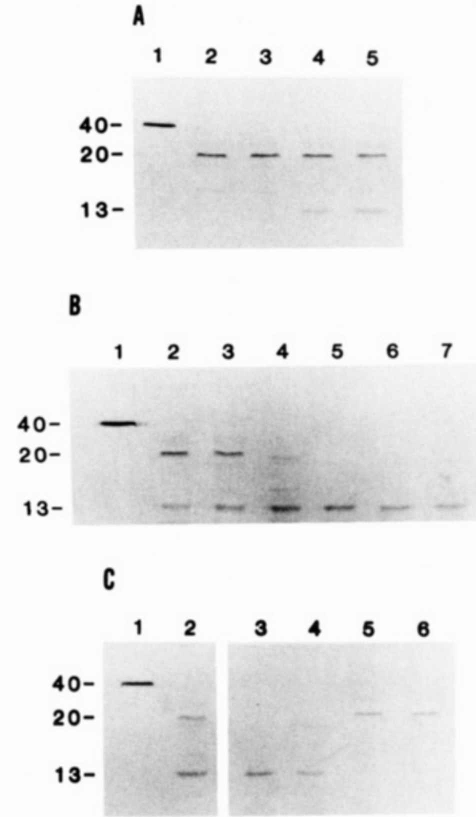

FIGURE 2: Trypsin proteolysis of TFIIIA free or bound to 5S RNA, DNA, or zinc. 7S particle purification, RNase A digestion, EDTA treatment, DNA binding, trypsin proteolysis, and SDS–PAGE were performed as described under Experimental Procedures. TFIIIA samples (5 μg of protein) were digested with trypsin (0.2 or 0.3 μg) in 50 μL of buffer B for 25 min at 23 °C. 50 μL of SDS sample buffer (buffer C) was added to the trypsin reaction followed by electrophoresis and staining of the gel with Coomassie blue. The photograph (gel illuminated on a white light box) was made with Polaroid 665 film. 40-, 20-, and 13- markers are the molecular masses (in kilodaltons) of intact TFIIIA (Ginsberg et al., 1984), trypsin-resistant domain (Smith et al., 1984), and RNase A, respectively. The 13-kDa band in all panels is RNase A which was added to the incubations to liberate TFIIIA from 5S RNA. (A) SDS–PAGE of 7S particles (lane 1, all panels), trypsin digest of TFIIIA bound to 5S RNA (lanes 2 and 3, 0.2 and 0.3 μg of trypsin), and trypsin digest of TFIIIA after removal of 5S RNA in 7S particles by RNase A digestion (lanes 4 and 5, 0.2 and 0.3 μg of trypsin). (B) Prior to trypsin digestion, free TFIIIA in DNA binding buffer (buffer B) was incubated with 10 μg of plasmid DNA ([protein]/[plasmid] = 25) for 15 min at 23 °C (lanes 4–7). TFIIIA in lanes 2, 4, and 6 was digested with 0.2 μg of trypsin and in lanes 3, 5, and 7 with 0.3 μg of trypsin. (C) Prior to addition of trypsin [0.2 μg (lanes 2, 3, and 5) and 0.3 μg (lanes 4 and 6)], samples electrophoresed in lanes 2–5 were incubated in 2 mM EDTA for 10 min at 23 °C in buffer B. (Lane 2) TFIIIA digested with trypsin; (lanes 3 and 4) TFIIIA treated with EDTA prior to trypsin digestion; (lanes 5 and 6) 7S particle treated with EDTA prior to trypsin digestion.

extension of the polypeptide chain over DNA could also explain how such a relatively small protein as TFIIIA extends over a large ICR (170 Å). The increased trypsin sensitivity of the 20-kDa domain upon binding of TFIIIA to DNA (Figure 2B) is not observed in the TFIIIA–5S RNA interaction (Figure 2A) and points to a fundamental difference between the two types of protein–nucleic acid interactions, in agreement with a previous observation (Fiser-Littell & Hanas, 1987).

Zinc Removal Increases Trypsin Sensitivity of the N-Terminal Half of TFIIIA. TFIIIA bound to 5S RNA in the 7S particle contains two to three zinc atoms that are resistant to chelation by EDTA (Hanas et al., 1983a). TFIIIA isolated from EDTA-treated 7S particles is capable of specific binding to the 5S RNA gene as assayed by DNase I protection. In contrast, the zinc in free TFIIIA is easily removed by brief exposure to EDTA, resulting in an apoprotein devoid of specific DNA binding ability (Hanas et al., 1983a). To test whether zinc in TFIIIA is required for maintaining the proper conformational state of the protein necessary for specific DNA binding, the 5S RNA in the 7S particle was digested with RNase, and the free TFIIIA was chelated with EDTA (a procedure previously shown to remove all the zinc in the protein and inhibit specific binding). When a TFIIIA sample treated in this manner (TFIIIA in these studies is indirectly shown to contain or lack zinc by its ability or inability to bind DNA specifically, not shown) is digested with trypsin, the N-terminal half is now degraded (loss of 20-kDa band; lanes 3 and 4, Figure 2C). Degradation products of the zinc-depleted 20-kDa fragment are 3 kDa or less in size and migrate at the ion front. If the TFIIIA–5S RNA complex is exposed to EDTA (a procedure previously shown to leave zinc bound to TFIIIA), the 20-kDa fragment generated by trypsin digestion remains intact (lanes 5 and 6, Figure 2C). We conclude that the removal of zinc from the N-terminal half of free TFIIIA results in an alteration in the structure of the N-terminal half of the protein such that more trypsin-sensitive sites are exposed.

When TFIIIA is bound to 5S RNA in the 7S particle, two to three zinc atoms are not chelated by EDTA (Hanas et al., 1983a). These zinc atoms are most likely confined to the N-terminal half of the protein since the 20-kDa domain remains relatively trypsin resistant under these conditions (lanes 5 and 6, Figure 2C). It has been pointed out that EDTA treatment of the 7S particle removes zinc from TFIIIA (Miller et al., 1985). Our results indicate this zinc does not play a role in the maintenance of the conformation of the N-terminal half of TFIIIA. The N-terminal region of TFIIIA initiates binding to the 3′ region of the 5S RNA gene ICR (Fiser-Littell et al., 1988; Vrana et al., 1988). Because zinc (a) is required for DNA binding specificity of TFIIIA and (b) its removal alters the conformation of the N-terminal half of this protein (Figure 2C), we conclude that one role for this metal in TFIIIA is to maintain the proper conformation of polypeptide segments in the N-terminal half which initiate specific interactions with nucleotides in the 3′ region of the ICR.

Reaction of IAEDANS with TFIIIA in the 7S Particle. In order to substantiate the existence of the different conformational states of TFIIIA identified by trypsin digestion in Figure 2, an independent measure of protein conformation was sought. IAEDANS, a fluorescent reagent which reacts with free thiol groups (Hudson & Weber, 1973) and which was used previously to detect changes in ribosomal protein conformation (Hanas & Simpson, 1985), was chosen to possibly detect changes in TFIIIA conformation. Under mild conditions (15 min, 23 °C), the reaction of IAEDANS with the 7S particle is limited. Spectrophotometric quantitation of the amount of AEDANS reacting with the 7S particle under these conditions yields a value of one AEDANS group per 7S particle (Table I). This value is most likely approximate because the UV absorption of this fluorescent probe is dependent upon the polarity of its environment (25% change in extinction between a polar and a nonpolar solvent; Hudson & Weber, 1973).

As demonstrated in Figure 3, IAEDANS labels the TFIIIA moiety of the 7S ribonucleoprotein particle. Lane 1, panel A, of this figure exhibits the AEDANS fluorescence of the labeled 7S particle subjected to SDS–PAGE (panel B is the Coomassie-staining pattern of this gel). AEDANS fluorescence is observed only in one band in lane 1 (panel A) which, when

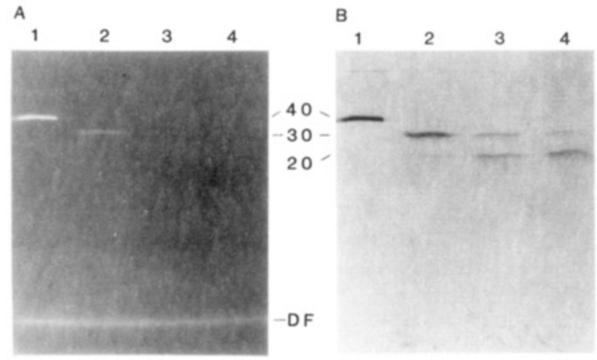

FIGURE 3: SDS–PAGE of AEDANS–TFIIIA. 7S particle purification, IAEDANS modification, trypsin proteolysis, and SDS–PAGE are described under Experimental Procedures. AEDANS–7S particle samples (6 μg of protein) were electrophoresed with no prior treatment (lane 1) or after digestion with trypsin (0.2 μg in 50 μL of buffer B) for 5 min (lane 2), 10 min (lane 3), or 15 min (lane 4) at 23°C. AEDANS fluorescence of the SDS gel (panel A) was photographed on a 360-nm light box, and the SDS gel stained with Coomassie blue (panel B) was photographed on a white light box.

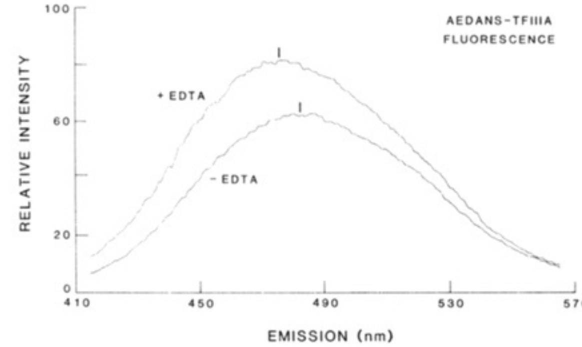

FIGURE 4: Fluorescence emission spectra of AEDANS–TFIIIA. 7S particle purification, IAEDANS modification, EDTA treatment, and recording of emission spectra were performed as described under Experimental Procedures. AEDANS–TFIIIA concentration was 5×10^{-7} M in 20 mM Tris-HCl, pH 7.5, 320 mM KCl, 1.5 mM $MgCl_2$, and 0.5 mM DTT. EDTA concentration was 2 mM. Emission spectra were recorded at 360-nm excitation. Verticle bars mark the emission wavelength maxima.

stained with Coomassie blue, is the 40-kDa TFIIIA protein (lane 1, panel B). Because of the denaturing conditions of SDS–PAGE, we conclude that the AEDANS group (fluorescence observed in lane 1, panel A) is covalently bound to TFIIIA. The mobility of the 5S RNA moiety (dissociated from TFIIIA in the 7S particle by SDS) is significantly greater than that of TFIIIA in this gel system and is not observed to be fluorescently labeled by IAEDANS. Lanes 2–4 exhibit the fluorescence (panel A) and Coomassie staining (panel B) of the AEDANS–7S particle when digested with trypsin for increasing times. The proteolysis pattern observed (panel B) is consistent with previous experiments demonstrating incremental digestion of two, 10-kDa domains at the C-terminal half of the protein (Smith et al., 1984). When the AEDANS–7S particle is digested with trypsin for increasing times (lanes 2–4, panel A), a decreasing amount of fluorescence is observed in the 30-kDa band, but a concomitant increase of fluorescence is not observed in the 20-kDa band. We conclude that IAEDANS is reacting to a significant degree with the C-terminal third of the 30-kDa fragment, a region located within the DNA binding domain of the protein (Miller et al., 1985).

Zinc Removal Enhances AEDANS–TFIIIA Fluorescence. The fluorescence emission spectrum of AEDANS-derivatized TFIIIA is illustrated in Figure 4 (lower tracing); this spectrum

Table II: Effect of Various Agents on the Fluorescence of AEDANS–7S Particles[a]

agent added	% fluorescence change[b]
RNase A[c]	−3.2
EDTA[c]	+1.3
RNase A + EDTA[c,d]	+21
RNase A + EDTA[d,e]	+28
RNase A + DNA[d,e]	−10
RNase A + heparin[d,e]	−11

[a] TFIIIA modification in the 7S particle with IAEDANS and measurement of AEDANS–TFIIIA fluorescence were described under Experimental Procedures. AEDANS–7S particles, RNase A, EDTA, plasmid DNA, and heparin were used at final concentrations of 5×10^{-7} M, 20 μg/mL, 3 mM, 5×10^{-8} M, and 30 μg/mL, respectively. Each agent was incubated with AEDANS–7S particles for 10 min at 23 °C. [b] Fluorescence relative to untreated or RNase A treated AEDANS–7S particles. [c] Reaction and fluorescence quantitation performed in buffer B. [d] Fluorescence change relative to RNase-treated AEDANS–7S particle. [e] Reaction and fluorescence quantitation performed in buffer A.

has an emission maximum at 482 nm and an excitation maximum at 360 nm. When AEDANS–TFIIIA is treated with EDTA under conditions which were previously shown to remove all the zinc in the protein (Hanas et al., 1983) and increase the trypsin sensitivity of the N-terminal half of the protein (Figure 2C), a large fluorescence enhancement (about 25%) as well as a blue-shift in the emission maximum (to 475 nm) is observed (Figure 4). These concomitant changes are consistent with the AEDANS group becoming restricted in motion and/or moving into a more hydrophobic environment (Hudson & Weber, 1973) and indicate a change in TFIIIA conformation upon zinc removal. EDTA does not alter the fluorescence emission of an AEDANS–mercaptoethanol adduct (experiment not shown). Table II lists the quantitative effects of EDTA and other agents on the fluorescence intensity of AEDANS–7S particles. Treatment of the derivatized particles (shown by SDS–PAGE to be labeled only in TFIIIA) with either RNase *or* EDTA results in only small changes in fluorescence intensity. The magnitude of these changes falls within the experimental error of these measurements (Hanas & Simpson, 1985), and these changes are probably not indicative of significant changes in TFIIIA structure upon removal of 5S RNA or zinc from the 7S particle, conclusions consistent with the trypsin digestion profiles seen under these conditions (Figure 2A, lanes 2–5, and Figure 2C, lanes 5 and 6). RNase treatment of AEDANS–7S particles (removes 5S RNA) followed by EDTA treatment (removes zinc) resulted in large fluorescence enhancements, consistent with a significant change in protein conformation. AEDANS–TFIIIA binding to plasmid DNA or to the linear polyanion heparin resulted in moderate fluorescence quenching, consistent with the AEDANS group on TFIIIA becoming more mobile and/or moving into a more hydrophilic environment. The similar change in AEDANS–TFIIIA fluorescence with DNA or heparin indicates lack of sequence specificity in the DNA-induced conformational change. We do not believe these fluorescence changes observed here are simply the result of TFIIIA binding to a negatively charged environment since a significant change is not observed when the protein is free or bound to the negatively charged, 5S RNA (Table II).

Conformational States of TFIIIA. Figure 5 depicts our interpretations of TFIIIA conformational states identified from the trypsin digestion patterns (Figure 2) and AEDANS–TFIIIA fluorescence measurements (Figure 4, Table II). TFIIIA conformational states I and II (bound and free of 5S RNA) are depicted as being similar in structure with a more compact N-terminal half. This is deduced from the relative

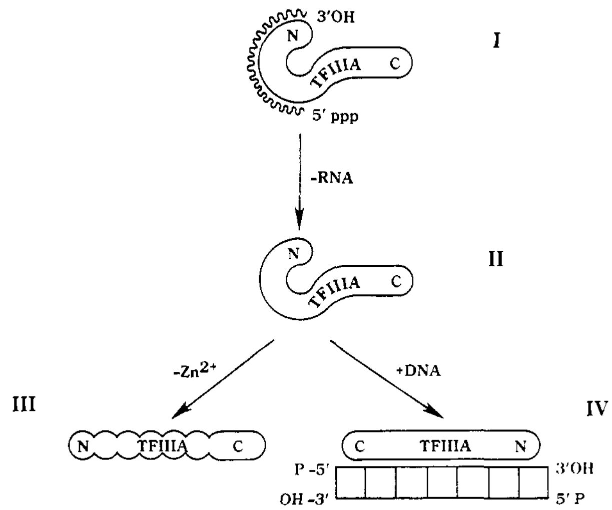

FIGURE 5: Conformational states of TFIIIA. N and C denote the N-terminal and C-terminal regions of TFIIIA, respectively. Roman numerals annotate the conformational states of TFIIIA referred to in the text. The double-stranded DNA template in state IV is subdivided every 10 base pairs.

trypsin insensitivity of this region whether free or bound with 5S RNA and the small AEDANS fluorescence change observed between these states. The 5S RNA is oriented in the 5′ to 3′ direction relative to the C to N termini of the protein, the same orientation maintained by the protein on the noncoding strand (5S RNA-like) of the 5S RNA gene. Previously published results suggested the TFIIIA binding site on 5S RNA and the 5S RNA gene are similar (Huber & Wool, 1986). Removal of zinc from free TFIIIA (state II) by EDTA chelation results in a change in conformation of the N-terminal half of the protein that is interpreted (in light of the increased trypsin sensitivity) as an opening or extension of the structure (state III). The large EDTA-dependent enhancement of AEDANS–TFIIIA fluorescence supports the existence of this structural change. Since 5S RNA is associated with the N-terminal half of TFIIIA, the observations that EDTA treatment of the 7S particle did not lead to trypsin sensitivity or to a significant AEDANS fluorescence change but chelation of free TFIIIA did are supportive results. As evidenced by trypsin sensitivity, the N-terminal half of TFIIIA undergoes extension and/or opening upon binding to template DNA (state IV). However, because the AEDANS–TFIIIA fluorescence is quenched upon DNA binding (not enhanced like EDTA treatment), some aspects of this conformational state (IV) are different than that produced by zinc removal (state III).

Registry No. Zn, 7440-66-6.

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Secretion of $^{35}SO_4$-Labeled Proteins from Isolated Rat Hepatocytes†

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ABSTRACT: Sulfation is a Golgi-specific modification of secretory proteins. We have characterized the proteins that are labeled with $^{35}SO_4$ in cultures of rat hepatocytes and studied their transport to the medium. Analysis by polyacrylamide gel electrophoresis showed that of the five most heavily labeled proteins, four had well-defined mobilities—apparent molecular masses of 188, 142, 125, and 82 kDa—whereas one was electrophoretically heterogeneous—apparent molecular mass of 35–45 kDa. Judging by their relatively high resistance to acid treatment, the sulfate residues in the 125- and 35–45-kDa proteins were linked to carbohydrate. Some of the secreted proteins were sialylated. In samples of pulse-labeled cells, there appeared to be no unsialylated forms, indicating that sulfation occurred after sialylation, presumably in the trans Golgi. Kinetic experiments showed that the cellular half-life was the same for all the sulfated proteins—about 8 min— consistent with the idea that transport from the Golgi complex to the cell surface occurs by liquid bulk flow.

Proteins destined for secretion are, during or after their synthesis, transferred into the lumen of the endoplasmic reticulum. They are then transported to the cell surface via the Golgi complex. The Golgi complex consists of three functionally different compartments—the cis, medial, and trans Golgi—which the proteins pass through successively. Most secretory proteins are modified at specific sites along the secretory pathway. Thus, e.g., many glycoproteins acquire galactose, fucose, and sialic acid residues in the trans Golgi. The transport between the different compartments of the secretory pathway is mediated by vesicles which have a characteristic coat on their surface [for reviews, see Burgess and Kelly (1987) and Pfeffer and Rothman (1987)]. Recent studies suggest that secretory proteins are transported merely by the bulk flow of liquid in the transport vesicles and that organelle-specific proteins are retained by binding to receptors (Munro & Pelham, 1987; Wieland et al., 1987). Only secretory proteins destined for secretory granules (Moore et al., 1983), or for one side in polarized cells (Urban et al., 1987), seem to be actively sorted. This sorting presumably takes place in a reticular extension of the trans Golgi (Griffiths & Simons, 1986).

We use isolated rat hepatocytes to study protein secretion (Fries et al., 1984; Fries & Lindström, 1986). Hepatocytes are polarized but appear to transport newly synthesized secretory and membrane proteins only to one side (Kloppel et al., 1986; Bartles, 1987). Furthermore, hepatocytes seem to lack regulated secretion, indicating that there is no active sorting of secretory proteins in these cells. To investigate the last steps in the secretory pathway, we have in the present study made use of the earlier observation that secretory proteins may incorporate sulfate in the Golgi complex.

Sulfation of proteins has been found in every tissue and cell line so far investigated [see, e.g., Heifetz et al. (1980), Liu et al. (1985), Paulsson et al. (1985), and Griswold et al. (1986)], including rat liver (Hille et al., 1984). Sulfate groups have been shown to be linked both to tyrosine (Huttner, 1987) and to carbohydrate residues (Slomiany & Meyer, 1972; Heifetz et al., 1980; Green et al., 1986). Autoradiographic and biochemical studies of different cell types have established that the sulfation of secretory proteins occurs in the Golgi complex (Young, 1973; Fessler et al., 1986; Lee & Huttner, 1984). In a recent study of a hybridoma cell line, it was shown that sulfation takes place in the trans Golgi (Baeuerle &

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