COMMUNICATION

Importance of Minor Groove Binding Zinc Fingers within the Transcription Factor IIIA-DNA Complex

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The gene-specific transcription factor IIIA (TFIIIA) binds to the internal promoter element of the 5 S rRNA gene through nine zinc fingers which make specific DNA contacts. Seven of the nine TFIIIA zinc fingers participate in major groove DNA contacts while two fingers, 4 and 6, have been proposed to bind in or across the minor groove. Pyrrole-imidazole polyamides are minor groove binding ligands that recognize predetermined DNA sequences with affinity and specificity comparable to natural DNA-binding proteins. We have examined the DNA binding activity of nine finger TFIIIA and shorter recombinant analogs in the presence of polyamides that bind six base-pair sequences (Kd ~ 0.03 to 1.7 nM) in the minor groove of the binding site for zinc finger 4. DNase I footprint titrations demonstrate that the polyamides and a recombinant protein containing the three amino-terminal zinc fingers of TFIIIA (zf1-3) co-occupy the TFIIIA binding site, in agreement with the known location of zf1-3 in the major groove. In contrast, the polyamides block the specific interaction of TFIIIA or zf1-4 with the 5 S RNA gene, supporting a model for minor groove occupancy by zinc finger 4. Minor groove binding polyamides targeted to specific DNA sequences may provide a novel chemical approach to probing multidomain protein-DNA interactions.

Keywords: DNA-binding protein; TFIIIA; 5 S RNA gene; zinc finger; pyrrole-imidazole polyamide

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Proteins with multisubunit DNA binding domains use a diverse structural repertoire to recognize their target sequences (Steitz, 1990). Proteins such as TBP bind exclusively in the minor groove (Kim et al., 1993a,b), others such as GCN-4 bind exclusively in the major groove (Ellenberger et al., 1992), and certain proteins such as Hın recombinase recognize both grooves (Feng et al., 1994; Sluka et al., 1990). In cases where protein-DNA complexes are too large for direct studies by NMR and X-ray methods, chemical approaches such as hydroxyl radical footprinting and methylation interference provide information concerning the groove location of individual DNA-binding subunits (for example see Draganescu et al., 1995; Lee et al., 1991). However, in the case of large proteins with multiple DNA-binding domains, footprinting results can in some cases be difficult to interpret. Pyrrole-imidazole polyamides bind to predetermined sequences in the minor groove of DNA (Wade et al., 1992), and we exploit them here to study a nine finger transcription factor to determine whether zinc fingers are located exclusively in the major groove or if zinc fingers bind in both grooves of the DNA helix.

The interaction of the zinc finger protein transcription factor IIIA (TFIIIA) with the internal control region (ICR) of the 5 S ribosomal RNA gene is the first step in the assembly of the active 5 S gene transcription complex (Bieker et al., 1985; Engelke et al., 1980). Zinc fingers 1 to 3, finger 5 and fingers 7 to 9, of the nine-finger protein, bind the ICR through base-specific interactions in the major groove (Clemens et al., 1992; Fairall et al., 1986; Sakonju & Brown, 1982). In contrast, methylation interference and protection experiments do not detect major groove interactions over the known binding sites for fingers 4 and 6. These DNA sequences each span about one full helical turn of

Abbreviations used: TFIIIA, transcription factor IIIA; ICR, internal control region; Py, N-methylpyrrole; Im, N-methylimidazole.
DNA (Clemens et al., 1992) and are strongly protected by TFIIIA from attack by hydroxyl radical (Churchill et al., 1990; Hayes & Clemens, 1992; Vrana et al., 1988). It has been proposed that fingers 4 and 6 each bind in or across the minor groove to bridge the major groove-binding fingers (see Figure 1A; Clemens et al., 1992; Hayes & Clemens, 1992; Hayes & Tullius, 1992). To provide direct evidence for minor groove occupancy by zinc finger 4, we have monitored the effect of two minor groove DNA-binding ligands on the DNA binding activity of TFIIIA and recombinant zinc finger proteins derived from TFIIIA.

Pyrrole-imidazole polyamides are a new class of synthetic DNA-binding ligands principally composed of N-methylpyrrole (Py) and N-methylimidazole (Im) amino acids. A binary code has been developed to correlate DNA-binding sequence-specificity with antiparallel side-by-side ring pairings in the minor groove (Mrksich et al., 1992, Wade et al., 1992). A pairing of Im opposite Py (Im/Py) targets a GC base-pair, while Py/Im targets CG (Wade et al., 1992). A Py/Py pairing is degenerate and targets TA and AT base-pairs (Pelton & Wemmer, 1989; Wade et al., 1992; White et al., 1996). Eight-ring hairpin polyamides recognize six base-pair (bp) target sequences with sub-nanomolar affinity (Trauger et al., 1996) and have recently been shown to inhibit TFIIIA-DNA binding and 5 S rRNA gene transcription in cell culture (Gottesfeld et al., 1997). We reasoned that a detailed analysis of the requirements for protein inhibition could determine if certain zinc fingers participate in minor groove DNA contacts.

Two eight-ring hairpin polyamides were synthesized to target the TFIIIA zinc finger 4 binding site within the somatic and oocyte-type 5 S gene ICR (Figure 1). Polyamide 1 is predicted to bind the 5'-TGGTTA-3' sequence (nucleotide positions +69 to +74) located within the binding sites for zinc fingers 4 and 5 (Clemens et al., 1992). Quantitative DNase I footprinting titrations (Trauger et al., 1996) on DNA restriction fragments containing either the oocyte-type or somatic 5 S genes reveals a dissociation constant (Kd) of 1.7 nM for 1. Polyamide 2 binds the 5'-AGTACT-3' sequence (Kd = 0.03 nM) found at nucleotide positions +74 to +79 of the somatic-type ICR. This sequence is located within the binding site for zinc finger 4, immediately adjacent to the binding site for finger 3 (Clemens et al., 1992).

We monitored the effect of polyamides 1 and 2 on TFIIIA binding. A recombinant protein containing the three amino-terminal zinc fingers of TFIIIA (zf1-3) was used as a control (Liao et al., 1992). NMR structure studies have shown that zf1-3 binds the ICR in the major groove and contacts nucleotides +80 to +92 of the 120-bp gene (Foster et al., 1997; Wuttke et al., 1997). Minor groove binding polyamides and certain major groove binding proteins have been found to co-occupy the DNA-helix (Oakley et al., 1992). Based on the major groove location of zf1-3, we expected that the polyamides used here would not interfere with the zf1-3-DNA interaction.

Polyamide 1 protects the ICR between nucleotides +69 to +78 on the non-coding (top) strand (Figure 2, lanes 3 to 5) and between nucleotides +66 to +74 on the coding (bottom) strand (not shown). An additional region of polyamide 1 protection is observed at a match 5'-TGGTAT-3'
sequence at nucleotide positions +101 to +106 downstream of the TFIIIA binding site. Occupancy of this sequence by polyamide 1 should not influence the binding of TFIIIA or recombinant analogs. Zf1-3 protects the ICR between nucleotides +77 to +95 on the non-coding strand of both the oocyte-type (Figure 2, lane 6) and somatic-type 5 S genes and induces a strong hypersensitive site between nucleotides +92 and +93 in both genes (Liao et al., 1992). DNase I footprinting clearly demonstrates that zf1-3 and polyamide 1 can co-occupy the same DNA molecules (Figure 2, lanes 7 to 9). When both reagents are present in the DNA-binding reaction, a footprint encompassing both the zf1-3 footprint and the polyamide footprint is generated (Figure 2, lanes 8-9).

TFIIIA protects the ICR from DNase I cleavage between nucleotides +45 and +95 on the non-coding strand (Figure 2, lane 11) and also introduces a hypersensitive site between nucleotides +92 and +93, as observed with zf1-3. Polyamide 1 is inhibitory to the binding of TFIIIA (Figure 2, lanes 12 to 14) such that when both polyamide 1 and TFIIIA are present only the polyamide 1 footprint is observed (lanes 13-14). In this experiment, increasing amounts of the polyamide were incubated with the DNA prior to the addition of TFIIIA, and substantial inhibition of binding was observed (>90%). When TFIIIA is added prior to polyamide 1 (lane 15) inhibition is still observed, although at a lower level. We also performed a TFIIIA titration experiment in the absence or presence of 50 nM polyamide 1 (Figure 3A). In this experiment, 2 nM TFIIIA produces full occupancy of the DNA in the absence of polyamide (lanes 6 and 13). In contrast, preincubation of the DNA with 50 nM polyamide 1 inhibits TFIIIA binding at all concentrations (lanes 8 to 11). Electrophoretic mobility shift assays also show that polyamide 1 inhibits the formation of stable TFIIIA-DNA complex (Figure 3B). These results suggest that when polyamide 1 is occupying the minor groove of the binding site for finger 4, TFIIIA cannot bind.

Zinc finger 5 of TFIIIA participates in direct contacts with the guanine pair at the intermediate element (nucleotides +70 and +71; Clemens et al., 1992). Since polyamide 1 was designed to contact this pair of guanines (Figure 1), inhibition of TFIIIA binding could result from interference with zinc finger 5, rather than zinc finger 4. As a control, we monitored the effect of polyamide 1 on the binding of a recombinant protein containing the amino-terminal zinc fingers 1 to 4 (zf1-4) (Figure 4). The zf1-4-DNA complex yields a similar DNase I footprint compared to that of zf1-3 (compare lanes 3 and 6 to 8 of Figure 4; see also Clemens et al., 1992, 1994). Finger 4-DNA contacts by zf1-4 have been detected by hydroxyl radical footprinting (Hayes & Clemens, 1992) and by gel mobility shift assays (Clemens et al., 1992). In contrast to the co-occupancy of the DNA by zf1-3 and the polyamide (Figure 2), compound 1 inhibits the binding of zf1-
4 to the ICR (lanes 9 to 11). These data suggest that occupancy of the finger 4 binding site by compound 1 blocks the interaction of finger 4 with this DNA sequence, for zf1-4 and full-length TFIIIA. A second polyamide targeted to the zinc finger 4 binding site, polyamide 2, protects the somatic-type 5 S gene ICR from cleavage by DNase between nucleotides +74 to +79 on the non-coding (top) strand (Figure 5, lane 7) and between nucleotides +73 to +79 on the coding (bottom) strand (not shown). Polyamide 2 and zf1-3 can co-occupy the same DNA molecules (Figure 5, lanes 8 to 12), while polyamide 2 inhibits binding of TFIIIA to the ICR (Gottesfeld et al., 1997). Since the binding sites for finger 3 (nucleotides +80 to +84; Wuttke et al., 1997) and polyamide 2 (+74 to +79) are immediately adjacent to one another, our data suggest that occupancy of the minor groove by a polyamide...
does not interfere with major groove-binding zinc fingers. Our results with these designed polyamides show that occupancy of the minor groove of the finger four binding site blocks TFIIIA binding, consistent with the proposal that finger four interacts with or crosses the minor groove (Clemens et al., 1992; Hayes & Tullius, 1992).

Inhibition of TFIIIA binding by the polyamides could be due either to direct steric blockage of the minor groove or to an alteration in DNA structure which prevents TFIIIA binding. Since TFIIIA binding is known to induce a significant (65°) bend in the ICR (Schroth et al., 1989, 1991), polyamides could conceivably stiffen the DNA (Wu & Crothers, 1984) and prevent bending. To distinguish between these possibilities, we monitored the effect of polyamide 2 on TFIIIA binding to linear and pre-bent circular DNAs (of the same length and sequence) using a gel mobility shift assay (Schroth et al., 1989). This gel system readily separates circular and linear DNAs and the TFIIIA complexes with these two DNAs. Identical TFIIIA binding inhibition curves were obtained for both DNAs (data not shown), suggesting that inhibition is due to blockage of the minor groove.

Our present results indicate that occupancy of the minor groove of the binding site for zinc finger 4 with a pyrrole-imidazole polyamide is not compatible with TFIIIA or zf1-4 binding. In contrast, major groove-binding zinc fingers (zf1-3) and the polyamides can co-occupy the same DNA molecules. The observation that polyamide 1 inhibits zf1-4 binding suggests that finger 4 may fold together with the other fingers to form a compact structure that would inhibit the entire domain (zf1-4) from binding. This possibility seems likely in light of the fact that all three fingers of zf1-3 form a compact structure when bound to DNA (Foster et al., 1997). Our current results suggest that zinc finger 4 binds in or across the minor groove, con-

Figure 4. Polyamide 1 inhibits zf1-4 binding to the ICR. Lane 1 shows a G-only sequencing ladder of a restriction fragment from the oocyte-type 5S RNA gene. The reaction of lane 2 shows the DNase digestion products of protein-free DNA. Lane 3 shows the reaction products in the presence of 10 nM zf1-3 while in lanes 4 and 5, 50 nM 1 was included in the reactions; the reaction of lane 5 contained both zf1-3 and polyamide. The reactions of lanes 6 to 8 and 9 to 11 contained increasing concentrations of zf1-4 (1, 5 and 10 nM, respectively) in the absence (lanes 6 to 8) or presence of 50 nM polyamide 1 (lanes 9 to 11). The polyamide was added to the DNA 30 minutes prior to adding zf1-3 or zf1-4.

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Consistent with models which proposed that these fingers use the minor groove to bridge the major groove-binding zinc fingers (Clemens et al., 1992; Hayes & Tullius, 1992). Future structural studies will be needed to determine the actual path of the zinc fingers in the major and minor grooves. Polyamide reagents are useful inhibitors of transcriptional activity by blocking transcription factor access to promoter elements for specific genes in vitro and in vivo (Gottesfeld et al., 1997). In addition to this activity, we have now shown that these reagents may be useful in dissecting major versus minor groove contacts between certain DNA-binding proteins and their target sites.

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Figure 5. Co-occupancy of zf1-3 and polyamide 2 on the somatic-type gene. Lane 1 shows the digestion products of protein-free DNA and lanes 2 to 6 show the digestion products obtained in the presence of increasing concentrations of zf1-3 (1, 2, 3, 4 and 6 nM final concentrations, respectively). Lane 7 shows the digestion products in the presence of 5 nM polyamide 2. Lanes 8 to 12 show digestion products of reactions where the DNA was first incubated with polyamide 2 for 30 minutes prior to the addition of zf1-3 (at the same concentrations as in lanes 2 to 6, respectively) and incubated for an additional 30 minutes prior to DNase treatment. Lane 13, G-only sequencing ladder.


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