2464–2471 Nucleic Acids Research, 1995, Vol. 23, No. 13

Zinc(II) ions selectively interact with DNA sequences present at the TFIIIA binding site of the *Xenopus* 5S-RNA gene

M. A. Martínez-Balbás+, E. Jiménez-García and F. Azorín*

Departament de Biologia Molecular i Cel.lular, Centre d'Investigació i Desenvolupament-CSIC, Jordi Girona Salgado 18-26, 08034 Barcelona, Spain

Received April 1, 1995; Revised and Accepted May 30, 1995

ABSTRACT

It has been known for some time that zinc, as well as most transition metal ions, is capable of binding to the DNA bases. However, little is known about the presence and distribution of metal binding sites along naturally occurring genomic DNA molecules. In this paper, the interaction of zinc with the *Xenopus* 5S-RNA gene has been studied and several metal binding sites have been identified on the basis of the changes in chemical reactivity observed in the presence of the metal. The strongest zinc-binding sites of the *Xenopus* 5S-RNA gene correspond to GGG trinucleotide repeats. Some GG dinucleotides also show a significant affinity for zinc. Interestingly, the binding site for TFIIIA, a zinc-finger transcription factor, contains several sites with strong zinc affinity. In particular, a TGGGA sequence which is essential for the binding of TFIIIA shows the strongest affinity for zinc. The conformational properties of this DNA sequence, together with the high electronegative potential of GGG runs, is likely to determine its strong affinity for zinc. The possible biological relevance of these results is discussed.

INTRODUCTION

Metal ions are important structural determinants of biological macromolecules. Though the importance of metal–DNA interactions has been recognised for some time, the degree of understanding of the mechanisms governing such interactions is still limited. Metal ions interact strongly with DNA influencing both the structure and stability of the macromolecule. In general, alkaline and alkaline-earth metals stabilise the double-stranded B-DNA conformation through electrostatic interactions with the phosphate backbone (see 1 for a review). On the other hand, most transition metal ions including zinc, bind also to the DNA bases, destabilising the B-DNA conformation (1). Finally, specific metal ions are known to promote the formation of a variety of non B-DNA conformations (i.e. Z-DNA, triple-stranded DNA, tetra-stranded DNA, four-way DNA junctions, etc.) (see 2 for a review).

Most of what is known about the binding of metal ions to DNA comes from studies performed with nucleosides, nucleotides and synthetic polynucleotides of repeated sequence (1,3,4). In this paper, the question of the presence and distribution of metal binding sites along naturally occurring genomic DNA molecules was addressed. The preferred site for metal ion co-ordination to the bases in DNA is the N7-position of the guanines (1) which is, in turn, the site of methylation by dimethylsulfate (DMS). Protection towards DMS methylation has been widely used to study the occupancy of this site in the complexes formed by DNA with proteins, drugs and other nucleic acids. DMS-reactivity is likely to be also strongly influenced by metal co-ordination at the guanine N7-position. Actually, protection from DMS methylation has been observed in the complex formed by a $d(GA{\cdot}GA)_n$ intramolecular hairpin with zinc (5). Here, we have used this experimental approach to study the interaction of zinc with a naturally occurring genomic DNA fragment, the *Xenopus* 5S-RNA gene, and the presence of several strong metal binding sites has been identified.

The importance of metal ions in nucleic acids processes is well established though their actual contribution is not completely understood. In general, the reactions in which nucleic acids participate are mediated by metal ions. Many DNA and RNA processing enzymes have metal ions as cofactors. DNA binding proteins also contain metal ions as structural components. In particular, a class of transcriptional activators, the zinc-finger proteins, contain zinc as a co-ordinated metal ion (6). TFIIIA is a member of the C_2H_2 family of zinc-finger proteins which is essential for transcription of the 5S-RNA genes. Interestingly, the strongest zinc binding sites of the *Xenopus* 5S-RNA gene cluster at the TFIIIA binding site. The possible biological relevance of these results is discussed.

MATERIALS AND METHODS

DNAs

The *Xenopus borealis* 5S-RNA gene was obtained from plasmid pXbsF201 which carries the somatic 5S-RNA gene inserted in the *Hin*dIII–*Bam*HI site of pUC9 (7).

* To whom correspondence should be addressed

+Present address: Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MA 20892, USA

When the behaviour of the TGGGA sequence was studied, oligomers of the oligonucleotide d(CTAGTGGGA·CTAGTC-CCA)$_2$ were obtained by self-ligation with T4 DNA ligase and cloned into the unique *Xba*I site of pUC19. Clones carrying different inserts were selected. Most of the results presented in this paper correspond to a plasmid carrying four TGGGA repeats. The complete sequence of the insert is 5′-CTAG**TGGGA**G-TAC**TGGGA**CTAG**TGGGA**CTAG**TGGGA**CT-3′.

Modification with dimethylsulfate (DMS)

For DMS-modification, ~3 µg of plasmid DNA, either linear or negatively supercoiled, was treated with 1 µl of DMS (Sigma), in a final volume of 500 µl, at room temperature for 1 min in a buffer containing 50 mM NaCl, 50 mM triethanolamine (pH 7.0) and the corresponding $MeCl_2$ at the concentration indicated in each case. Modification was stopped by the addition of 15 µl of 1 M β-mercaptoethanol, followed by ethanol precipitation. After modification, DNAs were cleaved with *Eco*RI and end-labelled with [α-^{32}P]dATP and the Klenow enzyme. After labelling, DNAs were cut with an appropriate restriction enzyme and the DNA fragments carrying the sequences of interest were purified by gel electrophoresis, treated with 1 M piperidine and the cleaved products resolved in denaturing polyacrylamide gels. When the modification was performed on linear DNA, plasmid DNAs were cleaved and end-labelled before treatment with the chemical reagent. For quantitative analysis, autoradiographs were recorded on Hyperfilm (Amersham) and scanned in a Molecular Dynamics laser densitometer. The intensity of each band was determined as the area underneath the corresponding peak on the densitometer scan, and the corresponding relative intensity (I_r) calculated after normalisation with respect to the sum of the intensities of all bands corresponding to the 5S-RNA gene sequence.

Modification with diethylpyrocarbonate (DEPC)

For DEPC-modification, ~3 µg of negatively supercoiled plasmid DNA was treated with 2 µl of DEPC, in a final volume of 50 µl, for 15 min at room temperature in the same buffer described above for DMS-modification. The reaction was stopped by ethanol precipitation and the modified samples were processed as described above.

Modification with osmium tetraoxide (OsO_4)

For OsO_4-modification, ~3 µg of negatively supercoiled plasmid was treated with 0.5 mM OsO_4 in the presence of 0.5% pyridine for 15 min at room temperature in the same buffer described above. The reaction was stopped by ethanol precipitation and the modified samples were processed as described above.

RESULTS

Figure 1 shows the patterns of DMS modification of the *Xenopus* 5S-RNA gene obtained in the presence of increasing $ZnCl_2$ concentration. Most of the guanines contained within the *Xenopus* 5S-RNA gene show a similar DMS-reactivity either in the absence or presence of zinc. However, in a few cases, addition of zinc results in a reduced reactivity. This is particularly evident for G_{86} and G_{98} (Fig. 1B). These two residues show a similar decrease in DMS-reactivity, as reflected by the very similar slopes (α) of $\ln I_r$ versus $\ln(1+[Zn])$ (Fig. 2A; Table 1). Both G_{86} and G_{98} occupy the central position on a GGG trinucleotide occurring at a TGGGA sequence. The DMS-reactivity of the two other guanine residues of the GGG trinucleotide does not decrease significantly in the presence of zinc (Fig. 2A). The *Xenopus* 5S-RNA gene contains two additional GGG trinucleotides, starting at positions 59 and 64. Also in these cases, the DMS-reactivity of the central guanine residues is reduced in the presence of zinc (Fig. 1). However, the decrease in reactivity is smaller than in the cases described above (Fig. 2A; Table 1). These results indicate that the interaction of zinc with the central guanine of GGG trinucleotide repeats is strongly influenced by the nature of the flanking nucleotides. Some GG dinucleotides contained within the 5S-RNA gene show also a decreasing DMS-reactivity on increasing zinc concentration (Fig. 1). This decrease is in general smaller than that observed at GGG trinucleotides and is also affected by the nature of the flanking sequences (Fig. 2B; Table 1). In particular, some GG dinucleotides, such as those starting at positions 7 and 116, do not show any significant decrease on DMS-reactivity upon increasing zinc concentration (Fig. 1B). The DMS reactivity of individual guanines do not show any significant decrease upon increasing zinc concentration (Fig. 1B). Table 1 summarises these results. The TGGGA sequence shows the strongest interaction with zinc, followed by the AGGGT and the AGGT sequences. A third group of sequences, which includes the fourth GGG trinucleotide and most of the GG dinucleotides, shows a significantly lower affinity for zinc.

Table 1.

Residue	Sequence	α
G_{98}	tgGga	0.19
G_{86}	tgGga	0.18
G_{60}	agGgt	0.15
G_{107}	aGgt	0.14
G_{70}	tGgt	0.11
G_{108}	agGt	0.10
G_{65}	cgGgc	0.09
G_{71}	tgGt	0.09
G_{48}	cgGa	0.08
G_{82}	tgGa	0.08
G_{59}	aGggt	0.07

The values of the slope (α) of $\ln I_r$ versus $\ln(1+[Zn])$ are presented for the guanine residues of the *Xenopus* 5S-RNA gene. Guanine residues showing $\alpha \leq 0.05$ are not presented. The position of the residue in the sequence is indicated in bold-face.

The effect described above is independent of the overall nucleotide sequence of the 5S-RNA gene. Figure 3 shows the patterns of DMS-modification of a DNA fragment containing four TGGGA-repeats. Also in this case, the DMS-reactivity of the central guanine residue of each repeat decreases strongly upon increasing zinc concentration (Fig. 3B). The relative magnitude of the decrease in reactivity is similar for the four repeats as indicated by the similar values of α obtained for the four repeats (Fig. 3B). These values, which are in the range 0.13–0.10, are

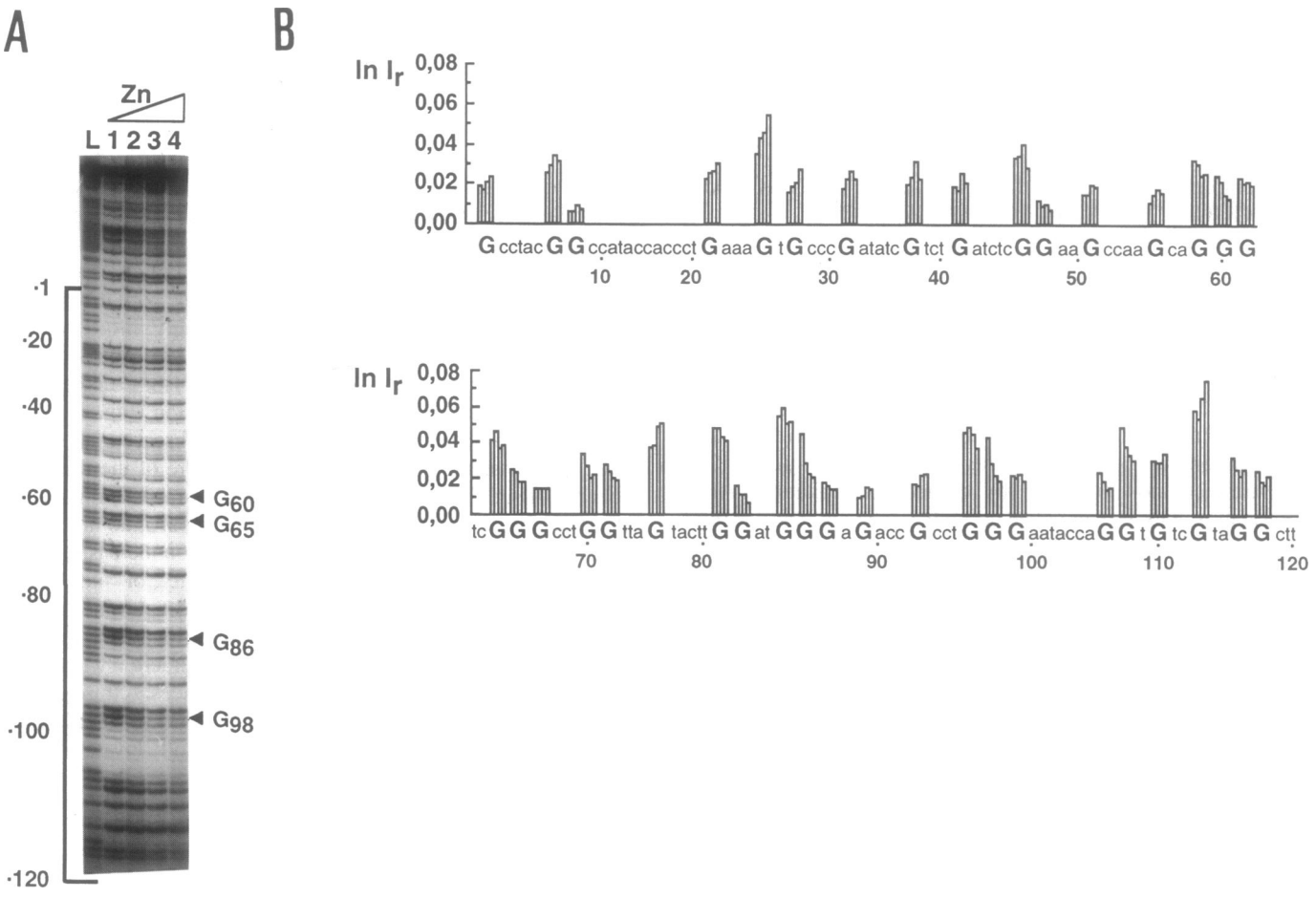

Figure 1. (**A**) Patterns of DMS-modification of the *Xenopus* 5S-RNA gene obtained in the presence of increasing zinc concentration: 0 (lane 1), 3 (lane 2), 30 (lane 3) and 60 mM (lane 4). Lane L corresponds to a G+A sequencing ladder. The 5′→3′ direction is top-to-bottom. The region of the gel corresponding to the 5S-RNA gene is indicated on the left. Residues G_{60}, G_{65}, G_{86} and G_{98}, showing strong decrease on DMS-reactivity are indicated on the right. (**B**) Quantitative analysis of the results shown in (A). The relative intensity (I_r) of each guanine residue of the *Xenopus* 5S-RNA gene is presented as a function of increasing zinc concentration from 0 mM (left) to 60 mM (right). Results are shown only for the non-coding strand. The coding strand contains two GGG trinucleotide repeats of sequence AGGGT and CGGGC respectively that also show a significant decrease on DMS-reactivity on increasing zinc concentration. Some GG dinucleotide repeats contained within the coding strand also show a decreasing DMS-reactivity.

significantly smaller than those observed for the TGGGA motives of the *Xenopus* 5S-RNA gene, suggesting that determinants other than the nucleotides immediately adjacent to the GGG trinucleotide also have an influence. A similar decrease on DMS-reactivity was obtained for molecules containing only two repeats of the TGGGA sequence (not shown).

The topological state of the DNA, whether linear or negatively supercoiled, does not have any significant effect on the decrease on DMS-reactivity observed at the TGGGA motives in the presence of zinc. Figure 3C shows the patterns of DMS-reactivity of linear DNA obtained upon increasing zinc concentration. Also in this case, the DMS-reactivity of the central guanine residue of the TGGGA-repeats decreases strongly even at low zinc concentration (Fig. 3C, lane 2). The values of α are also similar to those obtained with negatively supercoiled DNA.

That metal ion co-ordination to the guanine N7 position is responsible for the decrease on DMS-reactivity is also indicated by its metal ion specificity (Fig. 4). A similar decrease on the DMS-reactivity of the central guanine residue of the TGGGA sequence is also observed in the presence of transition metal ions such as Cd^{2+}, Co^{2+} and Mn^{2+}, that also bind to the DNA bases (1). On the other hand, this effect is much smaller in the presence of metals showing little or no co-ordination to the bases, such as Mg^{2+} (Fig. 4) (1). The relative decrease on DMS reactivity follows the order Co > Zn = Cd >> Mn > Mg (Fig. 4C) which correlates well with the relative stability of the corresponding metal-DNA complexes (1,3,4).

As judged by the patterns of DEPC and OsO_4 reactivity of the TGGGA sequence obtained in the presence of zinc, metal binding to this DNA sequence causes only minor conformational distortions. Regular right-handed B-DNA does not show any significant reactivity with either DEPC or OsO_4 (2). DEPC principally reacts at the N7 position of adenines when they are unpaired or highly distorted. OsO_4 reacts with pyrimidine residues and it is very sensitive to changes in stacking. As shown in Figure 5, no significant DEPC-reactivity of the adenine residues of the TGGGA motif is observed in the presence of the metal ion, indicating that no large structural distortion occurs upon zinc binding. On the other hand, thymine residues become moderately reactive to OsO_4 at high zinc concentration, indicating that some conformational change actually takes place upon zinc binding. OsO_4-reactivity is not constrained to the TGGGA

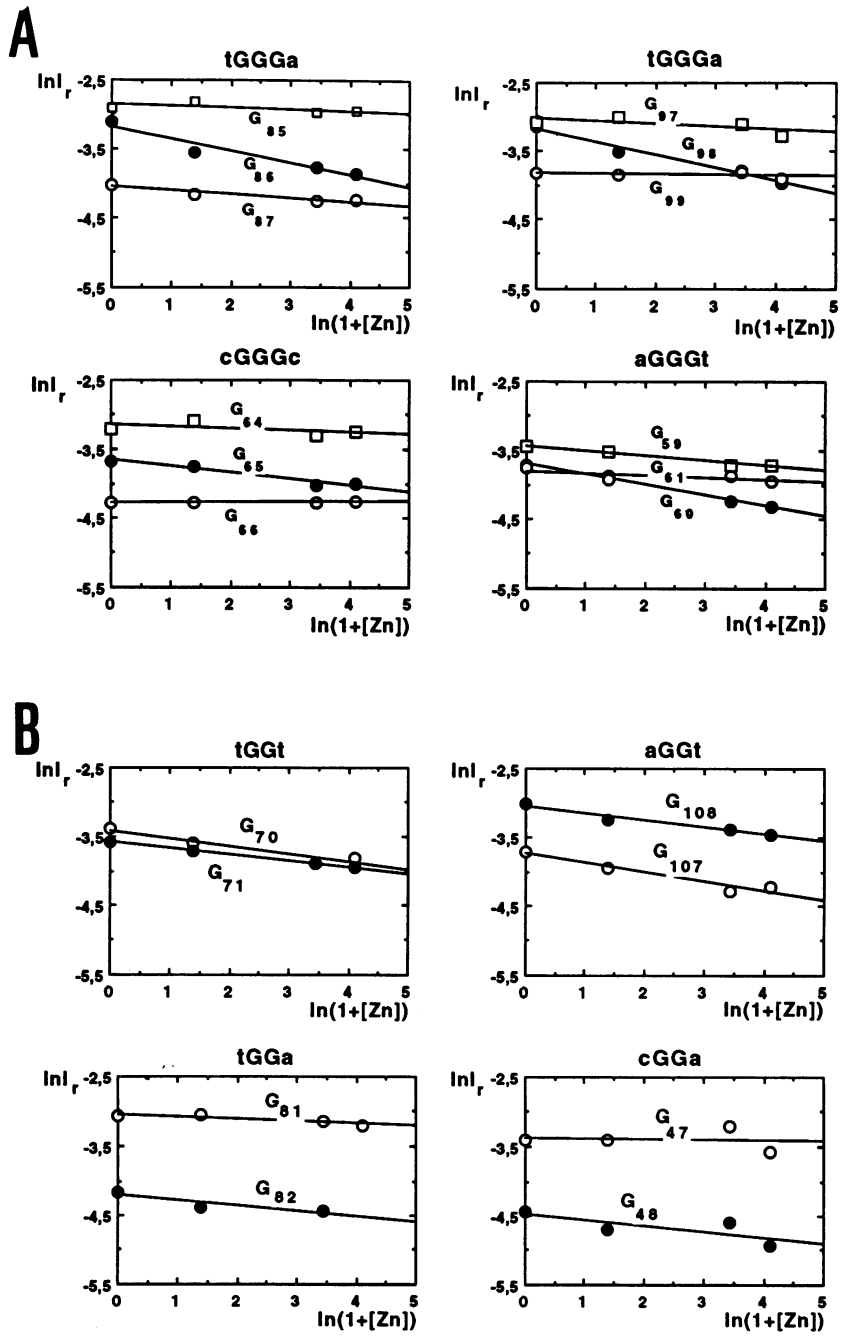

Figure 2. The decrease on DMS-reactivity as a function of increasing zinc concentration is presented for: (**A**) the four GGG trinucleotide repeats and (**B**) four GG dinucleotide repeats present in the *Xenopus* 5S-RNA gene. Results are shown only for the non-coding strand.

motives and thymine residues located 3 bp upstream from these motives are also significantly reactive with OsO_4, indicating that the whole DNA region is distorted. However, thymine residues of the TGGGA motives are always more reactive than the rest.

DISCUSSION

An important limitation on the study of metal–DNA interactions comes from the lack of experimental probes to analyse the binding of metals to long DNA molecules of randomised sequence. Here, we have explored the use of DMS to determine the distribution of zinc-binding sites along a naturally occurring genomic DNA sequence, the *Xenopus* 5S-RNA gene. Methylation by DMS occurs principally at the N7-group of the guanines which is known to be also the preferred site for metal ion co-ordination to the bases in DNA. Our results indicate that some of, but not all, the guanine residues contained within the *Xenopus* 5S-RNA gene become protected towards DMS methylation in the presence of zinc ions. The strongest decrease on DMS-reactivity is observed at the central guanine residue of the GGG trinucleotide repeats contained within the sequence TGGGA.

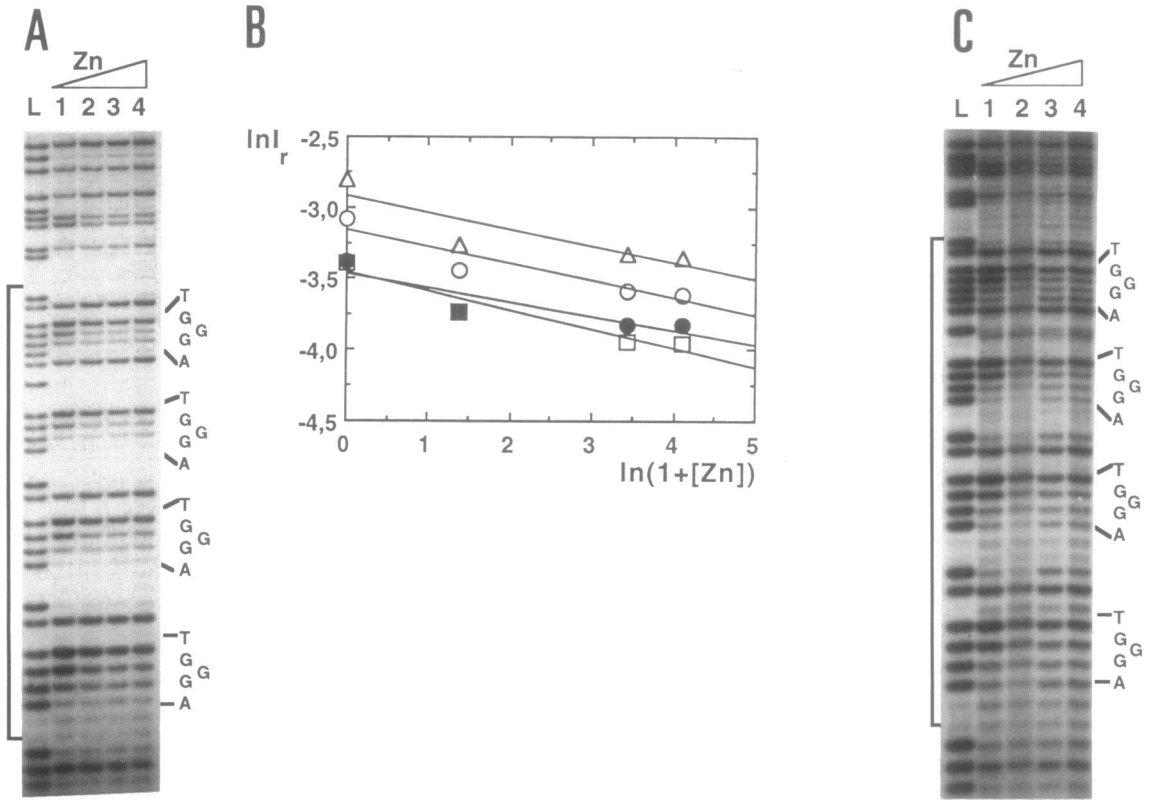

Figure 3. (**A**) Patterns of DMS-modification of a DNA fragment containing four TGGGA-repeats obtained in the presence of increasing zinc concentration: 0 (lane 1), 3 (lane 2), 30 (lane 3) and 60 mM (lane 4). Lane L corresponds to a G+A sequencing ladder. The 5′→3′ direction is top-to-bottom. The position of each TGGGA-repeat is indicated (right) as well as the region of the gel corresponding to the DNA fragment containing the four TGGGA-repeats (left). (**B**) Quantitative analysis of the results shown in (A). The decrease of DMS-reactivity is presented as a function of increasing zinc concentration for the central guanine residue of each TGGGA-repeat: (△) first, (●) second, (○) third and (□) fourth repeat. The slopes α are 0.13, 0.10, 0.12 and 0.12 respectively. (**C**) As in panel A but modification was performed on linear plasmid DNA.

The decrease of DMS-reactivity observed in the presence of zinc principally reflects binding of the metal to the DNA sequence rather than a conformational change on the DNA itself. On one hand, the effect does not depend on the topological state of the DNA substrate, whether linear or negatively supercoiled (Fig. 3). Second, the patterns of DEPC modification obtained in the presence of zinc indicate that no significant conformational change occurs on the DNA (Fig. 5). Third, metal ion co-ordination is also strongly suggested by the metal ion specificity of the effect (Fig. 4). Finally, our results are in accordance with what is known about the binding of zinc to DNA. It has been shown that GGG-runs have the most negative electrostatic potential of all possible three-base sequences (8) and direct binding of zinc to the central guanine of a GGG trinucleotide was shown by multinuclear NMR spectroscopy in the dodecamer d(ATGGGTACCCAT)$_2$ (9). Our results indicate that zinc binding to GGG-runs depends strongly on the nature of the flanking sequences, suggesting that, in addition to the high electronegative potential of the sequence, other factors are also contributing significantly to the strong zinc binding detected at the TGGGA sequences of the 5S-RNA gene.

From our results it is difficult to conclude the type of metal–DNA complex which is being formed. As judged by the results shown in Figure 5, binding of zinc to the TGGGA sequence causes only a minor structural distortion of the DNA binding site. It is known that zinc also coordinates to the phosphate oxygens in DNA (1) and it was proposed that zinc binding occurs via the formation of an N7/αPO_4 chelate (10). This type of co-ordination has been observed in several metal–nucleotide complexes (1,3,4), including the complexes formed by 5′-IMP with Zn^{2+} and Cd^{2+}. In all these complexes, the conformation of the nucleotide is significantly different from that observed in B-DNA (1,3,4). Actually, formation of an N7/αPO_4 chelate requires a C3′-endo puckering of the furanose ring (11–13), which is characteristic of A-DNA. In addition, in most metal–nucleotide complexes showing this type of co-ordination the torsion angles about the C1′–N and the C4′–C5′ bonds are in the *anti* and *+sc* regions, respectively (1,3,4). These conformational features are also characteristic of A-DNA. Evidence derived from DNaseI digestion experiments, X-ray crystallography and CD spectroscopy (14–16) indicates that the region of the *Xenopus* 5S-RNA gene spanning nucleotides 81–89, which encompasses the TGGGA sequence, adopts an A-like conformation. Adoption of an A-like conformation, together with the high electronegative potential of GGG-runs, would facilitate binding of zinc through the formation of a N7/αPO_4 chelate, providing a reasonable interpretation for our results.

Others have reported that zinc, at micromolar concentrations, specifically induces bending of the 5S-RNA gene (17). This bending is likely to result from the type of site-directed

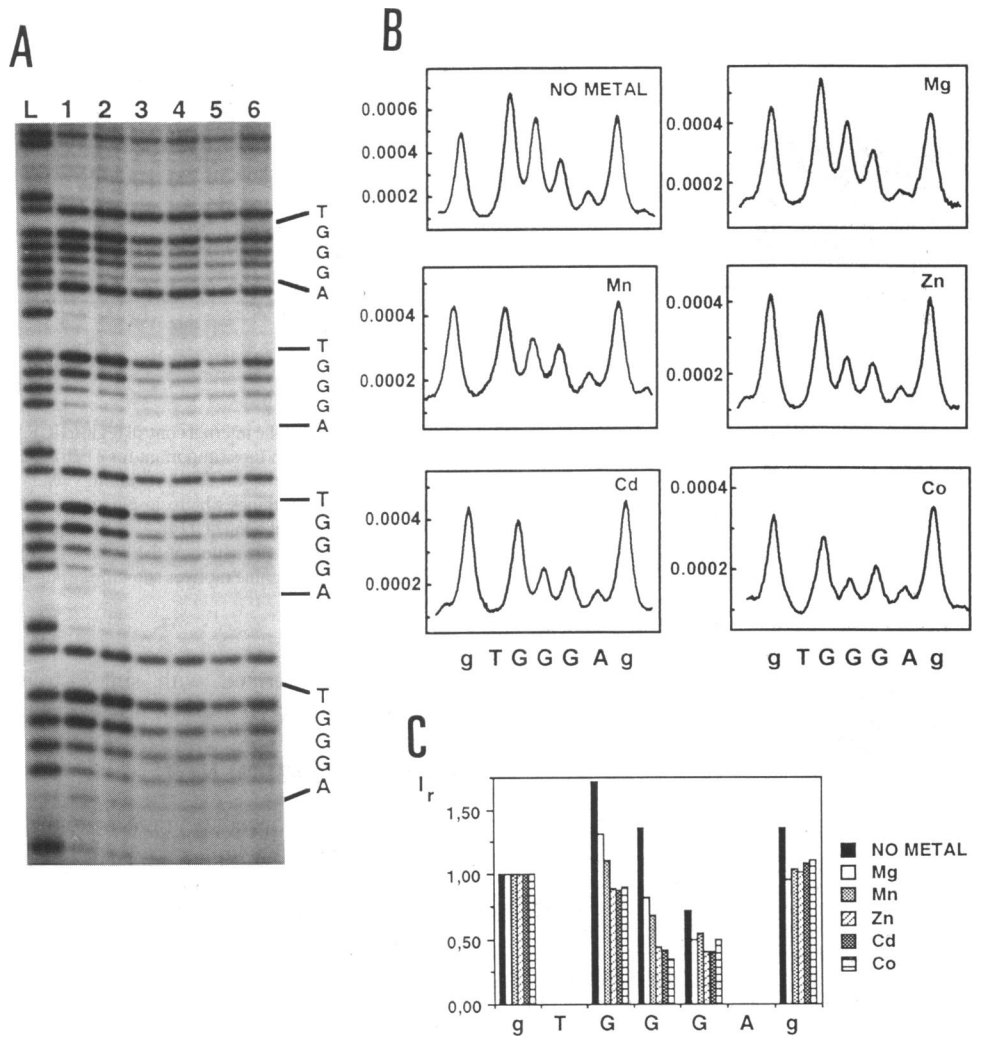

Figure 4. (**A**) Patterns of DMS-modification of the DNA fragment described in Figure 3, which contains four TGGGA-repeats, obtained in the presence of a 30 mM concentration of $MgCl_2$ (lane 2), $ZnCl_2$ (lane 3), $CdCl_2$ (lane 4), $CoCl_2$ (lane 5) and $MnCl_2$ (lane 6). Lane 1 shows the pattern of DMS-modification obtained in the absence of any added metal. Lane L corresponds to a G+A sequencing ladder. The 5′→3′ direction is top-to-bottom. (**B**) Densitometer scans corresponding to the gTGGGAg region, encompassing the first TGGGA-repeat. The optical density is shown in arbitrary units. Similar scans were obtained for the other three repeats shown in (A). (**C**) Comparative analysis of the results shown in (B). The relative intensity (I_r) of each guanine residue of the gTGGGAg sequence is shown as a function of the type of metal. The intensity of each guanine residue was normalised with respect to the intensity of the guanine found immediately 5′ from the TGGGA-motif. The extent of DMS-modification of the guanine located immediately 3′ from the TGGGA-motif is only slightly affected by the presence of any added metal.

interactions reported here. Interestingly, several strong metal binding sites occur within the binding site for TFIIIA, a zinc-finger transcription factor. The internal control region (ICR) of the *Xenopus* 5S-RNA gene, nucleotides 45–97, consists of three functional elements (Fig. 6) from which box C is the most important for TFIIIA binding (18–21). Box C contains a TGGGA motif, nucleotides 84–86, which is essential for TFIIIA binding, as shown by mutational and methylation studies (21–26) (Fig. 6). As shown in this paper, this site has a strong affinity for zinc. A second TFIIIA–DNA contact occurs in box C, from nucleotides 79–82, and a third strong protein–DNA contact is detected at nucleotides 70 and 71 (24). These two additional sites contain GG dinucleotides showing also a significant affinity for zinc (Fig. 2B; Table 1). Interestingly, as for TFIIIA, the specific DNA binding sites of many C_2H_2 zinc-finger proteins are enriched in sequences which are potential metal binding sites (6). The structure of the TFIIIA–DNA complex is not known. However, the DNA complexes formed by other C_2H_2 zinc-finger proteins have been solved by X-ray crystallography. These include the Zif268 protein, the human glioblastoma (GLI) protein and the *Drosophila* tramtrack protein (27–29). The three protein–DNA complexes mentioned above show some interesting features. On one hand, in all three complexes, some of the histidine residues that co-ordinate zinc make direct contacts with the phosphodiester oxygens and, secondly, the DNA molecule in the complex shows a distinctive conformation closer to that corresponding to A-form DNA (30). These results opens up the question of the hypothetical biological relevance of the preferential binding of zinc detected at the TFIIIA binding site. It is very well established that the main role of zinc in TFIIIA is to determine the appropriate folding of the protein domains involved in sequence specific DNA recognition. Furthermore, the crystal structures of the three protein–DNA

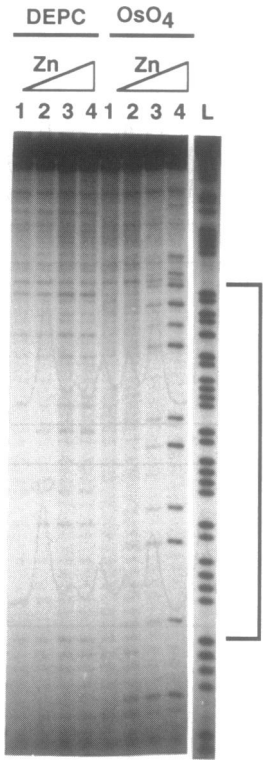

Figure 5. Patterns of DEPC and OsO_4 modification of a DNA fragment containing four TGGGA-repeats, obtained in the presence of increasing zinc concentration: 0 (lanes 1), 5 (lanes 2), 15 (lanes 3) and 30 mM (lanes 4). Lane L corresponds to a G+A sequencing ladder. The 5′→3′ direction is top-to-bottom. The region of the gel corresponding to the DNA fragment containing the four TGGGA-repeats is indicated. The DNA fragment used in this case was different from that described in Figures 3 and 4, showing a G to T mutation of the third guanine residue of the first TGGGA motif. This mutation generates a TGGT motif which, as shown in Figure 2B and Table 1, also binds zinc with high affinity.

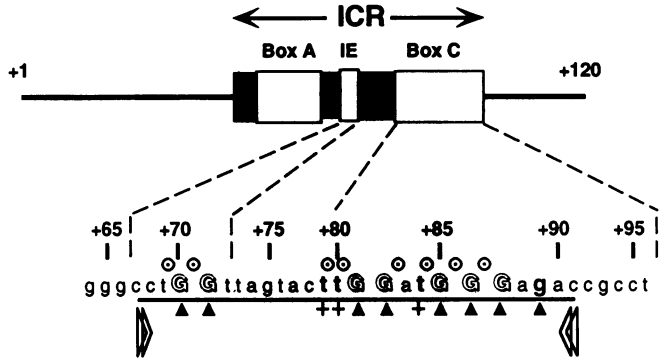

Figure 6. Structure of the internal control region (ICR) of the *Xenopus* 5S-RNA gene. The DNA region most important for TFIIIA binding is shown. Guanine bases which interfere with TFIIIA binding when methylated (24,25) are indicated by (▲). Thymines which closely approach TFIIIA (34) are indicated by (+). Phosphates that contact TFIIIA (22) are also indicated (⊙). The DNA region involved in the formation of the covalent complex with TFIIIA (31) is shown underlined. Guanine motives showing strong affinity for zinc are shown in outlined letters.

complexes described above rule out a direct participation of the metal ions in DNA binding. However, it might still be that, subsequent to the formation of the protein–DNA complex, the structural zinc ions contained within TFIIIA would directly interact with the DNA. In this respect, it is interesting to notice that TFIIIA remains bound to the DNA during transcription and, since the ICR occurs within the gene coding region, endures multiple passages of the RNA polymerase (32). It is possible that zinc ions could participate in the formation of this complex helping to maintain the protein bound to the DNA single-strand during transcription. Interestingly, all the sites with strong affinity for zinc of the TFIIIA binding site occur at the non-coding strand and the protein binds strongly to single-stranded DNA (33). If the structural zinc ions of TFIIIA directly contribute to its binding to single-stranded DNA, the structure of the protein must change profoundly. No large structural distortions have been reported to occur in TFIIIA after binding to DNA. However, it was reported that, under some circumstances, TFIIIA forms a very tight covalent complex with DNA which maps between nucleotides 68–90 including the DNA sequences of high zinc affinity described here (31). Interestingly, the DNA binding domain of TFIIIA, i.e. the zinc fingers, is sufficient to form the complex which is also formed with single-stranded DNA. Though speculative, this hypothesis could be addressed experimentally.

ACKNOWLEDGEMENTS

This work was financed by grants from the DGICYT (PB90-997 and PB93-102). The support of the CIRIT of the Generalitat de Catalunya through its Centre de Referència en Biotecnologia is also acknowledged. M.A.M-B. acknowledges receipt of a postdoctoral fellowship from the Spanish Research Council (CSIC) and E.J-G. of a doctoral fellowship from the CIRIT of the Generalitat de Catalunya. We are thankful to Dr A. Rodríguez-Campos for providing us with the plasmid DNA and to Drs J. Bernués, M. Coll, B. Piña and J. Portugal for carefully reading this manuscript.

REFERENCES

1 Saenger W. (1984) In *Principles of Nucleic Acid Structure* pp. 201–219. Springer-Verlag Edt. Berlin (1984).
2 Palecek, E. (1991) *Crit. Rev. Biochem. Mol. Biol.* **26**, 151–226.
3 Swaminathan, V., and Sundaralingam, M. (1979) *CRC Crt. Rev. Biochem.* **6**, 245–331.
4 Terrón, A. (1993) *Comments Inorg. Chem.* **14**, 63–88.
5 Beltrán, R., Martínez-Balbás, A., Bernués,J., Bowater, R., and Azorín, F. (1993) *J. Mol. Biol.* **230**, 966–978.
6 Coleman, J.E. (1992) *Annu. Rev. Biochem.* **61**, 897–946.
7 Ravzi, F., Gargiulo, G., and Worcel, A. (1983) *Gene* **23**, 175–183.
8 Pullman, A., and Pullman, B. (1981) *Q. Rev. Biophys.* **14**, 289–380.
9 Jia, X, Zon, G. and Marzilli, L.G. (1991) *Inorg. Chem.* **30**, 228–239.
10 Zimmer, Ch., Luck, G., and Triebel, H. (1974) *Biopolymers* **13**, 425–453.
11 Reily, M.D., and Marzilli, L.G. (1986) *J. Am. Chem. Soc.* **108**, 8299–8300.
12 Reily, M.D., Hambley, T.W. and Marzilli, L.G. (1988) *J. Am. Chem. Soc.* **110**, 2999–3007.
13 Alessio, E., Xu, Y., Cauci, S., Mestroni, G., Quadrifoglio, F., Viglino, P., and Marzilli, L.G (1989) *J. Am. Chem. Soc.* **111**, 7068–7071.
14 Rhodes, D., and Klug, A. (1986) *Cell* **46**, 123–132.
15 McCall, M., Brown, T., Hunter, W.N. and Kennard, O. (1986) *Nature* **322**, 661–664.
16 Fairall, L., Martin, S., and Rhodes, D. (1989) *EMBO J.* **8**, 1809–1817.
17 Nickol, J., and Rau, D.C. (1992) *J. Mol. Biol.* **228**, 1115–1123.
18 Pieler, T., Appel, B., Oei, S.-L., Mentzel, H., and Erdmann, V.A. (1985) *EMBO J.* **4**, 1847–1853.

Pieler, T., Oei, S.-L., Hamm,J., Engelke, U., and Erdmann, V.A (1985) *EMBO J.* **4**, 3751–3756.
Pieler, T., Hamm, J., and Roeder, R.G. (1987) *Cell* **48**, 91–100.
You, Q., Veldhoen, N., Baudin, F., and Romaniuk, P.J. (1991) *Biochemistry* **30**, 2495–2500.
Sakonju, S., Bogenhagen, D.F., and Brown, D.D. (1980) *Cell* **19**, 13–25.
Bogenhagen, D.F., Sakonju, S., and Brown, D.D. (1980) *Cell* **19**, 27–35.
Sakonju, S., and Brown, D.D. (1982) *Cell* **31**, 395–405.
Fairall, L., Rhodes, D. and Klug, A. (1986) *J. Mol. Biol.* **192**, 577–591.
Veldhoen, N., You, Q., Setzer, D.R., and Romaniuk, P.J. (1994) *Biochemistry* **33**, 7568–7575.
Pavletich, N.P., and Pabo, C.O. (1991) *Science* **252**, 809–817.
Pavletich, N.P., and Pabo, C.O. (1993) *Science* **261**, 1701–1707.
29 Fairfall, L., Schwabe, J.W.R., Chapman, L., Finch, J.T., and Rhodes, D. (1993) *Nature* **366**, 483–487.
30 Nekludova, L., and Pabo, C.O. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 6948–6952.
31 Blanco, J., and Gottesfeld, J.M. (1988) *Nucleic Acids Res.* **16**, 11267–11284.
32 Bogenhagen, D.F., Wormington, W.M., and Brown, D.D. (1982) *Cell* **28**, 413–421
33 Hanas, J.S., Bogenhagen, D.F., and Wu, C.-W. (1984) *Nucleic Acids Res.* **12**, 2745–2758.
34 Liao, X., Clemens, K.R., Tennant, L., Wright, P.E., and Gottesfeld, J.M. (1992) *J. Mol. Biol.* **223**, 857–871.