# DNA-sequence and metal-ion specificity of the formation of *H-DNA

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## ABSTRACT

**The homopyrimidine-homopurine sequence $d(CT/GA)_{22}$ undergoes, in the presence of zinc ions, transition to an altered DNA conformation (*H-DNA) which is neither H-DNA nor B-DNA. *H-DNA is characterized by a peculiar chemical reactivity pattern in which most of the polypyrimidine strand is hyperreactive to osmium tetroxide and the central part of the polypurine strand is sensitive to diethylpyrocarbonate. Formation of *H-DNA is specific of metal-ion. *H-DNA is detected in the presence of $Zn^{++}$, $Cd^{++}$ and $Mn^{++}$. The efficiency on promoting the transition is in the order of $Zn^{++} > Cd^{++} >> Mn^{++}$. Formation of *H-DNA is also specific of nucleotide sequence. From all the different homopolymeric sequences tested only the $d(CT/GA)_{22}$ sequence showed the zinc-induced transition to *H-DNA. These results suggest that stabilization of *H-DNA involves the formation of a specific complex between the metal-ion and the nucleotide sequence. The biological relevance of these results is discussed in view of the important role that zinc ions play on many nucleic acids processes.**

## INTRODUCTION

DNA in solution is structurally polymorphic. Studies of the conformational behaviour depicted by DNA sequences with a simple mono- or dinucleotide repeat have revealed the high degreee of structural flexibility of the DNA molecule. In particular, homopyrimidine-homopurine sequences are known to adopt several conformations that deviate significantly from the cannonical B-form. $d(T/A)_n$ sequences adopt a 'B-like' conformation (1-3), which appears to be associated with the regular bending of DNA fragments containing $d(T/A)_n$ stretches. Furthermore, $d(C/G)_n$ and $d(CT/GA)_n$ sequences are known to undergo transition to triple-stranded conformations in response to changing environmental conditions (i.e., increasing negative supercoiling, protonation, presence of metal ions, etc.). At low pH, these sequences exist as a homopyrimidine-homopurine-homopyrimidine triplex containing $CGC^+$ and , in the case of $d(CT/GA)_n$ sequences, TAT base triads (4-6). At neutral pH in the presence of magnesium ions, $d(C/G)_n$ sequences form a GGC triplex instead of the $CGC^+$ triplex found at low pH (6,7). In addition to triple-stranded helices, $d(CT/GA)_n$ sequences can also adopt other non-B conformations and at least six acid-induced conformations have been identified in linear poly $d(CT/GA)_n$ (8).

Recently, we reported a zinc-induced structural transition of a $d(CT/GA)_n$ sequence contained into negatively supercoiled DNA (9). This novel structure, called *H-DNA, is induced at neutral pH by moderate concentrations of zinc and it requires negative superhelicity. *H-DNA is characterized by a peculiar chemical reactivity. In *H-DNA, most of the polypyrimidine strand is sensitive to osmium tetroxide ($OsO_4$) modification while only the central part of the polypurine strand is reactive to diethylpyrocarbonate (DEPC). Formation of a homopurine-homopurine-homopyrimidine triplex might account for these results.

Here, we analyze the nucleotide and ion requirements for the formation of *H-DNA. Stabilization of this altered conformation appears to be specific of nucleotide sequence and metal-ion. From all the different divalent cations investigated ($Ca^{++}$, $Mg^{++}$, $Mn^{++}$, $Co^{++}$, $Ni^{++}$, $Cu^{++}$, $Zn^{++}$, $Cd^{++}$, $Hg^{++}$) only three ($Zn^{++}$, $Cd^{++}$, $Mn^{++}$) were shown to be efficient in the stabilization of the *H-DNA conformation ($Zn^{++} > Cd^{++} > > Mn^{++}$). Similarly, from all the different homopolymeric sequences tested ($d(CT/GA)_{22}$, $d(C/G)_{29}$, $d(T/A)_{60}$, $d(CG/GC)_5$, $d(CA/GT)_{30}$, $d(TA/AT)_{20}$) only the $d(CT/GA)_{22}$ sequence showed the zinc-induced transition to *H-DNA. These results suggest that stabilization of *H-DNA is achieved through the formation of a specific complex between the metal-ion and the nucleotide sequence.

The biological relevance of these results is discussed. Zinc ions play an important role as cofactors on many biological processes. In particular, a class of transcriptional regulators in eukaryotic cells contain zinc as a coordinated metal-ion (see 10, for a review). Upon DNA-binding, the zinc ions contained within these trans-acting factors might influence the conformation of the regulatory sequence. At this respect, is worth noting that $d(CT/GA)_n$ sequences are frequently located at the 5'-flanking region of many eukaryotic genes (11-14).

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## MATERIALS AND METHODS

### DNAs

Plasmids pE/$CT_{22}$, pE/$CA_{30}$, pE/$CG_5$ and pE/$T_{60}$ derivate from plasmid vector pE/O (15). pE/$CT_{22}$, pE/$CA_{30}$ and pE/$CG_5$ contain respectively d(CT/GA)$_{22}$, d(CA/GT)$_{30}$ and d(CG/GC)$_5$ inserts cloned at the second Sma I site of the polylinker of pE/O. pE/$T_{60}$ contains a d(T/A)$_{60}$ insert cloned at the unique Hind III site of pE/O. Plasmid pAT29 is a pBR322 derivative which contains a d(TA/AT)$_{20}$ insert cloned at the unique Bam HI site of pBR322 (16). pUC12T29 is a pUC12 derivative which contains a d(C/G)$_{29}$ insert cloned at the PstI site of pUC12 (17).

### Chemical modifications

Osmium tetroxide ($OsO_4$) and diethylpyrocarbonate (DEPC) modifications were carried out essentially as described previously (9). For $OsO_4$ modification, 3$\mu$g of DNA were subjected to treatment with 1% pyridine (Fluka), 1 mM $OsO_4$ (Agar-Aids) in a final volume of 50 $\mu$l in a buffer containing 50 mM NaCl, 50 mM Triethanolamine pH=7.0 and the appropriate metal-ion at the concentration indicated in each case. The reaction was allowed to proceed for 15 minutes at 25°C. DNAs were then ethanol precipitated twice and restricted with Eco RI (Boehringer-Manheim). After restriction, DNAs were end-labelled with either, $\alpha$-$^{32}$P-ATP (Amersham) and the Klenow enzyme (Boehringer-Manheim) or with, $\gamma$-$^{32}$P-ATP (Amersham) and T4-polynucleotide kinase (New England Nuclear) after dephosphorilation with CIP-alkaline phospatase (Boehringer-Manheim). After labelling, DNAs were digested with a convenient restriction endonuclease (Rsa I for plasmids pE/$CT_{22}$, pE/$CA_{30}$, pE/$CG_5$ and pE/$T_{60}$; Dra I for plasmid pUC12T29 and Bam HI for plasmid pAT29) and the fragments containing the repeated sequences purified by agarose gel electrophoresis. The purified DNAs were then subjected to cleavage with 1M piperidine at 90°C for 30 minutes and analyzed on 6% polyacrilamide-7M urea sequencing gels. When the extent of $OsO_4$-modification was analyzed, DNAs were digested with P1-nuclease (Boehringer-Manheim) after $OsO_4$-modification and linearization with BglI. Digestion was carried out at 37°C for 30 minutes at an enzyme/DNA ratio of 0.25 U/$\mu$g in a buffer containing 50mM NaCl, 10m $MgCl_2$, 10mM Tris-HCl, pH=8.0.

DEPC modifications were carried out exactly as described for $OsO_4$, except that 2$\mu$l of DEPC (Fluka) were added instead of 1% pyridine-1mM $OsO_4$. Sequencing ladders were obtained as described in (18).

## RESULTS

### Metal-ion specificity of the formation of *H-DNA

The effect of different metal-ions on the stabilization of *H-DNA was investigated. Several alkaline-earth ($Mg^{++}$, $Ca^{++}$) and transition metals ($Mn^{++}$, $Co^{++}$, $Ni^{++}$, $Cu^{++}$, $Zn^{++}$, $Cd^{++}$, $Hg^{++}$) were tested for their ability to induce formation of *H-DNA at a d(CT/GA)$_{22}$ sequence. To avoid possible charge and anion effects, all metal-ions were tested as their divalent chloride salts. *H-DNA was originally described as a non-B DNA conformation that a d(CT/GA)$_{22}$ sequence adopts in the presence of zinc ions (9). Formation of *H-DNA is detected at $Zn^{++}/P \geq 11$ (9) that, under our experimental conditions, corresponds to a zinc concentration of 2mM or higher. *H-DNA is characterized by a high sensitivity of the polypyrimidine strand to chemical modification by osmium tetroxide ($OsO_4$). This provides a general method for the evaluation of the amount of *H-DNA present in any given sample. $OsO_4$-modified DNA sequences are known to be sensitive to cleavage by single-stranded specific nucleases, such as P1 or S1 (9,19–21). Determination of the percentage of molecules cut at the d(CT/GA)$_{22}$ sequence by the nuclease will be a direct evaluation of the percentage of DNA molecules containing the d(CT/GA)$_{22}$ sequence stabilized as *H-DNA. To investigate the effect of the various metal-ions indicated above on the stabilization of *H-DNA, purified negatively supercoiled pE/$CT_{22}$ DNA ($\sigma = -0.05$) was modified with $OsO_4$-pyridine at pH = 7.0 in the presence of the various metal-ions at a concentration of either 2.5 mM or 5 mM. After modification, DNA was first linearized with Bgl I restriction endonuclease and then digested with P1-nuclease at pH = 8.0. P1-cleavage at the d(CT/GA)$_{22}$ insert of the Bgl I-linearized pE/$CT_{22}$ DNA should produce two DNA fragments of about 1388 bp and 926 bp in length, which are indicated by the asterisks in Figure 1. As shown in Figure 1, a significant proportion of the molecules are cut by P1 at the d(CT/GA)$_{22}$ insert when $OsO_4$-modification is carried out in the presence of either 5 mM $ZnCl_2$, 5 mM $CdCl_2$ or 2.5 mM $MnCl_2$. Around 65–70% of the molecules are cleaved by P1 when modification is carried out at 5 mM $ZnCl_2$ (Figure 1, lane 2). On the other hand, when modification is performed at 5 mM $CdCl_2$ (Figure 1, lane 3) only about 30–35% of the molecules become sensitive to P1 and an even lower percentage, around 5%, are cleaved when $OsO_4$-modification is carried out at 2.5 mM $MnCl_2$ (Figure 1, lane 9). Higher concentrations of $MnCl_2$ could not be tested, since precipitation occurred under the conditions of chemical modification.

On the other hand, no significant P1-cleavage at the d(CT/GA)$_{22}$ sequence is detected when $OsO_4$-modification is carried out in the presence of either 5 mM $MgCl_2$, 5 mM

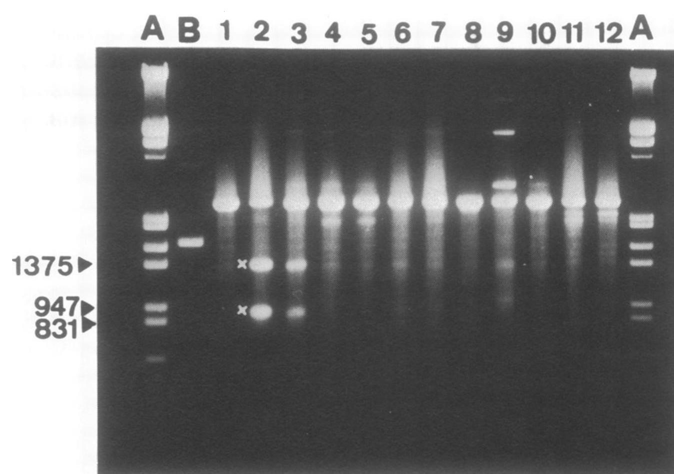


**Figure 1.** The effect of different metal-ions on the stabilization of *H-DNA. Negatively supercoiled pE/$CT_{22}$ DNA was modified with $OsO_4$, then subjected to digestion with BglI and P1 as described under Materials and Methods, and analized on a 1% agarose-TBE gel. Modification was carried out in the presence of: no added metal-ion (lane 1); $ZnCl_2$ (lane 2); $CdCl_2$ (lane 3); $CaCl_2$ (lane 4); $MgCl_2$ (lane 5); $CuCl_2$ (lane 6); $NiCl_2$ (lane 7); $CoCl_2$ (lane 8); $MnCl_2$ (lane 9); CuCl (lane 10) and $HgCl_2$ (lanes 11 and 12). The metal-ion concentration was either 5mM (lanes 2–8 and 12) or 2.5mM (lanes 9 and 11). In the case of CuCl modification was carried out in the presence of a saturated solution. Lanes A correspond to $\lambda$-EcoRI+HindIII molecular weight markers. Lane B corresponds to native negatively supercoiled pE/$CT_{22}$ DNA. DNA fragments originated by $OsO_4$-modification at the d(CT/GA)$_{22}$ sequence are indicated by the asterisks.

$CaCl_2$, 5 mM $CoCl_2$, 5 mM $NiCl_2$, 5 mM $CuCl_2$ or 5 mM $HgCl_2$ (Figure 1, lanes 4−8, 12). Under these conditions all chloride salts tested are fairly soluble except $HgCl_2$ that at a concentration of 5 mM precipitates slightly under the reaction conditions. A similar result was also obtained at a lower $HgCl_2$ concentration (2.5 mM) (Figure 1, lane 11). In these cases a low background of unspecific cleavage is observed that is similar to that obtained in the absence of any added metal-ion (Figure 1, lane 1). In addition, bands reflecting specific modification at DNA regions other than the $d(CT/GA)_{22}$ sequence are also detected when $OsO_4$-modification is carried out in the presence of $Mg^{++}$ or $Ca^{++}$ (Figure 1, lanes 4 and 5). These bands are likely to correspond to the extrusion of the main cruciform of pBR322 which is contained in plasmid pE/$CT_{22}$. A large number of bands is also observed when modification is carried out in the presence of $Hg^{++}$ (Figure 1, lanes 11 and 12). These bands are likely to arise from the interaction of $Hg^{++}$ with AT-rich regions. Mercurium ions are known to bind strongly to thymine residues in AT-rich DNA causing its partial melting (31,34). The monovalent form of copper was also tested but, given the high insolubility of its chloride salt, modification in this case was carried out in the presence of a saturated CuCl solution. Again, no significant P1-cleavage is detected under these conditions (Figure 1, lane 10).

In agreement with the results shown in Figure 1, the patterns of modification with $OsO_4$ and DEPC obtained at neutral pH in the presence of $Cd^{++}$ and $Mn^{++}$ are similar to those associated with the zinc-induced *H-DNA conformation (Figure 2). The patterns of modification obtained in the presence of 5 mM $CdCl_2$ or 2.5 mM $MnCl_2$ are very similar to those obtained in the presence of 5 mM $ZnCl_2$. A characteristic DEPC-hyperreactivity of the central part of the polypurine strand is observed together with an extensive $OsO_4$-modification of the polypyrimidine strand. However, the patterns of $OsO_4$-modification show some minor differences depending on the precise metal-ion present during the reaction. In the presence of 5 mM $CdCl_2$ or 2.5 mM $MnCl_2$, though the whole $d(CT/GA)_{22}$ sequence is hyperreactive to $OsO_4$, its 3′-half is less modified than when modification is carried out in the presence of 5 mM $ZnCl_2$. These results suggest that, although the general structural features of the conformation adopted by the $d(CT/GA)_{22}$ sequence in the presence of $Zn^{++}$, $Cd^{++}$ or $Mn^{++}$ are the same, some details of the structure must be different.

Also in agreement with the results shown in Figure 1 the patterns of modification obtained in the presence of 5 mM $CuCl_2$ or 5 mM $MgCl_2$ are close to what would be expected for B-DNA (Figure 2). In the presence of either $Cu^{++}$ or $Mg^{++}$, the polypyrimidine strand is not reactive to $OsO_4$, similarly to what is observed in the absence of any added metal-ion. On the other hand, the pattern of DEPC-modification obtained at neutral pH in the absence of any added metal-ion corresponds to the protonated H-form of the $d(CT/GA)_{22}$ sequence as it has been described before (9,22). Apparently, H-DNA is more stable under DEPC-modification conditions than under $OsO_4$-modification conditions (9). These results have also been interpreted as indicative of the existance at neutral pH of an additional altered DNA conformation (J-DNA) which is neither B-DNA nor H-DNA (22). Addition of 5 mM $MgCl_2$ or 5mM $CuCl_2$ changes significantly the pattern of DEPC-modification which becomes more 'B-like'. In the presence of either $Mg^{++}$ or $Cu^{++}$, the polypurine strand is less hyperreactive to DEPC being more uniformly modified than in the absence of any added metal-ion. These results suggest that the H-DNA conformation of the $d(CT/GA)_{22}$ sequence is destabilized in the presence of $Cu^{++}$ or $Mg^{++}$.

These results indicate that transition to the *H-DNA conformation of the $d(CT/GA)_{22}$ sequence can be induced by either $Zn^{++}$, $Cd^{++}$ or $Mn^{++}$. The efficiency on promoting the transition is in the order of $Zn^{++} > Cd^{++} >> Mn^{++}$. None

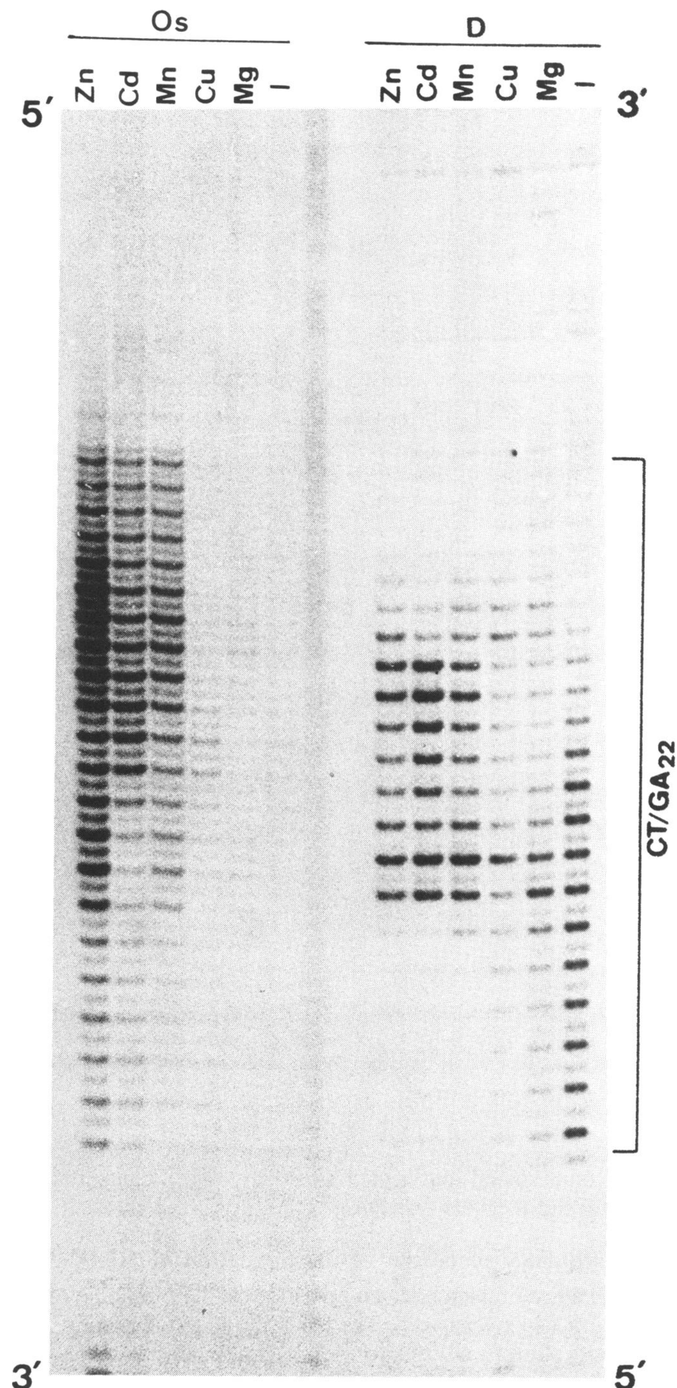


**Figure 2.** Patterns of $OsO_4$ (Os) and DEPC (D) modification of the $d(CT/GA)_{22}$ sequence obtained in the presence of: no added metal-ion (−); 5mM $ZnCl_2$ (Zn); 5mM $CdCl_2$ (Cd); 2.5mM $MnCl_2$ (Mn); 5mM $CuCl_2$ (Cu) and 5mM $MgCl_2$ (Mg). The 5′-to-3′ direction is indicated.

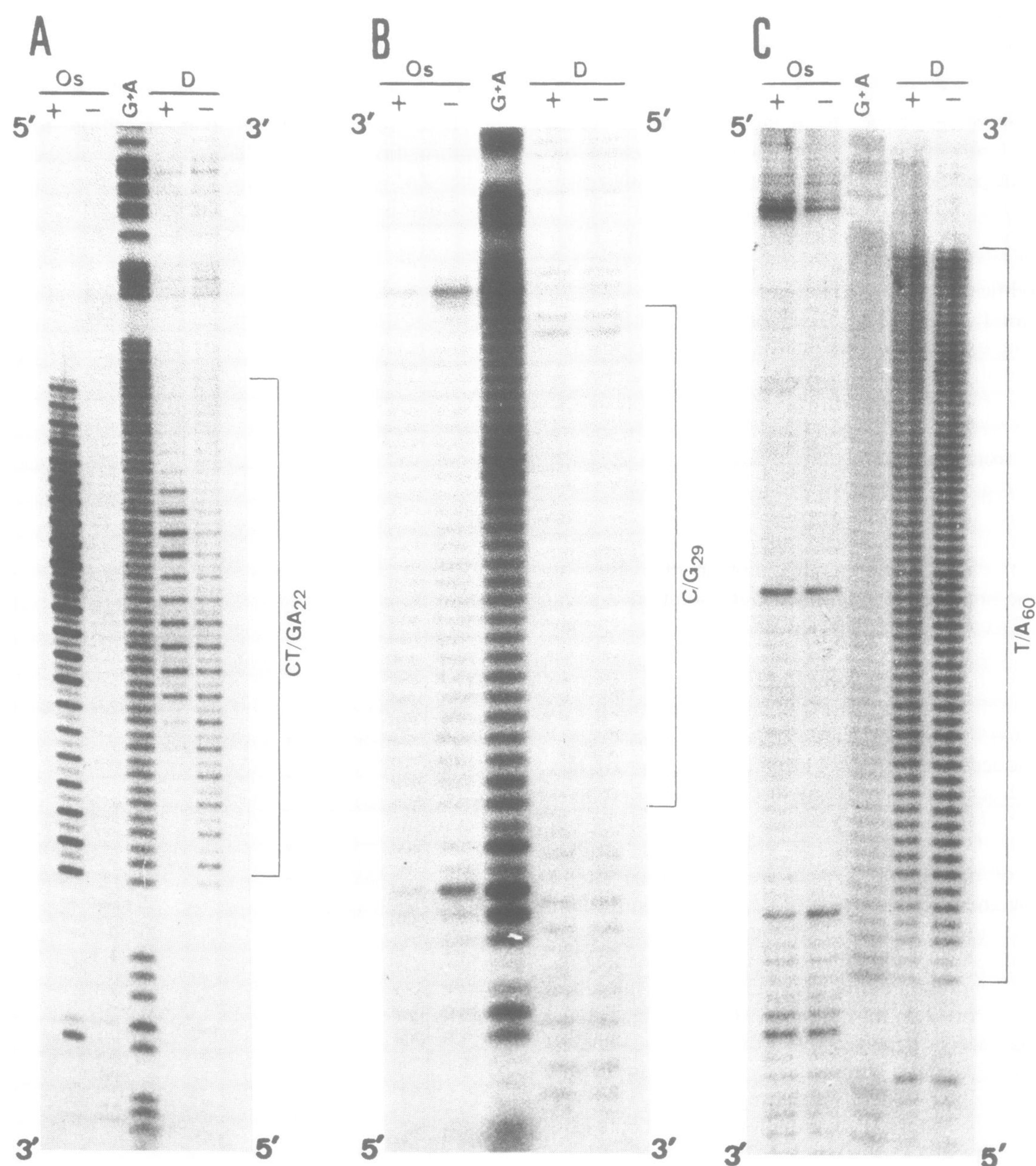


**Figure 3.** Patterns of chemical modification of the homopyrimidine-homopurine sequences: d(CT/GA)$_{22}$ (panel A); d(C/G)$_{29}$ (panel B) and d(T/A)$_{60}$ (panel C). The patterns of $OsO_4$-modification (Os) of the polypyrimidine strand and, of DEPC-modification (D) of the polypurine strand are shown for each sequence. Modification was carried out either in the presence (+) or absence (−) of 5mM $ZnCl_2$. G+A corresponds to a purine ladder obtained according to (18). The 5′-to-3′ directions are indicated.

of the other metal-ions tested show any significant effect on inducing the B-to-*H transition.

### DNA sequence specificity of the formation of *H-DNA

The influence of nucleotide composition on the formation of *H-DNA was also investigated. The following simple repeating DNA sequences were analyzed in view of their ability to undergo transition to *H-DNA induced by $Zn^{++}$: d(CT/GA)$_{22}$, d(T/A)$_{60}$, d(C/G)$_{29}$, d(CA/GT)$_{30}$, d(CG/GC)$_5$ and d(TA/AT)$_{20}$. For this purpose negatively supercoiled DNAs containing the DNA sequences described before were subjected to either $OsO_4$-modification or DEPC-modification in the presence of 5 mM $ZnCl_2$. Figure 3, shows the patterns of chemical modification obtained in the presence (+) or absence (−) of 5 mM $ZnCl_2$, corresponding to the three homopyrimidine-homopurine sequences investigated. Addition of $Zn^{++}$ promotes transition to *H-DNA of the d(CT/GA)$_{22}$ sequence. (Figure 3, panel A). On the other hand, neither a d(C/G)$_{29}$ sequence (Figure 3, panel B) nor a d(T/A)$_{60}$ sequence (Figure 3, panel C) show any signs of transition to *H-DNA. The patterns of chemical modification obtained in these cases are not affected significantly by the presence of $Zn^{++}$. The polypyrimidine strands are basically unmodified by $OsO_4$ either in the absence or presence of 5 mM $ZnCl_2$. A base particularly hyperreactive to $OsO_4$ is found at the center of the d(T/A)$_{60}$ sequence. Similarly, the polypurine strand of d(C/G)$_{29}$ is unmodified by

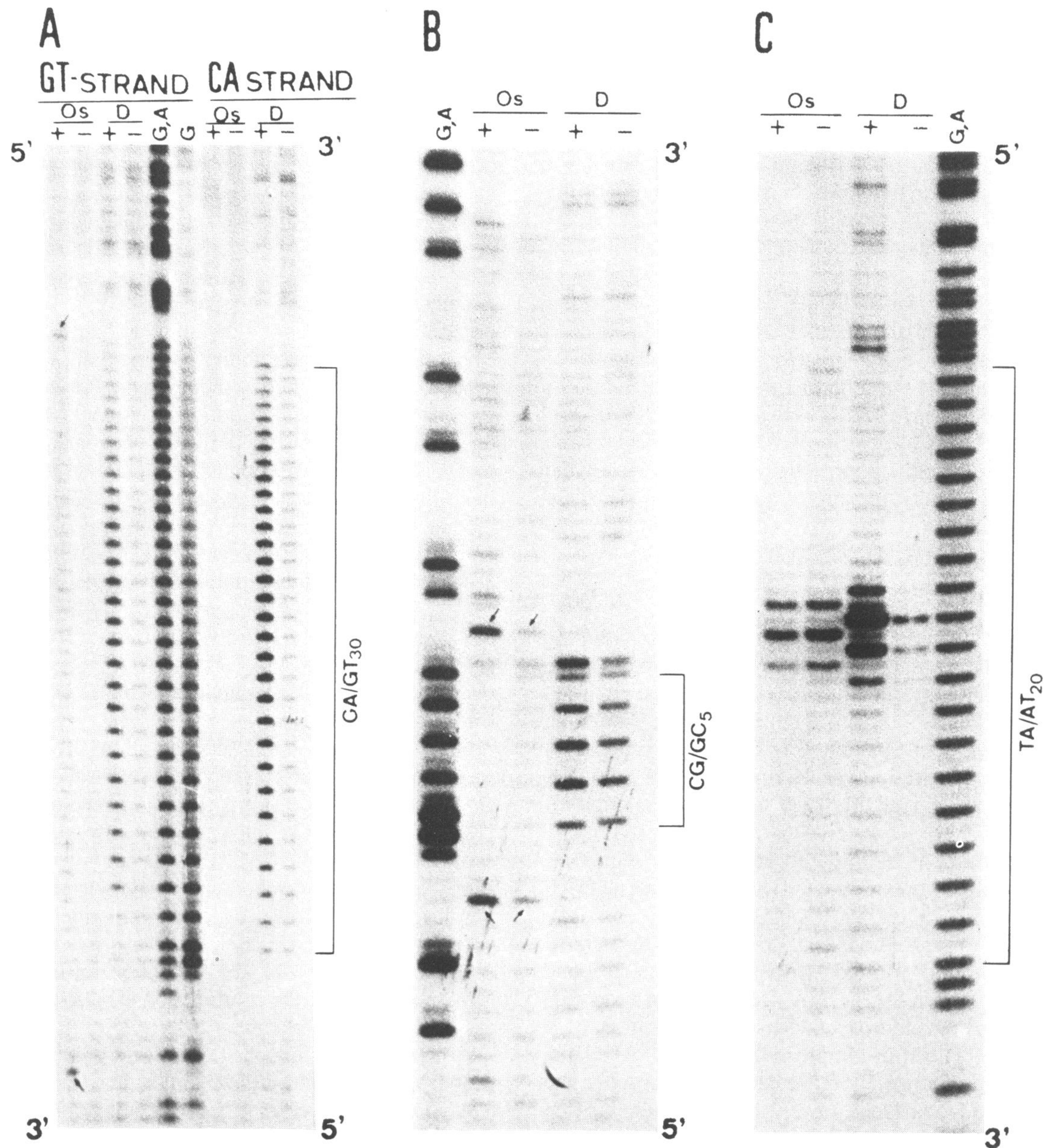


**Figure 4**. Patterns of chemical modification of the pyrimidine-purine alternating sequences: d(CA/GT)$_{30}$ (Panel A); d(CG/GC)$_5$ (panel B) and d(TA/AT)$_{20}$ (panel C). The patterns of $OsO_4$ (Os) and DEPC (D) modification of both strands are shown in (A). In (B) and (C), the patterns of modification are shown only for the GC- and the TA-strand, respectively. Similar patterns were obtained for the complementary strands. Modification was carried out either in the presence (+) or absence (−) of 5mM $ZnCl_2$. The arrows in (A) and (B), indicate pyrimidines located at the B-Z junctions which are particularly hyperreactive to $OsO_4$. G+A corresponds to a purine ladder obtained according to (18). The 5'-to-3' directions are indicated.

DEPC. However, the polypurine strand of the d(T/A)$_{60}$ sequence appears to be hyperreactive to DEPC, being extensively modified both in the presence and absence of $Zn^{++}$.

Figure 4 shows the results obtained for the pyrimidine-purine alternating sequences. Similarly to what is observed with d(C/G)$_{29}$ and d(T/A)$_{60}$, addition of 5 mM $ZnCl_2$ does not have any significant effect on the patterns of $OsO_4$- and DEPC-modification of either a d(CA/GT)$_{30}$ (Figure 4, panel A), a d(CG/GC)$_5$ (Figure 4, panel B) or a d(TA/AT)$_{20}$ (Figure 4, panel C) sequence. Formation of *H-DNA requires negative superhelicity (9). Therefore, the ability to form *H-DNA of the sequences mentioned above was determined when contained into negatively supercoiled plasmids ($\sigma = -0.05$). The actual minimum negative superhelical density above which transition to *H-DNA is detected at a d(CT/GA)$_{22}$ sequence is of $-0.03$ (unpublished results). Under these conditions the alternating d(CA/GT)$_{30}$ and d(CG/GC)$_5$ sequences exist as Z-DNA (23,24). Consequently the patterns of chemical modification obtained in the absence of zinc reflect the presence of Z-DNA. As it has been reported earlier (25,26), purines in Z-DNA show a fairly uniform pattern of DEPC-modification. In the case of the d(CA/GT)$_{30}$ sequence both the GT-strand and the CA-strand are hyperreactive to DEPC. Also in agreement with the results reported by others (26,27) a significant hyperreactive to $OsO_4$ of thymines located at the B-Z junctions is observed in the case of the d(CG/GC)$_5$ sequence. No such $OsO_4$-hyperreactivity is

detected in the case of the d(CA/GT)$_{30}$ sequence. The patterns of chemical modification obtained in the presence of $Zn^{++}$ are extremely similar to those obtained in the absence of the metal-ion. A similar DEPC-hyperreactive of the purines is observed. In addition, a more apparent $OsO_4$-modification is detected at the B-Z junctions. In particular thymine residues showing a significant $OsO_4$-hyperreactivity are found in the vicinity of the d(CA/GT)$_{30}$ sequence, suggesting that the left-handed Z-conformation of this sequence is stabilized by the addition of $Zn^{++}$.

A similar situation is found in the case of the alternating d(TA/AT)$_{20}$ sequence. In the absence of $Zn^{++}$ the sequence exist as a cruciform, showing a characteristic hyperreactivity of the nucleotides located in the loop of the cruciform (Figure 4, panel C). Similar results have been obtained by others (19,28,29). Addition of 5 mM $ZnCl_2$ does not change significantly the patterns of chemical modification (Figure 4, panel C) of the d(TA/AT)$_{20}$ sequence which remains as a cruciform under these experimental conditions.

## DISCUSSION

Homopyrimidine-homopurine sequences are highly polymorphic DNA sequences. They have been shown to be capable of adopting various non-B DNA conformations (4–9). We have reported recently that a d(CT/GA)$_{22}$ sequence undergoes transition to an altered non-B DNA conformation (*H-DNA) in response to increasing $Zn^{++}$ concentration (9). Here we analyze the DNA sequence and metal-ion requirements for the formation of *H-DNA.

From all the different simple repeating DNA sequences analyzed in this paper, only a d(CT/GA)$_{22}$ sequence showed transition to *H-DNA. Neither a d(C/G)$_{29}$ nor a d(T/A)$_{60}$ sequence appear to undergo the B to *H transition. The patterns of chemical modification of the d(C/G)$_{29}$ sequence correspond to what would be expected for B-DNA. Kowhi (7), has reported recently the pattern of chloroacetaldehide modification of a d(C/G)$_n$ sequence obtained in the presence of $Zn^{++}$ which, in good agreement with the results reported here, corresponds to B-form DNA. Actually, zinc ions prevent the magnesium induced transition to the GGC triplex at these sequences (7). On the other hand, the d(T/A)$_{60}$ sequence shows an altered chemical reactivity pattern which might be related to its peculiar DNA conformation (1–3). In this case, the polypurine strand is hyperreactive to DEPC suggesting that, as a consequence of the increased width of the major groove, the N7 group of adenines is more exposed in d(T/A)$_n$ tracts than in regular right-handed B-DNA. Similarly, the patterns of reactivity of the pyrimidine-purine alternating d(CA/GT)$_{30}$, d(CG/GC)$_5$ and d(TA/AT)$_{20}$ sequences, are not significantly affected by the presence of zinc ions. Stabilization of *H-DNA requires negative superhelicity (9). Therefore all experiments were performed on negatively supercoiled substrates. Under these conditions, d(CA/GT)$_{30}$ and d(CG/GC)$_5$ exist as Z-DNA, while d(TA/AT)$_{20}$ forms a cruciform. Addition of zinc does not affect the stability of both Z-DNA and cruciforms. Actually, Z-DNA appears to be more stable in the presence of zinc. A similar stabilizing effect of zinc ions upon the Z-DNA conformation has been reported by others (7,30). Consequently, we cannot exclude the possibility that any of these pyrimidine-purine alternating sequences could undergo transition to *H-DNA when initially adopting a B-DNA conformation. However, our results clearly indicate that the preferred DNA conformation adopted by these sequences when contained into negatively supercoiled DNA in the presence of zinc ions is either Z-DNA or cruciforms.

Formation of *H-DNA is also specific of metal-ion. Only zinc, cadmium and manganese appear to be efficient in the stabilization of *H-DNA. These three metal-ions are known to interact with the bases, in particular with the N7 group of purines (see 31–34, for reviews), suggesting that cation binding to the bases—maybe at the N7 position of purines—is an important factor for the formation of *H-DNA. At this respect, it is interesting that guanines in *H-DNA are not sensitive to DEPC that reacts precisely at this position. On the other hand, metal-ions such as $Mg^{++}$ or $Ca^{++}$, that interact preferentially with the phosphate groups, are not capable of inducing the formation of *H-DNA. Even at very high concentrations (32 mM), $Mg^{++}$ is shown to be inefficient in stabilizing *H-DNA (data not shown). Moreover, manganese that, in addition to its interaction with the bases, also binds to the phosphates shows the lowest stabilizing effect. Cation binding to the bases destabilizes the DNA double helix facilitating its melting (31,34). However, formation of *H-DNA is not just a consequence of this destabilizing effect. Metal-ions such as $Cu^{++}$, $Cu^{+}$ or $Hg^{++}$, that are known to interact strongly with the bases, do not promote transition to *H-DNA. Moreover, sequences as d(T/A)$_{60}$ or d(TA/AT)$_{20}$ that, given their nucleotide composition should melt more easily, do not show transition to *H-DNA.

Our results suggest that stabilization of *H-DNA involves the formation of a specific complex between the metal-ion and the DNA sequence. At this respect, it is interesting that complexes formed by zinc and cadmium with different mononucleotides show a similar geometry (35). In the majority of cases, the metal forms a distorted octahedral complex which, in the case of purines, always involves coordination to their N7 position. The coordination of manganese involves principally the phosphate oxygens. However in the complex form with guanosine-5′-monophosphate, the coordination is octahedral involving also the N7 group of the base.

A homopurine-homopurine-homopyrimidine triplex has been proposed to account for the chemical reactivity of *H-DNA (9). Zinc ions are known to facilitate the rewinding of heat denatured DNA (34), probably through the formation of crosslinks between the bases of the single strands. It is possible that, in *H-DNA, concurrence of the metal-ion would be required for the pairing of the two homopurine stretches which migth take place via the formation of metal-bridges. The patterns of $OsO_4$-modification of the d(CT/GA)$_{22}$ sequence obtained in the presence of either $Zn^{++}$, $Cd^{++}$ or $Mn^{++}$, show some minor differences which mainly involve the extent of modification of its 3′-half. These differences might reflect a diferential stabilization of the two posible isomeric homopurine-homopurine-homopyrimidine triplexes. In the presence of $Zn^{++}$, the triplex involving the 5′ part of the polypyrimidine strand appears to be majority (9). On the other hand, the patterns of $OsO_4$-modification obtained in the presence of $Cd^{++}$ and $Mn^{++}$ suggest that in these cases the triplex formed involves the 3′ part of the polypyrimidine strand.

The importance of metal-ions in nucleic acids processes is well established. However, their exact mechanism of action is still little understood in many cases. Metal-ions are known to play an important role on the stabilization of altered DNA conformations. Several metal-ions are very efficient on stabilizing Z-DNA (see,36 for a review). Magnesium is also known to promote transition to a triple-stranded conformation on d(C/G)$_n$

sequences (6,7) and is required for the correct folding of four-way DNA junctions (37). Potasium is known to induce a structural transition on telomeric DNA (38,39). On the other hand, the reactions in which nucleic acids participate in biological systems are generally mediated by metal-ions. Enzymes, and in general proteins working on DNA, have metal-ions as cofactors. In particular, a class of trans-acting transcriptional factors —the zinc finger proteins—contain zinc as a coordinated metal-ion. Here, we have shown results which suggest that some metal-ions could form a specific complex with a $d(CT/GA)_{22}$ sequence promoting a structural transition within it. Zinc ions are by large the most efficient on promoting such structural transition. Although it is highly speculative, the possibility exists that the zinc ions contained within such trans-activators will play a definite role on the modification of the conformation of the DNA double helix which ultimately should take place during transcriptional activation. Interestingly, $d(CT/GA)_n$ sequences are frequently found upstream of many eukaryotic genes (11–14). Experimental approaches will be developed to address this question.

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