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Supercoil-stabilized left-handed DNA in the plasmid $(dA\text{-}dT)_{16}$ insert formed in the presence of Ni^{2+}

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The $(dA\text{-}dT)_{16}$ insert of the plasmid pAT32 was probed with diethyl pyrocarbonate (DEPC) and nuclease *Bal*31 in the presence of Ni^{2+} known to be able to induce transition to left-handed conformation in the synthetic poly(dA-dT)·poly (dA-T). It has been shown that this insert in a supercoiled plasmid displays a DEPC modification pattern characteristic of left-handed DNA under conditions not sufficient to induce a left-handed structure in the linear plasmid and poly(dA-dT)·poly(dA-T).

Supercoiled DNA; $(dA\text{-}dT)_{16}$ tract structure; Chemical modification; Diethyl pyrocarbonate; Nuclease *Bal*31

1. INTRODUCTION

The ability of $(dG\text{-}dC)_n$ and $(dA\text{-}dC)_n$ sequences to form the left-handed Z conformation in natural DNAs was recognized at the beginning of 80s [1,2]. In contrast, with the $(dA\text{-}dT)_n$ sequences formation of the Z conformation under the same conditions has not been reported [3–6]. It has been shown that $(dA\text{-}dT)_n$ inserts in supercoiled plasmids adopt a cruciform structure [3–5], while in linearized plasmids $(dA\text{-}dT)_n$ inserts assume an unusual right-handed structure which is hypersensitive to chemical modification and cleavage with various nucleases [7,8]. Recently Taillandier and co-workers [9–11] have shown that poly(dA-dT)·poly(dA-dT) at high NaCl concentrations in the presence of Ni^{2+} displays CD, UV absorption and IR spectra characteristic of the left-handed Z DNA. If the structure studied by Taillandier and colleagues [9–11] is really left-handed, its stabilization by negative supercoiling can be expected.

In this paper we attempted to learn whether Ni^{2+} could induce a left-handed conformation in the $(dA\text{-}dT)_{16}$ insert of a pAT32 plasmid. We applied diethyl pyrocarbonate (DEPC), which has been shown [12,13] to react preferentially with purines involved in the left-handed Z DNA and has been successfully applied in various studies of left-handed DNAs and their interactions [14,15]. Our results show that in the presence of $NiCl_2$ the $(dA\text{-}dT)_{16}$ insert in a supercoiled plasmid displays the DEPC modification pattern characteristic of left-handed DNA at ionic strengths substantially lower than those necessary for the transition to the left-handed DNA in poly(dA-dT)·poly(dA-dT) [11].

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2. MATERIALS AND METHODS

2.1. *Plasmid DNA*

The pAT32 plasmid (a pUC19 derivative containing a $(dA\text{-}dT)_{16}$ insert cloned into the *Sma*I site; [3]) was isolated from chloramphenicol amplified cells as described previously [16].

2.2. *DEPC modification*

5 or 10 μg of plasmid DNA were placed in 50 μl total volumes. Reactions proceeded in 25 mM Tris-HCl (pH 7.6) plus indicated NaCl and/or $NiCl_2$ concentrations after 30 min pre-equilibration at room temperature. Modification was performed by adding 5 μl of diethyl pyrocarbonate (Serva) and vigorous agitating (repeated at 5 min intervals) at room temperature for

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30 min. The reactions were terminated by ethanol precipitation, the pellets were rinsed with 70% ethanol.

2.3. *Mapping of DEPC-modified nucleotide residues*

The fragment obtained after *Eco*RI and *Pst*I cleavage was labelled on the 3'-end of the bottom strand by a 'filling-in' reaction [17] using the Klenow fragment of *E. coli* DNA polymerase, TTP, and [α-^{32}P]dATP. After polyacrylamide gel electrophoresis, the *Eco*RI-*Pst*I fragment was recovered, dissolved in 30 μl of 0.75 M piperidine, and incubated at 90°C for 30 min. Piperidine was evaporated (repeated three times) and the DNA was dissolved in formamide and loaded on the sequencing gel next to the Maxam and Gilbert sequencing reactions [18] of the unreacted fragment.

2.4. *Nuclease* Bal31 *cleavage*

1.3 μg equivalents of plasmid DNA were placed in 25 μl total volumes containing nuclease *Bal*31 buffer plus NaCl and/or $NiCl_2$ up to indicated concentrations. After 30 min preincubation at room temperature 0.16 units of nuclease *Bal*31 (BRL) was added and cleaved at 37°C for 30 min. Cleavage was terminated by the addition of EGTA and DNA was purified by triple precipitation and cleaved with *Bgl*I.

2.5. *Polynucleotide*

Poly(dA-dT)·poly(dA-dT) was purchased from Pharmacia, Uppsala, and used without further purification. It was dissolved in 25 mM Tris-HCl (pH 7.8) buffer and added into the concentrated solutions of NaCl and $NiCl_2$ as indicated. UV absorption spectra were recorded at room temperature with a Specord M40 spectrophotometer. CD spectra measurements were performed on a Jobin Yvon Mark IV dichrograph at room temperature.

3. RESULTS AND DISCUSSION

Supercoiled plasmid pAT32 (at native superhelical density) was treated with DEPC in 25 mM Tris-HCl (pH 7.6) at various concentrations of $NiCl_2$ and NaCl, purified by ethanol precipitation, cleaved with restrictases and subjected to nucleotide sequencing as previously described [7,19,20]. Plasmid modification with DEPC in buffer only resulted in a modification pattern characteristic of the cruciform structure [21,22], i.e. in a strong modification of three adenines in the middle of the $(dA\text{-}dT)_{16}$ insert (cruciform loop) (fig.1, lane 1). Similar patterns were obtained also after modification in 25 mM Tris-HCl with either 0.2 M (lane 2) or 0.5 M $NiCl_2$ (lane 3). Addition of NaCl into the reaction mixture containing 0.2 M $NiCl_2$ and increasing its concentration up to 1.5 M did not result in any marked change of the modification pattern (fig.1, lanes 5–8). Increasing the $NiCl_2$ concentration to 1.0 M (in the absence of NaCl) resulted in a striking change of the modification pattern (fig.1, lane 4) with a uniform modification of all adenine residues in the $(dA\text{-}dT)_{16}$ insert. The same pattern was observed with 0.2 M $NiCl_2$ in the presence of 2.0 M NaCl (fig.2, lane 5), while modification in 2.0 M NaCl itself displayed the characteristic cruciform loop modification (fig.2, lane 4) similar to that obtained at lower ionic strengths (fig.1, lanes 1–3, 5–8). Treatment of the linearized (*Pst*I) plasmid with DEPC in 0.2 M $NiCl_2$, 2.0 M NaCl,

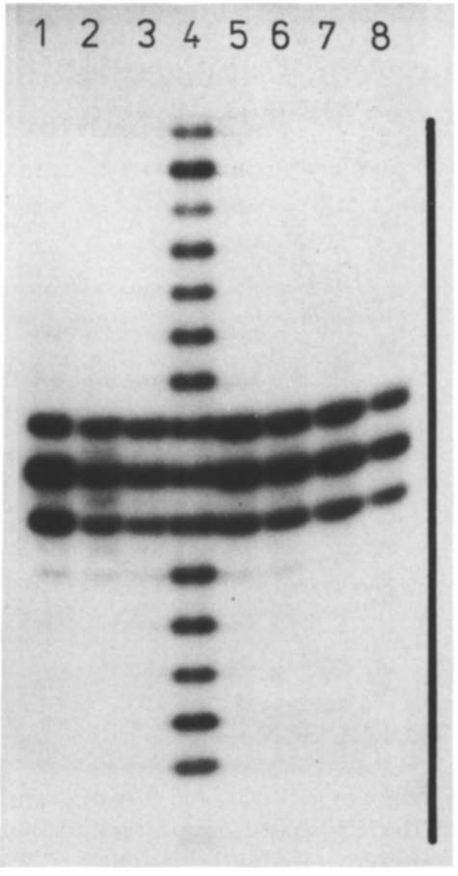

Fig.1. Mapping of DEPC modification of the *Eco*RI-*Pst*I fragment (containing the $(dA\text{-}dT)_{16}$ insert) of supercoiled pAT32 plasmid in dependence on ionic composition of reaction mixture. Supercoiled plasmid was modified with DEPC (30 min, room temperature) in 25 mM Tris-HCl, pH 7.6 (lane 1), plus either 0.2, 0.5, 1.0 M $NiCl_2$, respectively (lanes 2–4), or 0.2 M $NiCl_2$ plus 0.1, 0.5, 1.0, 1.5 M NaCl, respectively (lanes 5–8). After ethanol precipitation DNA was cleaved by *Eco*RI and *Pst*I restrictases, the *Eco*RI-*Pst*I fragment was handled as described in section 2. The vertical line indicates the $(dA\text{-}dT)_{16}$ segment.

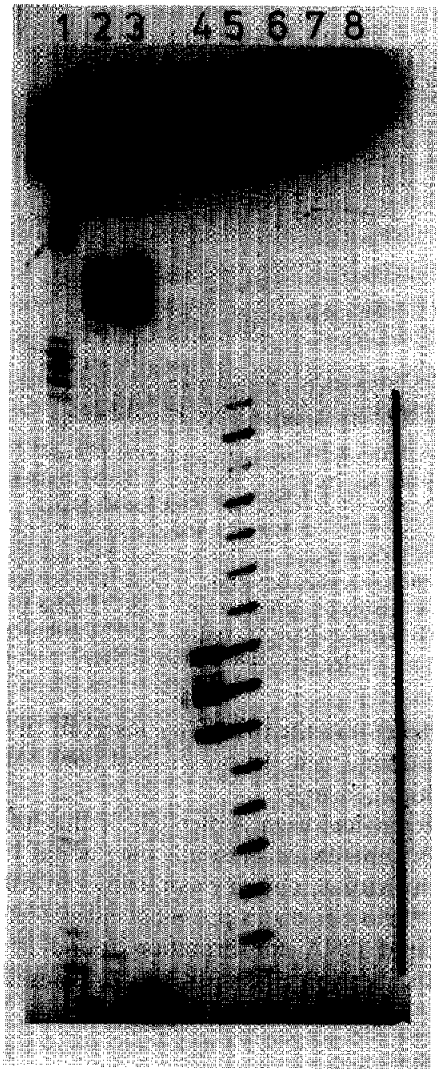

Fig.2. Mapping of DEPC modification of the $(dA\text{-}dT)_{16}$ sequence in supercoiled and linearized (*PstI*) pAT32. Supercoiled (lanes 4,5) and linearized (lanes 6–8) plasmid was modified with DEPC in 25 mM Tris-HCl, pH 7.6 (lane 6) plus either 2.0 M NaCl (lanes 4,7) or 2.0 M NaCl and 0.2 M $NiCl_2$ (lanes 5,8). Other details as in fig.1. Lanes 1–3 show sequencing reactions of C + T, A + G and G, respectively. The vertical line indicates the $(dA\text{-}dT)_{16}$ segment.

25 mM Tris-HCl, pH 7.8, did not show any significant modification differing from the background (fig.2, lane 8). Similar results were obtained after the DEPC treatment of the linearized plasmid either in the absence (lane 6) or presence (lane 7) of 2.0 M NaCl. In 2.0 M NaCl, 0.2 M $NiCl_2$, 25 mM Tris-HCl, pH 7.8 (i.e. under conditions where the linearized plasmid showed no sign of left-handed DNA in the insert), poly(dA-dT)·poly(dA-dT) displayed CD (fig.3) and UV absorption spectra (not shown) characteristic of B DNA. UV absorption and CD spectra characteristic of left-handed poly(dA-dT)·poly(dA-dT) were obtained [11] in 5.0 M NaCl, 90 mM $NiCl_2$, 10 mM Tris-HCl, pH 7.8; in similar media with lower $NiCl_2$ (up to 85 mM $NiCl_2$) spectra typical of right-handed B DNA were observed. Our measurements of UV absorption and CD spectra of poly(dA-dT)·poly(dA-dT) in 5.0 M NaCl, 95 mM $NiCl_2$, 25 mM Tris-HCl, pH 7.8 (not shown) agreed well with the results of Bourtayre et al. [11].

We may thus conclude that under conditions not sufficient to induce left-handed conformation in $(dA\text{-}dT)_n$ sequences of linear synthetic (fig.3) and natural DNAs (fig.2, lane 8) the $(dA\text{-}dT)_{16}$ insert in the negatively supercoiled plasmid undergoes a structural transition accompanied by a striking change in sensitivity to the DEPC modification (fig.2, lane 5). Is it a transition to a left-handed structure?

The DEPC modification pattern of the $(dA\text{-}dT)_{16}$ insert obtained with supercoiled pAT32 DNA is in agreement with the patterns obtained earlier with left-handed $(dG\text{-}dC)_n$ and $(dA\text{-}dC)_n$ inserts [12,13,20], thus supporting the notion of a left-handed structure. On the other hand DEPC is not a probe rigorously specific to left-handed DNA. This can clearly be seen from the $(dA\text{-}dT)_{16}$ modification pattern obtained with supercoiled DNA in the absence of $NiCl_2$ (fig.1, lane 1 and fig.2, lane 4) showing DEPC hypersensitivity of bases in the cruciform loop in agreement with the earlier data [21,22]. If DEPC reacts preferentially with bases only in cruciform loops and left-handed DNAs, then the obtained modification patterns of $(dA\text{-}dT)_{16}$ (fig.1, lane 4 and fig.2, lane 5) can be due to either left-handed or single-stranded regions. Occurrence of a single-stranded DNA region in 2.0 M NaCl, 0.2 M $NiCl_2$ at pH 7.6 is very unlikely, nevertheless we tested its presence by a single-strand selective nuclease *Bal*31 which is known to retain its enzymatic activity even at extremely high ionic strengths [23]. This nuclease site-specifically cleaved the cruciform (fig.4, lane 3) formed in 2.0 M NaCl as detected by DEPC (fig.2, lane 4) but practically no site-specific cleavage within the $(dA\text{-}dT)_{16}$ insert was observed

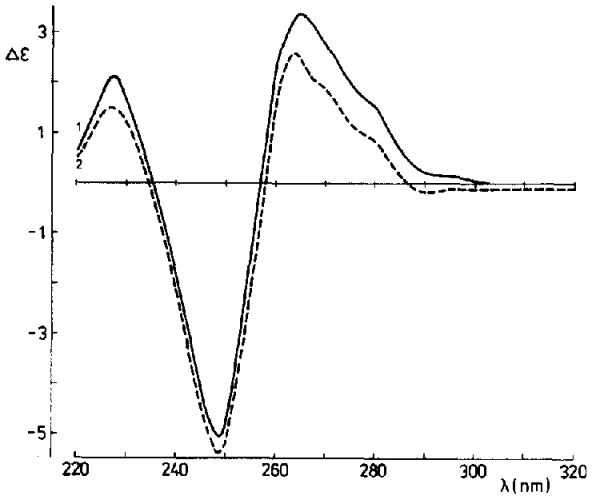

Fig.3. CD spectra of poly(dA-dT)·poly(dA-dT) in (1) 25 mM Tris-HCl, pH 7.8, and (2) 2.0 M NaCl, 0.2 M $NiCl_2$ plus 25 mM Tris-HCl, pH 7.8.

in 2.0 M NaCl, 0.2 M $NiCl_2$ (fig.4, lane 1) where the DEPC modification characteristic of Z DNA was observed (fig.2, lane 5). The enzymatic activity of *Bal*31 was not inhibited due to the presence of $NiCl_2$ as the enzyme cleaved the cruciform within the $(dA\text{-}dT)_{16}$ insert in the presence of 0.2 M $NiCl_2$ (fig.4, lane 2) and recognized and cleaved at the B-Z junction of the supercoiled pRW751 DNA (containing $(dC\text{-}dG)_n$ segments; [16,24]) in 2.0 M NaCl, 0.2 M $NiCl_2$ (not shown). Thus the formation of left-handed DNA appears to be a better explanation of the observed DEPC modification patterns of the $(dA\text{-}dT)_{16}$ insert in 2.0 M NaCl, 0.2 M $NiCl_2$ (fig.2, lane 5).

While preparing this paper the results of Raman spectroscopy were published [25] which provided strong evidence of the left-handed Z form of poly(dA-dT)·poly(dA-dT) in 5.0 M NaCl, 95 mM $NiCl_2$. Considering these spectroscopic data [25] we believe that the results of DEPC modification presented in this paper (figs 1 and 2) can be interpreted as the formation of supercoil-stabilized left-handed DNA within the $(dA\text{-}dT)_{16}$ insert in the presence of either 2.0 M NaCl, 0.2 M $NiCl_2$, or 1.0 M $NiCl_2$. Attempts to induce left-handed Z DNA in poly(dA-dT)·poly(dA-dT) in the absence of NaCl by increasing the $NiCl_2$ concentration failed [11] due to the precipitation of the polynucleotide. Here we show that $NiCl_2$ alone can induce the same DEPC modification pattern

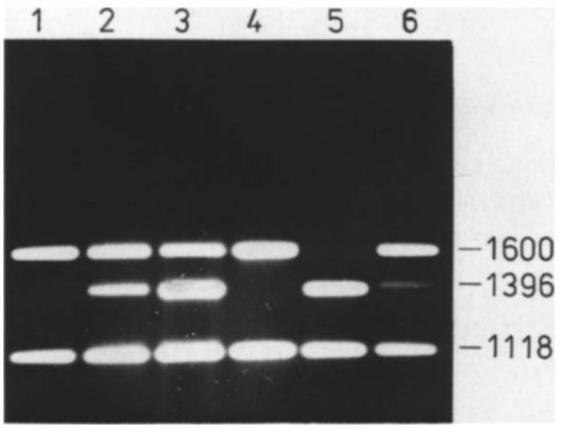

Fig.4. Nuclease *Bal*31 cleavage within the $(dA\text{-}dT)_{16}$ insert of pAT32 plasmid. Nuclease cleavage in *Bal*31 buffer (20 mM Tris-HCl, pH 7.6, 0.6 M NaCl, 12.5 mM $MgCl_2$, 12.5 mM $CaCl_2$) plus either 1.4 M NaCl and 0.2 M $NiCl_2$ (lane 1), or 0.2 M $NiCl_2$ (lane 2), or 1.4 M NaCl (lane 3), respectively, was performed as described in section 2. Lanes 4–6 contain marker fragments derived by *Bgl*I cleavage of pAT32 (lane 4) followed by *Bam*HI digestion (lane 5), and nuclease S1 followed by *Bgl*I cleavage (lane 6), respectively.

(fig.1, lane 4) in the $(dA\text{-}dT)_{16}$ insert of the supercoiled plasmid as in the presence of both $NiCl_2$ and NaCl (fig.2, lane 5).

Earlier it has been shown [3–5] that $(dA\text{-}dT)_n$ tracts in supercoiled plasmids may adopt two different structures, one of them being the cruciform (fig.5); the other one is probably closely related to the altered DNA conformation found in linear, $(dA\text{-}dT)_n$ bearing plasmids [7]. A transition be-

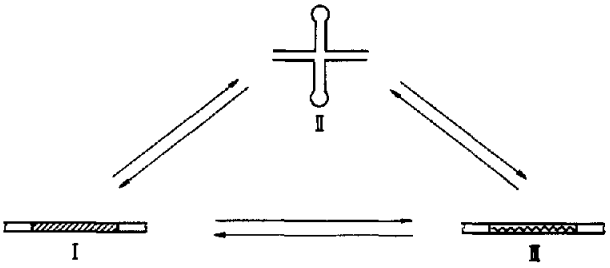

Fig.5. Schematic representation of a model of the equilibria in the $(dA\text{-}dT)_{16}$ segment in supercoiled DNA. Ionic strength increases from left to right. Increase of ionic strength or decrease in temperature results in an equilibrium shift from the conformation I (probably alternating structure with disturbed A-upon-T stacking, characterized by uniform modification with osmium tetroxide, pyridine) to the cruciform II [29]. Addition of Ni^{2+} at higher ionic strengths may result in the formation of left-handed DNA (III) which is (similarly to the cruciform structure) stabilized by negative supercoiling.

tween these two forms is dependent on the ionic strength, the nature of ions and temperature at temperatures far below melting. The behaviour of the $(dA\text{-}dT)_n$ tracts is thus highly reminiscent of DNA premelting observed earlier by means of physical and physico-chemical methods [26]. Our results show that in the presence of Ni^{2+} the $(dA\text{-}dT)_n$ segment in supercoiled DNA may undergo another transition, probably to the left-handed DNA (fig.5). To our knowledge no such structural versatility has been found in any other nucleotide sequence in supercoiled DNA.

4. CONCLUSIONS

We have shown that the $(dA\text{-}dT)_{16}$ tract adopts an unusual structure in the presence of $NiCl_2$ stabilized by negative supercoiling. Hypersensitivity of this structure to DEPC, stabilization by supercoiling and requirement for Ni^{2+} suggest that this structure is left-handed. If it is true, this is the first demonstration of left-handed structure of a $(dA\text{-}dT)_n$ segment in a natural DNA. Recently we have found [27] that the left-handed helix is formed in the presence of 10 mM $NiCl_2$ and 0.2 M NaCl within the 32 bp self-complementary alternating purine-pyrimidine insert, containing the block of $(dA\text{-}dT)_5$ in the centre. These findings, together with the quite recent discovery [28] of the ability of histone H5 to induce left-handed Z DNA in poly(dA-dT)·poly(dA-dT) under physiological conditions as well as the frequent location of $(dA\text{-}dT)_n$ elements 5′ to expressed genes [7], suggest that left-handed $(dA\text{-}dT)_n$ segments may play a biological role. The relation between the known carcinogenicity of Ni^{2+} and its specific influence on DNA conformation represents an interesting question connected with this role.

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