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Effect of intermolecular triplex formation on the yield of cyclobutane photodimers in DNA

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ABSTRACT

We have studied the effect of intermolecular triplexes formation on the yield of cyclobutane photodimers in DNA. DNA duplex within the pyrimidine-purine-pyrimidine triplex $d(TC)_nd(GA)_nd(CT)_n$ is protected from the formation of cyclobutane photodimers in the case of the stabilization of this triplex by acid pH, and in the case of supplementary stabilization by Mg^{2+} or Zn^{2+}. We have studied pH-independent pyrimidine-purine-purine triplexes stabilized by bivalent cations. In such triplexes, the protection from the formation of [6-4] photodimers is observed, whereas the protection from cyclobutane dimer formation does not take place. The formation of the $d(TC)_nd(GA)_nd(GA)_n$ triplex leads to an inversion of the intensities of cyclobutane CT and TC photodimers. We observed a sharp decrease in photoreactivity with respect to cyclobutane dimers in the duplex tract $d(C)_{18}d(G)_{18}$ in the presence of Ba^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+} and Ni^{2+}. The formation of the $d(C)_nd(G)_nd(G)_n$ triplex leads to 'antifootprinting', i.e. an increase in the yield of cyclobutane photodimers.

INTRODUCTION

With the irradiation of DNA by 240–300 nm of ultraviolet light, a large quantity of diverse photoproducts is formed. Reactions of the photodimerization of adjacent bases have the greatest quantum yield in double-stranded DNA, although photoreactions are also possible with separate nucleotides, for example, the formation of photohydrates. The formation of photodimers is a structure-dependent process, since only those two pyrimidines can be photoreactive which, while being in the electronically excited state as a result of the photon absorption, are appropriately situated with the respect to one another. This appropriate disposition differs significantly from the equilibrium geometry of B-DNA and is attained due to fluctuations of angles from their equilibrium values. Because of this, the yield of photodimers is believed to depend on the local torsion rigidity of DNA.

Among the photodimers, cyclobutane dimers predominate in duplex DNA at low doses of UV-irradiation (1). These dimers can be formed between any of the adjacent pyrimidines, TT, TC, CT or CC. The yield of TT dimers is the greatest, while the yield of CC dimers is the least. With the formation of cyclobutane dimers, the double bonds, C5-C6, of both pyrimidines cleave and link with the adjacent base with the formation of a four-member cyclobutane ring. The reaction of the formation of cyclobutane dimers is reversible and, at large doses of UV-irradiation, the quantity of these dimers levels off.

The quantum yield of [6-4] dimers at low doses is several fold lower than of the cyclobutane dimers (1). With increasing doses, the fraction of [6-4] dimers will grow, since the reaction of their formation is irreversible. In the duplex, the [6-4] dimers are formed between 5′-TC and 5′-CC and, at very high doses, between TT (2). Clearly, the [6-4] dimers create greater distortions in the double-helix structure than do cyclobutane dimers.

The use of UV-light for footprinting at the nucleotide level was suggested for the first time in 1984 (3). The method includes the following stages: irradiation, the transfer of photodamages to single-stranded breaks with the subsequent analysis of the products in sequencing gel. Since that time, it has been shown by the method of UV footprinting that, with an increase in the torsional rigidity of DNA, as for example, with the B–A transition (4) and in site-specific nucleoprotein complexes (3,5,6), a decrease in the photoreactivity of DNA occurs. And inversely, with a decrease in the torsional rigidity of DNA, as for example, with melting of DNA, in premelting of the dA dT tracts (7,8), UV photoreactivity of DNA increases. When the torsional rigidity does not change in solutions of various salts and organic solvents, with a change of temperature outside the interval of the helix-coil transition and at the sites of interaction of chromosomal proteins with DNA (6), UV photoreactivity remains unchanged.

The only exception known today is the alteration of the photoreactivity of DNA with the binding of Cu, Ag and Hg (9). Decrease of the yield of photoproducts in case of Hg^{2+} and increase in case of Ag^{+} and Cu^{2+} were observed (9).

We investigated the influence of triplexes on the yield of [6-4] photoproducts earlier (10–13) and showed that, in case of intra- and intermolecular triplexes, for pyrimidine-purine-pyrimidine and for pyrimidine-purine-purine triplexes, virtually complete protection from [6-4] photodimerization was observed. Tang *et al.* (14) studied the effect of H-DNA formation on the yield of

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both, [6-4] and cyclobutane, photoproducts and arrived at the same conclusion.

In the present paper we study how the formation of cyclobutane photodimers is affected by the formation of intermolecular triplexes of various types. The cyclobutane photodimers were transferred to the chain breaks by UV endonuclease from *M.luteus*.

The method of UV footprinting of triplexes is a convenient assay for studying of triplexes *in vitro* and, possibly, *in vivo*. The advantages of the use of cyclobutane dimer-tracking over [6-4] dimers seems obvious: a significantly greater quantum yield, simplicity, and also the possibility of tracking the formation of any pyrimidine dimers, not only TC and CC. However, the data presented below show that the effect of triplex formation on the yield of cyclobutane dimers is much more complicated than just simple protection observed in the case of [6-4] photoproducts.

MATERIALS AND METHODS

Plasmids

The plasmids pTC33.4, pG18 carrying $d(TC)_{16}d(GA)_{16}$ and $d(G)_{18}d(C)_{18}$, respectively, were prepared as described (10). The inserts were incorporated into the polylinker of the pUC19 plasmid. The sequences of inserts, plus a flanking polylinker, between boundary EcoRI and HindIII sites, are listed. Only strands carrying the homopyrimidine tract of the inserts are shown: pTC33.4:

5′-AGCTTGCATGCCTGCAGGTCGACTCTAGAGGATC-CCCGATCC$(TC)_{16}$GAATTGATCC$(TC)_{16}$GAATTGGGTAC-CGAGCTCGAATT-3′

pG18: 5′-AGCTTGCATGCCTGCA$(C)_{18}$TGCAGGTCGACT-CTAGAGGATCCCCGGGTACCGAGCTCGAATT-3′

Oligonucleotides

$d(CT)_5$, $d(AG)_5$ and $d(G)_{10}$ were synthesized on an oligonucleotide synthesizer at The OSU Biochemical Instrument Center by Jane Tolley.

UV-endonuclease crude extract from *Micrococcus luteus*

An *M.luteus* strain was generously donated by Dr. N.V.Tomilin. Preparation of a crude extract from *M.luteus* was performed as described in (15). *M.luteus* cells (0.5 ml) from one LB agar plate were washed twice with 50 mM Tris-HCl (pH 7.5) 0.1 mM dithiothreitol and collected by centrifugation. The cells were resuspended in 0.5 ml of the same buffer and lysed by adding 100 μg of lysozyme and incubating at 37°C for 30 min. The lysed cells were chilled in an ice bath and sonicated with MSE sonifier (England) at maximum output for 4×20 s with 3 min. intervals to maintain temperature below 4°C. The suspension was centrifugated at 30,000 rpm (100,000×g), 4°C for 30 min and the supernatant liquid (0.5 ml) was retained.

DNA samples and UV-irradiation

The plasmids were cut with the EcoRI and HindIII restriction endonuclease and the 3′-ends of the EcoRI site were labelled with α^{32}P dTTP and Klenow fragment of DNA polymerase I. The fragments carrying the inserts were isolated by electrophoresis on a 6% polyacrylamide gel, eluted from a gel slice into 100mM NaCl, 10mM Tris-HCl (pH 8.0), 1mM EDTA, concentrated by ethanol precipitation, and redissolved in 5mM Tris-HCl (pH 8.0), 0.5mM EDTA. About 0.1 pmols of labelled fragments were incubated with 3 μM of purine oligonucleotides or 1 μM of pyrimidine oligonucleotide in 20 μl of appropriate buffer (see legends to figures) for 2−3 hours at room temperature.

The samples were irradiated by two germicidal lamps (15W, 254nm) for 30−120 sec for cyclobutane photofootprinting and 5−7 min. for [6-4] photoproducts photofootprinting with an intensity 60 J sec^{-1} m^{-2}. Irradiated DNA was twice ethanol precipitated.

UV-footprinting analysis

To reveal [6-4] photoproducts, the DNA was dissolved in 100 μl of 1M piperidine. Reaction with piperidine was carried out at 90°C for 30 min.

To reveal cyclobutane photodimers, irradiated samples were dissolved in 15 μl of buffer M (10mM Tris-HCl (pH 8.0), 3.3mM EDTA). The *M.luteus* crude extract (1 μl) was added to each sample and incubation was performed for 3 h at 37°C. We checked that incubation with more enzyme did not induce additional scissions. Therefore, in our experiments all cyclobutane dimers were converted into chain breaks.

In both cases, the products of the DNA scission were resolved on an 8% or a 10% polyacrylamide denaturing gel.

RESULTS

UV footprinting of PyPuPy triplex $d(TC)_nd(GA)_nd(CT)_n$ with the use of cyclobutane dimers

For the study of the effect of triplexes of this type on the yield of cyclobutane dimers, a labelled fragment was taken which contained two $d(TC)_{16}d(GA)_{16}$ inserts, and the $d(CT)_5$ oligonucleotide formed a triple helix with them.

Initially, 150mM NaCit buffer at pH 4.5 and pH 6.5 was used (Fig. 1). In the absence of the oligonucleotide at pH 4.5 (lane 1) and pH 6.5 (lane 3), the yield of dimers which is typical for the B-DNA was observed. In the presence of the $d(CT)_5$ oligonucleotide, at pH 6.5, as has already been shown, a triplex does not form (11); DNA is in the B-form and yield of photodimers is the same (lane 4). Note that the bands in denaturing gel form doublets, in which the lower, stronger band corresponds to the TC dimer, and the upper, weaker band corresponds to the CT dimer (lanes 1, 2, 4). At pH 4.5 in 150mM Na^+, the $d(CT)_5$ oligonucleotide binds with the $d(TC)_nd(GA)_n$ insert and forms triplex. This is accompanied by a practically complete inhibition of the formation of cyclobutane dimers (lane 2), just as in the case of [6-4] photoproducts (11). A decrease in intensity can not be attributed to the influence of oligonucleotides on the UV endonuclease, since the intensity of the bands outside the $d(TC)_{16}d(GA)_{16}$ inserts (lane 2) is equal to the intensity of these dimers in the B form (lanes 1, 2, 4). Additionally, in lane 4, where the oligonucleotide was also present during incubation with UV-endonuclease, there would then have to be a decrease in band intensity, which is not observed. Thus, Fig. 1 demonstrates the inhibition of cyclobutane dimers formation in the duplex within the triplex zone. These our data correlate very well with the results of Tang *et al.* (14) for H-DNA formed by the $d(TC)_{18}d(GA)_{18}$ insert.

We obtained the same results when stabilized the PyPuPy triplexes by bivalent cations (Fig. 2). As was shown in (11), in the presence of 10mM Me^{2+}, the $d(CT)_5$ oligonucleotide forms a stable complex at pH 6.0 whereas without Me^{2+} no triplex is formed at that pH. At pH 6.0 in 30mM NaMes buffer, without the $d(CT)_5$ oligonucleotide, the normal yield of photodimers is observed in the presence of 7mM Mg^{2+} (Fig.2, lane 2) and of

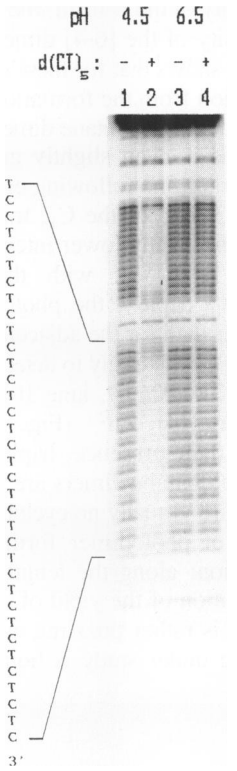

Fig. 1. Photofootprinting with detection of cyclobutane dimers of the intermolecular triplex formed between two $d(TC)_{16}d(GA)_{16}$ inserts and a $d(CT)_5$ oligonucleotide. The strand carrying the pyrimidine portion of the $d(TC)_{16}d(GA)_{16}$ inserts in the duplex DNA was studied. Irradiation was performed in 150mM of sodium citrate, the pH values are indicated. The 5'-end is at the top.

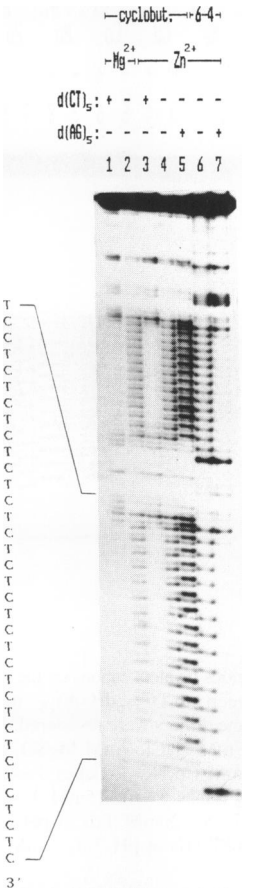

Fig. 2. UV-damage of intermolecular PyPuPy and PyPuPu triplexes in the presence of Mg^{2+} (lanes 1,2) and Zn^{2+} (lanes 3–7) between two $d(TC)_{16}d(GA)_{16}$ inserts and $d(CT)_5$ and $d(AG)_5$. The added oligonucleotide and type of registered photoproduct are shown. Irradiation was conducted in 30 mM NaMes, 7mM $MgSO_4$ at pH 6.0 (lanes 1–2); 30mM NaMes, 7mM $ZnSO_4$ at pH 6.0 (lanes 3–4); 20mM Tris, 50mM NaCl, 10mM $ZnSO_4$ at pH 7.0 (lanes 5–7). The 5'-end is at the top.

7mM Zn^{2+} (lane 4), with the addition of the $d(CT)_5$ oligonucleotide, a triplex is formed and the yield of CT and TC photodimers in the triplex zone sharply decreases (lanes 1 and 3). Virtually complete protection is observed for the case of magnesium ions, which are known to preferentially bind with phosphates, and for the case of zinc ions, which are known to preferentially bind with bases.

So, regardless of the mechanism of stabilization in the $d(TC)_nd(GA)_nd(CT)_n$ triplex, virtually complete protection from the formation of cyclobutane dimers is observed.

Photoreactivity of Intermolecular Triplexes Between the $d(TC)_{16}d(GA)_{16}$ Insert and the $d(AG)_5$ Oligonucleotide

We have shown previously (11) that in the presence of Zn^{2+}, but not Mg^{2+}, at pH 5.5, a triplex is formed between the $d(TC)_{16}d(GA)_{16}$ insert and the $d(AG)_5$ oligonucleotide. As a result, a decrease in the yield of the [6-4] photoproducts in the insert was observed.

The formation of PyPuPu triplexes is known to be pH-independent, and pH 5.5 was chosen in (11) because of precipitation of the Zn^{2+} ions with increasing pH. We have managed to obtain a stable triplex at pH 7.0 in the presence of Zn^{2+} with the addition of 50mM Na^+ (Fig. 2, lanes 5, 7). In the absence of the oligonucleotide, a normal yield of both, cyclobutane and [6-4], photodimers is observed (lanes 4, 6) while the addition of the $d(AG)_5$ oligonucleotide leads to inhibition of the [6-4] dimers (lane 7).

The pattern of the yield of cyclobutane dimers in the triplex zone turned out in an unexpected one (lane 5). Instead of expected inhibition of photoreactivity only a slight decrease in the yield of the TC dimers is observed accompanied by a dramatic increase in the yield of the CT dimers. This is manifested as the 'intensity inversion' within the band doublets.

Note that the UV endonuclease from *M. luteus* has two activities : 1) of pyrimidine dimer DNA-glycosylase, which hydrolyzes N-glycosyl bond of the 5'-member of the cyclobutane dimer, and 2) of apurine/apyrimidine endonuclease, which cleaves the phosphodiester bond at the site of hydrolysis. As a result, the 3' end-labelled fragment, which contained a cyclobutane dimer before treatment, lags in denaturing gel by two nucleotides the corresponding fragment, which contained the [6-4] photoproduct at the same site (compare lanes 5 and 6, Fig. 2).

As we showed earlier by the method photofootprinting with the [6-4] dimers (12, 13), the triplex between $d(TC)_{16}d(GA)_{16}$ and $d(AG)_5$ is stabilized not only by the Zn^{2+} ions, but also by a series of other bivalent cations: Cd^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}. Figure 3 shows that in all these cases, the 'band inversion' effect is observed. In the presence of Mg^{2+}, the pattern does not

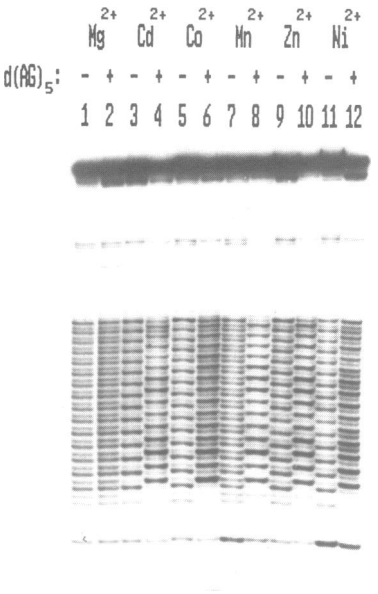

Fig. 3. The influence of various bivalent cations on the formation of intermolecular PyPuPu triplexes between $d(TC)_{16}d(GA)_{16}$ inserts and the $d(AG)_5$ oligonucleotide. Cyclobutane dimers were registered. Irradiation was conducted in 15mM Tris at pH7.7, 7mM NaCl, 7mM $MgSO_4$ (lanes 1–2); 15mM Tris at pH 7.1, 7mM NaCl, 7mM $Cd(NO_3)_2$ (lanes 3–4); 15mM Tris at pH 7.1, 7mM NaCl, 7mM $CoSO_4$ (lanes 5–6); 15mM Tris at pH 7.0, 7mM NaCl, 7mM Mn Acetate (lanes 7–8); 20mM Tris at pH 7.0, 50mM NaCl, 10mM $ZnSO_4$ (lanes 9–10); 15mM Tris at pH 7.0, 7 mM $NiCl_2$ (lanes 11–12).

depend on whether or not the oligonucleotide is added. This attests to the absence of the $d(TC)_nd(GA)_nd(GA)_n$ triplex in the presence of Mg^{2+}.

Note that our above arguments against the direct effect of oligonucleotide on the specificity of UV-endonuclease remain valid in the case of the 'band inversion' effect. Indeed, if the effect were due to a change in the specificity of UV-endonuclease under the influence of the $d(AG)_5$ oligonucleotide a similar effect would be observed in the presence of the Mg^{2+} ions, which is not the case (see Fig. 3, lane 2).

Photoreactivity of Intermolecular Triplexes Between the $d(G)_{18}d(C)_{18}$ Insert and the $d(G)_{10}$ Oligonucleotide

We have demonstrated that in the presence of the Mg^{2+} ions, intermolecular triplex is formed between $d(G)_{18}d(C)_{18}$ and $d(G)_{10}$ (11); the [6-4] photodimers are virtually completely inhibited. We have studied the effect of this triplex formation on the yield of cyclobutane dimers, and also, the formation of this triplex in the presence of various bivalent cations.

Figure 4 shows that a surprising phenomenon is observed. In the duplex, in the absence of an oligonucleotide, without bivalent cations (lane 1) and in the presence of Ca^{2+} (lane 5) and Mg^{2+} (lane 7), a normal pattern of cyclobutane photodimers formation is observed. When Ba^{2+} (lane 3), Cd^{2+} (lane 9), Co^{2+} (lane 11), Mn^{2+} (lane 13), Zn^{2+} (lane 15) or Ni^{2+} (lane 17) are present, the yield of cyclobutane dimers in duplex DNA in the absence of any oligonucleotide sharply decreases. Note that such effect was not observed in case of the $d(TC)_nd(GA)_n$ insert (Fig.3).

In the presence of Ca^{2+} (Fig. 5, lane 4) and Mg^{2+} (Fig. 5, lane 6), for which the photoreactivity of the duplex does not differ from the photoreactivity of the B form, the oligonucleotide forms triplex and the intensity of the [6-4] dimers sharply decreases. Fig. 4 (lanes 6 and 8) shows that for these cations under identical conditions no protection from the formation cyclobutane dimers is observed. The yield of cyclobutane dimers in the $d(G)_{18}d(C)_{18} + d(G)_{10}$ triplex zone is even slightly greater than for the B form. In the densitogram, the following effect is clearly visible: the CT dimer at the 3′ end of the C_{18} tract, which was in the B form, exhibited significantly lower intensity than the adjacent CC dimers (lanes 1, 2, 5); with the formation of the $d(C)_{18}d(G)_{18} + d(G)_{10}$ triplex, the photoreactivity of the CT dimer becomes greater than for the adjacent CC dimers (lane 6).

Cyclobutane dimers permit easily to detect the triplex formation in the presence of Cd^{2+} (Fig. 4, lane 10), Co^{2+} (Fig. 4, lane 12), Mn^{2+}(Fig. 4, lane 14), Zn^{2+} (Fig. 4, lane 16) and Ni^{2+} (Fig. 4, lane 18). In their presence, triplex formation leads to 'antifootprinting': cyclobutane dimers are formed in the triplex; without triplex, there are virtually no cyclobutane dimers formed. The 'fine structure' of photodimer formation throughout the triplex attracts attention: along the length of the $d(C)_{18}d(G)_{18}$ tract, significant variation of the yield of cyclobutane dimers is observed. This effect is rather puzzling taking into account the fact that the sequence under study is homogeneous.

DISCUSSION

The most probable scheme of TA*A and CG*G base triads is presented in Fig.6A. The available NMR data indicate that all bases in the triads are in the *anti* conformation (21). More precise structural data, including X-ray cristallography, are still unavailable.

Along with intermolecular PyPuPu triplexes formed between purine oligonucleotides and duplex DNA, in superhelical DNA an unusual structure, which includes this type of triplex, is formed in the presence of bivalent cations (16–20). These structures belong to the family of H forms and are often labelled as H*-DNA (see Fig. 6B).

Before we have shown that in case of intermolecular PyPuPu triplexes different sequences behave differently in the presence of bivalent cations. Our present data indicate that the same sequence is affected in different way by bivalent cations when it forms intermolecular triplex or H*-DNA. Table 1 shows that in the case of $d(TC)_nd(GA)_nd(AG)_n$ triplex bivalent cations affect H*-DNA and intermolecular triplex in a similar way. The only exception is Ni^{2+} ions, for which the data seem less reliable.

By contrast, in case of poly dG poly dC Table 1 shows that the H* form is formed in the presence of Mg^{2+}, Ca^{2+} or Mn^{2+}, but not Co^{2+} or Zn^{2+}; the intermolecular triplex is formed in the presence of all bivalent cations studied, with exception for Ba^{2+}. Moreover, the addition of Zn^{2+} ions to a buffer containing Mg^{2+} inhibits the formation of the H* form. This discrepancy can be explained in a following manner. It is possible that transferred Me^{2+} ions stabilize not only Hoogsteen pairing, but, even more significantly, Watson–Crick pairing. The energy difference of the formation of the H* form in the presence of Me^{2+} includes the energy gain due to Hoogsteen pairing and the energy loss due to partial disruption of Watson–Crick pairs within the insert. And this means that if Me^{2+} better stabilizes the Watson–Crick pairs than Hoogsteen ones, then the energy loss due to disruption of Watson–Crick pairs can prevails over the energy gain due to the formation of Hoogsteen pairs and the

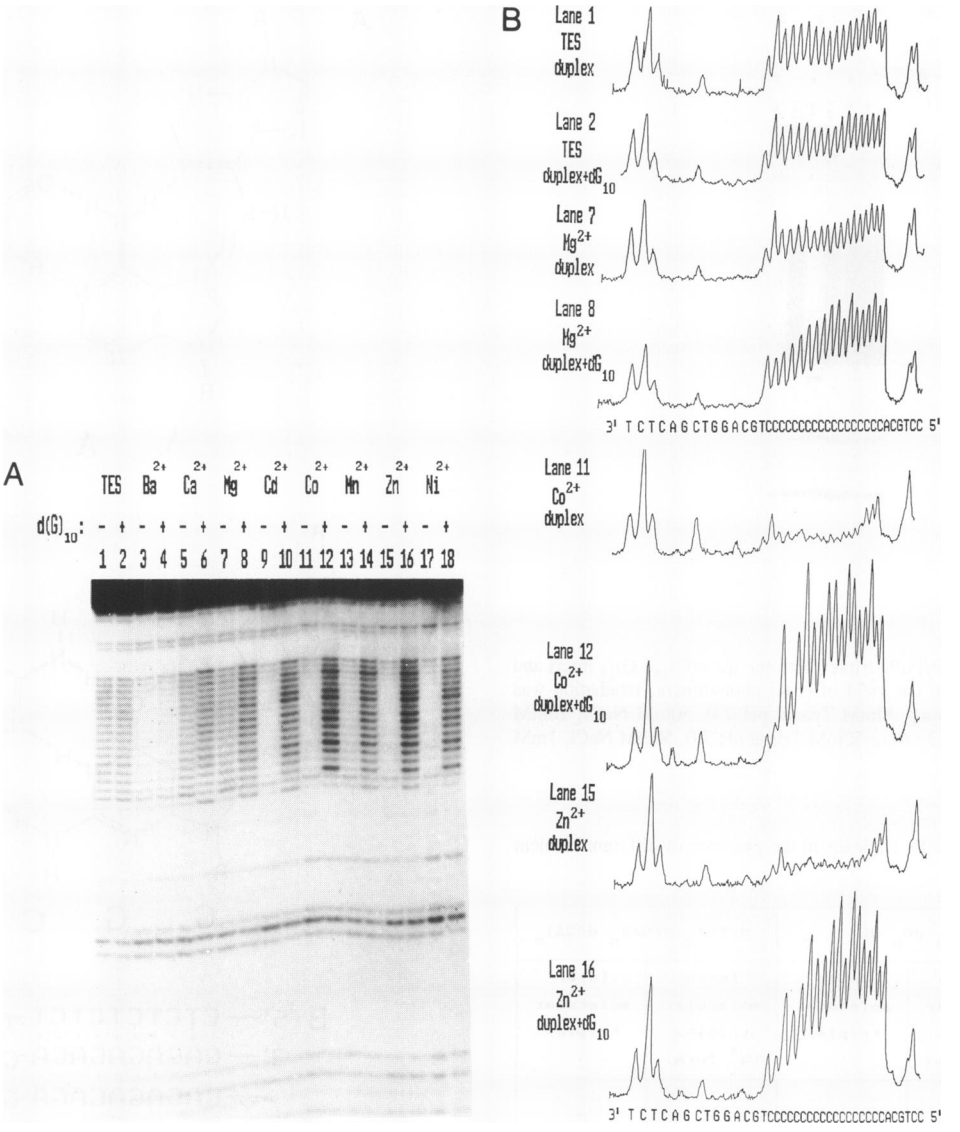

Fig. 4. UV photofootprinting with the use of cyclobutane photodimers of the intermolecular triplex between the $d(C)_{18}d(G)_{18}$ insert and the $d(G)_{10}$ oligonucleotide in the presence of various bivalent cations. Irradiation was conducted in 50mM Tris at pH 7.0, 50mM NaCl, 1mM EDTA (lanes 1,2), 50mM Tris at pH 7.0, 50mM NaCl, 10mM of corresponding metal (lanes 3–18). Panel A: radioautograph of gel. Panel B: densitometry of selected lanes.

H* form will be destabilized although the intermolecular triplex will become more stable in the presence of bivalent cations. Another possible explanation is that the bivalent cations bind to single-stranded hairpin of the H* form and significantly deteriorate its energetics.

In the absence of structural data on bivalent cations binding with triplexes it is hard to explain the data in Table 1 in terms of structure. Therefore we can put forward only very general reasons. Ba^{2+} ions destabilize triplex probably due to their large size. We also believe that $dC_n\ dG_n\ dG_n$ triplex can exist in two conformations: one in the presence of Cd^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+} or Ni^{2+} and the other one in the presence of Mg^{2+} or Ca^{2+}. The former conformation can match the incorporation of TA*A triads whereas the latter one can not. This difference between transition metal ions and the alkaline-earth cations stems, most probably, from the fact that the former bind preferentially with bases while the latter one bind preferentially with phosphates.

There are two main variants of the DNA photofootprinting assay. One consists in the registration of the [6-4] photoproducts and the other one consists in the registration of cyclobutane photodimers. Although in both cases a remarkable sensitivity of the yield of photoproducts to the triplex formation has been demonstrated, there is a striking variety of effects. In case of the [6-4] photoproducts the situation is quite simple. Namely, the triplex virtually prevents the formation of this type of photoproducts in the duplex in both major types of triplexes—PyPuPy and PyPuPu. Similar simple behavior is observed in the case of the formation of cyclobutane dimers in intermolecular (see Figs. 1–2) as well as intramolecular (within H-DNA (14)) PyPuPy triplexes.

All these date are consistent with a simple explanation of the effect of damping of the yield of photodimers due to triplex formation. This explanation stems from the idea that these photoproducts cannot be formed in DNA without significant fluctuational violation of the duplex. These fluctuations are inhibited by triplex formation and because of this, the yield of both types of photodimers is damped down. Although this explanation remains probably valid for the [6-4] photoproducts,

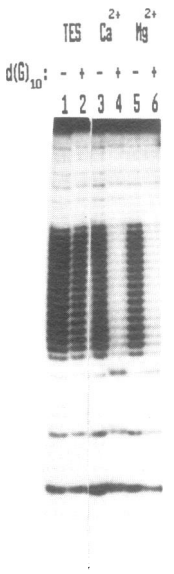

Fig. 5. The influence of the PyPuPu triplex between the $d(C)_{18}d(G)_{18}$ insert and the $d(G)_{10}$ oligonucleotide on the yield of [6-4] photodimers. Irradiation was conducted in a buffer containing 50mM Tris at pH 7.0, 50mM NaCl, 10mM of corresponding metal (lanes 3–6) or 50mM Tris at pH 7.0, 50mM NaCl, 1mM EDTA (lanes 1,2).

Table 1. Formation of Py-Pu-Pu triplexes in the presence of different bivalent cations

Bivalent metal cations	dC_n dG_n dG_n		$d(TC)_n$ $d(GA)_n$ $d(GA)_n$	
	Intra-molecular triplex (H^* form)	Inter-molecular triplex	Intra-molecular triplex (H^* form)	Inter-molecular triplex
Without	–	–	–	–
Ba^{2+}	No data	–	No data	–
Ca^{2+}	+ [20]	+	– [17]	–
Mg^{2+}	+ [19]	+	– [17]	–
Cd^{2+}	No data	+	+ [17]	+
Co^{2+}	– [20]	+	+ [18]	+
Mn^{2+}	+ [20]	+	+ [17]	+
Zn^{2+}	– [20]	+	+ [16]	+
Ni^{2+}	No data	+	– [17]	+

whose geometry seems to be totally inconsistent with the triplex geometry, for the case of cyclobutane dimers, the situation is much more complicated.

This is especially true for the case of PyPuPu triplexes. Our data clearly demonstrate that, rather than inhibiting the formation of cyclobutane dimers, the PyPuPu triplex formation may change the relative yield of TC and CT dimers (in case of the $d(TC)_nd(GA)_nd(GA)_n$ triplex).

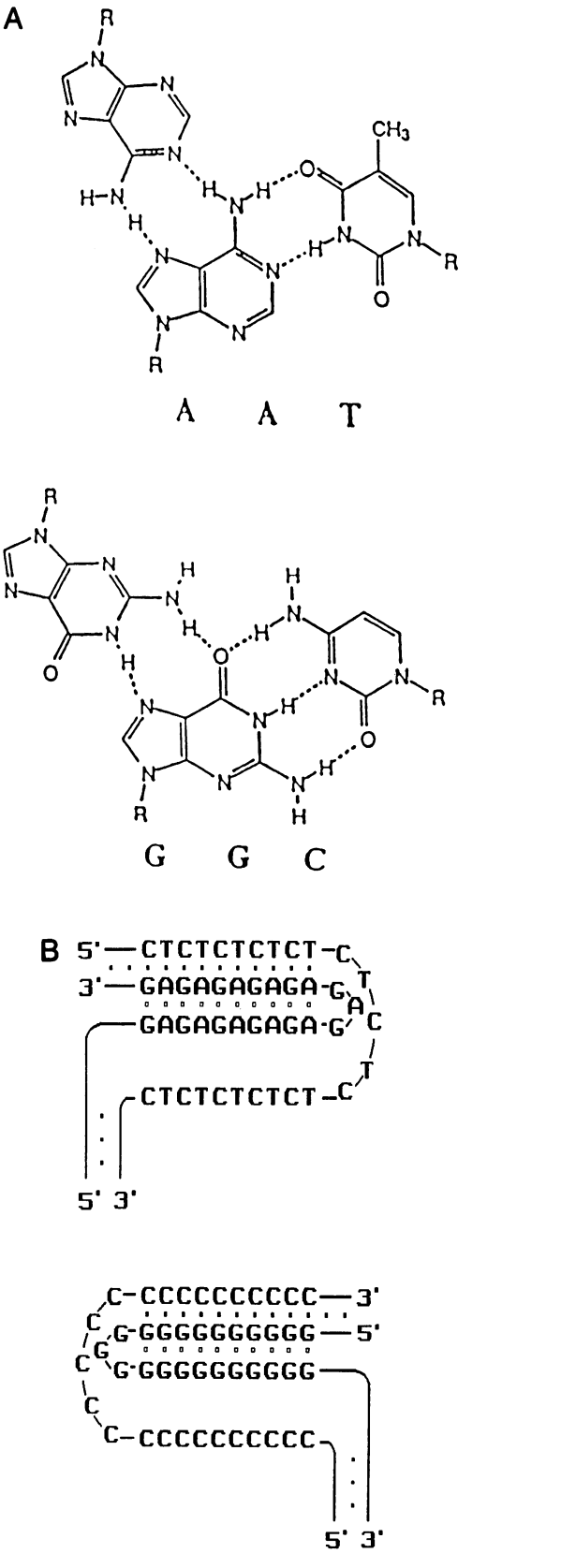

Fig. 6. A. A scheme of PyPuPu base triads. All bases are in *anti* conformation. **B.** Prevailing isomeric forms of H*-DNA for the $d(CT)_n/d(AG)_n$ (16–18) and dC_n/dG_n (19–20).

In explaining such unusual behavior, one has to bear in mind that PyPuPu triplexes in our experiments were always stabilized by bivalent cations. Therefore, the yield of photoproducts could be affected not only by DNA structure, but also by the direct influence of cations on DNA photochemistry. This direct influence could be different in cases of the duplex and of the triplex because the cation may have a different binding mode in duplex and in triplex.

Nevertheless, the data on the influence of different cations on the yield of cyclobutane dimers presented in Fig. 3 do not indicate that such direct effect makes any significant contribution in case of the 'band inversion' phenomenon. Indeed, in the absence of oligonucleotides, the patterns of formation of cyclobutane dimers are virtually identical for all cations used, and the pattern in the absence of bivalent cations is also the same (cf. odd lanes in Fig. 3 and lane 3 Fig. 1). Again, when the PyPuPu triplex is formed, the patterns are very similar independently of the type of bivalent cation (cf. lanes 4, 6, 8, 10, 12). Therefore, we attribute the 'band inversion' effect to some structural changes of the duplex within the PyPuPu triplex.

We believe that the peculiarities of DNA structure, rather than the direct effect of cations, are responsible for the effect of inhibition of cyclobutane dimer formation within the $d(G)_nd(C)_n$ insert in the presence of some cations (see Fig. 4). In this case our major argument against direct influence stems from the fact that we have never observed a significant influence of cations on the yield of dimers outside the insert (see Figs.3,4). Therefore, our data indicate that $d(G)_nd(C)_n$ tracts adopts a non-B-DNA structure in the presence of Ba^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+} and Ni^{2+}, but not in the case of Ca^{2+} and Mg^{2+}. The nature of this structure requires a special study.

In the light of the data for the $d(TC)_nd(GA)_nd(GA)_n$ triplex, the effect of enhancement of the yield of cyclobutane dimers in case of the $d(C)_nd(G)_nd(G)_n$ triplex does not look unexpected. Again, the data indicate that it is a structural effect, rather than the direct effect of cations on DNA photochemistry.

The fact that the 'band inversion' effect is associated with the structure rather than direct effect of ions on DNA photochemistry does not mean that it can be easily explained in terms of structure. Prior to the final photodimerization electronic excitation is known to undergo transfer between bases. As a result in case of duplex the yield of photodimers significantly depends on the neighboring bases (2). This effect is easily seen in Fig. 4B: the yield of T↑C dimer in sequence CTCT exceeds the yield in sequence GTCG by a factor of four. Similarly, the yield of different photodimers may strongly depend on structure (triplex versus duplex). This provides, in very general terms, the reasons for the 'band inversion' effect.

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