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Conserved guanine-guanine stacking in tetraplex and duplex DNA

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Abstract Using a series of suitably chosen oligonucleotides, we demonstrate that the DNA duplex of d(CCCCGGGG) provides an almost identical CD spectrum as the parallel-stranded tetraplex of d(GGGG). The CD spectra are very sensitive to base stacking in DNA so that the above observation indicates that guanine-guanine stacking is essentially the same within the duplex of d(CCCCGGGG) and the tetraplex of d(GGGG). A very similar CD spectrum is also provided by the A-form of d(CCCCGGGG) induced by trifluoroethanol. These results reveal that guanine-guanine stacking is a structural invariant conserved in various nucleic acid conformers. The structural invariance is likely to cohere with evolution of the genetic molecules and be important for fundamental functions, e.g. initiation of transcription.

Keywords Tetraplex DNA · Duplex DNA · Circular dichroism spectra · Conserved stacking · Guanine

Introduction

DNA can adopt numerous conformations, depending on the primary structure, hydration, ions, proteins, drugs and superhelical stress. The primary structure dependence is far from being fully understood, but it is, for example, evident that alternating pyrimidine-purine runs confer much more conformational flexibility on the duplex of DNA than the purine d(R) runs bound to the runs of the complementary pyrimidines d(Y) (Vorlíčková and Kypr 1985).

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The purine runs confer remarkable properties on DNA irrespective of the base composition or sequence. For example, the runs of adenine cause DNA bending by a mechanism whose essence is still not known (Jerkovic and Bolton2000). The runs of guanine introduce biologically relevant (transcription initiation) properties into DNA (Rhodes and Klug 1986), whose structural explanation was a matter of controversy (McCall et al. 1986; Gottesfeld et al. 1987; Fairall et al. 1989; Galat 1990; Huber et al. 1991). Below we present data contributing to an understanding of the origin of the peculiarity that the runs of guanine introduce into DNA.

Instead of being flexible within duplexes of DNA, the $d(R)_n.d(Y)_n$ blocks switch into triplexes or tetraplexes (reviewed in Shafer 1998) under conditions when their duplexes are no longer stable. The $d(R)_n$ run rigidity inspires an idea that conformations adopted by the strongly interacting purine bases in the duplex DNA might be conserved in the multi-stranded conformers. Here we show that base stacking of a run of four consecutive guanines is indeed conserved in the duplex of $d(C_4G_4)$ and the parallel-stranded tetraplex of $d(G_4)$.

Materials and methods

The DNA fragments used in this study were from the Laboratory of Plant Molecular Physiology, Faculty of Science, Masaryk University, Brno, Czech Republic. They were originally dissolved in a very weak buffer (1 mM sodium phosphate, 0.3 mM EDTA, pH 7.5) and these stock solutions were then used to prepare the samples described below. The temperature dependences of the UV absorption spectra of the samples were measured using a Unicam 6265 spectrophotometer. The spectra were also used to determine the oligonucleotide concentrations using the extinction coefficients (at 260 nm in 1 mM sodium phosphate, 0.3 mM EDTA, pH 7.5, at 90 °C) of 11,060, 10,180, 9720, 9390 and 9150 $M^{-1}\,cm^{-1}$ with $d(G_4)$, $d(CG_4)$, $d(C_2G_4)$, $d(C_3G_4)$ and $d(C_4G_4)$, respectively.

The samples were electrophoresed in 16% (29:1 monomer/bis ratio) polyacrylamide gels (14×16×0.1 cm in size) using a thermostatted SE-600 (Hoefer Scientific) slab at 60 V for 20 h. After the electrophoresis, the gels were stained with Stains All (Sigma) and scanned by a Molecular Dynamics Personal Densitometer using the ImageQuant software.

Circular dichroism (CD) spectra were recorded using a Jobin Yvon, mark VI, dichrograph in thermostatted 0.1 cm pathlength quartz cells (Hellma). Sample absorptions were about 0.8, giving an optimum signal-to-noise ratio.

Results

The tetramer $d(G_4)$ associates into a stable parallel-stranded tetraplex DNA that has been characterized in detail by various methods (Sen and Gilbert 1988; Aboul-ela et al. 1992; Wang and Patel 1993; Giraldo et al. 1994; Phillips et al. 1997; reviewed by Guschlbauer et al. 1990). Its CD spectrum is dominated by two strong positive bands in the vicinity of 205 nm and 260 nm (Fig. 1). CD spectra of the B-forms DNA are substantially different. Especially, the B-form CD bands are much weaker (Johnson 1994; Vorlíčková and Kypr 1985). Addition of one or two cytosine residues to the 5′-end of $d(G_4)$ does not change the CD spectrum shape, which is not surprising because PAGE shows (Fig. 2) that both $d(CG_4)$ and $d(C_2G_4)$ predominate in the tetraplex. However, $d(C_3G_4)$ and especially $d(C_4G_4)$ prefer to form a duplex (Trantírek et al. 2000) and not the tetraplex (Fig. 2). Hence it is remarkable how small the changes are in the CD spectrum accompanying the tetraplex-to-duplex transition. Especially notice the striking similarity of the CD spectra of the tetraplex of $d(G_4)$ and the duplex of $d(C_4G_4)$ expressed per molarity of G in the DNA (Fig. 1). The similarity suggests that the CD spectrum of the duplex of $d(C_4G_4)$ is almost entirely governed by its $d(G_4)$ runs. The $d(C_4)$ runs contribute negligibly, presumably because they are unstacked in the aqueous duplex of $d(C_4G_4)$ (Trantírek et al. 2000). In addition, the CD spectral contribution of $d(G_4)$ is essentially the same in the antiparallel duplex of $d(C_4G_4)$ and the parallel tetraplex of $d(G_4)$, which implies that the CD spectra are dominantly determined by the intrastrand guanine-guanine interactions which are very similar in the duplex and the tetraplex.

Trifluoroethanol (TFE) induces the usual cooperative B-A transition of the duplex of d(CCCCGGGG) (Fig. 3) that is accompanied by the appearance of the negative CD band close to 210 nm (Vorlíčková 1995). However, the strong CD band at 260 nm is but little changed during this transition, suggesting A-like guanine-guanine stacking in the aqueous duplex of d(CCCCGGGG). This view has been confirmed by independent molecular dynamics (MD) simulations (Trantírek et al. 2000). In addition, the MD simulations showed that the most prominent A-like feature was a guanine shift towards the double helix exterior to generate a cavity in the double helix center typical of

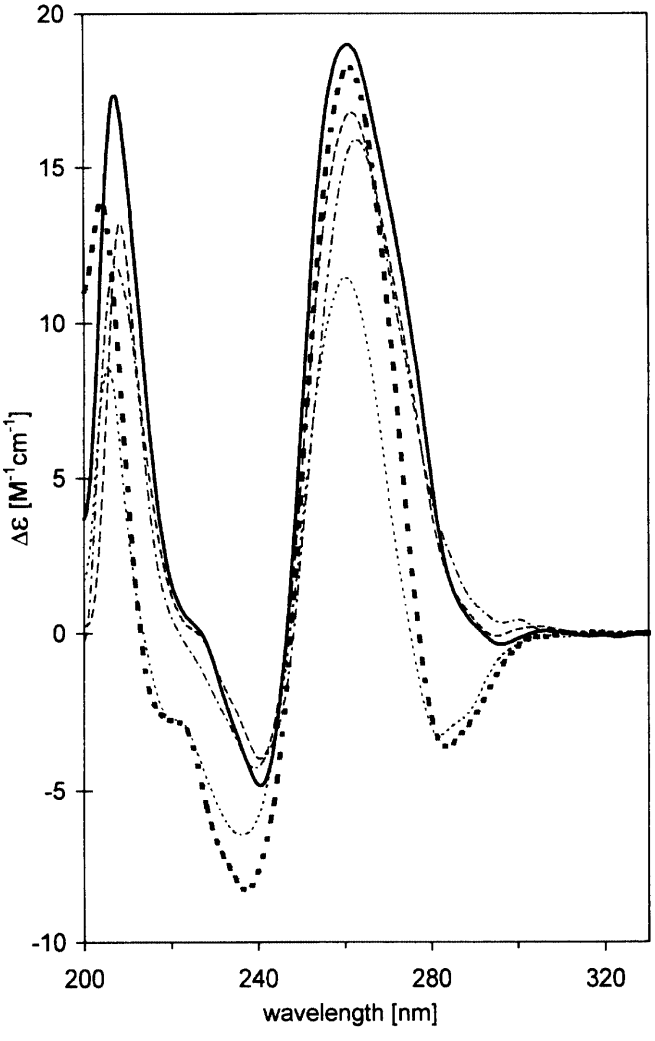

Fig. 1 CD spectra of the tetraplexes of $d(G_4)$ (*bold trace*), $d(CG_4)$ (*dashes*) and $d(C_2G_4)$ (*dash-dot*), and duplexes of $d(C_3G_4)$ (*dots*) and $d(C_4G_4)$ (*bold dashes*) in 0.1 M KCl, 10 mM $K_3(PO_4)_3$, pH 7.5, at room temperature. The ellipticities are expressed per mole of G in the DNA molecules

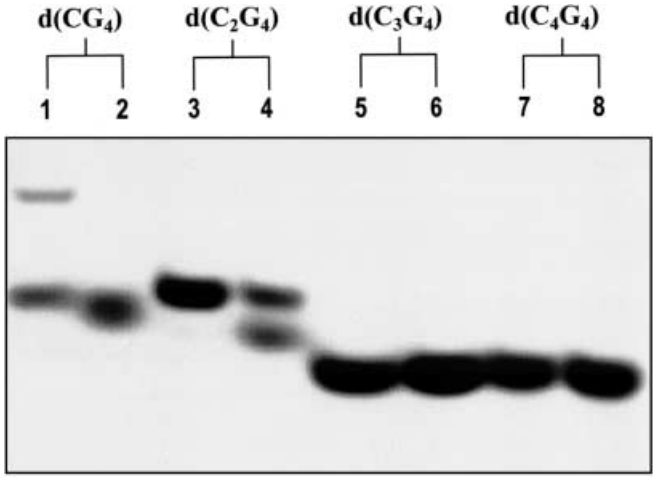

Fig. 2 A non-denaturing polyacrylamide gel showing migration of (*from left to right*) the tetraplexes of $d(CG_4)$ and $d(C_2G_4)$, and duplexes of $d(C_3G_4)$ and $d(C_4G_4)$. Each sample was loaded on two neighbouring lanes, the even samples being exposed to 90 °C for 5 min. The gel shows that this treatment eliminated higher aggregates of $d(CG_4)$ and partially destabilized the tetraplex of $d(C_2G_4)$, but had no significant effect on the samples of $d(C_3G_4)$ and $d(C_4G_4)$ forming a duplex

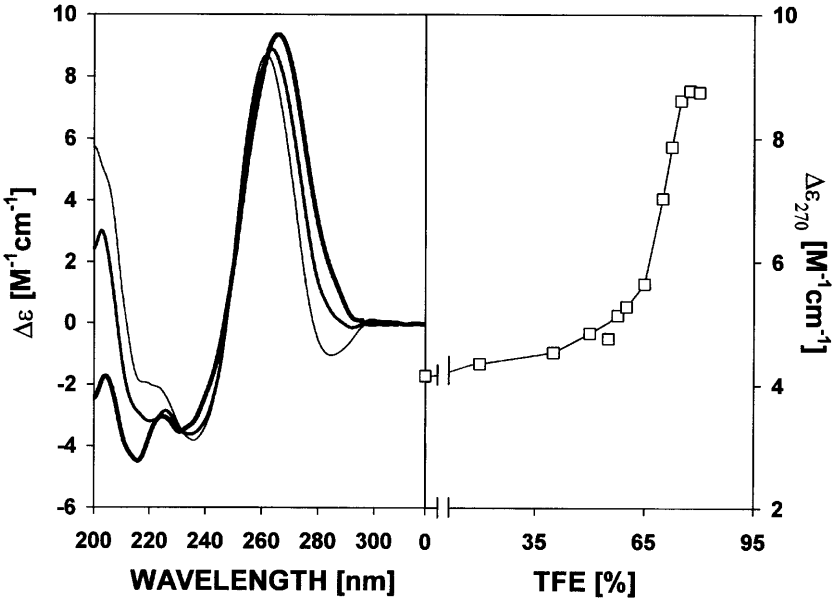

Fig. 3 The trifluoroethanol-induced B-A transition of the duplex of $d(C_4G_4)$. The concentrations of TFE were (*from the thinnest to the thickest trace*) 60, 70 and 80%. TFE was added to the octamer dissolved in 10 mM $Na_3(PO_4)_3$, 0.3 mM EDTA, pH 7. The CD spectra were measured at 0 °C

A-DNA (Trantírek et al. 2000). In the tetraplex the guanines are shifted towards the exterior as well (Phillips et al. 1997), confirming the above conclusion that guanine-guanine stacking is very similar and A-like in the DNA duplex of $d(C_4G_4)$ and the DNA tetraplex of $d(G_4)$.

Discussion

CD spectroscopy is sensitive to base stacking in DNA (Gray and Tinoco 1970; Johnson 1994). Here we show that the CD spectrum is essentially the same with the tetraplex of $d(G_4)$ and the duplex of $d(C_4G_4)$. This CD spectrum is unique, especially with the strong band in the vicinity of 260 nm which is also characteristic for the CD spectrum of the A-form. We suggest that the above reflects a very close similarity of guanine-guanine stacking in the parallel-stranded tetraplex, the antiparallel aqueous duplex of $d(C_4G_4)$ and the A-form of DNA. In other words, guanine-guanine base stacking is a structural invariant of DNA.

Structural invariants are important and interesting. Many of them have already been identified in RNA or proteins, but DNA has so far seemed to be a molecule where no invariants exist. However, we have recently discovered that the ApT steps have an essentially invariant base-base stacking geometry in the crystal structures of B-DNA as well as A-DNA (Neugebauerová and Kypr 2000). Now we see that the GpG steps are invariant even within different conformers of DNA including the tetraplex, the aqueous duplex and the A-form. We have recently generated a molecular model of the aqueous DNA duplex of $d(C_4G_4)$ that was remarkably consistent with the CD spectroscopy as well as NMR spectroscopy data (Trantírek et al. 2000). The most impressive feature of this duplex was the central hole that is characteristic for A-DNA or RNA (Conner et al. 1984; Heinemann et al. 1990), whereas the hole does not exist in B-DNA because the base pairs cross its center. The hole also exists in the tetraplex (Phillips et al. 1997), which supports the conclusion following from the present comparative CD spectroscopy studies that the successive guanines stack essentially in the same way irrespective of whether they are a part of the parallel-stranded tetraplex, the antiparallel aqueous duplex of $d(C_4G_4)$ or its A-form. This means that base stacking is A-like in the parallel-stranded tetraplex of DNA, which is in nice correspondence with the fact that the guanine tetrads stabilize the parallel-stranded tetraplex in RNA as well (Cheong and Moore 1992). Yeast G4p1 protein binds G-RNA and G-DNA equivalently (Frantz and Gilbert 1995), providing further support for the idea that guanine-guanine stacking is the same in DNA and RNA.

The present communication is important from the structural point of view because the invariant building blocks will improve our understanding of DNA and enhance the possibilities of DNA modeling. The invariant building blocks can also be used to check model reliability. The third aspect is the evolutionary history of the conserved $d(G_4)$ block. The fact that it is compatible with various DNA conformations could be important during transmission of the evolved properties between various replicators. In particular, the $d(G_4)$ and related blocks could have facilitated the transition from the RNA world to the DNA world (Jeffares et al. 1998; Trantírek et al. 2000) because the transition does not change the properties of the $d(G_4)$ block. Transcription initiation is dependent on the RNA-like properties of G-rich DNA regions (Rhodes and Klug 1986), which further supports the above views.

The fact that base stacking is a structural invariant at the GpG steps in DNA does not mean that the GpG

step always adopts the same geometry. For example, the so-called antiparallel tetraplex of $d(G_4T_4G_4)$, i.e. a dimer of two $d(G_4T_4G_4)$ foldbacks, provides quite a different CD spectrum than the parallel tetraplex of $d(G_4)$ (Balagurumoorthy et al. 1992; Guo et al. 1993; Vorlíčková et al. unpublished). In addition, we do not yet know how the tetraplex-like guanine-guanine stacking depends on the number of consecutive guanines in DNA and, in particular, on the $d(G_n)$ flanking regions. It will also be interesting to see in the forthcoming work how the base stacking geometries differ in the parallel and antiparallel tetraplex in solution, what causes the difference and whether base stacking in the antiparallel tetraplex also has a counterpart in a duplex DNA.

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