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Probing DNA triple helix structure by chemical ligation

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A series of oligomeric double and triple helical DNAs with irregular sequences of homopurine and homopyrimidine strands were prepared. DNA triplexes were identified by CD spectroscopy and thermal denaturation profiles (biphasic helix-coil transition). Condensation of oligonucleotides on single and double-stranded DNA templates was performed using water-soluble carbodiimide, phosphodiester and pyrophosphate internucleotide bonds being newly formed. Such chemical ligation proved to be a sensitive monitor of changes in the sugar-phosphate backbone resulting from conversion of double to triple helix and of third-strand binding.

DNA triplex; Chemical ligation of nucleic acids

1. INTRODUCTION

Renewed interest in DNA triple helices has recently intensified because of their potential role in the regulation of the eukaryotic genome [1,2]. There is also interest in generating nucleic acid ligands that recognize double-stranded targets. Such probes could serve as artificial gene repressors [3] and as DNA cleaving agents in sequencing procedures or in chromosome mapping [4]. Triple helices are believed to adopt an A-form structure, the purine strand and one pyrimidine strand being Watson-Crick paired and the other pyrimidine strand being Hoogsteen base-paired parallel to the purine strand along the major grooves [5–9]. In addition to physical methods [5–7], chemical approaches such as chemical and enzymatic cleavage-protection assays [8] and sequence-specific cleavage of double helical DNA [9] have been used for studying the molecular structure of triplexes. Recently, it was also reported that double-stranded DNA can serve as a template to promote BrCN-induced condensation of homopyrimidine Hoogsteen paired third strands [10]. Using a different condensing agent, the present study demonstrates the potentialities of chemical ligation for monitoring changes in the sugar-phosphate backbone on converting a DNA duplex to triplex and for following third-strand binding. Because the two homopyrimidine strands of a triplex are antiparallel, they cannot replace each other in the triplex structure when irregular sequences are used. This makes it possible to independently observe chemical ligation in the two homopyrimidine strands and to compare the reactivity and hence the arrangement of adjacent phosphate and hydroxyl groups at the ligation junction in the triplex and in the parent duplex.

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Abbreviations: CDI, 1-ethyl-3-(3'-dimethyl-aminopropyl) carbodiimide hydrochloride; MES, 2-morpholinoethanesulfonate

2. MATERIALS AND METHODS

Oligodeoxyribonucleotides were synthesized by the phosphoramidite method on a Cyclon (Biosearch) DNA synthesizer. TTCCCTC and TCCTCTCTA were phosphorylated at the 5' end with polynucleotide kinase and [γ-^{32}P]ATP. ACTCCCTTCp was obtained from ACTCCCTTCrU as described in [11]. CDI and MES were from Merck.

2.1. *Optical measurements*

Equimolar solutions of oligonucleotides 1–7 (Scheme 1) were mixed to give complexes I–VI (Scheme 2). ϵ_{260} values for oligomers 3–5 were assumed to be 8200, 13300 and 9000 (per monomer), respectively. Melting curves of oligomer mixtures in 0.05 M MES, pH 6.0, 0.02 M $MgCl_2$ (buffer A) were determined on a Cary 219 spectrophotometer. The data were processed on a Hewlett Packard 9825A computer. Circular dichroism measurements were made using a Jasco J-500C spectrometer.

2.2. *Chemical ligation reaction*

Oligomer mixtures (0.5×10^{-3} M (per monomer) forming duplex I or triplexes II, III, IIIa in buffer A were cooled to 0°C, and CDI added to a final concentration of 0.2 M. 5'-^{32}P labelled oligomers 2 or 7 were added in a 1.5-fold deficiency relative to the unlabelled ones. After 2 days (0°C, in the dark), reaction products were separated by 20% denaturing polyacrylamide gel electrophoresis.

3. RESULTS AND DISCUSSION

A series of DNA duplexes and triplexes (Schemes 1 and 2), including nick-containing ones, was obtained by mixing oligomers TCTCTCCTC (1), pTTCCCTC (2), TCTCTCCTCTTCCCTC (3), GAGGGAAGAGGAGAGA (4), ACTCCCTTCTCCTCTCTA (5), ACTCCC-

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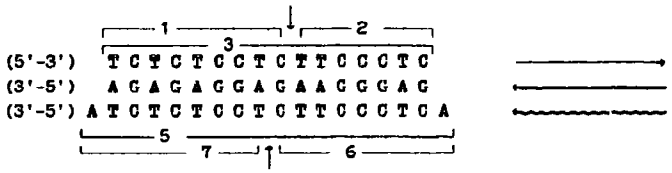


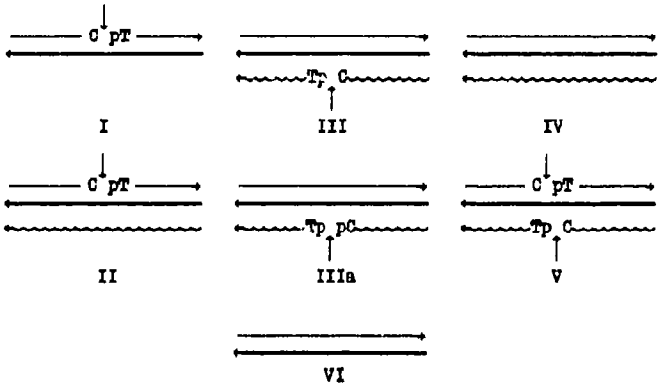
Scheme 1. The symbol d is omitted here and throughout the paper; the vertical arrows mark the site of the oligonucleotide junction. Pyrimidine oligomers 3 or 1 + 2 (designed →) and purine oligomer 4 (⟵) are Watson-Crick paired; oligomers 5 or 6 + 7 (⟵∿∿) are the Hoogsteen paired third strands; horizontal arrows indicate the 5′ → 3′ direction.

TTC(p) (6), pTCCTCTCTA (7), in different combinations.

The interaction of oligomers was dictated by the third strand binding code [12] and the assumption that the third strand must be parallel to the purine strand of the core duplex. Dangling A bases may stabilize triplex structure by stacking interactions with the bases of the core duplex. The irregular sequences of the oligomers provide for easy control of the strandedness of complexes formed. Diagrammatic representation of the complexes studied are shown in Scheme 2.

A preliminary study was made of the stability of the DNA triplexes under chemical ligation conditions. CD spectra (Fig. 1) confirm the presence of a new helical state in equimolar mixture of oligomers 3–5 (triplex IV, Scheme 2). Thus, the negative band at 215 nm, which is a useful indication of triplex formation [13], is a dominant feature of the CD spectrum of triplex IV. Moreover, the spectrum is significantly different from that of the normalized sum of its oligomeric components (Fig. 1).

Thermal denaturation studies also provide evidence supporting the existence at low temperature of triple-stranded DNA in buffer A (Fig. 2). All triplex-forming mixture exhibit a biphasic helix → coil transition at 260 nm (the biphasic nature of these transitions is much more apparent in the first derivative plots (Fig. 2).



Scheme 2. For explanations see Scheme 1. Nucleoside residues and phosphate groups facing the nick are indicated. In triplex IIIa, both ends of the 'nicked' chain are phosphorylated.

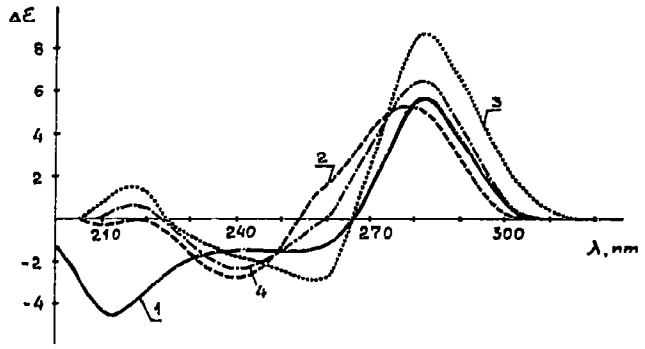


Fig. 1. CD spectra of triplex IV (1), duplex VI (2), oligomer 5 (3); normalized sum of spectra from duplex VI and oligomer 5 (4). Buffer A, 20°C, $C = 10^{-4}$ M, per monomer.

Where the core duplex is the same, as in a and b, the profiles show the same sharp transition with T_m of 62°C. A similar transition was observed for the parent duplex VI (data not shown). By elimination then, the lower T_m (13°C in a for nick-containing triplex III and 45°C in b for triplex IV) must correspond to the dissociation of the third strand. As expected, the nick in the third strand (as in a) considerably decreases T_m (by 32°C) for this transition. A more complicated picture is observed with triplexes V and II (Fig. 2c,d) which contain a nick in the Watson-Crick base-paired pyrimidine strand. The transition with a T_m of 33°C, corresponding to the melting of duplex I, was found in both profiles. In c, with both pyrimidine strands nicked, the transition with T_m at 9°C obviously corresponds to the dissociation of the third strand. In d, the additional transition occurs with T_m at 43°C, above that for the duplex, which at first may seem strange. However, this T_m value may be rationalized by recognizing that the remaining purine and pyrimidine intact strands are Watson-Crick sequence-related in the parallel orienta-

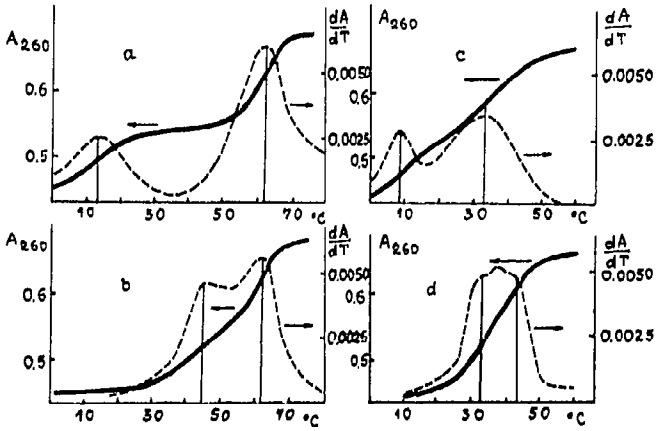


Fig. 2. Melting profiles of triple-stranded DNA in buffer A, $C = 0.5 \times 10^{-4}$ M: III (a), IV (b), V (c), II (d). First derivative plots of profiles are shown by dotted lines.

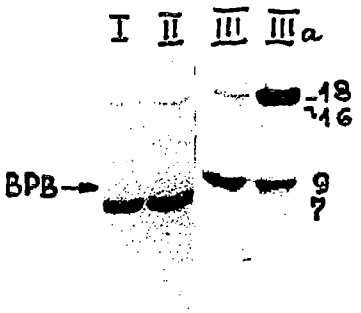


Fig. 3. Polyacrylamide gel electrophoretic analysis of reaction mixture containing double and triple-stranded oligomers after CDI-induced nick-ligation. For conditions see Materials and Methods. Lane numbers correspond to complex numbers (Scheme 2). The chainlength of oligomers is indicated to the right. XC and BPB, xylene cyanol and Bromphenol blue markers, respectively.

tion; and such parallel stranded helices containing both A · T and G · C pairs have been recently observed (J.R. Fresco, H. Shih and M. Thomas, personal communication).

Duplex I and triplexes II, III and IIIa were chosen as suitable models for CDI-induced condensation of pyrimidine oligonucleotides on single or double-stranded DNA templates. The results of chemical ligation experiments, presented in Fig. 3 and Table I, show that the condensation efficiency of Watson-Crick base-paired oligonucleotides in both double and triple-stranded complexes is approximately the same. Since a difference in sugar puckering, C3′-endo for the A family and C2′-endo for the B-family, clearly affects the extent of chemical ligation such that ligation for the A family is substantially less [14,15], this result indicates that a switch from B to A-type helix geometry does not occur in this case upon binding of the third strand to the DNA duplex. Instead, the low level of ligation, like that for an A type helix, shows that the target duplex sequences are probably already A-like. This result may be particular to the sequences studied here, as other cases have been reported of B-type duplex to A-type triplex transformation [6].

In the triplexes III and IIIa the two reacting groups must be in close proximity since both components of the third strand are bound to the core duplex. If they were not, the coupling reaction, which requires a stable complex, could not take place. The coupling yield was 14% for the formation of a new phosphodiester bond (triplex III) and 90% for pyrophosphate bond (triplex IIIa). Similar dependence of reaction efficiencies on the nature of the group that attacks the activated phosphate (3′-OH or 3′-phosphate) was noted earlier for DNA duplexes [14]. It is interesting that the product yields of nick-ligation in different pyrimidine strands of the same triplex are similar (compare triplexes II and III in Table I). It should be noted that in both chains identical nucleotide residues face the nick −3′C and 5′pT. The present chemical ligation results strongly support the idea that arrangements of 3′-OH and neighboring 5′-phosphate groups in both pyrimidine strands of triple-helical DNA are the same despite the difference in their hydrogen bonding to the purine strand. This conclusion is consistent with the X-ray fiber diffraction [5] and NMR [6] data concerning triplex sugar puckering mode and also results of T4 DNA ligase catalyzed ligation of two pyrimidine strands of a triplex [16].

Table I

Water-soluble carbodiimide-induced ligation of nicks in DNA double and triple helices

Complex	I	II	III	IIIa
Yield of chemical ligation product (%)	16±2	18±2	14±3	90±3

For reaction conditions see Materials and Methods. For complex composition see Schemes 1 and 2

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