SYNTHESIS OF A NOVEL POLYNUCLEOTIDE: POTENTIAL R-L MODEL

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ABSTRACT

Synthesis of a novel polynucleotide with potential alternating B and Z segments is described. This is the first report of a polynucleotide where the double helix undergoes change in handedness after every half a turn.

INTRODUCTION

Ever since the original proposal[1] of left-handed double helical structure of DNA and the subsequent discovery[2] of Z DNA in single crystals, extensive work has been carried out on left-handed Z DNA[3,4]. Using stereochemical guidelines and taking advantage of the inherent conformational flexibility of DNA, Sasisekharan and co-workers[5] could generate double helical DNA structures by joining five residues in right-handed and five residues in left-handed helical conformation. A composite RU and LZ helix with a stable link was proposed[6,7] as a special case of the generalised R-L model. Crick *et al*[8] ruled out by their studies on circular DNA, the possibility of the R-L model representing a generalized structure for DNA but the feasibility of such a structure in local segments was not tested experimentally.

Subsequent experimental studies have shown that B→Z transition can take place in oligo- and polynucleotides under a variety of conditions[3,4]. B→Z transition in the solid state under mild conditions, first reported from this laboratory, indicated the possibility of the coexistence of these two structures[9].

In cases where a potential Z DNA sequence is inserted in a covalently closed circular plasmid, B and Z conformations have been shown to coexist with a junction of ≥5 nucleotides[10–12]. The basic difference between Watson-Crick and R-L models is that the linking number is one order of magnitude higher in the former. Recently, it has been shown[13] that because of topological constraints several sequences adopt altered conformation in pBR322 Form V molecule with zero linking number. In one stretch of sequence starting from the *Eco* RI site of pBR322 Form V, shown below, altered (L) and normal (R) structures appear, alternately, four times in less than 40 nucleotides. Residues whose structures were probed are underlined. However, the fine details of the altered structure (L) are not known.

GAATTCTCATGTTTGACAGCTTATCATCGATAAGCTTTAA
R L R L

So far, no attempt has been made to study a polymer half of which is in right and half in left-handed conformation. For the first time we report a strategy to synthesize a polynucleotide with potential R-L conformation. This polymer, obtained by the block polymerization of d(CGCGCGATCGAT), has alternating six-base-pair Z- and B-helicogenic regions. Preliminary studies on the dodecamer, in concatamer and polymer form, indicate the presence of R-L conformation and open up new possibilities for the investigation of the role of unusual structures in the biological functions of DNA.

MATERIALS AND METHODS

2′ Deoxynucleosides, dimethoxytritylchloride, tetrazole, etc. used in the synthesis of the dodecamer were from Sigma Chemical Co., USA. Solvents were purified before use in the synthesis. T4 DNA ligase and polynucleotide kinase were from New England Biolabs, USA. Other reagents were of analytical grade.

Synthesis of d(CGCGCGATCGAT)

5′-Protected nucleosides were prepared according to Jones' procedure[14]. The corresponding amidites were prepared using methoxy (N,N-diisopropyl)-aminochlorophosphine and checked by ^{31}P NMR[15]. Coupling reactions were done manually in an open column system using standard procedure[15,16]. The dodecamer was deprotected and purified on a denaturing polyacrylamide gel[17].

CD measurements

A Jasco J 500A CD spectropolarimeter was used to measure the CD of the oligonucleotide. Samples were preheated and allowed to cool slowly before