DISULPHIDE BOND CONTAINING OLIGONUCLEOTIDE STABILIZES TRIPLEX DNA STRUCTURE

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Abstract: The triplex formation of disulphide containing oligonucleotide, unmodified and C-5 propyne deoxyuridine substituted oligonucleotides with duplex DNA using T_m was studied. The disulphide containing oligonucleotide stabilized triplex structure when targeted to duplex DNA at 7 pH whereas unmodified oligonucleotide and propyne deoxyuridine containing oligonucleotide did not stabilize triplex structure.

Key Words: C-5 Thiopropyne substituted oligonucleotides, T_m studies, triplex formation, C-5 propyne deoxyuridine oligonucleotides, disulphide containing oligonucleotide

INTRODUCTION

The high affinity sequence specific recognition of double stranded (ds) DNA is an area of biological and chemical research interest. High affinity sequence specific recognition of double stranded DNA by oligonucleotide analogues is important for the selective control of gene expression (Uhlman et. al. 1990; Goodchild, et.al., 1990; English, et.al. 1991) · The formation of triple helices from an RNA single strand represents a way to interference with transcription in DNA and RNA and translation in RNA (Thuong, et. al 1993; Felsenfeld, 1957). Since the discovery of the poly U, poly A poly U nucleic acid triple helix variety of base triplex motif others than U A U have been characterized (Howard et.al .1964; Morgan, et. al. 1968; Le Doan, et.al. 1987; Moser,et.al. 1987) However, two (Francois, et.al., 1988; Cooney, et.al 1988) structural motifs have become popular, they differ in the composition of base triplet and strand polarity. In the pu-pu py motif (Beal, et.al. 1991) the third strand is purine rich and poured in an antiparallel orientation. to the Hoogsteen side of the purine strand of the Watson Crick duplex and exhibits G-G: C, A-A: T and T-A: T base triplet (. In the py-pu-py motif the third strand consisting only of pyrimidine bases is paired to the Hoogsteen side of purine strand in a parallel fashion, T-A: T and C+-G: C (Le Doan, et.al. 1987; Moser,et.al. 1987) base triplets. Formation of the C- G: C triplet requires condition of low pH (<6.0) necessary for protonation of the third strand cytosine. The free nucleotide has a pK of 4.5, through this is elevated at isolated cytosine's within triplex forming oligonucleotides, depending on their number and location (Assensio, et. al., 1988). Several cytosine analogues have been synthesized in attempt to overcome this restriction (Povsic,et.al., 1989; Xodo,et.al 1991; Frochler, et.al., 1992; Huang,et.al., 1996; Ono,et.al. , 1991; Miller, et.al., 1992; Jetter, et.al., 1993; Bates, et.al., 1996; Hildbrand, et.al., 1997; Hildbrand,et.al., 1996; Barawkar, et.al., 1996; Rajeev, et. al . 1997; Tung, et. al . 1993; Sund, et.al., 1996; Sund, et.al., 1997; Cuenoud , et.al., 1998; Blommers, et.al., 1998; Nara, et.al . 1995).

Till now most of the bases analogues which have been prepared for triplex formation are derivatives of cytosine and have been designed to overcome the requirement for conditions of low pH. However, C+- G: C is more stable that T-A: T (1+). An approach different for increasing triplex stability is to modify the T-A: T triplet. Few efforts have been made to improve the stability of TAT triplet (Gryaznov, et.al., 1995; Jones, et.al., 1993; Froehler, et.al., 1992; Bijapur, et.al. 1999; Gowers, et.al., 1999; Osborne, et. al., 2004; Puri, et.al ., 2004; Sollogoub, et. al., 2002; Brennan, et.al 2007; Hong, et.al., 2008, Kumar et. al., 2010) . Experiments with S- propargylamino- dU as a charged analogue of thymine to stabilize T-A: T triplet has been described (Bijapur, et.al ·1999). The aim of our studies was to design an oligonucleotide containing purine and pyrimidine, which can stabilize triplex structure at neutral pH. There for using an earlier described method (Chaudhuri, et. al., 1995) thiol groups were incorporated at two adjacent thymidines of GGTGGGTTGGTG (Table-1). There after disulphide bond (Table-1) was made by oxidizing thiol groups. The disulphide containing oligonucleotide thus prepared was targeted to double stranded oligonucleotide.

For comparison unmodified as well as propyne modified oligonucleotide were also studied. Thermal denaturation studies showed that disulphide containing oligonucleotide formed the triplex structure whereas unmodified as well as propyne deoxyuridine modified oligonucleotides did not, clearly showed that we achieved our aim

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Material and Methods

Synthesis of Oligonucleotides : The unmodified oligonucleotides and C-5 thiopropyne substituted deoxyuridine containing oligonucleotide were synthesized , deprotected , purified , and also base composition analysis of oligonucleotides was carried out as described else where (Chaudhuri, et. al., 1995). Propyne deoxyuridine containing oligonucleotide was synthesized using 5-(1- propyne) -2'- deoxyuridine phosphoramidite (Glen Research). The propyne deoxyuridine containing oligonucleotide was deprotected and purified as the unmodified oligonucleotides. The preparation of disulphide containing oligonucleotide and purification was carried out as described earlier (Kumar, 2009). The preparation of the oligonucleotide, 2, was carried out using 5' GGAATTCCACCCAACCACTGCGCC3', 4, as template. The 15 mole of 3 and 15 mole of purified 4 were mixed in buffer (100 mM NaCl , 10 mM $MgCl_2$ and 10 mM PIPES) and exposed to air for 5-6 hrs and left overnight at 4 ^{o}C. The solution was dialyzed against water (4X 2.0 L) for 16 hrs and dried in Speed Vac concentrator. The dried oligonucleotides thus obtained were purified by gel electrophoresis on 20 % polyacrylamide containing 7 M urea, followed by crush, soak and dialysis methods. The oligonucleotide, 3, thus obtained was quantitated by UV absorbance at 260 nm. The molar extinction coefficient for oligonucleotide containing the non – natural residues was determined by taking molar extinction coefficient of 5- (1-propyl)-2'- deoxy uridine (Glen Research), $\varepsilon = 3.2 \text{ X} 10^{-3}$.

Thermal Denaturation Studies: Solutions for the thermal denaturation studies of triplex contained one is to one ratio of a given DNA oligomers and double stranded DNA target oligomer (1.5 μ M each) in a buffer (100 mM , NaCl , 100 m M $MgCl_2$, 100 m M NaPIPES) at 6 and 7 pH were prepared . The mixtures were heated to 90^{o} C allowed to cool down slowly to room temperature prior to the melting experiments. The melting studies were carried out in Teflon –Stoppered 1 cm path length quarts cells under nitrogen atmosphere on a Varian Carry UV- VIS Spectrophotometer equipped with thermo programmer. Absorbance (260 nm) was monitored while temperature was raised from 10^{o}C at a rate of 0.5^{o}C/ min.

Result: The disulphide bond formed between two adjacent C-5 thiopropyne deoxyuridine is shown in Figure (i). The oligonucleotide sequences synthesized and T_m of the triplex determined by thermal denaturation studies are tabulated in Table-1. The T_m profiles are shown in Figure (ii). The T_m values of oligonucleotide, 1to 3, were measured against the duplex D and the penetration resulted in two transition in case of oligonucleotide 2, the first transition corresponds to the melting of triple helix at 45^{0}C and the second transition to the duplex target at 75^{o}C, as shown in Table-1 and Figure-(ii).

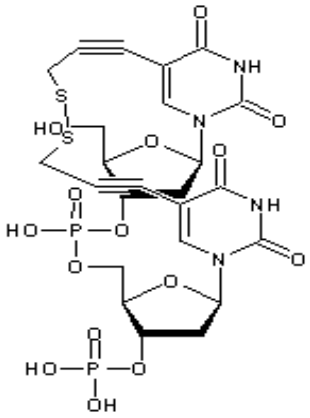

Figure (i): The disulphide bond formed between two adjacent C-5 thiopropyne deoxyuridine

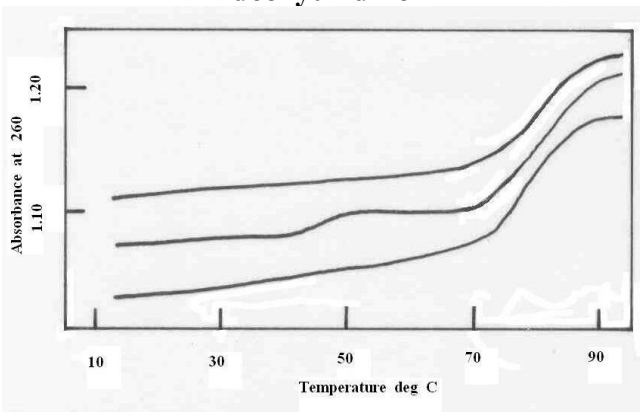

Figure-(ii) Tm profiles of D+3, lower ; D+2 , middle ; D+1 , upper at 7 pH.

Table -1: T_m data of Triple helix

S.No	Oligonucleotide Sequence	Triplex	Tm of duplex ^oC	Tm of Triplex ^oC
1	3' G G T G G G T T G G T G 5'	D+1	75	Not formed
2	S – S I I 3'G G T G G G T T G G T G 5'	D+2	75	45.0
3	P P I I 3' G G T G G G T T G G T G 5' P= Propyne	D+3	75	Not formed
4	5'CAATTTAGTCCAGGAGGGAAGGAGGACGTTACTACT3' 3' GTTAAATCAGGTCCTCCCTTCCTCCTGCAATGATGA5'	.....	75	.....
5	5' GGAATTCCACCCAACCACTGCGCC3'	.....	.....	.....

DISCUSSION: The triplex formation in case of oligonucleotide 2 , and not with unmodified oligonucleotide 1, as well as not with propyne deoxyuridine modified oligonucleotide, 3 at 7 pH shows that the disulphide bond makes geometry favorable for triplex helix formation. Our result is an example of triplex formation by purine and pyrimidine containing oligonucleotides sequence at neutral pH. Earlier Glick et.al. (Glick,et.al., 1992; 1991a) have reported synthesis of conformationally restricted DNA hairpin and showed by optical melting studies that disulphide cross linked increases T m by 21 ° C relative to the wild type sequence. Kool et. al.(Chaudhuri, et. al., 1995) have synthesized of bicyclic oligonucleotides by making a disulphide bond in between the circular DNA molecule and have shown that bicylic oligonucleotide has very high affinity for single stranded oligonucleotide. Jone et. al. (Jones, et. al. 1993) has made use of oligonucleotides containing a covalent conformationally restricted phosphodiester analog for high-affinity triple helix formation: the riboacetal internucleotide linkage. Froehler et. al. (Froehler, et.al. 1992) has used oligodeoxynucleotides containing C-5 propyne analogs of 2′-deoxyuridine and 2′-deoxycytidine for stabilization of triplex. From this it become clear that conformation restriction as well as π - π^* interactions promote triplex stabilization. In the present case we have made use of both the techniques. The thio propyne modified oligonucleotide contains triple bonds as well as a disulphide bond between the two adjacent deoxyuridines to restrict the conformation and hence stabilized triple helix.

Conclusion : Since the disulphide containing oligonucleotide stabilizes the triplex with Tm , 45° C , which is quite above 37 ° C therefore this oligonucleotide can be used as transcription inhibitor.

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