Chem Sci Trans., 2012, **1(1),** 162-165 Chemical Science Transactions
DOI: 10.7598/cst 2012.123 ISSN: 2278-3458

RESEARCH ARTICLE

Disulphide Bond Containing Oligodeoxynucleotide Stabilizes DNA:RNA Duplex Structure

AJAY KUMAR

Galgotias University, Plot No. 2, Sector17A, Yamuna Expressway, Distt. Gautam Budh Nagar, UP, India

ak.gupta59@rediffmail.com

Received 6 May 2012 / Accepted 20 May 2012

Abstract: Thermal stability of DNA: RNA duplex formation of disulphide bond containing oligodeoxynucleotide was studied using UV thermal melting (T_m). The disulphide bond containing oligodeoxynucleotide increased the melting temperature of DNA:RNA duplex by 9.5 ^oC to duplex containing unmodified oligodeoxynucleotide. The DNA: RNA duplex stability of disulphide bond containing oligodeoxynucleotide was also enhanced by 3 ^oC relative to duplex containing C-5 propyne deoxyuridine substituted oligodeoxynucleotide.

Keywords: C-5 Thiopropyne thymidine substituted oligodeoxynucleotides, T_m, C-5 propynedeoxyuridine oligodeoxynucleotide, Disulphide bond containing oligonucleotide.

Introduction

Synthetic oligodeoxynucleotides have greater potential to become a new type of rationally designed therapeutic agent[1-3]. These compounds interfere with the expression of selected genes through interactions with m RNA, genome DNA[4,5] or regulatory proteins[6-10]. Antisense oligo deoxynucleotides recognize target mRNA sequence through Watson- Crick hydrogen bonding between A and T and G and C. This recognition is highly specific and may lead to development of less toxic and more site specific therapeutic agents[11]. The use of oligodeoxynucleotides as antisense inhibitor of gene expression[4,12] and probes for RNA processes[13-15] requires high affinity for RNA. It has been shown in the past that chemical modification of oligodeoxynucleotides can improve their therapeutic potential[16,17]. The modifications may be carried out at base, sugar or backbone of oligodeoxynucleotides. The C-5 propyne analogs of 2'- deoxycytidine significantly enhanced the affinity to double helix formation with single strand RNA, relative to thymidine and 5- methyl-2-deoxycytidine[18]. To improve the affinity of single strand ligand towards single strand RNA various other successful modifications have been carried out in the past[19-27]. Recent candidates of such chemically modified oligonucleotide analogs include HNA[28], locked nucleic acid (LNA)[29,30], 2′-MOE-RNA and related 2′-*O*-modified RNA[31], PNA and modifications thereof[32], Morpholino-NAs[33] and tricyclo-DNA[34-36]. Earlier we have shown that disulphide bond containing oligodeo xynu- cleotide stabilizes triplex structure[41,42] as well as DNA: DNA double helix structure[43]. Here in, we describe binding of disulphide bond containing oligodeoxynucleotide with ribonucleic acid sequence.

Experimental

Synthesis of oligonucleotides

The unmodified oligonucleotides (deoxy- as well as ribo-) and C-5 thiopropyne substituted thymidine containing oligonucleotide were synthesized, deprotected, purified as described else where[37]. Base composition analysis of oligonucleotides was carried out as described else where[37]. Propyne oligonucleotide was synthesized using C-5-(1-propyne) -2'- deoxyuridine phosphoramidite (Glen Research). Preparation and purification of disulphide bond containing oligonucleotide was carried out as described earlier[37,38] .

*Synthesis of free thiol containing oligonucleotide (**2a**)*

The duplex containing free thiol groups was obtained by reducing the disulphide bond containing duplex. A portion of duplex (R+2) was separately treated with 100 mM DTT overnight and dialyzed against water (4X2L) and dried. Duplex was dissolved in 500 uL of PIPES buffer (100 mM, NaCl, 100 mM $MgCl_2$, 100 mM NaPIPES) and solution was stored at 4 ^oC overnight.

Thermal denaturation studies

The duplexes were prepared by mixing one is to one ratio of a DNA oligomers and complementary RNA target oligomer (1.5 u M each) in PIPES buffer at 7 pH. The mixtures were heated to 90 ^oC and allowed to cool down slowly to room temperature. The melting studies were carried out on a Varian Carry UV- VIS Spectrophotometer equipped with thermoprogammer. Teflon –Stoppered 1 cm path length quartz cells were used. The melting was carried out under nitrogen atmosphere. Absorbance (260 nm) was monitored while temperature was raised from 8 ^oC at a rate of 0.5 ^oC/ min.

Results and Discussion

The oligonucleotides synthesized are listed in Table 1. T_m of the duplexes determined is listed in Table 2. T_m profiles of the duplexes are shown in Figure 1. Ball and stick model of disulphide bond formed between two adjacent free thiol groups of C-5 Thiopropyne thymidines is shown in Figure 2. The duplexes were derived from oligonucleotides 1 to 3 and complementary single stranded RNA. The melting temperature (Table 2) of the duplex (R+2) is higher by 9.5 ^oC and 3.0 ^oC relative to (R+1) duplex and (R+3) duplex respectively. It is interesting to note that duplex containing disulphide bonded oligonucleotide is relatively more stable to duplex containing propyne –substituted oligonucleotide. This stabilization may be due to the favorable geometry of disulphide bond present in the center of the oligonucleotide.

Table 1. Oligonucleotides synthesized

S. No	Oligonucleotide Sequence
R	3' AAGAAAGAAAA 5'
1	5' TTC TTT CTTTTC 3'
2	S-S I I 5' TTCT T TCTTTTC 3'
2a	HS SH I I 5'TTCTT TCTTTTC 3'
3	rP Pr I I 5' TTCTT TCTTTTC 3'

Table 2. T_m data of RNA/ DNA Duplexes

Duplexes	T_m °C
R+1	42.50
R+2	52.00
ΔT_m	09.50
R+2a	46.00
ΔT_m	03.50
R+3	49.00
ΔT_m	06.50

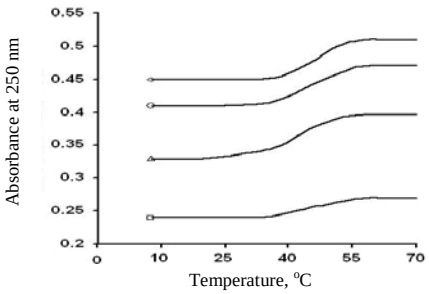

Figure 1. T_m profile of RNA / DNA duplexes (Δ) R+1, (○) R+2, (▫) R+2a, (◊) R+3

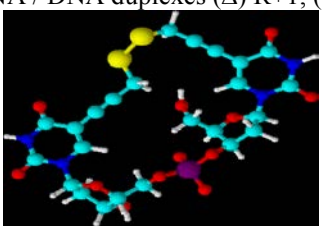

Figure 2. Disulhpide bond between two adjacent C-5 Thiopropyne thymidines

The oligodeoxynucleotide, 2a containing two free thiol groups increased the Tm of (R+2a) duplex by 3.5 °C relative to (R+1) duplex. It has been shown that DNA hairpin containing disulphide cross link increased the T_m by 21 °C relative to the wild type sequence[39,40]. The oligodeoxynucleotides containing propyne analogs of 2-deoxyuridine and 2-deoxycytidine have been used to stabilize the duplexes[18]. The stabilization of duplex in case of cross linked oligonucleotide was due to the restriction in flexibility of duplex. The oligodeoxynucleotides containing C-5 propyne analogs of 2-deoxyuridine and 2-deoxycytidine stabilized duplexes due to π- π^* interactions. The disulphide modified oligonucleotide contains triple bonds to promote π- π^* interactions as well as a disulphide bond between the two adjacent deoxyuridines to restrict the conformation.

Acknowledgement

I acknowledge DST (India) for BOYSCAST Fellowship.

References

1. Bell N M and Micklefield J, *Chem Bio Chem*., 2010, **10,** 2691-2703.
2. Tiemann K and Rossi J J, RNAi-based therapeutics - current status, Challenges and prospects, *EMBO Mol Med*., 2009, **1,** 142-151.
3. Sibley C R, Seow Y and Wood M J A, *Mol Ther*., 2010, **18,** 466-476.
4. Uhlmann E and Peyman A, *Chem Rev.*, 1990, **90,** 544-584.

5. Helene C and Toulme J J, *Biochem Biophys Acta*, 1990, **1049**, 99-105.
6. Tuerk C and Gold I, *Science*, 1990, **249(4968),** 505-510.
7. Bock L C, Griffin L C, Lathans J A, VermassE H and Tool J J, *Nature*, 1992, **355(6360),** 564- 566.
8. Wu H, Holecnberg J S, Tomich J, Chen J, Jones P A, Huang S H and Calame K L, *Gene.*, 1990, **89,** 203-209.
9. Harel –Bellan A, Brini A, Ferris D F, Robin P, Farrar W L, *Nucleic Acids Res.*, 1989, **17(11),** 4077- 4087.
10. Bielinska A, Shivdasani R A, Zhang L and Nabel G J, *Science*, 1990, **250,** 997-1001.
11. Stepenson M L, Zamecinik P C, *Proc Nalt Acad Sci* (USA), 1978, **75(1)**, 285-288.
12. Goodchild J, *Bioconjugate Chem.*, 1990, **1(3),** 165-187.
13. Lamond A I, Sproat B S, Ryder U and Hamm J, *Cell.*, 1989, **58,** 383-390.
14. Blencowe B J, Spoart B S, Ryder U, Barabino S and Lamond A I, *Cell.*, 1989, **59,** 531-539.
15. Barabino S M L, Spoart B S, Ryder U, Blencowe B J and Lamond A I, *EMBO J.*, 1989, **8,** 4171-4178.
16. Bennett C F and Swayze E E, *Annu Rev Pharmacol Toxicol*., 2010, **50,** 259-293.
17. Kool E T, *Acc Chem Res.*, 2002, **35,** 936-943.
18. Froehler B C, Wadwani S, Terhorst T J and Gerrard S R, *Tetrahedron Lett.*, 1992, **33(37),** 5307-5310.
19. Gryaznov S and Chen J, *J Am Chem Soc.*, 1995, **116,** 3143-3144.
20. Jones R J, Swaminathan S, Milligan J F, Wadwani S, Froehler B C and Metteucci D, *J Am Chem Soc.*, 1993, **115(21),** 9816-9817.
21. Beaucage S L and Iyer R P, *Tetrahedron*, 1993, **49,** 6123-6194.
22. Verma R S, *Syn Lett.*, 1993, 621-637.
23. Sanghvi Y S and Cook P D, *Carbohydrate Modification in Antisense Research*, ACS Symp Series, 1994, 580-581.
24. Egholm M, Buchardt O, Nielsen P E and Berg R H, *J Am Chem Soc*., 1992, **14,** 1895-1897.
25. Nielsen P E, Egholm M, Berg R H and Buchardt O, *Science*, 1990, **254,** 1497-1500.
26. Gryaznov S and Chen J K P, *J Am Chem Soc*., 1994, **116,** 3143-3144.
27. Aerschot A V, Verheggen I, Hendrix C and Herdewijn P, *Angew Chem Ind Ed Engel.*, 1995, **34,** 1338-1339.
28. Kang H, Fisher M H, Xu D, Miyamoto Y J, Marchand A, Van Aerschot A, Herdewijn P and Juliano R L, *Nucleic Acids Res.*, 2004, **32,** 4411-4419.
29. Veedu R N and Wengel J, *Chem Biodivers.*, 2010, **7,** 536- 542.
30. Imanishi T and Obika S, *Chem Commun*., 2002, 1653-1659.
31. Prakash T P, Kawasaki A M, Wancewicz E V, Shen L, Monia B P, Ross B S, Bhat B and Manoharan M, *J Med Chem*., 2008, **51,** 2766- 2776.
32. Nielsen P E, *Chem Biodivers.*, 2010, **7,** 786-804.
33. Moulton J D and Jiang S, *Molecules*, 2009, **14,** 1304-1323.
34. Ittig D, Liu S, Renneberg D, Schümperli D and Leumann C J, *Nucleic Acids Res.*, 2004, **32,** 346-353.
35. Renneberg D, Schümperli D and Leumann C J, *Nucleic Acids Res.*, 2002, **30,** 2751-2757.
36. Ittig D, Gerber A B and Leumann C J, *Nucleic Acids Res.*, 2011, **39,** 373-380.
37. Chaudhuri N C and Kool E T, *J Am Chem Soc.*, 1995, **11,** 10434-10442.
38. Kumar A, *IUP J Chem.*, 2009, Vol. **II(4),** 47-54.
39. Glick G D, Osborne S E, Knitt D S and Marino Jr J P, *J Am Chem Soc.*, 1992, **114,** 5447–5448.
40. Glick G D, *J Org Chem*., 1991, **56**, 6746-6747.
41. Kumar A, *E- J Chem.*, 2011, **8(2),** 507-512.
42. Kumar A, *Inter J Appl Bio Pharm Technol*., 2010, **1(2),** 404-409.
43. Kumar A, *IUP J Chem.*, 2011, **4(1),** 91-97.