CHEMISTRY LETTERS, pp. 2209-2212, 1992. 

# Temperature Dependent Duplex Formation of 3'-Phosphate Bearing α-2'-Deoxyoctathymidylate Phosphorothioate and Polyriboadenylic Acid Complex

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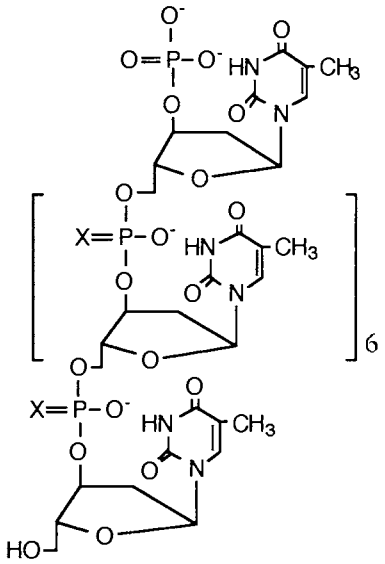
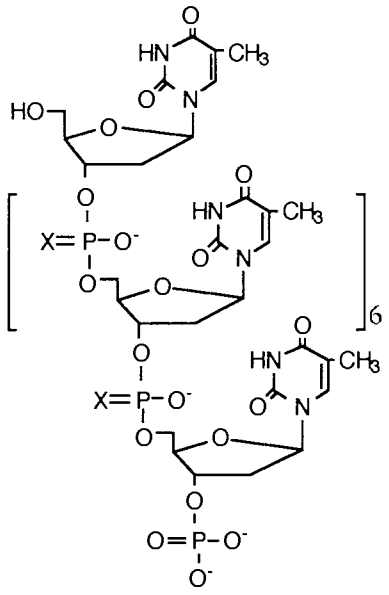
The temperature dependent duplex formation of α-2'-deoxyoctathymidilate phosphorothioate(α-dTps7Tpo) with polyriboadenylate{poly(rA)} was studied and compared with the duplex formation of the corresponding α-dTpo7Tpo, β-dTpo7Tpo and β-dTps7Tpo with poly(rA). The DNA analog α-dTps7Tpo forms stable duplex with poly(rA) and the duplex exhibited greater stability compared to β-dTps7Tpo and poly(rA) duplex. The obtained results also indicate that the configuration of glycoside bond has greater effect of the stability of duplexes than the modification of internucleotide linkages.

A research focused on the use of oligonucleotide or its analog as a sequence specific gene controlling agent has been attracting a growing attention.[1,2] These oligonucleotide or its analog is called an antisense agent which is believed to bind to its complementary RNA thus prohibit the expression of gene information through the blocking of normal translation process. Previously, we have reported that phosphorothioate analogs of oligodreoxynucleotide which were complementary to mRNA of Human Immunodeficiency Virus(HIV) possess strong anti-HIV activity as well as showing nuclease resistant property.[3] The anti-HIV activity of these DNA analogs was proved to be brought by the sequence specific inhibition of translation process.[4] This means that the thermodynamic stability of antisense agent and target RNA complex is one of the critical factors for the action of such agent and, therefore, the study about stability of antisense DNA • target RNA complex is quite important for any potential antisense agent. Recently we have made a brief report on the convenient synthesis of novel DNA analog, 3'-phosphate bearing α-2'-deoxyoctathymidylate phosphorothioate(α-dTps7Tpo) as a potential antisense agent.[5,6] In this DNA analog, glycoside bonds were exclusively consisted of α-anomeric configuration and every internucleotide bond has phosphorothioate diester linkages. Here, we wish to make the first report of the thermodynamic stability of this DNA analog and its complementary polyriboadenylate duplex. The comparisons were also made with the corresponding oligomers, α-2'-deoxyoctathymidylate(α-dTpo7Tpo), β-2'-deoxyoctathymidylate(β-dTpo7Tpo) and β-2'-deoxyoctathymidylate phosphorothioate(β-dTps7Tpo).

The thermodynamic stabilities of 2'-deoxyoctathymidylate analogs and polyriboadenylate duplexes were followed by the change of UV absorption at 260 nm using HITACHI 200-10 spectrophotometer connected with TOKYO RIKAKIKAI NCB-221 water bath thermocontroller. Cuvettes were 0.1 mm path length quartz cells and nitrogen was continuously circulated through the cuvettes compartment. All duplexes



α-dTpo7Tpo (X=O) and α-dTps7Tpo (X=S).



β-dTpo7Tpo (X=O) and β-dTps7Tpo (X=S).

Fig. 1. The structures of 3'-phosphate bearing oligodeoxynucleotide analogs.

were formed 1:1 mixture of a strand with its complement in a buffer solution(pH7.0) containing 20 mM sodium cacodilate and 0.1 M sodium chloride. All samples were pre-melted at 75-80 °C and allowed to thermally equilibrate. The temperature of the compartment was increased steadily with the rate of 0.2 °C/min. Each melt curve is composed of more than 40 individual temperature points and the values of melting points(Tm) for each samples were determined by calculating ΔA/ΔT obtained from melting curves.[7]

The melting curves thus obtained are shown in Fig. 2 which exhibited monophasic sigmoidal curves. The values of melting temperature(Tm) obtained from these data are listed in Table 1. These results indicate that all samples tested were forming stable double strands at low temperature and melting(dissociating) at higher temperature in a two-state transition mode.[8] However, the values of Tm obtained by these data were quite different in each other. The complex of α-dTpo7Tpo • poly(rA) formed the most stable complex with the value of Tm 37 °C, while the complex of β-dTps7Tpo • poly(rA) formed the least stable complex with the value of Tm 14 °C, which is 23 °C lower than the former case. The order of the complex stability was α-dTpo7Tpo • poly(rA) >, β-dTpo7Tpo • poly(rA) >, α-dTps7Tpo • poly(rA) > and β-dTps7Tpo • poly(rA). In general, oligomers consisted of α-anomeric nucleotides are tend to give higher Tm values compared to the corresponding oligomers consisted of β-anomeric nucleotides. Helene and his co-workers reported that α-dTpo7T • poly(rA) mixture forms more stable complex than the corresponding β-dTpo7T • poly(rA) mixture.[9] As described above, our result is parallel with their observation. When the comparisons are made, on the other hand, with respect to the nature of phosphodiester linkages, all phosphorothioate oligomers

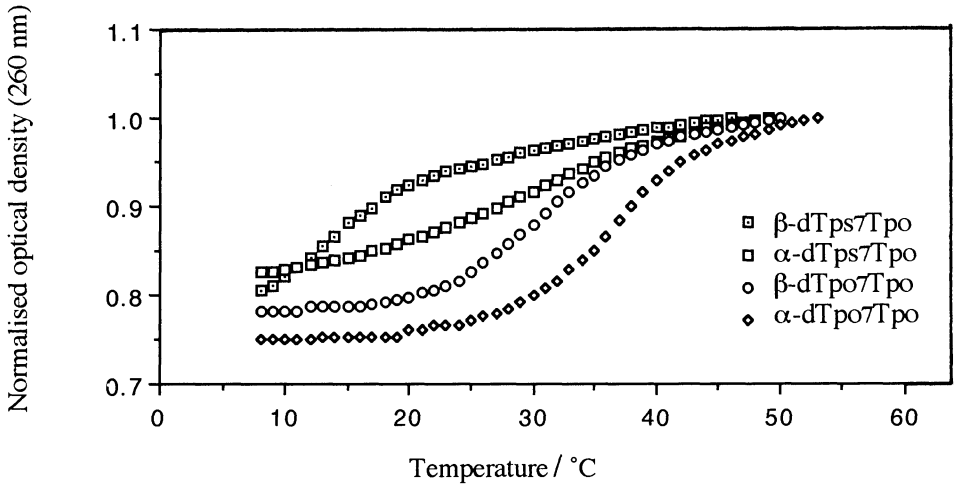



Fig. 2. Melting curves of oligoDNAs and poly(rA) duplexes. Total strand concentration was 3 X $10^{-4}$M for each samples.

Table 1. Melting temperature(Tm) of 2'-deoxyoctathymidilate analogs and polyadenilate duplexs

| Duplex | β-dTpo7Tpo + poly(rA) | β-dTps7Tpo + poly(rA) | α-dTpo7Tpo + poly(rA) | α-dTps7Tpo + poly(rA) |
|---|---|---|---|---|
| Tm / °C | 32 | 14 | 37 | 28 |

tend to give more relaxed melting curves compared to their counterparts. This may be due to the presence of steric isomers in phosphorothioate oligomers.[10] Compared to the corresponding oligomers having normal phosphodiester linkages, the suppression of Tm for phosphorothioate oligomers were also substantial. These results are again parallel to the former findings.[11] However, the interesting observations here are that the degree of Tm suppression caused by the substitution of normal phosphodiester linkages to phosphorothioate diester linkages was in smaller scale than Tm suppression caused by the substitution of glycoside bond configuration from α to β. For example, the difference of the Tm value between β-dTps7Tpo and β-dTpo7Tpo was 18 °C while the difference between α-dTps7Tpo and α-dTpo7Tpo was 9 °C. These results lead the conclusion that the substitution of glycoside bond in DNA strands from naturally occurring β-form to α-form has greater effect on the stability of DNA • RNA double strand than the modification of internucleotide linkages in the same DNA strand, at least in the case of homo-pyrimidine oligoDNA and homo-purine oligoRNA complex. The results obtained here also clearly demonstrate that α-dTpo7Tpo forms more stable complex with poly(rA) than the corresponding β-dTps7Tpo, analogues compounds of which were once proved to be useful antisense agents.[3,4]

The abilities to make stable double strand with its complementary RNA strand and the enhanced stability towards the action of common nucleases[5] would make α-DNA phosphorothioate analogs as potential antisense agents.

This work was partially supported by a Grant-in Aid from IWAKI SCHOLARSHIP and by a grant from Gilead Sciences.

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( Received July 30, 1992)