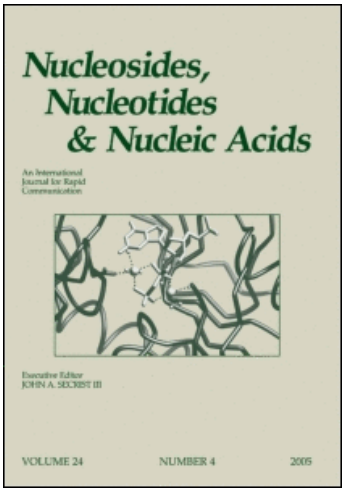


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The Influence of the Non-Nucleotide Insert on the Hybridization Properties of Oligonucleotides

Inna A. Pyshnaya[a]; Dmitrii V. Pyshnyi[a]; Alexander A. Lomzov[a]; Valentina F. Zarytova[a]; Eugenia M. Ivanova[a]
[a] Institute of Chemical Biology and Fundamental Medicine SD RAS, Novosibirsk, Russia

Online publication date: 10 July 2004

To cite this Article Pyshnaya, Inna A. , Pyshnyi, Dmitrii V. , Lomzov, Alexander A. , Zarytova, Valentina F. and Ivanova, Eugenia M.(2004) 'The Influence of the Non-Nucleotide Insert on the Hybridization Properties of Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 23: 6, 1065 — 1071
To link to this Article: DOI: 10.1081/NCN-200026073
URL: http://dx.doi.org/10.1081/NCN-200026073

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 6 & 7, pp. 1065–1071, 2004

The Influence of the Non-Nucleotide Insert on the Hybridization Properties of Oligonucleotides

Inna A. Pyshnaya,* Dmitrii V. Pyshnyi, Alexander A. Lomzov, Valentina F. Zarytova, and Eugenia M. Ivanova

Institute of Chemical Biology and Fundamental Medicine SD RAS, Novosibirsk, Russia

ABSTRACT

The effect of different non-nucleotide inserts incorporated into oligonucleotide chains on their hybridization properties was studied by the method of thermal denaturation. Various types of alkyldiols and oligoethylene glycols were used as inserts modifying oligonucleotide backbone. Such modification of oligonucleotides caused the destabilization of their complementary complexes. It was shown that the hybridization properties of the modified oligonucleotides depend on several features of inserts: the type, number, length of insertions, and positions of interrupted dinucleotide steps in oligonucleotide chain.

Key Words: Oligonucleotide; Thermodynamics; Non-nucleotide insert; Complex formation; Antisense.

INTRODUCTION

The ability of oligonucleotides to bind specifically on nucleic acids attracts to them a steadfast interest of researchers as tools for studying of various processes involving

*Correspondence: Inna A. Pyshnaya, Institute of Chemical Biology and Fundamental Medicine SD RAS, 8 Lavrentiev Ave., Novosibirsk, 630090, Russia; E-mail: pyshnaya@niboch.ncs.ru.

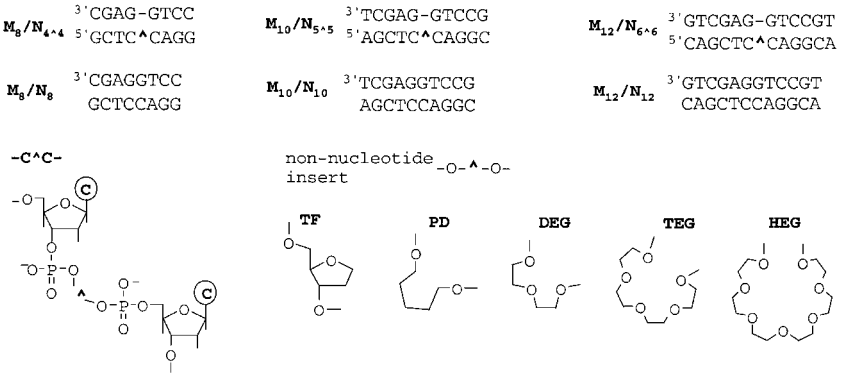
DOI: 10.1081/NCN-200026073 1525-7770 (Print); 1532-2335 (Online)

nucleic acids. Most work done on this topic has concentrated on achieving the highest stability of complexes of oligonucleotides with the target nucleic acid,[1–3] which means that mismatch discrimination will require hybridization at temperatures well above 37°C (physiological condition). This limits their applicability in vivo, and any other than extracorporal treatment of patients would bear a risk of unspecific action. In order to be applicable as therapeutics, oligonucleotides should possess preset hybridization properties. From this point of view derivatives of native oligonucleotides containing a local modification in the sugar-phosphate backbone, which decreases the thermal stability of their complementary complexes, are promising.[4,5] We have shown previously that the ability of oligonucleotides to form the complementary complexes with a DNA template can be varied by incorporation of non-nucleotide inserts into sugar phosphate backbone of oligonucleotide.[6,7]

Here we have studied the effect of different non-nucleotide insertions in an oligonucleotide chain on the efficiency of duplex formation. It was shown that the hybridization properties of the modified oligonucleotides depend on the type and number of inserts.

RESULTS AND DISCUSSION

We studied hybridization properties of modified oligonucleotides by the method of thermal denaturation of complexes. Thermodynamic parameters of the duplex formation (ΔH° and ΔS°) were obtained by melting curve fitting procedure to a ''two-state'' model with sloping baselines[8–10] and were used to calculate the free-energy ΔG° at 37°C. The thermal denaturation of oligonucleotide complexes was carried out under the commonly used conditions (0.01 M sodium phosphate (pH 7.3), 1 M NaCl, and 0.1 mM EDTA, total concentration of oligonucleotides is 26 μM) using a special device based on the UV-detector of Milichrom liquid chromatograph (Russia) in thermoregulated cell. Each melting curve comprised not less than 600 absorbance values with a frequency of 10 points/°C and was recorded with a heating rate 0.7–1°C/min. For all melting curves, correction on the thermal expansion of water was made. The heating curves coincided with the cooling curves.



Scheme 1. The structure of the modified and ordinary complexes and monomeric non-nucleotide insertions.

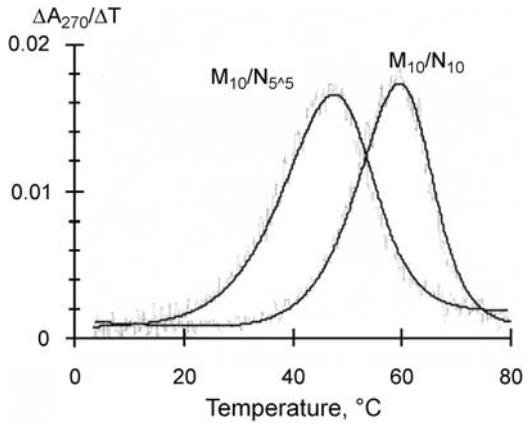


Figure 1. The differentiated experimental (gray) and fitting (solid) melting curves of modified and ordinary complexes.

At the first step we examined the influence of various types of alkyldiol and oligoethylene glycol residues on the hybridization properties of oligonucleotides using model complexes formed by octa-, deca-, and dodecademers consisting of inserts with various number of atoms in their linear structure: isostructural tetrahydrofuran analog (TF), pentamethylendiol (PD), diethylene glycol (DEG), tetraethylene glycol (TEG), hexaethylene glycol (HEG) (Scheme 1).

Modified oligonucleotides were synthesized by a common phosphoramidate protocol on the synthesizer ASM-700 using synthons of diols obtained accordingly to.[11]

For each complex $M_n/N_{m^\wedge m}$ of the modified oligonucleotide, a reference complex M_n/N_m of the ordinary oligomer was studied for comparison. The experimental melting curves of all studied complexes were well described by fitting procedure to a ''two-state'' model. As an example, differential melting curves of the complex $M_{10}/N_{5^\wedge 5}$ and its reference complex M_{10}/N_{10} are shown in Fig. 1.

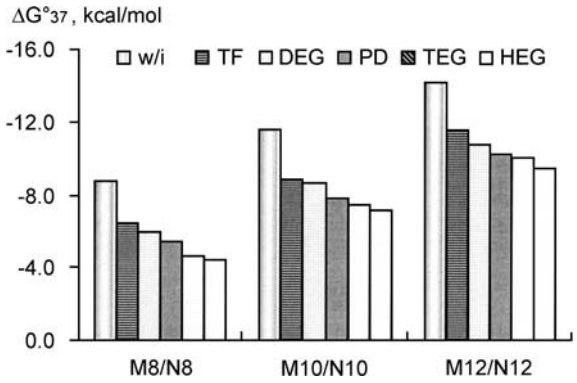
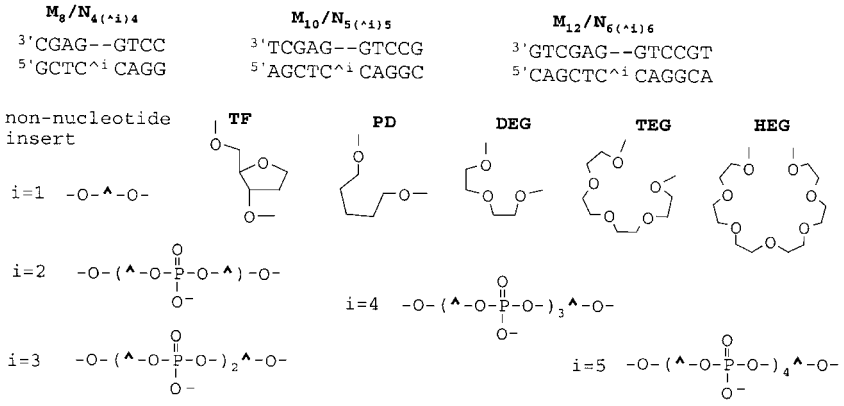


Figure 2. The decrease of the free-energy of M_n/N_n duplex formation depending on the type of inserts in modified oligonucleotide $N_{m^\wedge m}$; w/i-without insert (ordinary oligomers).



Scheme 2. The structure of modified complexes and oligomeric non-nucleotide insertions.

The incorporation into the oligonucleotides of any of the proposed inserts resulted in a decrease of free-energy of the complex formation (Fig. 2).

The TF insert causes the minimum destabilization of complexes $Mn/N_{m\text{^}m}$. The destabilizing effect of the inserts is enhanced when their contour lengths are increased. For example, the ΔG°_{37} values of all complexes formed by DEG containing oligomers are approximately -1.4 kcal/mol higher than corresponding values for HEG containing oligomers.

Hybridization properties of the modified oligonucleotides depend also on the type of the insert. The PD- and DEG-inserts, which have an equal number of atoms in their backbones but differ in rigidity, bring reliably different influence on duplex stability. The difference between values of free-energy for complexes $M_n/N_{m\text{^}m}$ formed by DEG- or PD-containing oligomers is (~ -0.6 kcal/mol), respectively.

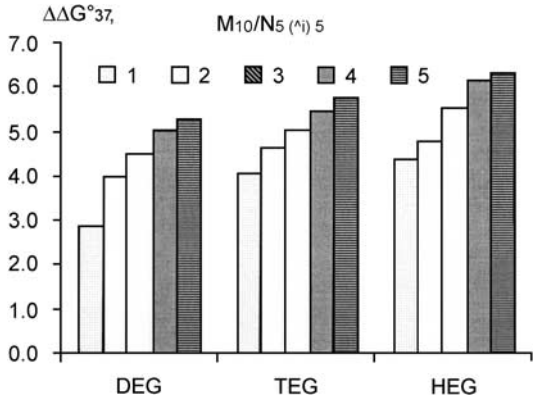
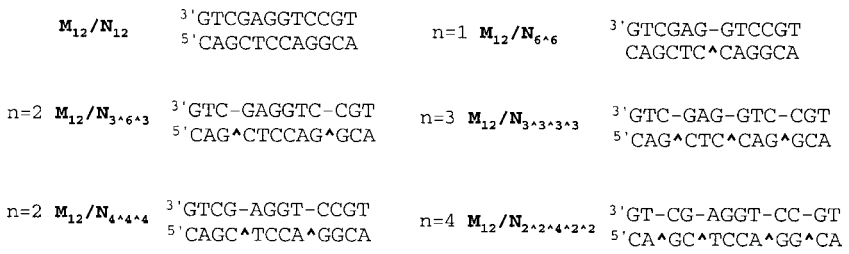


Figure 3. The destabilizing contribution of the oligomeric inserts to free-energy of formation of complex $M_{10}/N_{5(\text{^}i)5}$ for modified oligonucleotide $N_{5(\text{^}i)5}$ depending on the number of units in the insert.



Scheme 3. The structure of complexes formed by DNA template and modified oligonucleotides containing various number of insertions in the different position.

We have also investigated the effect of the lengthening of the inserts by their amplification from 1 to 5 units (Scheme 2).

It was shown that the introduction of the single unit of the insert gives the highest effect for all complexes. The addition of each next monomer unit (up to 5) in the insert results only in a slight stepwise decrease of the values $\Delta\Delta G^\circ_{37} = \Delta G^\circ_{37}(M_n/N_{m(\hat{}i)m} - \Delta G^\circ_{37}(M_n/N_m)$ of the modified complexes (for example, Fig. 3). This regularity is retained with a variation of oligonucleotide length.

Note that effects of inserts HEG, TEG_2, and DEG_3 containing equal number of ethylene glicol units are similar (4.4, 4.6, and 4.5 kcal/mol respectively).

Thus, the change of the thermal stability of the complexes formed by oligonucleotides containing non-nucleotide inserts depends on the nature of the insert, namely on its length and rigidity. The influence of several inserts incorporated into different positions of the oligonucleotide on its hybridization properties was investigated with an example of the complexes formed by 12-mer oligonucleotide (Scheme 3).

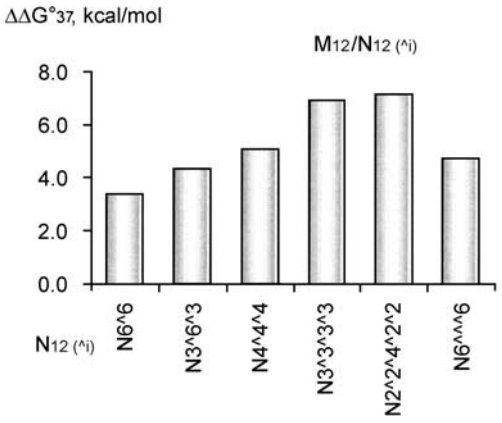


Figure 4. The destabilizing free-energy contribution of the oligomeric inserts to formation of complex $M_{12}/N_{12(\hat{}i)}$ for modified oligonucleotide depending on the number of DEG units at single or multipe positions in oligonucleotide.

The data obtained show (Fig. 4) that the simultaneous insertion of several DEG residues in different positions of oligonucleotide sequence results in the further lowering of the thermal stability of the modified complexes. However, the effect of each next insert is diminished, possibly due to dinucleotide steps breakage by inserts.

It is interesting that the effect of an insert containing three units of DEG in single site is lower than three DEG incorporated into three different positions of oligomer.

The sum of the results obtained allow us to conclude, that the following characteristics are of great importance for the designing of modified oligomers: 1) the type and the length of an insert, 2) the location of the modified dinucleotide, 3) the number of inserts in different positions of an oligonucleotide sequence.

Thus, using non-nucleotide inserts in structure of oligonucleotides it is possible to change their hybridization properties and enhance the site-specificity of their binding with the targeted nucleic acids at physiological conditions.

ACKNOWLEDGMENTS

This work was supported by grant of the program for young scientists of Siberian Division of RAS (#50), Grant of MCB Program of RAS (#10.6) and integration grant of SD RAS (#126).

REFERENCES

1. Cook, P.D. *Antisense Research and Applications*; Crooke, S.T., Lebleu, B., Eds. CRC Press: Boca Raton, 1993; 169–176.
2. Agrawal, S.; Kandimalla, E.R. Antisense therapeutics: is it as simple as complementary base recognition? Mol. Med. Today **2000**, *6*, 72–81.
3. Good, L. Translation repression by antisense sequences. Cell. Mol. Life Sci. **2003**, *60*, 854–861.
4. Freier, S.M.; Altmann, K.H. The ups and downs of nucleic acid duplex stability: structure-stability studies on chemically-modified DNA:RNA duplexes. Nucleic Acids Res. **1997**, *25*, 4429–4443.
5. Mangos, M.M.; Min, K.-L.; Viazovkina, E.; Galarneau, A.; Elzagheid, M.I.; Parniak, M.A.; Damha, M.J. Efficient RNase H-directed cleavage of RNA promoted by antisense DNA or 2′F-ANA constructs containing acyclic nucleotide inserts. J. Am. Chem. Soc. **2003**, *125*, 654–661.
6. Pyshnaya, I.A.; Pyshnyi, D.V.; Ivanova, E.M.; Zarytova, V.F.; Bonora, G.M.; Scalfi Happ, C.; Seliger, H. Oligonucleotide conjugates with non-nucleotidic spacers designed for discriminative hybridization at physiological temperature. Nucleosides Nucleotides **1998**, *17*, 1289–1297.
7. Vorobjev, P.E.; Pyshnaya, I.A.; Pyshnyi, D.V.; Venyaminova, A.G.; Ivanova, E.M.; Zarytova, V.F.; Bonora, G.M.; Scalfi-Happ, C.; Seliger, H. Nuclease resistance and RNase H sensitivity of oligonucleotides bridged by oligomethylenediol and oligoethylene glycol linkers. Antisense Nucleic Acid Drug Dev. **2001**, *11*, 77–85.
8. Petersheim, M.; Turner, D.H. Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp,

CCGGAp, ACCGGp, CCGGUp, and ACCGGUp. Biochemistry **1983**, *22*, 256–263.
9. Pyshnyi, D.V.; Pyshnaya, I.A.; Levina, A.S.; Goldberg, E.L.; Zarytova, V.F.; Knore, D.G.; Ivanova, E.M. Thermodynamic analysis of stacking hybridization of oligonucleotides with DNA template. J. Biomol. Struct. Dyn. **2001**, *19*, 555–570.
10. Lokhov, S.G.; Pyshnyi, D.V. Thermodynamic and spectral properties of DNA miniduplexes with the terminal G·A mispairs and 3′ or 5′ dangling bases. FEBS Lett. **1977**, *420*, 134–138.
11. Durand, M.; Chevrie, K.; Chassignol, M.; Thuong, N.T.; Maurizot, J.C. Circular dichroism studies of an oligodeoxyribonucleotide containing a hairpin loop made of a hexaethylene glycol chain: conformation and stability. Nucleic Acids Res. **1990**, *18*, 6353–6359.

Received December 2, 2003
Accepted April 23, 2004