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On DNA

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Abstract: The study of oligonucleotide analogs today is mainly targeted at therapeutic or diagnostic applications. However, the detailed knowledge of the structural features of such analogs in comparison to natural DNA may in the future also lead to novel structural design principles in supramolecular chemistry and materials research.

Keywords: Conformational restriction · Glycosylated DNA · Oligonucleotide analogs · Supramolecular chemistry

Towards DNA-Selective Oligonucleotide Analogs

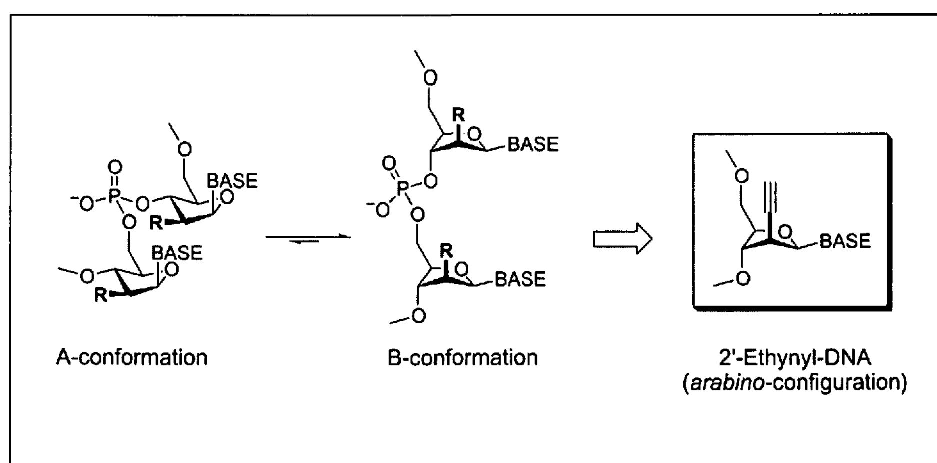
In general, modified oligonucleotides [1], like regular oligodeoxynucleotides, form slightly stronger hybrids with complementary RNA than with DNA [2]. Only a few attempts have been made to design oligonucleotides which exclusively bind a DNA complement but not an RNA one. DNA specificity would be highly desirable to improve the efficiency of oligonucleotide drugs. There is exactly one copy of a disease-related gene within a cell but many more RNA transcripts. Other possible applications include oligonucleotide-based DNA-sensors in probes where both nucleic acids might be present or DNA-selective priming.

DNA double helices are capable of adopting several different conformations whereas RNA is confined to A-form duplexes [3]. Selectivity for a DNA complement should thus be possible if an A-form conformation of the resulting double helix is not accessible (Scheme 1). The exclusion of an A-form double helix should in principle be possible at the nucleoside level since the overall duplex conformation is dictated by the sugar-phosphate backbone conformation. Preorganization of the (deoxy)ribose moiety towards a C(2')-*endo*-conformation should additionally lead to more stable duplexes. The freezing of internal degrees of freedom will render the loss in entropy upon complexation less negative [1].

The flexible furanose ring can be rigidified by extending it to a pyranose ring or by bridging two of the ring positions. Most of these efforts have yielded oligonucleotide analogs which mimic an C(3')-*endo* conformation. Since both RNA and DNA can form A-form duplexes this only led to marginal selectivities for complementary RNA-strands.

To restrict the conformational freedom of individual nucleosides in a B-form double helix we introduced an *arabino*-configured ethynyl substituent at the C(2')-position of deoxynucleosides (Scheme 1). We assumed that in such an oligodeoxynucleotide the individual nucleosides adopt a C(2')-*endo* conformation, typical of B-form DNA double helices, because the alternative C(3')-*endo* (A-form RNA or DNA) conformation would lead to sterically unfavorable interactions of the ethynyl group with the phosphodiester residue or the nucleobase of the 3'-neighbouring nucleotide. Hence, this modification is expected to maintain a certain duplex conformation through steric interactions in a dinucleotide step within the duplex.

The pairing properties of 2'-*arabino*-ethynyl modified oligonucleotides can be summarized as follows [4]: 1) The 2'-*arabino*-ethynyl modification of pyrimidine nucleosides leads to a strong destabilization in duplexes with DNA as well as with RNA. The likely reason is that the ethynyl group sterically influences the torsional preferences around the glycosidic bond leading to a conformation unsuitable for duplex formation. 2) If the modification is introduced in purine nucleosides no such influence is observed. The pairing properties are not or only slightly changed and in some cases (deoxyadenosine homo-polymers) the



Scheme 1. Design rationale for 2'-ethynyl-DNA

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desired stabilization of the pairing with a DNA complementary strand and destabilization with an RNA complement is observed. 3) In oligonucleotides of alternating deoxycytidine-deoxyguanosine sequence the incorporation of 2'-*arabino*-ethynyl deoxyguanosine surprisingly leads to the formation of a left-handed double helix irrespective of salt concentration. The rationalization for this behavior is that the ethynyl group locks such duplexes in a left-handed conformation through steric blockade.

The results from the study on 2'-ethynyl-DNA meant that we had to turn our attention to a different design principle in the quest to develop a DNA-selective oligonucleotide analog. In this context a particular DNA analog caught our attention, namely the 1,5-anhydrohexitol nucleic acids described recently [5]. We refer to them as O(4')-homo-DNA and -RNA. They are formally related to natural DNA or RNA by the introduction of an additional methylene unit between the ring oxygen, O(4'), and the anomeric center, C(1'), in the furanose ring of a nucleotide. Oligonucleotides of this type bind to complementary RNA sequences with significantly higher thermal stability than the corresponding DNA sequences. In addition, a slight preference for an RNA as opposed to a DNA complement is found.

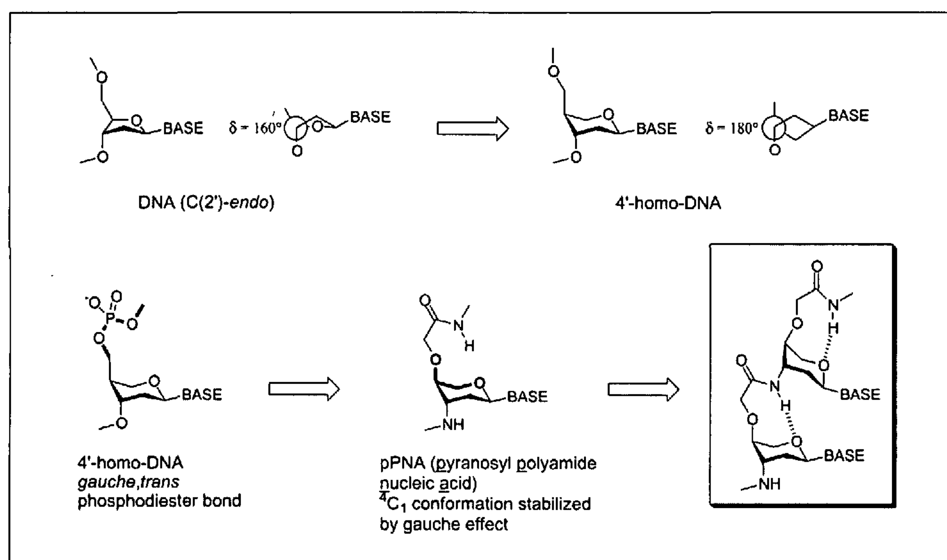
Following the same rationale, one would expect a hexopyranosyl oligonucleotide formally derived from DNA by introducing an additional methylene group between O(4') and *C(4')* to correspond to DNA rather than RNA in its conformational behavior (Scheme 2). Closer inspection of this DNA-mimic by molecular modeling studies suggests that for the internucleosidic phosphodiester bond a *gauche,trans* rather than the energetically slightly more favorable *gauche, gauche* conformation is required in order to maintain an optimal helicity. To help stabilize the desired $^4C_1$ conformation of the pyranose as well as the *gauche,trans* conformation of the phosphodiester functionality we sought for a substitute of the internucleosidic linkage. The *trans*-conformation of one of the phosphodiester bonds could be ideally approximated by an amide function. Since a carbamate linkage in this position is not desirable one also has to interchange the oxo and the methylene groups leading to the monomer structure shown in Scheme 2. The synthesis of such pPNA (pyranosyl polyamide nucleic acid) oligomers is currently under way. Preliminary results suggest that the assumptions concerning the conformational preferences in pPNA are indeed correct.

Glycosylated DNA

The improvement of desired pairing properties of oligonucleotide analogs should not only be possible through rational design. In many areas of chemistry the study of natural products has been an extremely successful strategy to develop new concepts. RNA, especially transfer-RNA, is a rich source of altered nucleoside structures which mostly help to stabilize a particular tertiary structure. On the other hand, only a few modifications are found in DNA, the most prominent being 5-methyl-deoxycytidine for the silencing of genes. A highly unusual modification is found in certain bacteriophages, *e.g.* in T4-phages which affect *E. coli.* In the whole genome of this phage, the deoxycytidine residues are replaced by 5-hydroxymethyl-deoxycytidine. About 75% of these nucleosides are additionally modified with glucose residues (Fig.). The analogous deoxyuridine derivative is found in different phages as well as in the single cell parasite *Trypanosoma brucei.* The glucose prevents access to the major groove and thus protects the viral DNA from degradation. This nuclease protection is highly desirable in antisense oligonucleotides and warranted a closer look at the biophysical properties of oligonucleotides containing such modifications.

5-Glucosyloxymethyl-2'-deoxyuridine incorporated into oligonucleotides causes a slight decrease in pairing affinity compared to unmodified reference duplexes [6][7]. This behavior is found for duplexes with complementary DNA as well as complementary RNA. By determining the thermodynamic parameters of duplex formation it was shown that the destabilization is due to a smaller change in enthalpy. The negative pairing enthalpy cannot be compensated for by a favorable entropic term, which most likely reflects the displacement of ordered water within the major groove. In the case of the corresponding glucosylated deoxycytidine a small increase in duplex stability is found, again caused by the more favorable pairing entropy.

These results led us to investigate the effect of other moieties placed within the major groove which are similar in size to a monosaccharide. The phosphoramidite building blocks of 5-benzyloxymethyl-deoxyuridine and -cytidine were prepared and built into oligonucleotides [7]. These display decreased pairing affinity. Again, the destabilization is enthalpic in



Scheme 2. In the same manner as hexitol nucleic acid (O(4')-homo-DNA) imitates the C(3')-*endo* sugar conformation in A-form duplexes, 4'-homo-DNA should be a mimic of the C(2')-*endo* conformation of B-DNA. To imitate the presumed *gauche,trans* conformation of the phosphodiester bond in 4'-homo-DNA oligomers an amide group is ideally suited. The inversion of the oxygen atom and the methylene group should stabilize the desired $^4C_1$ conformation *via* an additional *gauche* effect.

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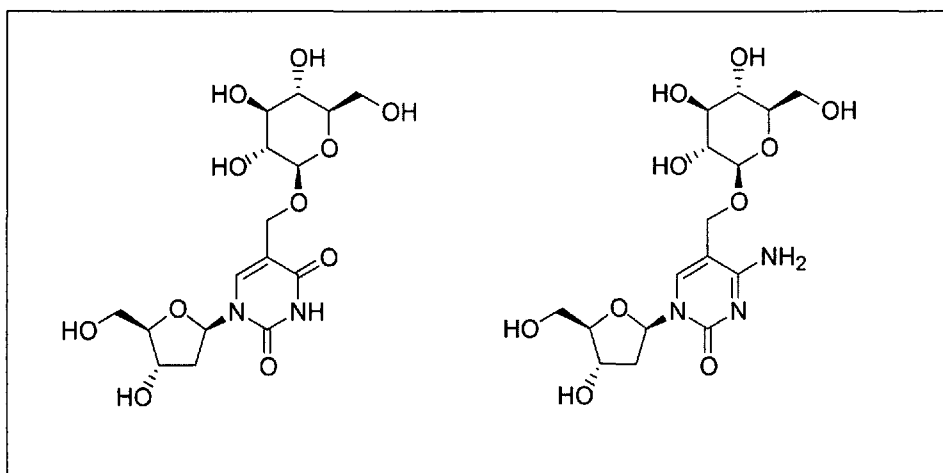

Fig. Structures of β-D-glucosylated 5-hydroxymethyl-deoxyuridine (left) and –cytidine (right) which are part of the genomic DNA in certain bacteriophages and *Trypanosoma brucei*.

nature and the unfavorable pairing enthalpy cannot be overcome by the displacement of the first hydration shell. To improve the thermal stability and yet retain the desired protection from nucleases as observed for the glucose modification, we synthesized oligodeoxynucleotides containing diaminoglucose instead [6]. The amino groups, positively charged at neutral pH, strongly improved binding to a complementary strand by electrostatic interactions with the negatively charged phosphodiester groups of the opposite strand.

Future Prospects

A problem often encountered in supramolecular chemistry is the fact that synthesis becomes a limiting factor the more complex molecular assemblies grow. In most cases, the inherent difficulties associated with solution phase C–C bond forming reactions preclude the formation of discrete molecules with a molecular weight in excess of a few 1000 Da. Even when changing to the most efficient covalent bond forming processes, the slow kinetics of large molecules put a limit to the degree of complexity achievable. On the other hand, nucleic acids and proteins are formidable precedents for the organization of functional subunits into a larger supermolecule. Over the last 40 years, chemists have developed the most efficient procedures known for the synthesis of these classes of macromolecules. In solid-phase DNA synthesis repetitive coupling yields in excess of 99% are routinely achieved. It seems therefore fair to say that a system based on the oligonucleotide assembly strategy would make an ideal platform for sophisticated molecular devices or novel materials. The possibility to synthesize an oligomer chain one monomer at a time allows for the synthesis of spatially controlled assemblies with graded properties. The combination of two complementary single strands into a structurally uniform rod-like complex allows for a persistence length of several dozen nanometers. With so many structurally diverse oligonucleotide analogs at hand today it is time to harness the power of DNA chemistry towards this goal.

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[1] a) J. Hunziker, C. Leumann, in 'Modern Synthetic Methods', Eds. B. Ernst, C. Leumann, Verlag Helvetica Chimica Acta, Basel, and VCH, Weinheim, **1995**, Vol. 7, p. 331; b) S.M. Freier, K.-H. Altmann, *Nucleic Acids Res.* **1997**, *25*, 4429; c) O. Seitz, *Angew. Chem.* **1999**, *111*, 3674.

[2] K.-H. Altmann, R. Imwinkelried, R. Kesselring, G. Rihs, *Tetrahedron Lett.* **1994**, *35*, 7625.

[3] W. Saenger, 'Principles of Nucleic Acid Structure', Springer-Verlag, New York, **1984**.

[4] a) R. Buff, J. Hunziker, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 521; b) R. Buff, J. Hunziker, *Synlett* **1999**, 905; c) R. Buff, J. Hunziker, *Helv. Chim. Acta*, submitted.

[5] a) C. Hendrix, H. Rosemeyer, I. Verheggen, F. Seela, A. van Aerschot, P. Herdewijn, *Chem. Eur. J.* **1997**, *3*, 110; b) C. Hendrix, H. Rosemeyer, B. de Bouvere, A. van Aerschot, F. Seela, P. Herdewijn, *Chem. Eur. J.* **1997**, *3*, 1513; c)B. Allart, K. Khan, H. Rosemeyer, G. Schepers, C. Hendrix, K. Rothenbacher, F. Seela, A. van Aerschot, P. Herdewijn, *Chem. Eur. J.* **1999**, *5*, 2424.

[6] a) J. Hunziker, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 201; b) R. Tona, R. Bertolini, J. Hunziker, *Org. Lett.* **2000**, 2, 1693.

[7] R. Bertolini, J. Hunziker, *Helv. Chim. Acta* **2000**, *83*, 1962.