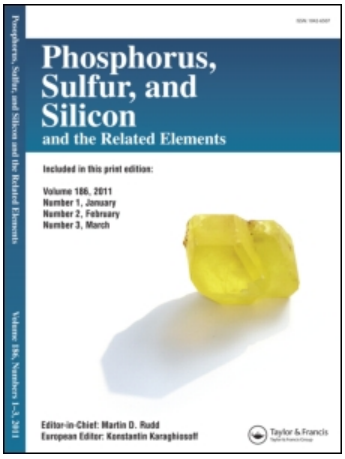

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Phosphate-Methylated DNA Fragments Involved in Parallel and Antiparallel Duplex Formation Novel Aspects of Structure Stability and Biological Activity

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PHOSPHATE-METHYLATED DNA FRAGMENTS INVOLVED IN PARALLEL AND ANTIPARALLEL DUPLEX FORMATION. NOVEL ASPECTS OF STRUCTURE, STABILITY AND BIOLOGICAL ACTIVITY

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Abstract Parallel duplexes are encountered for the phosphate-methylated DNA dinucleotides d(CpC) and d(TpC) for the S_P configuration exclusively, since inward location of the methyl group (R_P) encounters severe steric interactions in the groove. A parallel duplex with T-T base pairs is found for the natural system $d(T_{10})$ only after complexation of the cationic peptides polylysine and polyornithine with the phosphate groups, which diminishes phosphate-phosphate repulsions. For natural $d(C_{10})$, parallel duplex formation is seen exclusively after complexation with polylysine, which only uses pro-S phosphate oxygens in the complexation. Polyornithine complexates with both pro-S and pro-R phosphate oxygens, leading to steric hindrance in the groove of the C-C duplex.
For antiparallel duplexes of phosphate-methylated DNA ('antisense') with natural polynucleotides ('sense') it is found that the hybridization has a cooperative character. Furthermore, it is mentioned that long phosphate-methylated DNA fragments can now be routinely synthesized. Biological experiments revealed that these systems specifically inhibit DNA replication and transcription in vivo and in vitro. Thus, phosphate-methylated DNAs are potentially useful as a new class of antisense antiviral or cytostatic agents.

INTRODUCTION

It is well known that the stability of natural Watson & Crick type DNA duplex structures represents an energetic balance between attractive base-base interactions (i.e., hydrogen bonding between the complementary bases and vertical base stacking), and electrostatic phosphate-phosphate repulsion. In our recent work, we have shown that methylation of the phosphate groups in one or both strands leads to an extra stabilization of the duplex since the interstrand phosphate-phosphate repulsions are cancelled.[1] Using deoxythymidine strands (dT_n, n= 2-10), it was found that methylation of the phosphate groups leads to the formation of a stable non-Watson & Crick duplex with

T-T base pairs in a parallel alignment.[2,3] In this paper we present a brief review of our new results on parallel DNA structures. Furthermore, the structure and stability of Watson & Grick-like hybrids of natural DNA ('sense') with complementary phosphate-methylated DNA ('antisense') have been examined. The possible utility of phosphate-methylated DNA fragments as site-specific antisense inhibitors of DNA replication and/or transcription is discussed.

PARALLEL PHOSPHATE-METHYLATED DNA

Recent experiments have shown that also the phosphate-methylated d(CpC) with S_P configuration forms a right-handed parallel miniduplex (T_m = 33 °C), whereas the corresponding R_P diastereoisomer is only present in the single strand form for temperatures as low as 5 °C.[4] Since phosphate-methylated d(TpT) shows a duplex independent of the chirality on phosphorus (T_m = 30 °C), we have performed AMBER molecular mechanics calculations to make the chiral differences on phosphorus visible. These calculations clearly show that the inward orientation of the R_P configuration in d(CpC) encounters severe steric interactions in the groove of the duplex.[5] This exclusive effect of chirality on the formation and stability was also encountered in phosphate-methylated d(TpC) demonstrating a parallel duplex via T-T and C-C base pairs for the S_P configuration exclusively (T_m = 25 °C). In general, this parallel hybrid formation is only encountered in pyrimidine bases.[6]

PROTEIN COMPLEXATION LEADING TO PARALLEL DNA DUPLEXES

Using the natural DNA oligomers $d(T_{10})$ and $d(C_{10})$, it was found that addition of the cationic peptide polylysine also induces formation of a parallel duplex with T-T (T_m = 22 °C) and C-C (T_m = 21 °C) base pairs.[7,8] Apparently, partial shielding of the negative phosphate groups is sufficient for the formation of a parallel duplex. A detailed structural study revealed that the positive terminal ammonium groups ($-(CH_2)_4-NH_3^+$) of the polylysine reside in a perfect orientation for complexation with DNA phosphates.[4] Using the peptide polyornithine with one methylene less in the side-chains ($-(CH_2)_3-NH_3^+$), no duplex formation occurs for $d(C_{10})$, while $d(T_{10})$ still forms a parallel duplex (T_m = 22 °C). AMBER calculations have made clear that polylysine shields the pro-S oxygens of the DNA phosphate groups, whereas polyornithine uses both pro-S and pro-R phosphate oxygens. Apparently, the involvement of pro-R oxygen in $d(C_{10})$-polyornithine complexation prohibits hybridization of the $d(C_{10})$ strands. It can be concluded that polyornithine complexation and R_P methylation both involve unfavourable steric interactions in the groove for duplexes with C-C base pairs.

ANTIPARALLEL HYBRIDS OF NATURAL AND PHOSPHATE-METHYLATED DNA (SENSE-ANTISENSE DNA)

We have studied antiparallel hybrids of phosphate-methylated $d(A_n)$ (n= 2,3,4) with natural poly dT.[9,10] Two conclusions could be drawn: (i) the duplex stability strongly depends on the length of the phosphate-methylated fragment (the T_m-values for complexation with poly dT are 30, 41, and 57 °C for n= 2,3, and 4, respectively); (ii) the length of the template of the natural DNA is of essential importance: phosphate-methylated $d(A_3)$ shows a T_m-value of 41 °C with poly dT, and 27 °C with $d(T_{30})$, while no melting transition is observed for e.g. $d(T_{10})$. These results show that hybridization of short phosphate-methylated DNA fragments with long strands of natural DNA has a cooperative character.[11,12]

USE OF PHOSPHATE-METHYLATED DNA FRAGMENTS AS ANTISENSE INHIBITORS OF DNA REPLICATION

We have recently improved the synthesis of long phosphate-methylated DNA fragments.[13] Currently, natural DNAs of an arbitrary base sequence, prepared on an automated DNA synthesizer, can be routinely converted into their phosphate-methylated counterparts. These phosphate-methylated DNAs form highly stable hybrids with a complementary region in natural polynucleotides. As a consequence of this hybridization, phosphate-methylated DNAs may inhibit the replication and/or transcription of cellular DNA. We have tested this possibility by studying the influence of a site-specific 18-mer on the in vitro replication of an *E. coli* gene incorporated in a single strand DNA virus. It was indeed found that the replication process stopped exactly at the site of the hybridization.[13] Also, the transcription process in *E. coli* cells in vivo was influenced via phosphate-methylated DNA.[14] A 22-mer, specific for the operator site in the lac operon was able to reduce β-galactosidase production by 70%. Since the oligomer was not complementary to the messenger RNA, but to the template DNA strand, inhibition can have occurred only at the transcription level. A similar inhibition has been seen for the alanine racemase enzyme of *Salmonella typhimurium*, which furnishes the D-alanine for the cell wall biosynthesis. A specific phosphate-methylated 21-mer gave rise to a 30% decrease of the growth rate at a concentration of 1 μM. These examples clearly demonstrates the possible utility of tailor-made phosphate-methylated DNA fragments for specific blocking of genetic information. Further research is in progress to establish the effectiveness of phosphate-methylated oligomers in eukaryotic systems. This involves studies in the field of growth inhibition of leukemia cells with the *myc* gene, viral infections (HIV) and rheuma.

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