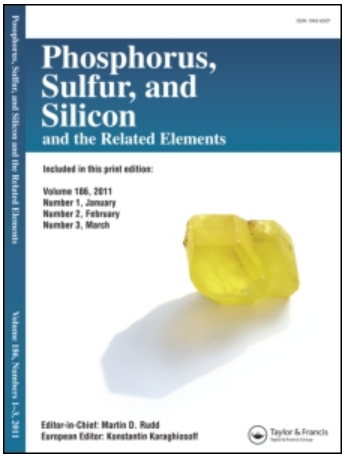

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The Use of ^{31}P Relaxation Experiments to Probe the Effects of Nucleoside Analogs on DNA Dynamics

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In the present study, we examine the effects of two nucleoside analogs on the structure and dynamics of self-complementary duplexes and model primer/template duplexes.

Keywords: DNA; phosphorus; relaxation; dynamics; cytosine arabinoside; ganciclovir

INTRODUCTION

Solution NMR studies of ^{15}N and ^{13}C relaxation rates have been applied to many protein systems to examine backbone and side-chain dynamics. Investigations of the relaxation properties of other biologically relevant spin-1/2 nuclei, including phosphorus-31, could benefit from a similar approach. ^{31}P is present in the phosphodiester backbones of RNA and DNA, a key location for probing dynamics along the length of these molecules. Previous ^{31}P relaxation measurements have been hampered by difficulties in resolving resonances within the narrow range of phosphorus chemical shifts. One solution to this problem is offered by the heteronuclear J cross-polarization spectroscopy (heteroTOCSY) experiment[1,2], which has been used to obtain ^{31}P-^{1}H correlations in both oligoribonucleotides[3] and oligodeoxyribonucleotides[4]. We have adapted the heteroTOCSY experiment for measuring ^{31}P T_1 and T_2 values[5]. In the present study, we examine the effects of two nucleoside analogs on the structure and dynamics of self-complementary duplexes and model primer/template duplexes.

MATERIALS AND METHODS

Oligonucleotides containing araC and ganciclovir were synthesized and purified as previously described[6-7]. NMR experiments were carried out and analyzed as previously described[6-7] . Structures of primer/template duplexes were calculated from random extended strands using a torsion angle dynamics protocol described by Brunger and coworkers[8] . Structures calculated using this protocol were deemed to be acceptable if there were no distance violations greater than 0.5 Å.

RESULTS

Cytosine Arabinoside

Cytosine arabinoside (AraC) is a potent agent in the treatment of various forms of human leukemia. The total amount of araC misincorporation into DNA has been correlated with the cytotoxicity of the drug, suggesting that misincorporation of araC into DNA is the lethal event in cells. Investigations into the biochemical mechanism(s) by which misincorporation of araC into DNA interferes with DNA synthesis have revealed that araC at the 3' primer terminus greatly reduces the rate of addition of the next nucleotide by a variety of bacterial, viral, and mammalian DNA polymerases[9] .

We have determined the solution structures of an araC-containing (d(CGCGAATT)(araC)d(GCG)$_2$) and a control (d(CGCGAATTCGCG)$_2$) duplex using restrained molecular dynamics calculations and back-calculation of NOESY data. An inspection of the final control and araC structures revealed a global similarity between the two structures. A closer examination of the lesion site revealed that the hydroxyl group of araC formed a hydrogen bond with either the O1P or O5' oxygen of G(10)[6] .

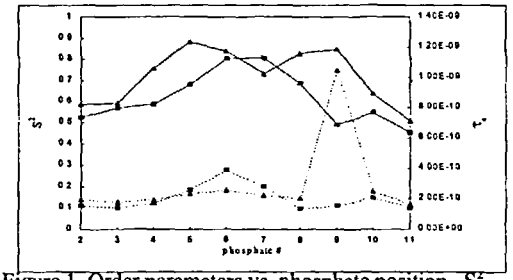

Figure 1. Order parameters vs. phosphate position. S^2 - solid lines; τ_e -dashed lines; AraC - triangles; Control - squares.

We have developed several NMR pulse oligonucleotides sequences for measuring phosphorus relaxation rates in moderately sized[5]. Analysis of the phosphorus relaxation data using the model-free formalism with fast internal motions[10] indicated that the S^2 order parameter, which describes the spatial restriction on the motion, and the effective internal correlation time, τ_e, are markedly increased at the site (Figure 1). *To the best of our knowledge, this is the first direct evidence that a nucleoside analog can produce this type of effect on DNA, and it suggests that the biochemical perturbations produced by araC may be due to dynamic as well as structural effects.*

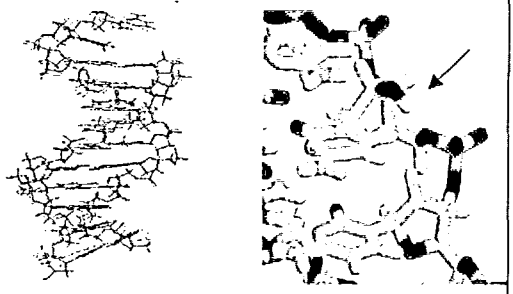

Figure 2. (A) Restrained molecular dynamics structures of araC primer-template duplex. (B) Intramolecular hydrogen bond in araC primer.

We have determined the solution structure for a primer-template duplex containing araC,

3'-GTA GGT TGG GTG-5'

5'-CAT CCA ACX C-3' where X represents the araC residue. This duplex was designed to model a product that DNA polymerases are apparently unable to form, that is, with one nucleotide added to an araC-terminated primer. As shown in Figure 2A, the overall structure of the duplex is not substantially different from normal B-type DNA. This finding suggests that the polymerization product with one base added onto an araC-terminated primer does not have a significantly perturbed structure. Interestingly, an intramolecular hydrogen bond can be observed between the 2' hydroxyl group of the araC residue and its own 5' oxygen (arrow, Figure 2B).

Ganciclovir

The compound 9-(1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir) is an effective antiviral agent used primarily in the clinic to treat CMV, an important pathogen in immunocompromised patients. Biochemical investigations employing a variety of DNA polymerases have shown that incorporation of ganciclovir by these polymerases does not result in absolute chain termination, but instead results in N+1 arrest, where one additional dNTP is added to ganciclovir followed by inhibition of further polymerization. Recently, we designed a chiral chemical synthesis of the phosphoramidite of ganciclovir, thus enabling the automated chemical synthesis of DNA containing only the biologically relevant isomer of ganciclovir[11]

To understand the structural effects of ganciclovir in DNA at a quantitative level, ganciclovir was incorporated into the decamer CTGXATCCAG (X=ganciclovir or deoxyguanosine). Restrained molecular dynamics simulations were performed using experimentally derived interproton distance and dihedral constraints to generate the structures[7] . The most striking change in the structure of the ganciclovir-modified duplex was a obvious kink in the backbone at the A(5) residue, immediately 3' to the site of ganciclovir incorporation. ^{31}P NMR data indicated an increase in backbone mobility at this site (not shown), which was not unexpected considering the increased degrees of freedom allowed by the acyclic sugar.

We have determined the solution structure for a primer-template duplex containing ganciclovir,

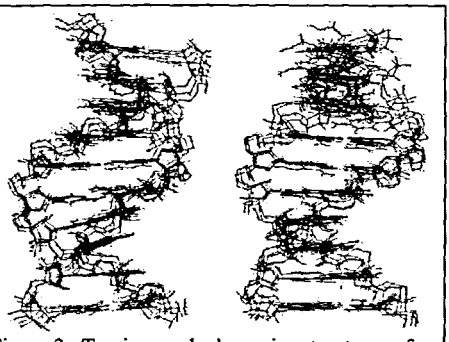

Figure 3. Torsion angle dynamics structures of control (left) and ganciclovir (right) duplexes.

3'-GTA GGT TCC GTG5'
5'-CAT CCA AGX C -3'

where X represents the ganciclovir residue. Inspection of Figure 3 shows that the duplex containing ganciclovir is disordered at the primer/template junction (the two base template overhang is not shown). A comparison of Figures 2 and 3 leads us to the conclusion that while the intramolecular hydrogen bond of araC leads to a stabilization of the duplex, the acyclic nature of ganciclovir produces the opposite effect.

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