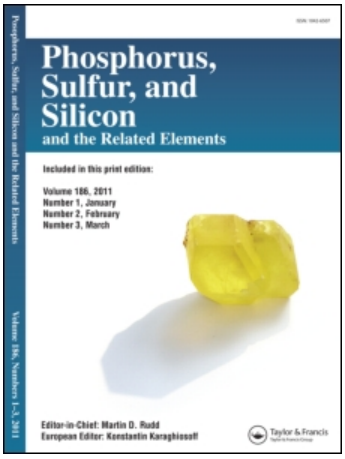

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BIOLOGICAL PROPERTIES OF DODECA(THYMIDINE PHOSPHATES) CONTAINING 5-(*o*-CARBORAN-1-YL)-2'-DEOXYURIDINE

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Abstract 5-(*o*-Carboran-1-yl)-2'-deoxyuridine containing dodecathymidylic acids (2-7) manifested increased lipophilicity, resistance to digestion by calf spleen phosphodiesterase, and snake venom phosphodiesterase. They were substrates for T4 polynucleotide kinase, primers for *E. coli* polymerase I, human α DNA polymerase, and HIV-1 reverse transcriptase. They also form heteroduplexes that are substrates for *E. coli* RNase H.

INTRODUCTION

Polymers and biopolymers such as boronated oligophosphates and oligonucleotides, are novel radiosensitizer for boron neutron capture therapy (BNCT) of cancers. Carboranyl containing oligonucleotides were designed as boron rich carriers for BNCT, and also as antisense agents for antisense oligonucleotide technology (AOT). They were produced from the monomer 5-(*o*-carboran-1-yl)-5'-dimethoxytrityl-2'-deoxyuridine-3'-[*N*,*N*-diisopropyl-β-cyanoethyl]phosphoramidite in an automated DNA synthesizer.[1] The physicochemical characteristics of model 12-mers (2-7) containing 5-(*o*-carboran-1-yl)-2'-deoxyuridine (CDU) were studied.[2] Herein, we report the biological properties of oligonucleotides **2-7** containing one or more CDU residues at different locations within the oligonucleotide chain.

RESULTS

Synthesis of 5-(o-carboran-1-yl)-2'-deoxyuridine containing dodecathymidylic acids (2-7). The synthesis of oligonucleotides **2-7** has previously been reported.[2] All compounds were characterised by UV, HPLC and ESI-MS.

Resistance to bovine spleen phosphodiesterase II (BSPDE). Pronounced effect of CDU modification on the oligonucleotide stability in the presence of phosphodiesterase II, 3'-exonuclease from calf spleen (BSPDE) was observed. The resistance increased in the order $d(T)_{12}$ (1) < $d(T)_{10}$CDUd(T) (5) ~ $d(T)_9(CDU)_2d(T)$ (6) < $CDUd(T)_{11}$ (2) ~ $d(T)CDUd(T)_{10}$ (3) ~ $^{5'}CDUd(T)_9CDUd(T)$ (7).

Resistance to snake venom phosphodiesterase (SVPDE). The presence of CDU at the 3'-end of oligonucleotides effectively improved their stability towards snake venom phosphodiesterase (SVPDE) from *Crotalus durissus terrificus*. The oligonucleotide resistance towards enzymatic hydrolysis of internucleotide phosphodiester linkages increased in the following order: unmodified 1 < 5 ~ 7 << 6. The fraction of nonhydrolyzed oligonucleotide after 10 min was 2.5, 44, 38, and 80 %, respectively.

Phosphorylation by T4 polynucleotide kinase. The phosphorylation experiments with T4 polynucleotide kinase showed that CDU-oligonucleotides 1-7 were efficiently phosphorylated at their 5'-ends by the enzyme. The efficacy of the 5'-end phosphorylation was comparable to the phosphorylation of unmodified, standard oligonucleotide $d(T)_{12}$ (1).

Priming the DNA polymerization. CDU-modified oligonucleotides 2-7 and unmodified dodecathymidylic acid 1 were tested for their ability to serve as a primers for DNA polymerases. Four different enzymes were studied, namely human polymerase α and β, *E. coli* bacterial polymerase I, and human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT). All oligonucleotides 1-7 functioned as primers for polymerases studied, except for human polymerase β.

Induction of RNase H activity. CDU-modified oligonucleotides 2-7 formed RNA-DNA heteroduplex substrates for *E. coli* RNase H with a poly rA template of 400-600 bases in length. These heteroduplexes were digested by RNase H in a fashion comparable to the digestion of the unmodified duplex formed by dodecathymidylic acid 1.

DISCUSSION

The enzyme SVPDE successively hydrolyzes 5'-mononucleotides from deoxyribooligonucleotides with free 3'-OH groups. The digestion of the DNA proceeds in the 3'->5' direction.[3] In contrast, BSPDE requires a free 5'-hydroxyl terminus, and digestion proceeds in the opposite 5'->3' direction. The nucleolytic activity of both enzymes towards oligonucleotides is substantially decreased by nucleic base modification for single stranded as well as double

stranded substrates.[4,5] The presence of two CDU residues at the 3'-end of the oligonucleotide substantially increased the oligomer stability towards 5'-exonucleolytic enzymes, such as phosphodiesterase I from snake venom (SVPDE).[2] The observed higher stability of **6** compared to **5** may be due to the slow hydrolysis of two internucleotide linkages beyond the modified base 5'-CDUpCDU-3' and 5'-TpCDU-3' or very slow cleavage of the phosphodiester linkage beyond modified dimer 5'-TpCDUpCDU-3' or trimer 5'-TpTpCDUpCDU-3'. A substantially pronounced effect on the oligonucleotide stability was observed for the 3'-exonuclease from calf spleen (BSPDE), which truncated the oligonucleotides from the 5'-end. It appears that the presence of one CDU residue at the 5'-end as in **2** or **7** protected the oligonucleotide against digestion. For oligomers **3-6** it is likely that the nucleolytic hydrolysis of the oligionucleotides proceeded fast until the first CDU residue or nucleoside directly preceding the CDU was reached.[5,6]

A variety of nucleic acid compounds can be phosphorylated in the polynucleotide kinase reaction provided they have a nucleotide bearing a free 5'-hydroxyl group with a phosphoryl group at the 3'-position.[7] As anticipated, phosphorylation of CDU-containing oligonucleotides with T4 polynucleotide kinase was shown for all oligonucleotides **2-7**. The efficacy of phosphorylation was comparable to the unmodified oligonucleotide **1**. Similarly results were obtained for the oligonucleotide **2** bearing a CDU modification at the 5'-end. The data demonstrate that CDU-oligonucleotides can be labeled at the 5'-end which is of practical importance.

CDU-modified oligonucleotides **2-7**, and unmodified dodecathymidylic acid **1** were tested for their priming activity in DNA polymerisation process catalyzed by four different DNA polymerases: human polymerase α and β, *E. coli* polymerase I and human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT). It was found that all oligonucleotides **1-7** were primers for the polymerases used although with different efficacy, except for human polymerase β. In general, the ability for priming the DNA synthesis changed in order **2** ~ **3** ≥ **1** > **4** ~ **5** ~ **6** ~ **7**. Elongation of CDU-modified oligonucleotide primers, using poly dA template, in the presence of human polymerase β did not occur. This is consistent with the enzyme requirement for the presence of phosphate at the template 5'-end in the short gap (less than 6 nucleotides) filing process.[8] Indeed, oligonucleotide **1-7** were used in fourfold excess (per base) relative to the template, which favors formation of short gaps.

RNase H recognizes RNA-DNA hybrids as a substrate and cleaves only the RNA in endonucleolytic manner. At least four base pair heteroduplex stretches are necessary for the substrate recognition. All CDU-modified oligonucleotides 2-7 RNA-DNA heteroduplexes with a 400-600 bases poly rA template were found to be substrate for RNase H. Despite the finding that the melting temperatures (T_m) of duplexes with CDU-oligonucleotides varied from 15°C to 29°C, the efficacy of poly rA template digestion seems independent of the T_m of the duplex formed with CDU-oligonucleotide.[2]

Based on the physicochemical and biological properties of these carboranyl containing oligonucleotides, we are currently designing oligomers that could be targeted against overexpressed genes in cancer and in virally infected cells. We also are developing methodologies for the synthesis of the oligonucleotides containing the carboran-1-yl modification within the internucleotide linkage.[9,10]

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