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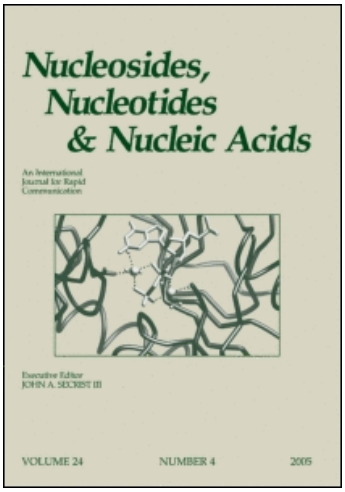

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Alkylation and Cleavage of a DNA Fragment with Trioligonucleotide Reagent

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Alkylation and Cleavage of a DNA Fragment with Trioligonucleotide Reagent

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ABSTRACT

A possibility of site-directed chemical modification of a ssDNA fragment with ''trioligonucleotide reagent'' (TOR), consisting of a central oligonucleotide derivative carrying N-(2-chloroethyl)-N-(p-formylphenyl)-N-propyl-N-3-ydeneamino groups at both 5′- and 3′-thiophosphate ends and two border derivatives with 4-carbohydrazidephenyl groups at their 3′- and 5′-phosphate ends, respectively, is shown. Products of site-directed fragment cleavage, more abundant than the alkylation products, were found at 50°C. The overall level of DNA modification by TOR reached 30% at a small excess of the oligonucleotide derivatives.

Key Words: Oligonucleotide derivatives; DNA alkylation; Cleavage.

INTRODUCTION

A variety of oligonucleotide analogs for the antisense approach bearing cross-linking, cut-off, and intercalating groups have been described.[1] A new strategy for the antisense approach, which has the advantage of ''hybridization-triggered cross-linking''[2] combined with the advantage of autoligation reactions of oligonucleotide

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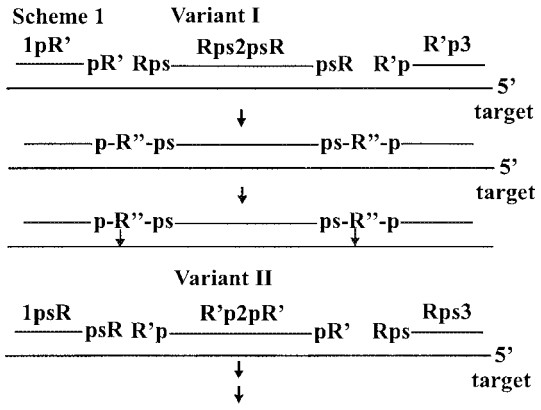

Scheme 1.

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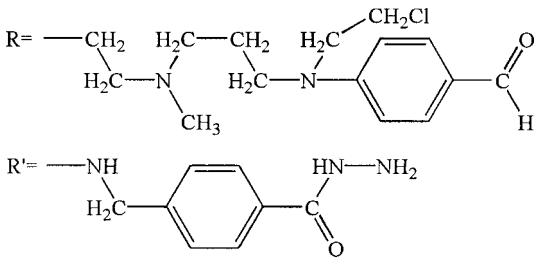
derivatives on an NA-target,[3] has been proposed.[4] This system is called ''binary oligonucleotide reagent'' (BOR). In terms of Scheme 1, it consists of 1pR′ and Rps2. Ligation of the groups in the perfect complementary complex yields a group, which is more reactive than the original ones and modifies the target. The operation of BOR has been demonstrated with the following R and R′ groups[4] shown in Scheme 2, which yield a Schiff-base in the complex.

The modification of a DNA fragment with the BOR results in a several times higher yield than with any of its components alone.[4] BOR is driven by autoligation reaction. The demonstrated and expected advantages of this strategy prompted me to further elaborate the BOR strategy and to investigate the possibility of a ''trioligonucleotide'' approach (Scheme 1, TOR).

The structures of the DNA target ([5′-^{32}P]-''58'') and starting oligonucleotides are: AGCTTGGGACCGAACCCAGGGCCTTGCGCTTCCTAGGTAAGCGCTCTAC-CACTGAGCG; Variant I: 1p (GGTAGAGCGCTTACCTp), ps2ps (psAGGAAGCG-CAAGGCCCps), p3 (pTGGGTTCGGTCCCAAG); Variant II: 1ps (GGTAGAGCGCTTACCTps), p2p (pAGGAAGCGCAAGGCCCp), ps3 (psTGGGTTCGGTCCCAAG).



Scheme 2.

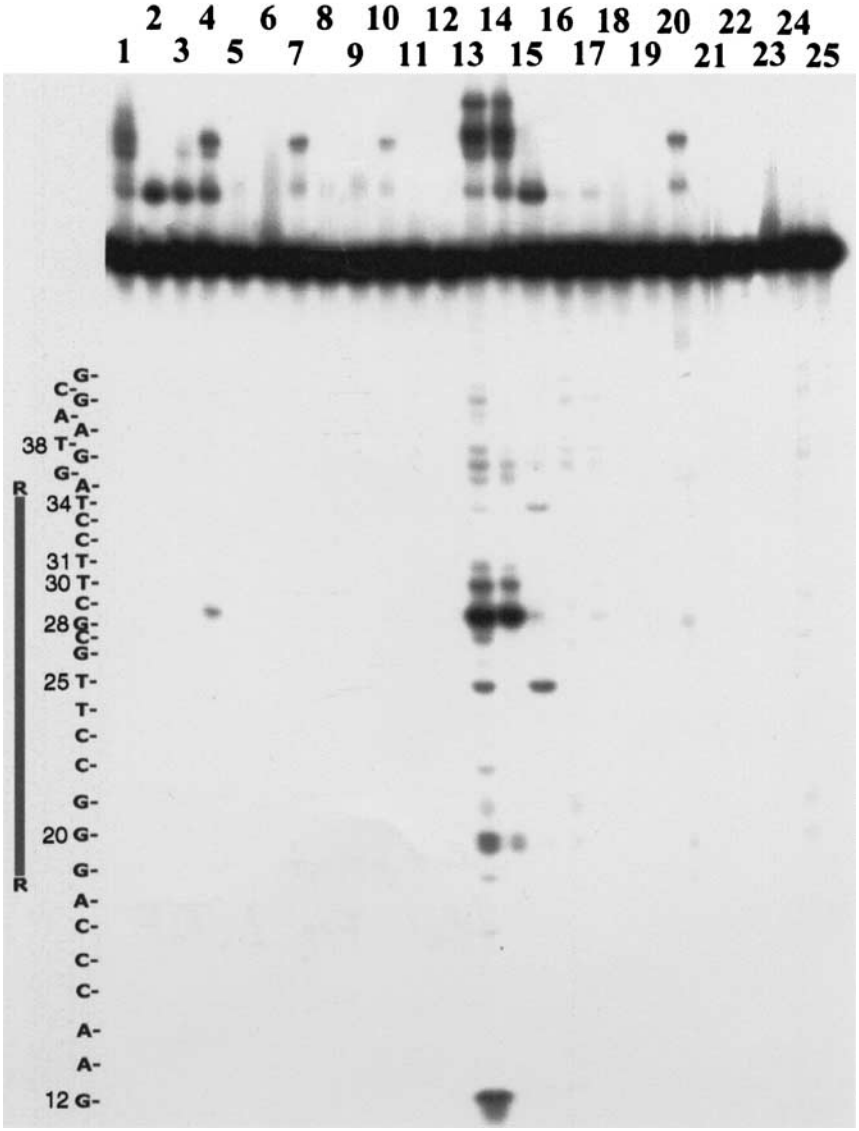


Figure 1. Radioautograph of the denaturating 20% gel. Lanes 1-12-37°C. Variant I: 1. 1pR′, Rps2psR, R′p3; 2. Rps2psR; 3. 1pR′, Rps2psR; 4. Rps2psR, R′p3; 5. 1pR′, R′p3; 6. [^{32}P]-''58'' alone; Variant II: 7. 1psR, R′p2pR′, Rps3; 8. R′p2pR′; 9. 1psR, R′p2pR′; 10. R′p2pR′, Rps3; 11. 1psR, Rps3; 12. [^{32}P]-''58'' alone; Lanes 13-25-50°C. Variant I: 13. 1pR′, Rps2psR, R′p3; 14. 1pR′, Rps2psR, R′p3; 15. Rps2psR; 16. 1pR′, Rps2psR; 17. Rps2psR, R′p3; 18. 1pR′, R′p3; 19. [^{32}P]-''58'' alone; Variant II: 20. 1psR, R′p2pR′, Rps3; 21. R′p2pR′; 22. 1psR, R′p2pR′; 23. R′p2pR′, Rps3; 24. 1psR, Rps3; 25. [^{32}P]-''58'' alone. The location of the derivative Rps2psR on the target and the products of the Maxam-Gilbert digestion of the target are at the left.

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RESULTS AND DISCUSSION

The modification was carried out at 37°C, when individual oligonucleotide components of TOR can form a stable complementary complex with the target, and at 50°C, when, supposedly, these individual components do not form complexes. Conditions, under which ligation of the derivatives had occurred only in the presence of a target but not in the solution (in the BOR variant), were used: 0.4 μM of [^{32}P]-''58'' and 1 μM oligonucleotide derivatives were incubated in 10 mM HEPES-KOH buffer, pH 7.3 at 37 or 50°C for 70 h. The results are shown in Fig. 1.

At 50°C (Lanes 13 and 14), the yield of alkylation with the TOR is higher that at 37°C (Lane 1). Comparison of alkylation products on lanes 13 and 14 with those in lanes 15; 3, 4; and 16, 17, 18 shows that 1) Lanes 13 and 14: The lower band above the starting [^{32}P]-''58'' is the product of alkylation by Rps2psR not activated within the TOR; the middle bands are the products of alkylation by the left and right TOR flanks; the upper band is the product of alkylation by the complete TOR; 2) At 50°C, alkylation by a pair of TOR components is observed only with the complete TOR. The presence of at least one of the flanking oligonucleotide components reduces the alkylation of the target by Rps2psR in an independent manner. ''Background'' alkylation is very weak. Thus, the alkylation observed on Lanes 13 and 14 results from the cooperative interaction of all the three components and corresponds to the TOR model. An intriguing result is efficient cleavage of the target by TOR at 50°C. The products of cleavage at G_{20} and G_{36}, G_{37}, T_{38} (TOR), as well as T_{34} (Rps2psR) occur in the vicinity of the modifying groups at a linear double-stranded structure of the complexes. Other cleavage sites, including the main one, could emerge only from distortions in the linear structures of the complexes. Moreover, the identical independently prepared reaction mixtures loaded onto Lanes 13 and 14 differ in some minor cleavage products. A possible explanation for these effects is formation of stem-and-loop structures, shown to be feasible by computation. Experiments in which the combinations 1p + Rps2psR + R′p3 and 1pR′ + Rps2psR + p3 were used at 50°C gave rise to addition products of binary pairs and major cleavage products (data not shown). Two activated R groups differently contribute to both alkylation and cleavage, which should be taken into account during choosing the site of target modification. No TOR modification is observed in variant II (Lane 20). The original radioautograph shows only a faint band of the product of such alkylation.

EXPERIMENTAL

1p, p2p, p3 and 1ps, ps2ps, ps3 were purchased from Biosan (Novosibirsk). 4-Carbohydrazidebenzylamine and N-methyl-N,N′-di(2-chloroethyl)-N′-(p-formylphenyl)-propylenediamine 1,3 were kindly provided by V. I. Vysochin and Prof. G. V. Shishkin (Novosibirsk Institute of Bioorganic Chemistry).

Synthesis of the oligonucleotide derivatives 1psR, Rps3, Rps2psR and 1pR′, R′p3, R′p2pR′.

The first group of the derivatives were synthesized by alkylation of 1ps, ps3, and ps2ps with N-methyl-N, N′-di(2-chloroethyl)-N′-(p-formylphenyl)propylenediamine 1,3[4] with the use of double concentrations of the alkylating reagent in the last case.

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The contents of 1psR, Rps3, and Rps2psR, as determined by PAGE were 85, 83, and 80%, respectively. The relative electrophoretic mobilities and UV spectra of the derivatives were in agreement with their structures.

Derivatives 1pR′, R′p3, and R′p2pR′ were synthesized from 1p, p3, p2p and 4-carbohydrazidebenzylamine similarly to Ref. [4]. The degrees of conversion of the starting oligonucleotides to 1pR′ and R′p3 according to ion-exchange HPLC (POROS 20 PI 4.6 × 100 column, linear gradient 0–0.2 M of potassium phosphate pH 7.5 in 30% acetonitrile, 2 ml/min) data (retention times 26.656 and 23.051) were 80 and 85%, respectively. The degree of conversion of p2p to R′p2pR′ was 40%. The product was isolated by ion-exchange HPLC (retention time 25.341) and desalted by reverse-phase HPLC. The presence of two acylhydrazide moieties in R′p2pR′ was confirmed by PAGE of the products of its treatment with p-nitrobenzaldehyde.[4]

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REFERENCES

1. Uhlmann, E.; Peyman, A. Antisense oligonucleotides: a new therapeutic principle. Chem. Rev. **1990**, *90*, 543–584.
2. Webb, T.R.; Matteucci, M.D. Sequence-specific cross-linking of deoxyoligonucleotides via hybridization-triggered alkylation. J. Am. Chem. Soc. **1986**, *108*, 2764–2765.
3. Goodwin, J.T.; Lunn, D.G. Template-directed synthesis: use of a reversible reaction. J. Am. Chem. Soc. **1992**, *114*, 9197–9198.
4. Oshevski, S.I. Concept of ''Binary Oligonucleotide Reagent.'' Nucleosides Nucleotides **1998**, *17*, 1969–1975.

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