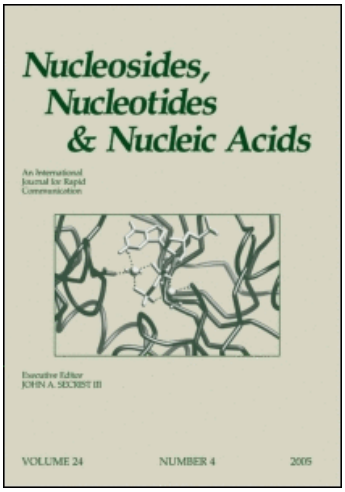

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RECOGNITION OF DNA BY STRAND INVASION WITH OLIGONUCLEOTIDE-SPERMINE CONJUGATES

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ABSTRACT

Modified oligonucleotides bearing spermine groups (ODN-sper) with increased binding affinity to DNA have been synthesized. The ability of these ODN-sper to bind within superhelical double-stranded DNA by strand invasion has been studied. The uptake by a supercoiled plasmid was 3 fold higher for the ODN-sper than for the unmodified oligonucleotides.

In recent years, many modifications have been introduced into oligonucleotides (ODNs) in order to improve their therapeutic properties (for examples see Ref 1). Several approaches to increase the binding affinity of ODNs have included the introduction of cationic groups. However, only a few of these have led to significant melting temperature enhancements, showing that the introduction of positive charges is not intrinsically sufficient. Spermine is an endogenous polyamine known to stabilize DNA against thermal denaturation (2). It has also been shown that spermine has a binding affinity for the minor groove of DNA (3). Therefore, we tethered the polyamine to ODNs in the place of the exocyclic amine of guanine, ordinarily present in the middle of the minor groove of double-stranded DNA (4). We (4–6) and others (7), have observed a strong thermal stabilization of the duplex structures of these ODN-sper. The groove location seems to be of importance, since many other alkylammonium and imidazolium groups, linked at the same position, have shown similar effects (7–10). On the other hand, spermine conjugation to the

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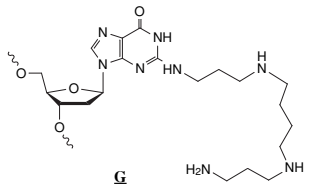
Table 1. Oligonucleotide Sequences



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Name	Sequence
22dGsp3-a	5′-ATGA**G** ATGTG AC**G**AA C**G**TGT AC-3′
22dGsp6-a	5′-ATGA**G** AT**G**T**G** AC**G**AA C**G**T**G**T AC-3′
22dGsp0-b	5′-TGGTA AAATG GAAGA CGCCA AA-3′
22dGsp3-b	5′-TG**G**TA AAAT**G** GAA**G**A CGCCA AA-3′
22dGsp0-c	5′-ATGAA GAGAT ACGCC CTGGT TC-3′
22dGsp4-c	5′-AT**G**AA **G**AGAT ACGCC CT**GG**T TC-3′

C-4 position of cytosine (in the major groove) led to decreased duplex stability (11).

The properties of ODN-sper led us to consider their ability to recognize specific sequences of DNA by strand invasion of the double helix. ODN-sper, by achieving this, would become useful therapeutic agents for diagnosis and the gene expression regulation.

We have obtained several ODN-sper, with up to six modifications (Table 1), using a 2-spermino-2′-deoxyinosine phosphoramidite (5,6). ODN-sper were isolated by reverse phase HPLC and the majority of them showed the correct mass by MALDI-TOF mass spectrometry. However, the highly modified oligonucleotides sometimes exhibited peculiar spectroscopic behavior. For instance, **22dGsp6-a** gave a broad peak of higher mass than expected. An explanation for these effect could be the conversion of the trifluoroacetamide groups (protecting the spermine moiety) into acetyl groups during the capping step (mass + 43 Da) (12). However, a mass spectrum of **22dGsp4-c** (Fig. 1-A) presented a set of peaks different by 38 Da in average, consistent with the formation of multiple potassium adducts. This observation is supported by the presence of at least one potassium adduct in almost all the ODN-sper mass spectra. Moreover, only a single band was observed for **22dGsp4-c** in polyacrylamide gel electrophoresis in denaturating conditions (Fig. 1-B). Acetyl groups on the polyamine would have led to some distinct bands, as the ODNsper electrophoretic mobility is strongly altered by the number of positive charges (5).

In the mid seventies two independent works described the uptake of single stranded fragments by superhelical DNA (13,14). At 37°C and in physiological salt concentrations, the strand invasion was almost undetectable. However, the invasion occurred spontaneously over a threshold temperature (∼60°C at 200 mM NaCl) or at 37°C for low salt concentrations. Beattie *et al*. have proposed a mechanism that explains the experimental data (Fig. 2) (15). The phenomenon is governed by two rate-limiting steps: first the disruption of a region in the superhelical DNA (**I** <=> **II**); and second, the nucleation between the ODN and the plasmid (**II** <=> **III**).

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Figure 1. Characterization of the ODN-sper. (A) MALDI-TOF mass spectroscopy of 22dGsp4-c (B) Denaturing 19% PAGE. Lane 1: 22dGsp4-c; lane 2: 22dGsp0-c (C) ^{32}P-labeled ODN-sper in the strand invasion conditions (0.8% agarose gel). Lane 1: 22dGsp3-a; lane 2: 22dGsp6-a.

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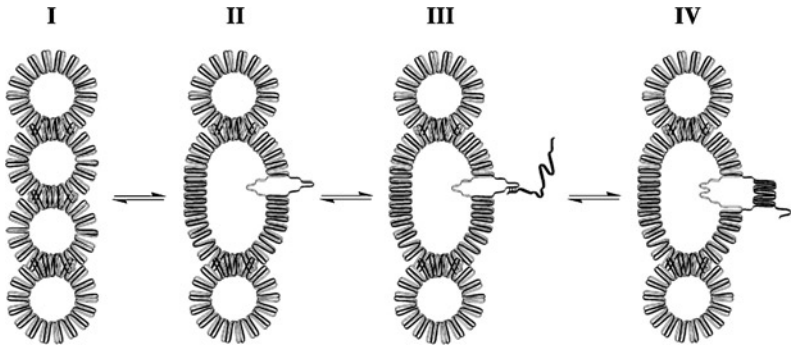

Figure 2. Strand invasion mechanism. (I) Supercoiled plasmid (II) Partially relaxed molecule with the formation of a single-stranded region. (III) Nucleation: formation of the first Watson-Crick base pairs between the ODN and the plasmid (IV) Strand invasion complex.

Then, the final complex **IV** is readily formed and is stable over weeks. We expect that ODN-sper can initiate strand invasion by accelerating the rate-limiting steps.

Since ODN-sper are able to displace homologous strands from preformed duplexes of the same size, they should, therefore, promote the disruption of the Watson-Crick hydrogen bonds in the targeted region. Furthermore, their partially zwitterionic constitution should also increase the association kinetic and the local concentration in the proximity of the superhelical DNA.

The oligonucleotides in Table 1 are complementary to sequences present in the luciferase gene. We have studied the uptake of these ODNs by two plasmids, pCMV-Luc and β-Gal, in which the targeted gene is present and absent, respectively. We were unable to assay the strand invasion ability of **22dGsp6-a** because, under the reaction conditions, **22dGsp6-a**, which is almost neutral, essentially aggregated (Fig. 1-C).

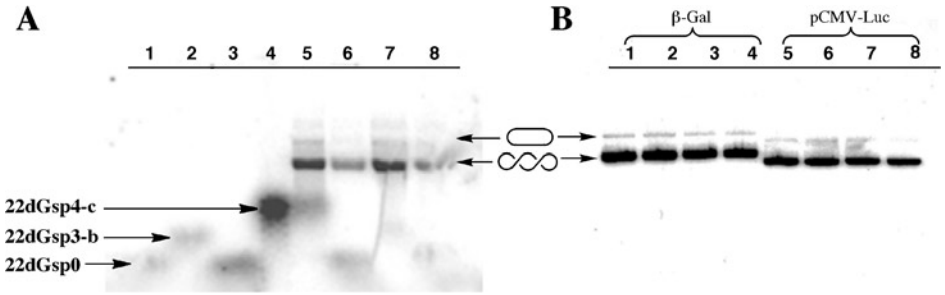

Figure 3. Strand invasion. (A and B) 0.7% agarose gel. Lanes: 1 to 4: β-Gal plasmid; 5 to 8: pCMV-Luc plasmid. (A) Plasmids (25 nM) were incubated 10 min at 95°C in MES buffer pH 6, 7, containing 150 mM of NaCl, in the presence of ^{32}P-labeled single-stranded ODNs (25 nM). Lanes: 1 + 8: 22 dGp0-b, 2 + 7: 22dGsp30-b, 3 + 6: 22dGsp0-c. 4 + 5: 22dGsp4-c. (B) Plasmids stained by ethydium bromide. The positions of the ODNs, the supercoiled and the circular relaxed plasmids are indicated by arrows.

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The plasmid and the oligonucleotide were incubated for 10 min at 95°C and slowly cooled to room temperature. The ODNs formed strand invasion complexes only with the plasmid containing their complementary sequences (Fig. 3). Quantification of the ODNs radioactivity present in the complexes revealed that ODN-sper were taken-up about 3 fold more efficiently than the unmodified ODNs. Surprisingly, we also observed uptake of the ODN-sper within the circular relaxed form of the plasmid.

Further investigation with highly modified ODN-sper would assess if they can invade DNA in physiological conditions. These ODN-sper should have a T_m close to the DNA melting temperature and, conversely to **22dGsp6-a**, a more pronounced overall charge.

REFERENCES

1) Freier, S. M.; Altmann, K. H. *Nucleic Acids Res*., **1997**, *25*, 4429.
2) Tabor, H. *Biochemistry*, **1962**, *1*, 496.
3) Schmid, N.; Behr, J.-P. *Biochemistry*, **1991**, *30*, 4357.
4) Schmid, N.; Behr, J.-P. *Tetrahedron Lett*., **1995**, *36*, 1447.
5) Potier, P. F.; Adib, A.; Behr, J.-P. *Chem. Eur. J*., **2000**, *in press*.
6) Potier, P.; Adib, A.; Kochkine, A.; Huc, I.; Behr, J.-P. *Nucleosides Nucleotides*, **1999**, *18*, 1467.
7) Diaz, A. R.; Eritja, R.; Garcia, R. G. *Nucleosides Nucleotides*, **1997**, *16*, 2035 and **2000**, *19*, 703.
8) Eritja, R.; Diaz, A. R.; Saison-Behmoaras, E. *Helv. Chim. Acta*, **2000**, *83*, 1417.
9) Manoharan, M.; Ramasamy, K. S.; Mohan, V.; Cook, P. D. *Tetrahedron Lett*., **1996**, *37*, 7675.
10) Ramasamy, K. S., Zounes, M., Gonzalez, C., Freier, S. M., Lesnik, E. A., Cummins, L. L., Griffey, R. H., Monia, B. P. and Cook, P. D. *Tetrahedron Lett*., **1994**, *35*, 215.
11) Prakash, T. P.; Barawkar, D. A.; Kumar, V.; Ganesh, K. N. *Bioorg. Med. Chem. Lett*., **1994**, *4*, 1733.
12) Ferrer, E.; Neubauer, G.; Mann, M.; Eritja, R. *J. Chem. Soc., Perkin Trans. 1*, **1997**, 2051.
13) Holloman, W. K.; Wiegand, R.; Hoessli, C.; Radding, C. M. *Proc. Natl. Acad. Sci. U.S.A*., **1975**, *72*, 2394.
14) Liu, L. F.; Wang, J. C. *Biochim. Biophys. Acta*, **1975**, *395*, 401.
15) Beattie, K. L.; Wiegand, R. C.; Radding, C. M. *J. Mol. Biol*., **1977**, *116*, 783.

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