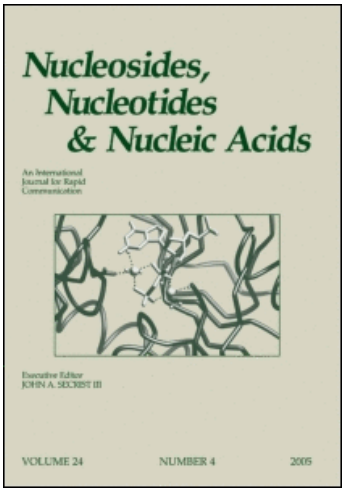

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Base Changes and Triple-Helix Hybridization Properties of GT Containing Third Strands: A Systematical Study

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BASE CHANGES AND TRIPLE-HELIX HYBRIDIZATION PROPERTIES OF GT CONTAINING THIRD STRANDS: A SYSTEMATICAL STUDY.

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ABSTRACT: Triple-helix recognition of the DNA major grove offers a powerful approach for the design of sequence specific DNA binding molecules. Although the triple-helix binding of pyrimidine containing third strands is well characterized now, the factors governing the recognition of double stranded DNA by GT containing third strands are poorly understood. Oligonucleotides with a variable amount of G and GpT or TpG steps have been synthesized here. A systematical study was performed to determine the influence of these changes on the triple-helix hybridization properties of these oligonucleotides. Large difference in the hybridization properties have been observed by band shift experiments.

For more than ten years the design of molecules that sequence specifically bind DNA has been a goal for chemists and biologists. Various small drugs exhibit such properties. However their specificity is still limited to a few base pairs, and differences between specific and nonspecific binding is low (1).

Dedicated to the memory of Dr. Roland K. Robins.

The triple-helix approach develops more recently (2). It is based on the ability of some DNA sequences to interact in the major groove with double helical DNA. Triple-helix forming sequences exhibit a high degree of specificity. They recognize sequences longer than 20 bases, and one mismatch greatly destabilizes their hybridization to the DNA duplex (3,4). Until recently the recognition was limited to a homopurine strand, however recognition of the four bases is possible now (5).

Various types of triple-helix hybridizations have been well characterized. Pyrimidine oligonucleotides bind in parallel orientation to the purine strand of the Watson-Crick double helix, through Hoogsteen hydrogen bounds. T recognizes the AT base pair, protonated cytosine recognizes the GC base pair, and G recognizes the TA base pair when the amount of G in the third strand is low (6). However cytosine are protonated at acidic pH only, and the hybridization properties of the pyrimidine third strand are greatly decreased at physiological pH (7).

Interestingly, triple-helix recognition by purine rich oligonucleotides occurs at physiological pH (8), but their hybridization properties are less well understood. Dervan and co-workers demonstrated that these oligonucleotides bind in the antiparallel orientation to the Watson-Crick purine strand; their hybridization are due to the recognition of the AT base pair by T and the GC base pair by G (8). These results were confirmed by band shift experiments and footprinting. However these data were established on sequences highly enriched for purines residues (84 to 92%) (9), and some sequence dependence is suspected (8,10).

As G recognizes the TA base pair on T rich third strand and the GC base pair on a G rich third strand, we have evaluated the hybridization properties of GT containing triplexes between these two extreme base compositions. Third strands have been base paired according to (8). Two sets of triplexes, in the parallel and the antiparallel orientation to the Watson-Crick purine strand, and their corresponding duplexes were synthesized. The first set contained an increasing amount of G residues and a fixed amount of GpT or TpG steps, and the second one contained a fixed amount of G and increasing amounts of GpT or TpG steps. Large differences

TABLE 1: Combination of oligonucleotides with a third strand variation of the amount of GT with a fixed amount of GpT steps.

NUMBER OF GUANINE	PARALLEL TO W.C. PURINE STRAND	NUMBER OF GUANINE	ANTIPARALLEL TO W.C. PURINE STRAND	G (%)
3G-P	5' TTTTCTTTCCTTTT 3' 3' AAAAGAAAGGAAAA 5' 3' *TTTTGTTTGGTTTT* 5'	3G-AP	5' TTTTCTTTCCTTTT 3' 3' AAAAGAAAGGAAAA 5' 5' *TTTTGTTTGGTTTT* 3'	21%
5G-P	5' CTTTTTTCTTTCCC 3' 3' GAAAAAAGAAAGGG 5' 3' *GTTTTTTGTTTGGG* 5'	5G-AP	5' CTTTTTTCTTTCCC 3' 3' GAAAAAAGAAAGGG 5' 5' *GTTTTTTGTTTGGG* 3'	36%
7G-P	5' CCTTTTTCTTCCCC 3' 3' GGAAAAAGAAGGGG 5' 3' *GGTTTTTGTTGGGG* 5'	7G-AP	5' CCTTTTTCTTCCCC 3' 3' GGAAAAAGAAGGGG 5' 5' *GGTTTTTGTTGGGG* 3'	50%
9G-P	5' CCCTTTCCTTCCCC 3' 3' GGGAAAGGAAGGGG 5' 3' *GGGTTTGGTTGGGG* 5'	9G-AP	5' CCCTTTCCTTCCCC 3' 3' GGGAAAGGAAGGGG 5' 5' *GGGTTTGGTTGGGG* 3'	64%
11G-P	5' CCCCTCCCTTCCCC 3' 3' GGGGAGGGAAGGGG 5' 3' *GGGGTGGGTTGGGG* 5'	11G-AP	5' CCCCTCCCTTCCCC 3' 3' GGGGAGGGAAGGGG 5' 5' *GGGGTGGGTTGGGG* 3'	79%

Oligonucleotide combinations were named with reference to the number of G in the third strand (5G to 11 G) and to its parallel (P) or antiparallel (AP) orientation. The third strand is printed in italic.

were observed in the hybridization properties of these triplexes as reported here.

MATERIAL AND METHODS

Oligonucleotides purification and labelling: The oligonucleotides (Table 1 and Table 2) were purchased from the Genset company. They were precipited with ethanol, and their purity was verified by 20% (W/V) acrylamide sequencing gel electrophoresis. Triplex forming strands were labelled with γ [^{32}-P]-ATP (ICN) using T4 polynucleotide kinase (Boehringer) according to published

TABLE 2 Combination of oligonucleotides with a third strand variation of the amount of GpT or TpG steps with a fixed amount of GT.

NUMBER OF GpT or TpG STEPS	PARALLEL TO W.C. PURINE STRAND	NUMBER OF GpT or TpG STEPS	ANTIPARALLEL TO W.C. PURINE STRAND	GpT or TpG (%)
4-GpT-p	5' CCCTTTCCTTCCCC 3' 3' GGGAAAGGAAGGGG 5' 3'*GGGTTTGGTTGGGG* 5'	4-GpT-ap	5' CCCTTTCCTTCCCC 3' 3' GGGAAAGGAAGGGG 5' 5'*GGGTTTGGTTGGGG* 3'	30
7-GpT-p	5' CTTCCTCCCTCCCT 3' 3' GAAGGAGGGAGGGA 5' 3'*GTTGGTGGGTGGGT* 5'	7-GpT-ap	5' CTTCCTCCCTCCCT 3' 3' GAAGGAGGGAGGGA 5' 5'*GTTGGTGGGTGGGT* 3'	54
10-GpT-p	5' CTCCTCCCTCTCTC 3' 3' GAGGAGGGAGAGAG 5' 3'*GTGGTGGGTGTGTG* 5'	10-GpT-ap	5' CTCCTCCCTCTCTC 3' 3' GAGGAGGGAGAGAG 5' 5'*GTGGTGGGTGTGTG* 3'	71

Oligonucleotide combinations were named with reference to the number of GpT or TpG step in the third strand (4-GpT to 10-GpT) and to its parallel (P) or antiparallel (AP) orientation. The third strand is printed in italic.

procedures (11). Non-incorporated nucleotides were removed by precipitation using ethanol saturated with ammonium acetate.

Band shift experiments: Band shift electrophoresis was performed to discriminate between duplexes and triplexes. The oligonucleotides were resupended in 35 mM pH 8 tris borate , 10mM $MgCl_2$, 10% (V/V) glycerol (incubation buffer). 0.1pmole of the third strand labelled oligonucleotide, in the parallel or in the antiparallel orientation to the Watson-Crick purine strand, were incubated alone or with increasing amounts (0, 40, 400, 4000 fold excess) of its corresponding duplex, in 10µl of incubation buffer. The oligonucleotide samples were cooled to 4°C after 30min incubation at 16°C. 5µl of each sample were analysed by

electrophoresis on a 10% (W/V) polyacrylamide gel buffered with 35 mM pH 8 Tris borate, 10mM $MgCl_2$ at 4°C. Gels were then fixed in ethanol/acetic acid/H_2O (5/1/4 V/V), dried, and submitted to autoradiography on Kodak Xomat films. In all experiments, duplex formation was verified by UV shadowing at the highest duplex concentration before fixation (5000 fold excess as compared to the third strand). All the experiments were repeated at least three times.

Autoradiogram quantification: Autoradiograms were digitalised and quantified with the Bio Image software after comparison to a standard gray scale. Data were obtained as the percentage of shifted material, and were plotted against log(duplex excess). After linear regression, the equation of the straight line was obtained and the amount of duplex sufficient to bind 50% of the third strand was deduced.

RESULTS AND DISCUSSION:

A series of oligonucleotide sequences have been designed in order to investigate the hybridization properties of GT containing third strands. The following parameters have been taken in consideration. All duplexes have been tested with two triple helix forming oligonucleotides hybridizing in parallel or antiparallel orientation to the Watson-Crick purine strand. Variation of the G content of the third strand (Table 1) was tested separately by varying the content of GpT or TpG steps in the third strand (Table 2).

The hybridization properties of these oligonucleotides have been compared by incubating a fixed amount of the labelled third strand with increasing concentrations of the unlabelled duplex. The non hybridized third strand was separated from the hybridized ones by non denaturing polyacrylamide gel electrophoresis at pH 7.5 in the presence of 10mM $MgCl_2$.

As expected third strand hybridization was observed with oligonucleotides of high G percentage (79 %) antiparallel to the Watson-Crick purine strand (Fig. 1). 50% of triplex formation was obtained with about 400 fold excess of the duplex (Table 3). In this

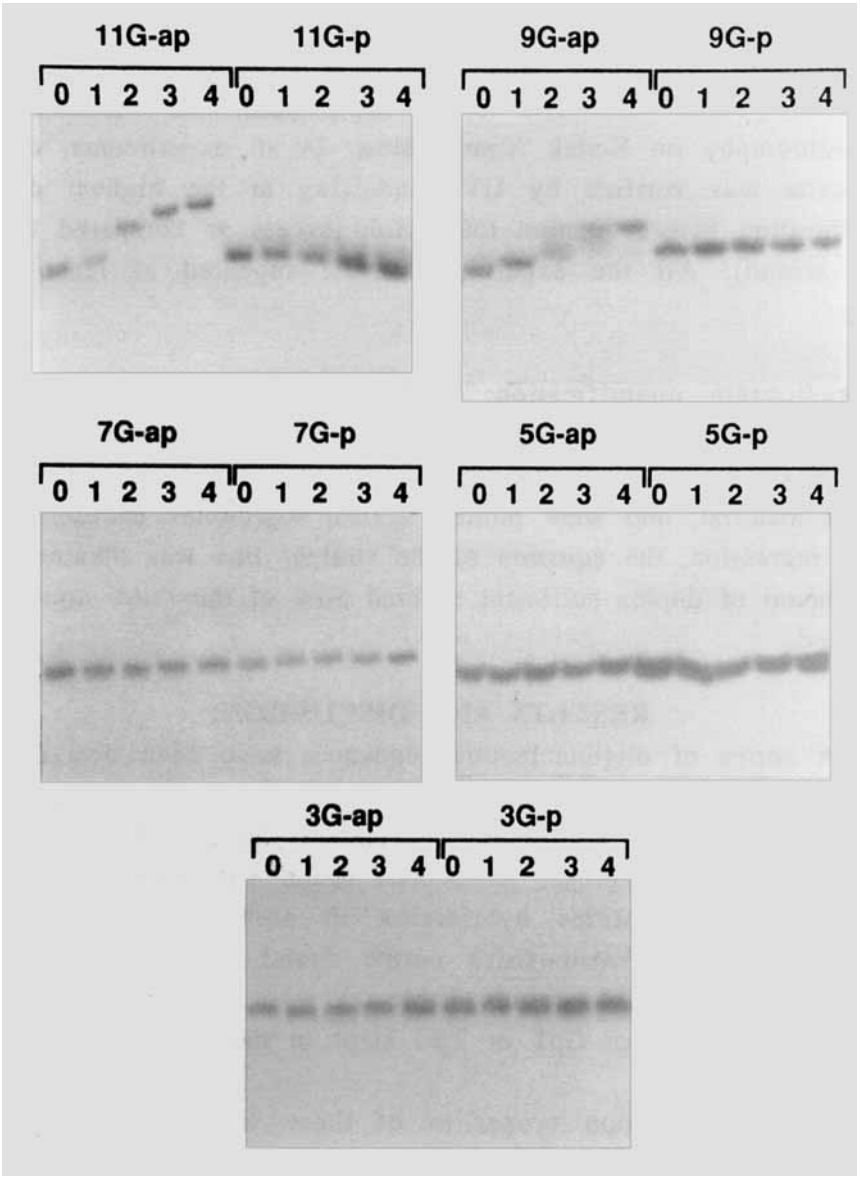

Fig. 1. Triple helix hybridization of third strands with changes in the percentage of G.

Band shift experiments were performed as described in Material and Methods, with a constant concentration of [^{32}-P] labelled third and an increasing amount of its corresponding duplex, at pH 8, and in the presence of 10mM $MgCl_2$. Gel were then fixed and submitted to autoradiography.

TABLE 3 Summary of the hybridization properties of the oligonucleotides combinations.

VARIATION IN THE NUMBER OF G ON THE THIRD STRAND			
NUMBER OF GUANINE	AFFINITY OF A THIRD STRAND ANTIPARALLEL TO W.C. PURINE STRAND (M)	NUMBER OF GUANINE	AFFINITY OF A THIRD STRAND PARALLEL TO W.C. PURINE STRAND (M)
3G-P	NDE	3G-AP	NDE
5G-P	NDE	5G-AP	NDE
7G-P	NDE	7G-AP	NDE
9G-P	$0.19\ 10^{-10}$	9G-AP	NDE
11G-P	$0.25\ 10^{-10}$	11G-AP	NDE

B

VARIATION IN THE NUMBER OF GpT OR TpG STEPS ON THE THIRD STRAND			
NUMBER OF GpT OR TpG STEPS	AFFINITY OF A THIRD STRAND ANTIPARALLEL TO W.C. PURINE STRAND (M)	NUMBER OF GpT OR TpG STEPS	AFFINITY OF A THIRD STRAND PARALLEL TO W.C. PURINE STRAND (M)
4-GpT-P	$0.19\ 10^{-10}$	4-GpT-AP	NDE
7-GpT-P	NDE	7-GpT-AP	NDE
10-GpT-P	$0.02\ 10^{-10}$	10-GpT-AP	$2,35\ 10^{-10}$*

Combination of oligonucleotides where no hybridization was detected are noted NDE. Results are given as the concentration of duplex sufficient to displace 50% of the labelled third strand. (*) the electrophoretic mobility of this complex is higher than those of triplex, and was not dependent of the presence of $MgCl_2$.

case, parallel and antiparallel third strand differed only by one mismatch. Under our experimental conditions, hybrid formation is highly specific, since a single mismatch is sufficient to abolish the interactions (Fig. 1).

The hybridization properties are characteristic of the GT third strands reported previously (8,12). They are strongly dependent on the presence of $MgCl_2$, and poorly dependent on the pH (between pH 5 and 9) (not shown).

The hybridization of the third strand was not affected when decreasing the G percentage to 65%. However at lower G percentage (from 50 to 22%) triplex formation could not be detected wathever the orientation of the third strand (Fig. 1). It could not result from a lack of duplex formation, since duplex hybridization was controlled by UV shadowing in all the experiments (not shown). Furthermore the results could not be due to differences in the kinetic of third strand hybridization between these oligonucleotide combinations. When a labelled third strand was incubated with a 500 fold excess of its unlabelled coresponding duplexes no hybridization was observed at a G percentage lower than 65%, even upon 6 hours incubation at 16°C. On the contrary oligonucleotides with a G percentage of 65% or higher are hybridized upon 1 min incubation (not shown).

These results point to the need of high G contents for the hybridization of a GT containing third strand. Decreasing the G content from 65 to 50 percent was sufficient to abolish the hybridization, and it was surprising that no regular decrease in the hybridization properties was observed between 79 to 22% G. These results also confirm the antiparallel orientation of GT containing third strands to the Watson-Crick purine strand reported by (8,9).

Third strand hybridization of oligonucleotides varying in the amount of GpT or TpG but carrying a constant G content have been tested in the same experimental conditions (Fig. 2). Triplex formation was tested with third strand containing 4 or 10 GpT and TpG steps. However no hybridization was observed with oligonucleotides carrying 7 GpT or TpG steps. A band shift was detected with the oligonucleotide 10-GpT-p; however it was not attributed to triplex formation since it was not dependent on the

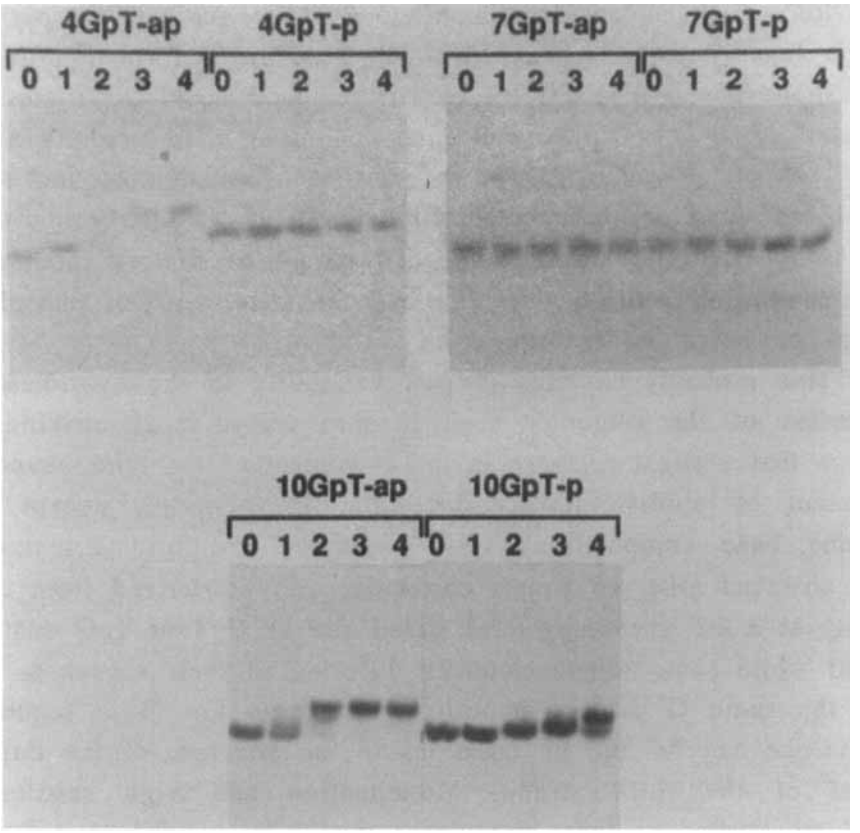

Fig. 2. Triple helix hybridization of third strands with changes in the percentage of GpT or TpG steps.

Band shift experiments were performed as described in Material and Methods, with a constant concentration of [^{32}-P] labelled third and an increasing amount of its corresponding duplex, at pH 8, and in the presence of 10mM $MgCl_2$. Gel were then fixed and submitted to autoradiography.

presence on $MgCl_2$ and its electrophoretic mobility is significantly higher than those of triplex.

Obviously, the oligonucleotides designed for these experiments will probably not cover all the possible combinations, and our results point to the complexity of GT triple-helix hybridization.

Recent molecular modelling studies (13) have pointed out the B-type sugar phosphate backbone, and the *anti* conformation of the third strand bases in G CG triplex. If some of the G CG triplet are changed by T AT triplet, molecular modelling also predicts a B-sugar phosphate backbone and an *anti* conformation of the third strand base at every base composition. The authors also predicts an increased sensitivity of G rich third strand to factors modifying electrostatic interactions, due to closer contacts between phosphate groups on neighbouring strands.

This probably explains in part variability in the hybridization properties of the oligomer used in this study. It is striking to observe that a slight decrease in the G content of the third strand is sufficient to abolish its hybridization. Furthermore, except for extreme base compositions were changes in hybrid orientation were observed (10), no simple correlation can be derived from the affinity of a GT containing third strand and its GpT or TpG content. Indeed while some oligonucleotides differing in their sequence, and with the same G content hybridize, others do not. This sequence dependence can be due to variations in the structure of the duplex and/or of the third strand. Modelisation and high resolution structural studies of these complexes would be helpful to a better understanding of these interactions.

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REFERENCES

1. Nielsen P.E., Bioconjugate Chemistry, 2, 1 (1991).
2. Thuong N.T., Héléne C., Angew. Chem. Int. Ed. Engl., 32, 666 (1993).
3. Mergny J.L., Sun J.-S., Rougèe M., Montenay-Garestier T., Barcelo F., Chomilier J., Hélène C., Biochemistry, 30, 9791 (1991).

4. Yoon K., Hobbs C., Koch J., Sardaro M., Kutny R., Weiss A.-L., Proc. Natl. Acad. Sci. USA, 89, 3840 (1992).
5. Griffin L.C., Kiessling L.L., Beal P.A., Gillespie P., Dervan P.B., J. Am. Chem. Soc., 114, 7976 (1992).
6. Griffin L.-C., Dervan P.-B., Science 245, 967 (1989).
7. Povsic T.J., Dervan P.B., J. Am. Chem. Soc., 111, 3059 (1989).
8. Beal P.A., Dervan P.B., Science, 251, 1360 (1991).
9. Durland R.H., Kessler D.J., Gunnell S., Duvic M., Pettitt B.M., Hogan M.E., Biochemistry, 30, 9246 (1991).
10. Sun J.S., De Bizemont T., Duval-Valentin G., Montenay-Garestier T., Hélène C., C. R. Acad. Sci. Paris, 313, 585 (1991).
11. Maniatis T., Fritsch E.F., Sambrook J. Molecular cloning, a laboratory manual.NY: Cold Spring Harbor Laboratory Press, (1982) Cold Spring Harbor.
12. Marck C., Thiele D., Nucl. Acids Res, 5, 1017 (1978).
13. Laughton C.A., Neidle S., Nucl. Acids Res., 20, 6553 (1992).

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